Chlamydia pneumoniae antibodies are associated with an atherogenic lipid profile

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Abstract

Objective—To determine, within a representative population group of men and women, whether alteration of the lipid profile might underlie the reported association between *Chlamydia pneumoniae* and ischaemic heart disease.

Design and setting—Cross sectional survey in an area with a high incidence of ischaemic heart disease.

Subjects—400 randomly selected participants in the World Health Organisation MONICA project's third population survey in Northern Ireland.

Main outcome measures—Stored sera were examined by microimmunofluorescence for IgG antibodies to *C pneumoniae* at a dilution of 1 in 64. Mean total and high density lipoprotein (HDL) cholesterol were compared between seropositive and seronegative individuals with adjustment for age, measures of socioeconomic status, smoking habit, alcohol consumption, body mass index, and the season during which blood had been taken.

Results—In seropositive men, adjusted mean serum total cholesterol and HDL cholesterol were 0.5 mmol/l (9.2%) higher and 0.11 mmol/l (9.3%) lower, respectively, than in seronegative men. Differences in women did not achieve statistical significance, but both total cholesterol and HDL cholesterol were higher (3.6% and 5.8%, respectively) in seropositive than in seronegative individuals.

Conclusions—There is serological evidence that *C pneumoniae* infection is associated with an atherogenic lipid profile in men. Altered lipid levels may underlie the association between *C pneumoniae* and ischaemic heart disease.

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Keywords: epidemiology; lipids; cholesterol; ischaemic heart disease

Chlamydia pneumoniae is a Gram negative obligate intracellular bacterium first described in 1986.¹ It is principally a respiratory pathogen and is a common cause of community acquired pneumonia,² but the widespread prevalence of serological evidence of infection^{3–5} indicates that most *C pneumoniae* infections may be subclinical.⁶ There is a substantial body of evidence relating *C pneumoniae* infection to the development of ischaemic heart disease. The organism has been identified in atheromatous lesions of the coronary arteries and aorta,^{7–8} and with few exceptions⁹ both retrospective^{10–14}

and prospective seroepidemiological studies^{15–16} have shown an association between evidence of *C pneumoniae* infection and ischaemic events. This observational evidence of association is further supported by pilot intervention studies which indicate substantial reductions in secondary ischaemic events in survivors of myocardial infarction treated with antibiotics effective against *C pneumoniae*.^{17–18}

Various mechanisms have been suggested whereby infection with *C pneumoniae* may affect the risk of cardiovascular disease. Animal models have shown that *C pneumoniae* easily gains access to the vascular system following pulmonary infection.¹⁹ Infection of endothelial or smooth muscle cells in vessel walls may occur,²⁰ resulting in local inflammation and fibrosis and subsequent atheroma formation. Alternatively, repeated or persistent *C pneumoniae* infection may stimulate the production of proinflammatory cytokines,²¹ which may increase the risk of cardiovascular disease^{22–23} by disturbing the basal thromboresistant function of vascular endothelial cells,²⁴ inhibiting endothelium derived relaxing factor,^{24–25} or affecting smooth muscle cell contractility.²⁶ Recent research indicates that the risk of cardiovascular disease in *C pneumoniae* infection may be mediated through a simpler process, namely the induction of an atherogenic lipid profile.^{27–28}

We have investigated the relation between total and high density lipoprotein (HDL) cholesterol and evidence of *C pneumoniae* infection in a population representative group of adult men and women from an area with a high incidence of ischaemic heart disease.

Methods

SURVEY METHODS

Subjects were drawn from those recruited in Northern Ireland into the World Health Organisation monitoring of trends and determinants in cardiovascular disease project's third population survey (MONICA 3). This survey employed a single stage random sampling procedure (sampling frame: the general practitioner patient register held by Central Services Agency, Belfast), with stratification by age and sex. Subjects were screened by trained observers adhering to a standard protocol²⁹ between October 1991 and June 1992. Information collected by questionnaire included data on education and socioeconomic status, past medical history, drug treatment, and lifestyle (smoking habit, alcohol consumption, and diet). Height and weight were measured and a blood sample (not fasting) was taken, centrifuged, and separated within four hours. Serum from all subjects was analysed for total

