Seroprevalence of *Chlamydophila pneumoniae* in ischaemic heart disease

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SUMMARY

We investigated the presence of *Chlamydophila pneumoniae* antibodies in 125 patients with cardiovascular disease and in 128 controls. *C. pneumoniae* antibodies were measured by microimmunofluorescence assay. A significantly high prevalence of IgG *C. pneumoniae* antibodies at titre ≥8 was found in patients (84%) in comparison to controls (47.6%). Considering as cut-off the IgG titre ≥32, 52% of patients with coronaropathies and 18.75% of controls resulted positive ($p < 0.0001$). IgA *C. pneumoniae* antibodies were found in patients and controls without statistically significant differences. High *C. pneumoniae* antibodies (titre ≥256) were found in 11% of patients with acute myocardial infarction (AMI) and in none of the controls. In patients, the percentage of IgG and IgA seropositivity increased with age and decreased in patients aged >70 years. Only patients with AMI are at risk of having antibodies against *C. pneumoniae* (OR=6.69). None of the known risk factors for cardiovascular disease was significantly associated with *C. pneumoniae* seropositivity IgG. This is the first report in our area on the possible association of *C. pneumoniae* IgG seropositivity and acute ischemic events.

KEY WORDS: Heart disease, *C. pneumoniae*, Serology

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INTRODUCTION

A number of well known risk factors have been discovered which enhance the rate of progression of atherosclerosis lesions and increase the frequency of its sequelae, such as myocardial infarction. However, these risk factors account for only about 50% of atherosclerosis (Dugan *et al.,* 2002). Recently elevated markers of acute-phase reactants of inflammation were observed in patients with acute myocardial infarction (AMI) (Ridker *et al.*, 1997). In recent years the possibility that infectious agents might play a role in the association between chronic inflammation and atherosclerosis has received increasing attention (Danesh *et al.*, 1997).

Several infectious agents have been investigated as possible stimuli for triggering chronic inflammation and atherosclerosis including Cytomegalovirus and *Helicobacter pylori* (Muhlestein *et al.*, 1998; Siscovick *et al.,* 2000). These studies have produced conflicting results (Zhu *et al.,* 2002). In particular, a growing body of evidence has linked chronic *C. pneumoniae* infection with an increased risk of atherosclerosis, chronic coronary heart disease, and acute myocardial infarction (Campbell *et al.,* 1998; Arcari *et al.,* 2005). The role of *C. pneumoniae* in vascular disease stems from seroepidemiologic studies, detection by isolation or molecular methods of the microorganism in atherosclerotic plaque and animal model experiments (Boman *et al.,* 2002). In addition, the role of *C. pneumoniae* is also supported by the beneficial effects of antibiotic therapy with macrolides on future cardiovascular events (O'Connor *et al.,* 2003; Jespersen *et al.*,

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2006). However, conflicting results are also reported for *C. pneumoniae,* (Nobel *et al.,* 1999; Altman *et al.,* 1999; Ridker *et al.,* 1999; Hoffmeister *et al.,* 2000). In Italy few studies have dealt with the possible association between *C. pneumoniae*, atherosclerosis and cardiovascular disease (Mazzoli *et al.,* 1998; Sessa *et al.,* 2001; Ciervo *et al.,* 2002). The current study was undertaken to investigate the possible association between infection with *C. pneumoniae*, as determined by the presence of specific serum antibodies, and coronary heart diseases.

MATERIALS AND METHODS

Study populations

A total of 125 (40 males and 85 females, mean age 59 years ±10 years) consecutive patients admitted to the Coronary Care Unit (CCU) of the University of Foggia, Southern Italy were investigated. Ninety-nine of these (32 men and 67 women, mean age 66±14 years) suffering from AMI, 19 patients (7 men and 12 women, mean age 59±11 years) had proven unstable angina and 7 further patients (1 man and 6 women, mean age 66±10 years) had a recurrent-AMI (reAMI). Patients with coronaropathies were enrolled within 24 h from the onset of symptoms. Diagnosis of AMI was made on the basis of alteration of the ST segment and specific enzymatic variation suggesting cardiac necrosis; unstable angina was diagnosed on the basis of occlusion of 70% of at least one vessel at coronary angiography.

One hundred and twenty-eight voluntary blood donors (mean age of 51±6 years) from the blood bank of the same hospital served as controls. They were matched for age and sex to patients with coronaropathies. Sera were stored at -20°C until tested. Informed consent was obtained from all participants. A standardized questionnaire was applied to all patients with coronary diseases which included a history of cardiovascular risk factors and heart disease, previous antibiotic therapy, alcohol intake, smoking, history of diabetes, family history of coronary heart diseases, history of hypertension and body mass index.

