clinical investigations

A Possible Association of Chlamydia pneumoniae Infection and Acute Myocardial Infarction in Patients Younger Than 65 Years of Age*

Francesco Blasi, MD, PhD; Roberto Cosentini, MD; Rita Raccanelli, MD; Ferdinando M. Massari, MD; Cristina Arosio, MD; Paolo Tarsia, MD; and Luigi Allegra, MD

Study objectives: We conducted a retrospective study on patients with acute myocardial infarction (AMI) and evaluated the incidence and prevalence of Chlamydia pneumoniae infection. Methods: Sixty-one consecutive patients with AMI aged younger than 65 years were enrolled. Within 24 h of hospital admission, serum samples and pharyngeal swab specimens were obtained from all patients. In 49 of 61 patients, after a mean of 28 days from hospital admission, a second serum sample was drawn. A third serum sample was obtained in 23 of 61 patients. Serologic testing for Chlamydia pneumoniae was performed by a microimmunofluorescence test. We applied a nested-polymerase chain reaction for C pneumoniae DNA detection to pharyngeal swab specimens. Simultaneously, we performed a serologic study for C pneumoniae infection on 61 serum samples obtained from blood donors, matched for age, sex, and smoking habits.

Results: Serologic test results for C pneumoniae were consistent with acute reinfection in 12 patients, with chronic infection in 23 patients, and results were negative in 26 patients with AMI. In 3 of 12 patients with acute reinfection pattern and in 3 of 23 patients with chronic infection pattern, C pneumoniae DNA was detected on pharyngeal swab specimens. A significantly higher prevalence of IgG titers was observed in patients with AMI (35/61) compared to blood donors (18/61) (p=0.003).

Conclusion: Our data confirm the possible role of C pneumoniae infection in coronary heart disease and suggest that reinfection may trigger the onset of AMI.

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Key words: acute myocardial infarction; Chlamydia pneumoniae; polymerase chain reaction; respiratory tract infections

Abbreviations: CHD=coronary heart disease; PCR=polymerase chain reaction; URTI=upper respiratory tract infection

Known risk factors, ie, smoking, hypercholesterolemia, lipoprotein (a) levels, hypertension, etc, explain 50 to 70% of the etiopathogenesis of myocardial infarction. Recent evidence indicates the possible role of viral and bacterial infections in the development of atherosclerosis and their association with myocardial infarction.1-7 Chlamydia pneumoniae, a common intracellular pathogen frequently involved in upper and lower respiratory tract infections, has been recently associated with atherosclerosis and coronary heart disease (CHD).8-10 Seroepidemiologic evidence indicates that most patients with CHD present an anti-C pneumoniae antibody

*From the Institute of Respiratory Diseases (Drs. Blasi, Raccanelli, Arosio, Tarsia, and Allegra), University of Milan, Emergency Medicine Department (Dr. Cosentini), and Department of Cardiology (Dr. Massari), IRCCS Ospedale Maggiore, Milan, Italy.

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Reprint requests: Francesco Blasi, MD, PhD, Istituto di Tistologia e Malattie dell’Apparato Respiratorio, Università degli Studi di Milano, Pad. Litta IRCCS Ospedale Maggiore di Milano, via F. Sforza 35, I-20122 Milano, Italy

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pattern consistent with chronic infection. Furthermore, C pneumoniae has been detected in athrocerotic coronary plaques by several methods, including immunocytochemistry, transmission electron microscopy, and molecular biology techniques.

Recently we detected C pneumoniae DNA in a high percentage of aortic aneurysm plaques. Furthermore, our serologic data support the hypothesis that chronic C pneumoniae antibody pattern may be a possible risk factor marker for atherosclerosis.

In order to clarify the role of C pneumoniae infection in acute myocardial infarction, we conducted a study on patients with acute myocardial infarction and evaluated the incidence and prevalence of C pneumoniae infection.

