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Chlamydia pneumoniae antibodies and angiographically demonstrated coronary artery disease in a sample population from Italy

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Abstract

Recent reports suggest an association between *Chlamydia pneumoniae* and chronic coronary heart disease. This case-control study investigates the relationship between the presence of immunoglobin G (IgG) and immunoglobin A (IgA) when measured by means of microimmunofluorescence (MIF) and angiographically diagnosed coronary disease. Cases ($n = 150$) were angiography patients with at least one coronary artery lesion occupying at least 50% of the luminal diameter. Controls ($n = 49$) were angiography patients with no detectable signs of coronary artery disease and patients ($n = 56$) without signs or symptoms of coronary disease and with normal ECG results. No significant differences were revealed between the seroprevalence of IgG and IgA and geometric mean titers (GMT) as measured in cases and controls. When cases were compared with controls whose angiographic results were normal, after adjusting for established risk factors (cholesterol, smoking, hypertension, diabetes, age, gender and family history), the estimated risk of coronary artery disease was 0.79 (95% confidence interval (C.I.), 0.31–1.99) for the presence of IgG and was 0.94 (95% C.I., 0.37–2.39) for IgA. When cases were compared with controls with normal ECG results, the adjusted odds ratio (O.R.) for coronary artery disease was 1.17 (95% C.I., 0.52–2.62) for the presence of IgG and 0.82 (95% C.I., 0.36–1.86) for the presence of IgA. These results do not support an association between *C. pneumoniae* infection and coronary disease.

Keywords: *Chlamydia pneumoniae*; Coronary disease; Coronary angiography; Microimmunofluorescence

1. Introduction

Coronary heart disease represents the principal cause of premature death among the male population of industrialised countries [1]. Atherosclerosis (ATS) is considered to be the most frequent pathologic basis for coronary disease [1,2]. Although numerous independent risk factors for ATS have been identified (advanced age, male gender, smoking, high serum cholesterol levels, hypertension, diabetes mellitus) [3,4], these factors account for the majority, but not for the entirety of observed cases. The incidence of coronary disease varies greatly among the various regions of the world. Genetically based factors have therefore been suggested to explain such variations of incidence among ethnic groups [1]. The mechanisms responsible for the onset and progression of the disease are as yet unknown. During the last two decades, infectious agents have been cited as potential cardiovascular pathogens. Numerous publications have suggested that viral (enterovirus, herpesvirus) and bacterial (*Helicobacter pylori*) agents may play a role in the development of ATS or in the activation of atherosclerotic plaques [1,3,5,6]. Recently, on the basis of results achieved through studies in seroepidemiology, ultrastructural

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Table 1
Prevalence and geometric mean titers (GMT) of immunoglobin G (IgG) and immunoglobin A (IgA) *Chlamydia pneumoniae* antibodies in subjects with angiographically demonstrated coronary stenosis and in controls

C. pneumoniae antibodies	Cases		Controls	
	Stenosis $\geq 50\%$ $n = 150$	Stenosis $< 50\%$ $n = 31$	Normal angiography $n = 49$	Negative history, normal ECG $n = 56$
IgG $\geq 1/32$	80 (53.3%)	18 (58.1%)	21 (42.9%)	28 (50%)
GMT	33.98	32.72	28.17	32.00
IgA $\geq 1/8$	70 (46.7%)	13 (41.9%)	16 (32.6%)	27 (48.2%)
GMT	9.76	7.65	6.85	8.94

analysis, immunocytochemistry, molecular biology and tissue culture, an association between *Chlamydia pneumoniae* (a newly identified respiratory pathogen) [7–9] and coronary disease has been proposed [1–7,10–17].

Investigation of this association has been performed mostly in the US and Northern Europe, while contributions from Mediterranean countries have been scarce [8]. Furthermore, although a considerable amount of seroepidemiological research has been carried out, it has only rarely involved angiographically demonstrated cases [4,15].

The objective is to evaluate the association between immunoglobin G (IgG) and immunoglobin A (IgA) anti-*C. pneumoniae* antibodies and angiographically demonstrated coronary disease by means of a case-control study.