Laboratory analysis

C-reactive protein (CRP), fibrinogen, erythrosedimentation rate, and blood glucose were determined by standard methods. Serum levels of triglycerides, serum total high density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were measured by enzymatic methods.

Serology

Serum IgM, IgG, and IgA class antibodies to *C. pneumoniae* were determined by microimmunofluorescence as previously described (Maggi *et al*. 2006). Briefly formalinized, purified *C. pneumoniae* elementary bodies were used (AR39 strain, distributed by the University of Washington, Washington, USA). AR 39 appears to be the strain most frequently involved in atherosclerosis (Movahed, 1999). Sera were tested for IgG, IgM, and IgA antibodies starting from a dilution of 1:8. Sera positive in screening tests were tested in two fold dilutions. Sera resulted positive for *C.pneumoniae* were also tested for antibodies to *C. trachomatis* and *C. psittaci*. All sera were tested in a blind fashion by two experienced investigators.

Statistical analysis

Data are summarized as count and percentage. Differences between independent groups were analyzed by chi-square test or Fisher exact test as appropriate; chi-square for trend was performed to evaluate trend in percentage. P-values for comparison in subgroups were adjusted by means of permutational test performed in the MULTTEST procedure of SAS STAT.

The evaluation of risk factor for antibody titre values >8, used as a dependent variable, was performed by logistic regression, whose independent variables were sex, age class and disease. All analyses were conducted with SAS 9.1 software for PC. Differences were considered as statistically significant for $p<0.05$.

RESULTS

A high overall prevalence of IgG *C. pneumoniae* antibodies (titre ≥8) was found in patients in comparison to controls (IgG: 84% vs 47.6%, $p<0.0001$). IgA were found at titre ≥8 in both patients (9.6%) and controls (11.7%) ($p> 0.73$).

IgG antibodies against *C. pneumoniae* were found at titre ≥1:32 in 52% (65/125) of patients with

coronary artery diseases and in 18.75% (24/128) of controls (p<0.0001). In addition, high IgG *C. pneumoniae* antibody titre ≥256 was found in 11% of patients with AMI and in none of the controls. IgA antibodies against *C. pneumoniae* were detected at titre ≥1: 16 in 9.6% of patients and in 11.7% of controls without statistically significant differences.

None of the subjects had IgM antibodies to *C. pneumoniae*. All sera positive for antibodies to *C. pneumoniae* were negative for antibodies to *C. trachomatis* and *C. psittaci*. Furthermore, IgG at titre ≥1: 32 were found in 53/99 (53.5%) of patients with AMI, in 7/19 (36.8%) of the patients with angina, in 5/7 (71.4%) of patients with reAMI and in 24/128 (18.7%) of controls. None of the comparisons among pair groups resulted statistically significant.

IgA at titre ≥16 were found in 10/99 (10.1%) of patients with AMI, in 2/19 (10.5%) of patients with angina, in no patients with reAMI and in 12/128 (9.3%) of controls without statistically significant differences among pair groups. Table 1 shows the results of the serological investigations in the subjects studied.

There was no evidence of association of IgG *C. pneumoniae* seropositivity with sex either among the three groups of cardiovascular diseases considered or between patients and controls.

A statistically significant association between IgA and sex was found (χ^2_{MH}=5.9763, p=0.0145) in AMI patients in which antibodies to *C. pneumoniae* were present more in the males compared to females (21.9% vs 4.5%, respectively, p=0.007).

In the group of patients admitted to CCU the percentage of IgG seropositivity increased with age in the age group 50-70 years and decreased in the >70 years group (χ^2=11.25, p=0.0238; test for trend p=0.02). A similar trend was also observed for IgA seropositivity but it was not statistically significant (χ^2=5.314; p=0.204).

In addition, none of other risk factors (high level of BMI, HDL, LDL, PCR, fibrinogen, VES, familiarity and diabetes) investigated in the present study were significantly associated with *C. pneumoniae* IgG seropositivity (titre ≥8). Taking the titre ≥32 as cut-off, the same result was observed with only age as a statistically significant associated factor (χ^2=25.6463). Sex (p=0.0128, χ^2=6.197), hypertension (p=0.041) and fibrinogen levels (p=0.0328) were the only statistically significant factors associated with IgA positivity at titre ≥8. Presence of IgA (titre ≥16) was not significantly associated with sex (χ^2=4.6102; p=0.0318) or hypertension (p=0.0173).