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Table 1 Social class distribution of study subjects and Northern Ireland residents (25 to 64 years) at 1991 census

Social class	Men		Women	
	Study population (%)	Northern Ireland population (%)	Study population (%)	Northern Ireland population (%)
I	8.4	5.1	4.8	1.7
II	17.9	24.7	29.0	26.9
IIINM	24.6	10.3	29.0	30.2
IIIM	29.6	29.7	17.2	5.8
IV	11.7	12.8	13.1	15.3
V	7.8	5.3	6.9	10.3

NM, non-manual; M, manual.

cholesterol (by the CHOD-PAP method) and HDL cholesterol (by precipitation employing phosphotungstic Mg^{2+} reagents). Serum not required for these assays was stored in aliquots at $-70^{\circ}C$.

In this study, sera (thawed on one previous occasion) from 400 randomly selected subjects were analysed for the presence of anti-*C pneumoniae* antibodies. When serum was not available for one of the selected subjects, a replacement was randomly chosen.

LABORATORY METHODS

The MRL Diagnostics *Chlamydia* MIF (micro-immunofluorescence) assay³⁰ was used to detect the presence of IgG antibodies to antigens from *C pneumoniae* (TW183), *C psittaci* (strains 6BC and DD34), and *C trachomatis* (serotypes D–K) at a serum dilution of 1/64. Slides were viewed at a magnification of 400 $\times$ on a Leitz fluorescence microscope within 24 hours by two independent observers. Results were graded on a scale 0, +/-, 1+, 2+, 3+ and 4+ with 0 and +/- interpreted as negative. If the observers disagreed on a result, the opinion of a third observer was sought. Subjects whose sera reacted with *C trachomatis* or *C psittaci* antigens were excluded from the analysis.

STATISTICAL/ANALYTICAL METHODS

Age, total cholesterol, HDL cholesterol, HDL/total cholesterol ratio (expressed as a percentage of total cholesterol), body mass index, systolic blood pressure, and diastolic blood pressure were used in the analyses as continuous untransformed variables. Social class of the head of the household, tenure of accommodation, and highest educational level achieved were used as measures of socioeconomic status. Occupational class was coded according to the OPCS standard occupational classification³¹ and was grouped into manual (IIIM, IV, and V) and non-manual (I, II, and IIINM) classes. Tenure was categorised into rented and owned, and highest educational level achieved categorised into elementary, primary or secondary school only, grammar school only, technical or nursing college, and third level education. Smoking habit was grouped into four categories: current smoker of 20 or more cigarettes a day, current smoker of less than 20 cigarettes a day, ex-smoker, and never smoked. Alcohol intake was categorised into lifetime abstinence, current abstinence, current alcohol intake less than 10 units per week, and current intake of more than 10 units per week. Month of screening was categorised

as winter (November, December, January) or not winter (all other months).

Both bivariable (independent samples *t* test, χ^2 test, and χ^2 test for trend) and multivariable analyses (multiple and logistic regression) were performed using SPSS for Windows. Multiple regression models were constructed with total cholesterol, HDL cholesterol, and HDL/total cholesterol ratio as dependent variables, and *C pneumoniae* status as the independent variable, with covariables age, sex, occupational class, tenure of accommodation, highest educational level achieved, smoking status, alcohol intake, body mass index, and season of screening. Models were constructed for all subjects (with a sex/seropositivity interaction term included) and for men and women separately. These models were also constructed excluding subjects with a previous diagnosis of angina, myocardial infarction, or heart attack, and subjects providing responses to the Rose angina questionnaire in keeping with a diagnosis of definite or possible angina.

Results

In MONICA 3, 565 of 5000 subjects randomly selected from the Central Services Agency register were ineligible because they were deceased, resided outside the MONICA project area (Belfast and surrounding districts), or because their age was outside the specified range (25–64 years). A further 226 were not located after at least two visits to the last known address and reference to other sources. Of 4435 eligible subjects, 2005 participated in the project, giving a response rate of 45.2%. Age and sex were available for all 2430 non-responders, but only 317 (13%) provided information on social class and so on. Overall, in MONICA 3 the male and female non-response rates were very similar—at 54.6% and 54.9%, respectively—and non-response rates were higher in the younger age groups (64.4% and 62.2% in 25–34 year old men and women, respectively, compared with 47.8% and 51.3% in the 55–64 year olds).

