**Chlamydia pneumoniae** Detection in Atherosclerotic Plaques in Italy

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Italian investigations have shown an association between *Chlamydia pneumoniae* infection and atherosclerosis. With the use of several diagnostic techniques, including serology, a microimmunofluorescence test, and nucleic acid amplification methods, a temporal association was found between acute *C. pneumoniae* reinfection and acute myocardial infarction, suggesting that an acute infection superimposed on a chronic or latent infection may trigger the onset of acute myocardial infarction. *C. pneumoniae* but not *Helicobacter pylori* or *Mycoplasma pneumoniae* was found in atherosclerotic plaques of abdominal aortic aneurysms and the carotid artery. A reverse transcriptase–polymerase chain reaction process confirmed the presence of viable *C. pneumoniae* in carotid atheromas. Nucleic amplification of peripheral blood mononuclear cells may enable the identification of subjects carrying *C. pneumoniae* in the vascular wall. Macrolide treatment reduced fibrinogen and C-reactive protein plasma levels and *C. pneumoniae* burden in patients with atherosclerotic diseases.

Methods

In all studies described, each patient had a serologic microimmunofluorescence (MIF) test for *C. pneumoniae* for IgG, IgM, and IgA. MIF results were classified as follows: past (chronic infection) pattern (IgG ≥16 and <512; IgA, ≥16 and <256), acute first infection (IgM ≥16 associated with IgG ≥512, IgA ≥256, or four-fold increase in IgG or IgA titers), and reinfection (IgG ≥512, IgA ≥256, or four-fold increase in IgG or IgA titers).

Different PCR techniques were used for detection of *C. pneumoniae* DNA in the studies. We used a nested PCR on pharyngeal swab specimens with a nested set of primers designed to detect a fragment of the 16s RNA gene of *C. pneumoniae* [3] in the studies on acute myocardial infarction [4] and aortic abdominal aneurysms [5]. In order to evaluate the possible presence of viable *C. pneumoniae* in atheromas [6], we applied two different molecular biology techniques with a PCR technique [7] that allowed the detection of ~10–100 elementary bodies and a reverse transcriptase (RT) PCR with the PCR primers designed to detect a fragment of the 16s RNA gene of *C. pneumoniae* [3]. The PCR technique described by Campbell et al. [7] was also applied in a treatment study on eradication of *C. pneumoniae* from carotid plaques [8].

In another study [9], *C. pneumoniae* DNA was detected by a touchdown nested PCR, the most sensitive PCR technique, which allowed the detection of ~1–5 elementary bodies [10]. For *Helicobacter pylori* DNA detection, we used a nested PCR that allowed the detection of ~5 *H. pylori* [11], and for *Mycoplasma pneumoniae* DNA detection we used a PCR technique that allowed the detection of ≤10 bacteria [12].

Results

**Study 1: acute myocardial infarction** [4]. We evaluated the possible association between *C. pneumoniae* infection and the onset of acute myocardial infarction in 61 patients ≤65 years old (49 men, 21 women; mean ages, 52.8 ± 8 years [range, 34–64]) admitted to the coronary unit with acute myocardial infarction. In this study, a positive history for symptoms indicating recent respiratory tract infections preceding acute myocardial infarction was recorded in 12 of 61 patients. Serologic
test results were consistent with *C. pneumoniae* acute reinfection in 12 patients and with chronic infection in 23 but were negative in 26 patients. *C. pneumoniae* was also identified by PCR in 3 of 12 patients with acute reinfection pattern and in 3 of 23 with chronic infection pattern. A significantly higher IgG seroprevalence was observed in the acute myocardial infarction group (35/61) compared with blood donors (18/61) matched for age (± 5 years), sex, and smoking habits. No difference was found in the IgA seroprevalence, and no *C. pneumoniae* DNA was detected on blood donor pharyngeal swab specimens. This study found further proof of the possible involvement of *C. pneumoniae* in coronary heart disease and suggested that an acute infection superimposed on a chronic or latent infection (reinfection) may trigger the onset of acute myocardial infarction.

**Study 2: atherosclerotic plaques of aortic aneurysms**[5]. The presence of *C. pneumoniae* but not *H. pylori* in atherosclerotic plaques of aortic aneurysms was demonstrated in another study by our group. *C. pneumoniae* DNA was detected by PCR[4] in 26 aortic plaque samples (51%) from 51 patients but not in lesion-free arterial specimens. Most patients (23/26) with PCR-positive plaques showed a *C. pneumoniae* antibody pattern of past or chronic infection, 2 had high titers, and 1 was seronegative. Among the 25 PCR-negative subjects, 9 were seronegative, 7 had high antibody titers, and 9 had antibody patterns indicating past or chronic infection. These findings suggested a possible role for *C. pneumoniae* chronic infection and the development of aneurysmal lesions and ruled out the possibility of a direct involvement of *H. pylori* infection in the pathogenesis of atherosclerosis.

**Study 3: atherosclerotic plaques of carotid arteries**[6]. We analyzed samples from 30 patients who underwent surgery for removal of atherosclerotic plaques from carotid arteries. During surgery, samples were obtained of carotid plaques, lingual veins, and superior thyroidal artery branches. We applied two molecular biology techniques to the samples: PCR (DNA detection) and RT-PCR (mRNA detection). The study confirmed the presence of viable (RT-PCR-positive) *C. pneumoniae* in atherosclerosis 10 of 30 carotid plaque specimens and showed good correlation between PCR and RT-PCR findings. Moreover, there was fairly good correlation between PCR and serology results. All PCR and RT-PCR results were negative on lingual vein and thyroidal artery samples.