In addition, no significant correlation between the presence of IgG and IgA antibody at titre ≥16 and a history of previous respiratory tract infections and previous use of antibiotics such as macrolides or fluoroquinolones was observed.

The logistic regression model to evaluate risk of IgG positivity (titre ≥8) adjusted for age and sex, gave a high odds ratio for AMI patients with respect to controls (OR=6.69; CI95% 3.057-14.64). For IgA seropositivity, only sex was a significant variable with OR=2.675 (CI95% 1.146-6.245), without an increased risk for each category of disease with respect to controls.

No other factors resulted statistically significant in the logistic model. Only age (χ^2=21.634;

TABLE 1 - *IgG antibody titres against C. pneumoniae based on microimmunofluorescence (MIF) in patients and controls.*

		IgG TITRES ≥8 %								*IgA TITRES ≥8 %*				
Group	*N*	*<8*	*8*	*16*	*32*	*64*	*128*	*256*	*512*	*<8*	*8*	*16*	*32*	*64*
AMI	99	10.5	0.8	25.6	18.4	11.2	4	7.2	1.6	71.2	0	2.4	3.2	0
reAMI	7	1.6	0	0	2.4	0	0.8	0.8	0	5.6	0	0	0	2.4
Angina	19	4	0	5.6	4	0.8	0	0.8	0	13.6	0	1.6	0	0
Controls	128	52.3	0.8	28.1	13.3	3.9	1.6	0	0	88.3	0	7.1	2.3	2.3

AMI: acute myocardial infarction; reAMI: recurrent acute myocardial infarction; N: number.

p=0.0002) was a significant factor correlated to the presence of *C. pneumoniae* IgG antibodies.

DISCUSSION

C. pneumoniae is responsible for respiratory tract infections and may play a co-factor role in the pathogenesis of atherosclerosis. The first report of a possible association between *C. pneumoniae* and atherosclerosis was described in 1988. Elevated IgG and IgA antibody titres to *C. pneumoniae* were found in 68% of the AMI patients (Saikku *et al.,* 1988).

A review of 18 published epidemiological studies including 2700 cases suggests an association between *C. pneumoniae* antibodies and coronary heart disease, even if the studies were rather heterogeneous, depending also on the assay applied (EIA or MIF) or on the antibody titre considered as cut-off (Danesh *et al.,* 1997). More recently, seropositivity for *C. pneumoniae* was associated with an increased risk for a future cardiovascular event (Fagerberg *et al.,* 1999). Other studies have not confirmed this association (Nobel *et al.,* 1999; Altman *et al.,* 1999; Ridker *et al.,* 1999; Hoffmeister *et al.,* 2000). Several studies have also been performed in Italy. Like our data, a significantly higher IgG but not IgA seroprevalence was observed in AMI patients compared to controls (57.4% and 29.5%, respectively) (Blasi *et al.,* 1997). The same authors demonstrated that atherosclerotic plaques may be colonized by viable *C. pneumoniae* (Blasi *et al.,* 2000). IgG *C. pneumoniae* seropositivity (IgG ≥32) was found in 58.3% of AMI patients and in 42.8% of patients with chronic ischemic heart disease compared to 38% of controls. This was also true for *C. pneumoniae* IgA (Varveri *et al.,* 1998).

High levels of antibodies to OMP-2 of *C. pneumoniae* were observed in patients with coronary artery diseases and this was well correlated to results of IgG anti *C. pneumoniae* determined by MIF (Ciervo *et al.,* 2002). *C. pneumoniae* DNA was detected in the peripheral blood mononuclear cells of 25.8% of the patients with acute ischemic heart disease and in 4.8% of the healthy subjects. IgG were found in 76.3% of patients and 33.3% of controls and this was true also for IgA (Sessa *et al.,* 1999; Sessa *et al.*, 2001). A high prevalence rate of high titres of IgG and IgA anti *C. pneumoniae* antibodies in AMI patients has also been demonstrated (Mazzoli *et al.,* 1998).

Our study is the first report, in our area, on the possible association of *C. pneumoniae* seropositivity and acute ischemic events and our results are in agreement with those reported in other Italian studies. Whether macrolide treatment that has antiinflammatory properties and antibacterial activity against *C. pneumoniae* may be used to reduce the risk of adverse cardiac outcome in *C. pneumoniae* seropositive patients remains to be established.

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