This report was based on sera from 393 subjects (199 men and 194 women). Seven of 400 randomly selected sera (52 replacements) were excluded, three and two because of cross reaction with *C psittaci* and *C trachomatis* antigens, respectively, and one sample had been tested twice with inconsistent results; therefore both samples were excluded. The random, sampling procedure used delivered a sample with characteristics (such as mean age, weight, total cholesterol, and so on) closely resembling all participants in MONICA 3.

The social class distribution of study subjects is compared to that of the Northern Ireland population at census in 1991³² (table 1). Professional occupations (both sexes), skilled non-manual occupations (in men) and skilled manual workers (women) were overrepresented in the study.

C. PNEUMONIAE INFECTION, SOCIODEMOGRAPHIC AND LIFESTYLE VARIABLES

Overall, 276 sera (70%) tested positively for anti-*C pneumoniae* IgG. The prevalence of anti-

Table 2 Chlamydia pneumoniae seropositivity and social, biological, and lifestyle risk factors for ischaemic heart disease

		Men		Women	
		Mean (SD) in seronegative men	Mean (SD) in seropositive men	Mean (SD) in seronegative women	Mean (SD) in seropositive women
Age (years)		44.9 (11.5)	45.3 (11.4)	42.6 (12.2)	45.6 (11.1)
Diastolic blood pressure (mm Hg)		83.7 (11.2)	83.5 (11.3)	78.4 (10.2)	78.3 (14.6)
Systolic blood pressure (mm Hg)		135.4 (19.1)	132.3 (18.4)	127.0 (19.3)	127.8 (23.1)
Body mass index (kg/m ²)		26.2 (4.4)	26.2 (3.7)	24.8 (5.0)	25.6 (4.0)
		Number (%) seronegative	Number (%) seropositive	Number (%) seronegative	Number (%) seropositive
Social class	Non-manual	28 (30.8)	63 (69.2)	30 (33.0)	61 (67.0)
	Manual	18 (20.5)	70 (79.5)	18 (33.3)	36 (66.7)
Tenure	Owned	44 (27.2)	118 (72.8)	50 (31.6)	108 (68.4)
	Rented	9 (27.3)	24 (72.7)	10 (38.5)	16 (61.5)
Highest educational level achieved	Primary, elementary or secondary school only	25 (21.0)	94 (79.0)	34 (30.4)	78 (69.6)
	Grammar school	6 (24.0)	19 (76.0)	6 (37.5)	10 (62.5)
	Technical/polytech/nursing college	12 (54.5)	10 (45.5)	13 (34.2)	25 (65.8)
	University	10 (32.3)	21 (67.7)	8 (34.8)	15 (65.2)
Smoking status	Never smoked	21 (28.4)	53 (71.6)	30 (32.6)	62 (67.4)
	Ex-smoker	14 (23.0)	47 (77.0)	16 (35.6)	29 (64.4)
	Current smoker less than 20/day	5 (20.8)	19 (79.2)	12 (34.3)	23 (65.7)
	Current smoker 20 or more/day	14 (35.0)	26 (65.0)	3 (16.7)	15 (83.3)
Alcohol consumption	Lifetime abstainer	6 (46.2)	7 (53.8)	10 (28.6)	25 (71.4)
	Current abstainer	1 (7.1)	13 (92.9)	3 (17.6)	14 (82.4)
	Current drinker < 10 units/week	18 (25.7)	52 (74.3)	36 (33.6)	71 (66.4)
	Current drinker 10 or more	27 (27.3)	72 (72.7)	12 (41.4)	17 (58.6)
Season	Not winter	42 (31.1)	93 (68.9)	42 (35.9)	75 (64.1)
	Winter	12 (18.7)	52 (81.3)	22 (28.6)	55 (71.4)

C. pneumoniae IgG was not related to age, but seropositivity was slightly (although not significantly) more common in men than in women (72.9% *v* 67.0%). Seropositivity was more common in subjects from manual than non-manual social classes, but statistical significance was not achieved for either sex (table 2). There was no association between seropositivity and tenure of accommodation but a significant trend towards lower prevalence of infection with higher level of educational achievement was evident in men (χ^2 5.3, df 1, *p* = 0.02).