**Materials and Methods**

Between September 1993 and September 1994, we enrolled 61 consecutive patients aged younger than 65 years (49 men; mean age, 52.8±8 years; age range, 34 to 64 years) with acute myocardial infarction admitted to the coronary unit of our 700-bed primary care hospital. Within 24 h of admission, serum samples and pharyngeal swab specimens were obtained from all patients and frozen at −20°C. After a mean of 28 days from admission, patients were recalled, and in 49 of 61 patients, a second serum sample was drawn. In 23 of 61, it was possible to obtain a third serum sample after 4 more weeks.

For each patient, a serologic test for C pneumoniae was performed using a microimmunofluorescence test for IgG, IgA, and IgM antibodies using a specific antigen (TW-183; Washington Research Foundation, Seattle). Microimmunofluorescence results were classified as follows: past (chronic) infection pattern (>16 IgG <512; >16 IgA <256); acute first infection (IgM ≥16 associated with IgG ≥512; IgA ≥256 or fourfold increase in IgG or IgA titers); and reinfection (IgG ≥512; IgA ≥256; or fourfold increase in IgG or IgA titers). We applied a nested-polymerase chain reaction (PCR) using a nested set of primers designed to detect a fragment of the 16s ribosomal RNA gene of C pneumoniae as reported by Black et al.

Pharyngeal swab specimens were treated with a solution containing Tris-HCl 20 mmol, polysorbate 20 (TWEEN 20; Sigma; St. Louis)-Nonidet P-40 (0.5% [vol/vol] each), and proteinase K 100 μg/mL, and incubated at 60°C for 1 h and at 98°C for 10 min. DNA was extracted twice with phenol/chloroform/isoamyl alcohol and precipitated with sodium acetate-ethanol by standard methods. DNA was amplified in 50-μL volumes containing 200 μmol of each deoxynucleoside triphosphate, 2 μmol of each primer, 1 unit Taq polymerase, 10 mmol Tris-HCl, 2 mmol MgCl2, and 50 mmol KCl.

First amplification was performed in an automated thermocycler (Hybaid Ltd; Teddington, UK) for 35 cycles at 94°C for 1 min (3 min for the first cycle), 45°C for 1 min, and 72°C for 1 min (10 min for the last cycle). Second amplification was performed in the same way starting with 2 μL of first amplification. Amplification products, 858 bp in size, were made visible by electrophoresis in 3% agarose gel containing ethidium bromide, 0.2 μg/mL. To avoid the risk of contamination, tissue preparation, PCR amplification, and electrophoresis were performed in separate rooms. In each assay, a negative and a positive control were run. The negative control contained all of the PCR reagents and sterile distilled water. As positive control, we used C pneumoniae purified elementary bodies at a concentration of 10^{9}/μL.

A systematic evaluation of history for recent respiratory tract infections (within 3 weeks prior to admission) was performed by questioning both the patients and relatives.

Simultaneously, as a normal control group, we analyzed 61 serum samples obtained from blood donors matched for age (±5 years), sex, and smoking habits. The blood samples had been drawn over the same time span covered by the study. In a subgroup (25 subjects), a pharyngeal swab was obtained and examined as described above.

Informed consent was obtained from all subjects prior to admission to the study.

**Results**

A positive history for symptoms indicating recent respiratory tract infections preceding acute myocardial infarction was recorded in 12 of 61 patients. Serologic test results for C pneumoniae were consistent with acute reinfection in 12 patients, with chronic infection in 23 patients, and findings were negative in 26 patients. The distribution of known risk factors in patients with acute myocardial infarction and according to serologic patterns is shown in Table 1 and 2, respectively. The number of smokers was significantly lower among the C pneumoniae-seropositive patients compared to seronegative patients (p=0.017).

In 3 of 12 patients with acute reinfection pattern and in 3 of 23 patients with chronic infection pattern, C pneumoniae DNA was detected on pharyngeal swab specimens. A further positive pharyngeal swab specimen was also found among the 26 seronegative cases in a patient with a positive history for recent upper respiratory tract infection (URTI). Unfortunately a single serum sample was available, thus preventing the detection of a possible seroconversion (Table 3).

A positive history for recent URTI was present in 3 of 12 patients with acute serologic pattern, in 4 of 23 patients with chronic serologic pattern, and in 5 of 26 seronegative patients.