**Study 4: *C. pneumoniae* DNA detection in peripheral blood mononuclear cells (PBMC)**[9]. This study was designed to define a method for the identification of chronic *C. pneumoniae* carriers. Serology, using MIF, is probably the diagnostic reference standard of the acute infection, but its inability to distinguish between chronic and prior infection precludes its use for identifying subjects with ongoing infection. Our data indicate the possible use of *C. pneumoniae* DNA detection in PBMC as a potential marker of chronic infection in persons with cardiovascular diseases[9]. Abdominal aortic aneurysm tissue and PBMC of 41 consecutive subjects undergoing abdominal aortic aneurysm surgery were analyzed by PCR for the presence of *C. pneumoniae*, *M. pneumoniae*, and *H. pylori* DNA. In no case was evidence of *H. pylori* or *M. pneumoniae* DNA found, thus adding further negative evidence to the possibility of direct involvement of these organisms in cardiovascular diseases. Similar numbers of patients had *C. pneumoniae* DNA detected in artery specimens and PBMC (17 of 41 and 19 of 41, respectively). Overall 20 patients (49%) were *C. pneumoniae* DNA positive, 16 (39%) in both PBMC and vascular tissue, 3 (7.3%) in PBMC only, and 1 (2.4%) in the artery specimen only (table 1). These results suggest that PCR of PBMC may be a good way to identify persons who carry *C. pneumoniae* in the vascular wall.

**Treatment studies**[8, 13]. We also performed two small treatment studies. The first was designed to evaluate changes in fibrinogen and C-reactive protein in 84 patients with ischemic heart diseases and *H. pylori* and/or *C. pneumoniae* seropositivity who were randomly assigned to antibiotic treatment or no treatment[13].

Fibrinogen and C-reactive protein plasma levels are markers of activation of inflammation and are strongly implicated in coronary heart disease. Treatment consisted of omeprazole, clarithromycin (500 mg twice a day for 14 days), and tinidazole in *H. pylori*-positive patients and clarithromycin alone in *C. pneumoniae*-positive patients. The effect of treatment and other baseline variables on inflammatory parameters levels, determined after 6 months, was evaluated by multivariate analysis. The main results of the study showed that treatment significantly reduced fibrinogen and C-reactive protein levels in ischemic heart disease patients and that this reduction was detectable 6 months after treatment. Of interest, patients seropositive for both organisms had the greatest reduction of fibrinogen levels (about 20%).

The second treatment study was designed to determine the effect of specific antibiotic treatment with roxithromycin (150 mg twice a day) in the eradication of *C. pneumoniae* from carotid artery plaques[8]. We analyzed 32 patients who underwent carotid endarterectomy. Patients were randomized to receive roxithromycin before surgery or no treatment. Sixteen were treated with antibiotic a mean of 26 days (range, 17–35). *C. pneumoniae* DNA was detected significantly more often among the nontreated patients than in subjects treated with roxith-

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<th>Table 1. <em>Chlamydia pneumoniae</em> serology and polymerase chain reaction (PCR) results in the 41 patients studied.</th>
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NOTE. –, minus; +, positive; PBMC, peripheral blood mononuclear cells.
bacterial burden of vascular lesions. Antibiotic treatment is effective in reducing the also indicate that bacterial colonization does not initiate vas-
ports the specificity of detection in atheromas, although it could
ure to detect in the development of vascular atherosclerotic lesions. The fail-
plaques may be taken to indicate that the organism plays a role
infection. In conclusion, even if an etiologic role of C. pneumoniae infection in atherosclerosis has not yet been firmly established, an infectious basis of atherosclerosis seems plausible, and C. pneumoniae is one of the most important candidates for the initiation or modulation of atherogenesis.

Discussion

Analysis of the studies described suggests that C. pneumoniae was identified in roughly 50% of atheromatous lesions. The interpretation of these findings may be different, as the organism may play a role in the development of vascular atherosclerotic lesions or may simply act as an innocent bystander or colonizer within previously existing atherosclerotic plaques. Our data show that atherosclerotic plaques may be colonized by viable C. pneumoniae, reinfection seems to be associated with acute myocardial infarction, possibly disturbing the balance in the plaque and activating C. pneumoniae, and finally that antibiotic treatment with a macrolide is associated with a reduced burden of bacteria interacting with some emerging risk factors such as fibrinogen and C-reactive protein.

The presence of viable C. pneumoniae within atherosclerotic plaques may be taken to indicate that the organism plays a role in the development of vascular atherosclerotic lesions. The failure to detect C. pneumoniae in atherosclerosis-free tissue supports the specificity of detection in atheromas, although it could also indicate that bacterial colonization does not initiate vascular lesions. Antibiotic treatment is effective in reducing the bacterial burden of C. pneumoniae within atherosclerotic plaques and induces a reduction of plasma levels of some of the most important risk factors for chronic heart disease (e.g., fibrinogen and C-reactive protein). We suggest that PCR of PBMC be considered the new reference standard for the diagnosis of chronic C. pneumoniae infection.

References