C. pneumoniae seropositivity was not related to smoking habit, alcohol consumption, anthropometric measurements (height, weight, and body mass index), or systolic or diastolic blood pressure in either sex. Infection was more common in men screened during winter than in men screened during the rest of the year (81.3% seropositive *v* 68.9%), although this relation did not reach conventional statistical significance (χ^2 3.35, df 1, *p* = 0.07).

C. PNEUMONIAE INFECTION AND LIPIDS

In this dataset, age, living in rented accommodation, and having high body mass index (> 25

kg/m²) were associated with raised total serum cholesterol, while being female, not smoking, and having low body mass index were associated with raised HDL cholesterol.

The unadjusted and adjusted associations between seropositivity for *C. pneumoniae* and lipids are shown in table 3. Total cholesterol was significantly higher and HDL cholesterol and the HDL/total cholesterol ratio significantly lower in seropositive men than in seronegative men. In women, being seropositive was also associated with raised total cholesterol, although the association did not reach levels of conventional statistical significance. Seropositive women tended to have higher, rather than lower, HDL cholesterol and a sex/seropositivity interaction term included in the model for HDL cholesterol involving all subjects achieved statistical significance.

Thirty four subjects (8.6%, 20 male and 14 female) had a history of angina, myocardial infarction, or heart attack or gave responses to the Rose angina questionnaire in keeping with definite or possible angina. Exclusion of these subjects from the regression models made a minimal difference to the adjusted relations

Table 3 Chlamydia seropositivity and lipid levels

	Men				Women			
	Serology status		Mean difference (95% CI)		Serology status		Mean difference (95% CI)	
	Positive	Negative	Unadjusted	Adjusted	Positive	Negative	Unadjusted	Adjusted
Total cholesterol (mmol/l)	5.46 (1.01)	5.86 (0.96)	0.4 (0.09 to 0.72)*	0.5 (0.15 to 0.87)**	5.76 (1.30)	6.00 (1.40)	0.24 (-0.18 to 0.62)	0.21 (-0.17 to 0.59)
HDL cholesterol (mmol/l)	1.18 (0.27)	1.07 (0.38)	-0.11 (-0.20 to -0.02)*	-0.11 (-0.21 to 0)*	1.37 (0.31)	1.39 (0.33)	0.02 (-0.08 to 0.11)	0.08 (-0.02 to 0.18)
HDL/total cholesterol ratio	22.1 (5.7)	18.7 (7.9)	-3.4 (-5.3 to -1.4)**	-3.5 (-5.7 to -1.4)**	25.4 (7.1)	24.3 (8.8)	-1.1 (-3.4 to 1.26)	-0.2 (-2.1 to 2.5)

Values are means (SD) or 95% confidence intervals (CI). Adjustments were made for age (years), sex, manual/non-manual occupational class, tenure of accommodation, highest educational level achieved, smoking habit, alcohol consumption, body mass index, and season of screening.

p* < 0.05; *p* < 0.01.

between seropositivity and lipid levels. The significance, magnitude, and direction of effects were unaltered with one exception. The adjusted mean difference in HDL cholesterol in seropositive compared with seronegative men (-0.11 , 95% confidence interval -0.22 to 0.01), although unaltered in magnitude and direction, no longer achieved conventional statistical significance ($p = 0.07$). The sex/seropositivity interaction term achieved statistical significance when included in the model for HDL cholesterol involving all subjects without symptomatic—or a history of symptomatic—ischaeamic heart disease.