IgM antibodies specific for C pneumoniae were absent in all patients with acute myocardial infarction.

| Table 1—Distribution of Risk Factors in 61 Patients With Acute Myocardial Infarction |
|-----------------------------------------------|-----------------|
| Risk Factors                                   | No. of Patients |
| Smokers                                       | 38              |
| Past smokers                                  | 12              |
| Nonsmokers                                    | 11              |
| Hypercholesterolemia (≥6 mmol/L)              | 15              |
| Hypertension (DAP* ≥95 mm Hg)                 | 19              |
| Smoking+hypercholesterolemia+hypertension     | 6               |

*DAP=diastolic arterial pressure.
The acute infections observed in these patients should therefore be interpreted as reinfections.

A significantly higher IgG seroprevalence was observed in the acute myocardial infarction group (35/61) compared to blood donors (18/61) (p = 0.003).

Conversely, there was no difference in the IgA seroprevalence between these two groups. *C. pneumoniae* DNA detection on pharyngeal swab specimen resulted in negative findings in all tested blood donors.

### CONCLUSION

The study of the pathogenesis of CHD has been expanded recently to include previously unexplored lines of research. It has been suggested that infections may be involved in the development of acute myocardial infarction. Herpes simplex virus, cytomegalovirus, *Helicobacter pylori*, and *C. pneumoniae* have been reported as possible cofactors of atherosclerosis and CHD, although the evidence so far presented is controversial.1-6,17,19-21

Among proposed infective agents, *C. pneumoniae* involvement has been suggested on the basis of seroepidemiologic evidence as well as by detection of the agent on atherosclerotic plaques in arterial specimens by immunocytochemistry and molecular biology.8-15 Recently, *C. pneumoniae* has been isolated by culture from the coronary artery of a patient with coronary atherosclerosis providing direct evidence of the presence of viable organism in atheromatous lesions.22 To our knowledge, no study on the role of *C. pneumoniae* infection in acute myocardial infarction has yet been performed.

The aim of the present study was the evaluation of a possible association between *C. pneumoniae* infection and the onset of acute myocardial infarction. The main finding of the study was a high incidence of acute *C. pneumoniae* infection in patients with acute myocardial infarction. Furthermore, our data confirm a high prevalence of respiratory symptoms consistent with URTI, which occurred in about 20% of our patients, in the 3 weeks prior to admission to hospital. In the 12 patients with serologic tests indicating acute *C. pneumoniae* infection, antibody titers consistent with reinfection23 were observed. In 3 of 12 patients, *C. pneumoniae* was also found in pharyngeal swab specimens by PCR.

These findings add further evidence to the hypothesis that *C. pneumoniae* infection may act as a trigger for acute myocardial infarction. Recent reports have identified this agent in atherosclerotic coronary plaques and have detected immune complexes containing chlamydial lipopolysaccharide in
patients with acute myocardial infarction.\textsuperscript{11, 12} \textit{C. pneumoniae} reinfection could lead to instability within atherosclerotic plaques via a reactivation of a chronic or latent infection and/or immune-mediated endothelial damage. The demonstration of an association between \textit{C. pneumoniae} reinfection and acute myocardial infarction is a new aspect that may be linked to the previously suggested role of chronic infection in the pathogenesis of atherosclerosis. The importance of chronic \textit{C. pneumoniae} infection in CHD is also confirmed by the high seroprevalence observed in our patients. The detection of \textit{C. pneumoniae} on swab specimens in 3 of 23 patients with chronic serologic pattern is further evidence of the possible persistence of this pathogen in CHD patients.

It has been suggested previously that smoking is a potential confounder of the \textit{C. pneumoniae}-CHD association.\textsuperscript{19} However, considering that we found a higher number of smokers among seronegative acute myocardial infarction patients compared to seropositive patients, smoking status does not seem to have influenced our results.

The hypothesis of a possible role for chronic \textit{C. pneumoniae} infection in the pathogenesis of CHD finds further proof in the results of the present study. Furthermore, our data suggest that an acute infection superimposed on a chronic or latent infection (reinfection) may trigger the onset of acute myocardial infarction.

**References**