2. Materials and methods

Testing was performed on a total of 286 subjects to determine the presence of *C. pneumoniae* antibodies. Of these, 230 underwent coronary angiography (primarily for angina and myocardial infarction, but also for stress dyspnea, ECG abnormalities and valve pathologies) at the Hemodynamics Unit of the Sienese Hospital Service from June 1995 to April 1997. Of those examined, 150 subjects (138 men with a mean age of 55.28 and 12 women with a mean age of 59.41) displayed a stenotic lesion $\geq 50\%$ of at least one principal coronary artery (anterior interventricular, circumflex, right). These patients formed one group of cases. A second group consisted of 31 subjects (24 men with a mean age of 56.12 and 7 women with a mean age of 58.85) with a stenotic lesion of at least one coronary artery $< 50\%$ (non-critical stenosis). A first control group was formed of 49 subjects (22 men with a mean age of 47.95 and 27 women with a mean age of 52.62) with no angiographic evidence of coronary lesions. A second control group consisted of 56 subjects (40 men with a mean age of 53.9 and 16 women with a mean age of 52.8) who did not undergo coronary angiography, had no history of heart disease and presented normal ECG. These were recruited during the same period from patients undergoing minor surgery or general check-ups. None of these patients suffered from chronic illnesses or recent or present respiratory infections.

Sera was assayed for class IgG and IgA *C. pneumoniae* antibodies by means of indirect microimmunofluorescence (MIF) (*C. pneumoniae* IgG/IgM; *C. pneumoniae* IgA Micro-IF test kit-Labsystems). Sera with titers of $\geq 1{:}32$ for IgG and $\geq 1{:}8$ for IgA were considered to be positive.

3. Statistical analysis

The seroprevalence of IgG and IgA *C. pneumoniae* antibodies was compared by means of Fisher's χ^2 test.

Geometric mean titers (GMT) were compared using the Kruskall–Wallis test.

The estimated relative risk of coronary disease for subjects displaying antibody, with respect to seronegative, was evaluated by calculating the adjusted odds ratio (O.R.) for the principal risk factors involved (gender, age, family medical history, cholesterol, diabetes, hypertension, smoking, alcohol consumption) by means of logistic regression.

Statistical analysis was performed using the following software: StatVieu (Abacus Concepts, Berkeley, CA, 1992); Statistica (StatSoft, Tulsa, OK, 1994) and Excell 5.01 (Microsoft Corporation, Cambridge, MA, 1994).

4. Results

The prevalence of IgG *C. pneumoniae* antibodies ($\geq 1/32$) was 53.3% among the cases with stenotic lesions of $\geq 50\%$ (Table 1) and 58.1% among the patients with non-critical stenosis. In the control group displaying normal angiographic results the seroprevalence was 42.9%, while it was 50% in the control group with no medical history of coronary disease and normal ECG (Table 1).The IgG *C. pneumoniae* GMT were 33.98 and 32.72, respectively, for the cases and 28.17 and 32.00 for the controls (Table 1).

Table 2
Estimated relative risk of coronary disease compared with immunoglobin G (IgG) and immunoglobin A (IgA) *Chlamydia pneumoniae* antibodies titers (in cases versus controls with normal angiography)

		Cases	Controls	Adjusted O.R.	95% C.I.
		Stenosis ≥ 50% $n = 150$	Normal angiography $n = 49$		
IgG titer	<1:32	70	28		
	≥1.32	80	21	0.79	0.31–1.99
	1:32	25	8	0.45	0.10–2.04
	≥1:64	55	13	0.75	0.27–2.11
IgA titer	<1:8	80	33		
	≥1:8	70	16	0.94	0.37–2.39
	1.8–1.32	46	12	1.04	0.36–3.04
	≥1:64	24	4	0.71	0.15–3.42

The IgA seroprevalence values were 46.7 and 41.9% in the two groups of cases, while in the control groups they proved to be respectively 32.6 and 48.2% (Table 1). The IgA *C. pneumoniae* GMT were 9.76 and 7.65 in the cases and 6.85 and 8.94 in the controls (Table 1).

No statistically significant differences were detected between cases and controls in all the studied groups.

After having standardised for the principal known risk factors (gender, age, positive family history, blood cholesterol, diabetes, hypertension, smoking, alcohol consumption) by means of logistic regression, the estimated relative risk of coronary disease (Table 2) for subjects with IgG class antibodies, with respect to seronegative, was 0.79. No significant increase in the relative risk of coronary disease was observed to correspond with increasing antibody levels (Table 2).

The estimated relative risk of coronary disease for subjects with class IgA antibodies, with respect to seronegative (after having standardised for the principal known risk factors by means of logistic regression), was found to be 0.94 (Table 2). No significant increase in the relative risk of coronary disease was found to associate with the increase in IgA *C. pneumoniae* titers (Table 2).

Furthermore, no significant association between class IgG and IgA *C. pneumoniae* antibodies and the relative risk of coronary disease was observed when the cases were compared with the controls having normal ECG and no history of coronary illness (Table 3).

5. Discussion

Serological analysis of the cases subjected to coronary angiography revealed a slightly higher level of seroprevalence and IgG antibodies to *C. pneumoniae* GMT in the cases than in the controls, but this difference was not statistically significant.

The estimated relative risk of coronary disease for the subjects with IgG antibodies, when compared to seronegative, was 0.79 (95% confidence interval (C.I.) = 0.31–1.99). No significant increase in the relative risk of coronary disease was found to correspond to higher antibody titer levels.