Discussion

In this study we examined the relation between seropositivity for IgG antibodies to *C pneumoniae* and lipid levels. Possession of IgG antibodies to *C pneumoniae* is evidence of exposure to the organism but a single measurement will not distinguish between a primary infection, a repeat infection, or a chronic infection. Reinfection with *C pneumoniae* is common and most acute infections in adulthood are reinfections.³³ Chronicity is a feature of the genus and there is evidence that infection with *C pneumoniae* may persist for many months.³⁴ The presence of IgG antibodies in the sera of subjects in this study (all adults) could therefore be interpreted as evidence of repeat, chronic, or latent infection with *C pneumoniae*, although resolved infection with persistence of IgG cannot be excluded. If the presence of anti-IgG antibodies to *C pneumoniae* merely denotes acute infection, then the relation between *C pneumoniae* and an atherogenic lipid profile found in this study may be entirely the product of an acute phase reaction following the acute infection. If this is the case the disturbance of the lipid profile could be expected to be short lived and with presumably no accompanying increased risk of atherosclerosis. However, it is unlikely that 70% of the subjects studied had a recent infection with *C pneumoniae*, even if it was asymptomatic. It may be more likely that possession of IgG antibody to *C pneumoniae* denotes either previous exposure (with antibody persistence) or chronic or latent infection. If IgG denotes only previous exposure—for example, in childhood, perhaps the relation between evidence of infection and the lipid profile is confounded by an unrecognised factor. But if IgG seropositivity denotes chronic, latent, or even frequent repeat infection, the alteration of the lipid profile we have observed in men may also be chronic and contribute to atherogenesis.

Previous studies showing an association between *C pneumoniae* infection and lipid levels^{27–28} largely ignored the role that confounding may have played in their findings. Several factors may confound this association—for example, low socioeconomic status has been related to both raised total cholesterol^{35–37} and *C pneumoniae* infection,³ although these are not consistent findings.^{12–38–40} Smoking is a recognised risk factor for *C pneumoniae* infection^{40–41} and is associated with low HDL cholesterol.^{42–43} Simi-

larly, alcohol intake^{44–45} and body mass index^{42–46} are strongly associated with HDL cholesterol and although there is no evidence that they are related to *C pneumoniae* infection, it was felt that the epidemiology of the infection was not sufficiently known to allow them to be excluded as potential confounders. Blood pressure is unlikely to confound a relation between seropositivity and lipids, as blood pressure is not known to influence lipid levels directly and, in this study, systolic and diastolic blood pressure were not associated with *C pneumoniae* seropositivity.

The date at which blood was collected may also have confounded the association. Seasonal variation in total cholesterol and HDL cholesterol is a recognised phenomenon, with the total cholesterol peak^{47–49} and HDL cholesterol trough^{50–51} usually occurring during the winter months. Although, seasonal variation in lipid levels was not observed in this study, subjects screened in winter had a higher prevalence of anti-*C pneumoniae* antibodies than those screened during the rest of the year. This is in keeping with the findings of other investigators.¹¹

The relations between *C pneumoniae* and lipid levels were therefore adjusted for available measures of socioeconomic status, smoking habit, alcohol intake, body mass index, and season of blood collection. The effect of the adjustments was to accentuate the relations in men, with adjustment for measures of socioeconomic status contributing most to the increase in strength.

It is theoretically possible that, if *C pneumoniae* infection is related to ischaemic heart disease by a mechanism other than disturbance of the lipid profiles, any disturbance in lipid levels seen in *C pneumoniae* positive individuals may be caused by abnormal lipid profiles occurring in those with ischaemic heart disease. If this were the case then the seropositivity/lipid relations observed in this study could be expected to be markedly attenuated by excluding from analyses those subjects with evidence of ischaemic heart disease. However, these associations remained substantially unaltered following the exclusion of subjects with symptomatic—or a history of symptomatic—ischaeamic heart disease. Residual confounding resulting from an inability to identify and exclude subjects with asymptomatic ischaemic heart disease remains a possibility.

It is conceivable that the observed association between *C pneumoniae* seropositivity and lipid levels may be the product of selection bias introduced through poor response rates. However, for this to occur, individuals with low total cholesterol (or high HDL cholesterol) who were positive for anti-*C pneumoniae* IgG or persons negative for anti-*C pneumoniae* IgG and with high total cholesterol (or low HDL cholesterol) would have to have opted out. Given the high prevalence of the infection and lack of association with social class, age, and sex (in this study), it is unlikely that this would have occurred. Residual confounding by an unrecognised factor, or inadequate adjustment for identified confounders, may also explain

our findings—a possibility which cannot be eliminated until the basic epidemiology of *C pneumoniae* infection is more clearly elucidated.

The findings of this study are consistent with the observation by Laurila *et al.*²⁷ in a cross sectional study of male reindeer herders in Northern Finland, of decreased HDL and HDL/total cholesterol ratio in subjects with serological evidence of *C pneumoniae* infection. However, as in other studies of *C pneumoniae* infection in which total cholesterol was measured,^{11 12 40 52} Laurila *et al.* did not observe an association between seropositivity and cholesterol levels, though in a follow up study total cholesterol was raised in subjects with evidence of chronic infection.²⁸ Raised triglyceride levels have also been observed in persons with evidence of *C pneumoniae* infection.^{27 40}

The most commonly reported infection induced lipid abnormalities in man and experimental animals have been decreased HDL cholesterol and raised triglycerides and very low density lipoprotein.^{53–55} These effects are part of the acute phase response, appear to be mediated by cytokines such as tumour necrosis factor, interleukin (IL)-1, and IL-6,⁵⁴ and have been observed in persons with serological evidence of *C pneumoniae* infection.²⁷ The effect of infection on total and LDL cholesterol is less clear. In rodent models, increases in total cholesterol have been seen following lipopolysaccharide challenge,^{53 56} but it has consistently been reported in man and primates that total and LDL cholesterol values decrease in severe bacterial infections, including community acquired pneumonias.^{53 55 57} Decreases in total and HDL cholesterol have also been shown in humans with minor illnesses (including minor infections),^{58 59} and these lipid changes are not the result of dietary changes.⁵⁹ Therefore it appears that in humans the lipid component of the acute phase response to infection involves an increase in triglycerides and a decrease in total, LDL cholesterol, and HDL cholesterol.

The acute phase response to infection may therefore explain the association observed in the present study between *C pneumoniae* seropositivity and lowered HDL cholesterol but does not convincingly explain raised total cholesterol. The lipid response to chronic infection may be different from that which occurs in acute infection. Laurila *et al.* have observed raised total cholesterol in Finnish men with serological evidence of chronic *C pneumoniae* infection,²⁸ but the relation was only seen in non-smokers and the association was not adjusted for other potential determinants of raised total cholesterol. It is possible that a significant proportion of our subjects testing positive for *C pneumoniae* antibodies have a chronic rather than an acute infection. If this is the case, our findings would be consistent with those of Laurila *et al.* but this would require the assumption that the lipid response to chronic infection is substantially different from that of acute infection. Studies of—for example, *Helicobacter pylori* infection (a chronic gastric infection) have not provided such evidence.^{40 60 61}

This is the first study to examine the relation between infection with *C pneumoniae* and lipid levels in both men and women. In both sexes we observed an effect on total cholesterol in the same direction, but infected men had lower HDL cholesterol concentrations while infected women had higher concentrations. Addition of a sex/seropositivity term to the HDL model confirmed this sex specific difference in direction of effect. As few biological processes show opposite effects between the sexes, and there is no evidence that the acute phase response is different in women and men, this finding must be regarded with caution until confirmed by other investigators.

We have, however, demonstrated an epidemiologically robust association between an atherogenic lipid profile and evidence of infection with *C pneumoniae* and, although the possible mechanism of action may not be clear, this finding may have substantial public health implications. If treatment of *C pneumoniae* infection resulted in a sustained reduction in total cholesterol similar in magnitude to the increase seen in infected subjects in this study, data from meta-analyses of cholesterol lowering trials^{62 63} indicate an expected decrease in the risk of ischaemic heart disease in treated individuals (potentially 70% of the population) of at least 20%. Further investigation in this area is essential to determine whether the atherogenic lipid profile in *C pneumoniae* infection is sustained or recurs with repeated infection sufficiently often to alter long term cardiovascular disease risk, and to determine if relatively simple antibiotic treatment can substantially reduce the risk of cardiovascular disease.

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