After having assayed the sera for IgA, thought to be a more reliable marker of chronic infection than IgG, an analysis of the cases and controls revealed no significant differences. The estimated relative risk of coronary disease displayed no significant association with the presence or the variation of IgA titers.

Comparison of the serological study proved difficult due to the various antibody fractions identified, the application of various seropositivity cut-offs and the different definition criteria in the cases.

The subdivision of the cases into two groups based on the extent of the coronary stenosis (at least 50% of the luminal diameter and < 50%) was conducted in order to conform with the case procedures found in literature [4,15].

Most previously conducted research, however, has been performed on the basis of cases lacking angiographic confirmation, and has demonstrated an association between seropositivity for *C. pneumoniae* and ATS [10,12,13,18]. In fact, research based on angiographically demonstrated cases and controls is quite rare; of the two surveys conducted in Seattle [4,15], only one [15] detected a significant association (adjusted O.R. = 1.6; 95% C.I. = 1.0–2.7). The second [4] revealed an association only when the cases were compared with a control group not subjected to angiography.

It has been suggested that controls with negative angiographic results represent a highly selected group of subjects which may include individuals with mild ATS (coronary angiography is not the gold standard for detecting minor lesions of the wall) [1,4]. The study also included a control group not subjected to angiography which again failed to reveal a significant associa-

Table 3
Estimated relative risk of coronary disease compared with immunoglobin G (IgG) and immunoglobin A (IgA) *Chlamydia pneumoniae* antibody titers (in cases vs controls with no medical history of coronary disease and with normal ECG)

		Cases	Controls	Adjusted O.R.	95% C.I.
		Stenosis ≥ 50% $n = 150$	Negative history, normal ECG $n = 56$		
IgG titer	< 1:32	70	28		
	≥ 1:32	80	28	1.17	0.52–2.62
	1:32	25	15	0.49	0.17–1.41
	≥ 1:64	55	13	0.46	0.15–1.36
IgA titer	< 1:8	80	29		
	≥ 1:8	70	27	0.82	0.36–1.86
	1.8–1.32	46	21	0.51	0.20–1.26
	≥ 1:64	24	6	0.71	0.18–2.76

tion between *C. pneumoniae* antibodies and coronary disease.

The use of elderly control patients or a recent epidemic might void the serological association between *C. pneumoniae* and ATS. However, in the study the mean age of the controls was, if only slightly, lower than that of the cases. Serologic investigation recently conducted in the same geographical area on an open population has demonstrated a seropositivity for *C. pneumoniae* of 53.6% in the 41–60 age group [19]. A serologic survey of all the cases of pneumonia and broncopneumonia admitted to the Institute of Infectious Diseases in Siena from January 1994 to March 1997 does not, however, support the hypothesis of a recent epidemic (data not shown).

Although a minority, a number of published serologic reports are in agreement with the results of the investigation [20,21].

Moreover, other studies have failed to detect a correlation between the presence of *C. pneumoniae* in coronary arteries and specific antibody titers. Some have found these levels to be minimal or absent in subjects presenting atheromae with *C. pneumoniae* and to be high even in cases which tested negatively for this pathogen [20,22]. This has led to speculation that high IgG titers may actually protect the cardiovascular apparatus from infection, in contrast with the original suggested association between *C. pneumoniae* and coronary disease which was based on the presence of high antibody levels in subjects with coronary pathology [20].

The results do not support an association between seropositivity for *C. pneumoniae* and coronary disease, but at the same time cannot exclude the existence of a link between *C. pneumoniae* infection and ATS. Even if the outcome of serological research remains inconclusive greater investigation in Mediterranean countries, which are characterised by reduced mortality due to coronary heart disease, may prove useful.

The limitations of serologic studies lie not only in the difficult task of selecting controls which are guaranteed to be free of ATS, but also in the wide range of antibody responses to *C. pneumoniae*, due to variations in the infective dose, mode of infection, previous exposure or anamnestic responses to other chlamydiae [1]. Furthermore, the presence or absence of antibodies does not represent a foolproof indicator of previous infection. Since case-control studies are cross sectional, they do not document if the infection precedes the onset of ATS.

Direct techniques such as electronic microscopy, immunohistochemistry and polymerase chain reaction (PCR) have revealed the presence of *C. pneumoniae* in coronary, carotid and other arteries in proportions varying from 35 to 100% of observed cases [11,22]. Recently this pathogen has also been isolated in carotid and coronary atheroma cultures, confirming the presence of viable bacteria within the plaques [23,24]. Nevertheless the role of *C. pneumoniae* in the pathogenesis of atheromatous lesions is yet unknown.

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