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Chlamydia pneumoniae infection and mortality from ischaemic heart disease: large prospective study

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Editorial by Koenig

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Abstract

Objective To determine whether there is an independent association between infection with *Chlamydia pneumoniae* and ischaemic heart disease.

Design Prospective study using a nested case-control design.

Setting Medical centre in London run by BUPA, a private medical organisation.

Participants 21 520 professional men aged 35-64 who attended for a medical examination in London between 1975 and 1982.

Main outcome measure Death from ischaemic heart disease.

Results The distributions of concentrations of IgG and IgA antibodies to *C pneumoniae* were similar in the 647 men who subsequently died of ischaemic heart disease and in 1294 age matched controls who did not. There was no material association with heart disease irrespective of the cut-off point chosen to define seropositivity. At a cut-off point that defines 15% of controls as positive, for example, the odds ratios were 1.26 (95% confidence interval 0.95 to 1.68) for IgG and 1.09 (0.82 to 1.43) for IgA.

Conclusions No material association was found between infection with *C pneumoniae* and ischaemic heart disease. The size and prospective design of the study and the socioeconomic homogeneity of the cohort minimise both random and systematic error.

Introduction

Several reports have linked ischaemic heart disease with various infections, notably those caused by *Helicobacter pylori* and *Chlamydia pneumoniae* (also called

Chlamydia pneumoniae).¹⁻² A review in 1997 emphasised the problems in interpreting these associations.² The studies showing associations tended to be small, retrospective, and susceptible to bias or confounding. It was concluded that large prospective studies of socially homogeneous cohorts were needed to resolve the issue.² We then reported a clearly negative result in much the largest study to report on the association between *H pylori* and ischaemic heart disease, with 648 deaths.³ The prospective design of this study reduced bias, and the likelihood of an indirect association arising through differences in social class (socioeconomically disadvantaged people being more likely to have the infection and to develop heart disease regardless of a possible causal link between the two) was minimised by the homogeneity of the study population; the participants were all professional men attending for a routine medical examination. Both random and systematic errors were therefore likely to be small.

The studies of *C pneumoniae* and heart disease have generated mixed results regardless of whether IgG or IgA was measured.^{1-2,4-11} For example, results from the Caerphilly prospective study were negative for IgG antibodies to chlamydia but suggested an association with IgA (odds ratio for fatal ischaemic heart disease 1.83, 95% confidence interval 1.17 to 2.85).⁷ We report on the relation between ischaemic heart disease and both IgG and IgA antibodies to *C pneumoniae* in the BUPA study.

Participants and methods

The study design was as reported previously for *H pylori* and ischaemic heart disease.³ Briefly, the BUPA

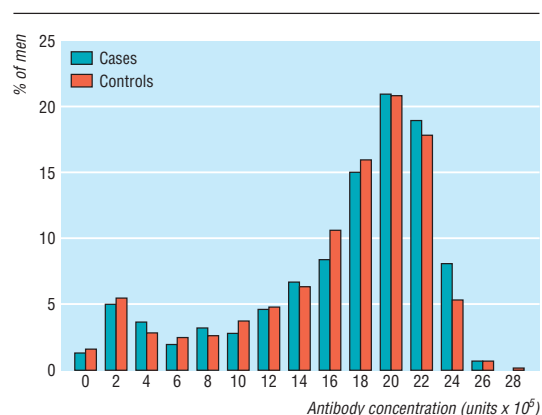


Fig 1 Distribution of serum concentration of IgG antibodies to *Chlamydia pneumoniae*, expressed as fluorescent counts (arbitrary units), in 647 men who died of ischaemic heart disease and 1294 matched controls who did not

study is a prospective (cohort) study of 21 520 healthy professional men aged 35–64 who attended the BUPA (a private medical organisation) centre in London for a routine medical examination between 1975 and 1982. Risk factors for ischaemic heart disease were measured, and serum samples were stored at -40°C . At the end of 1994 (average follow up 15.6 years), 648 men with no history of heart disease on entry had died from ischaemic heart disease as defined by codes 410–414 of ICD-9 (international classification of diseases, 9th revision). Two controls were selected for each case, matched for age and duration of storage of the serum sample. For one case there was an insufficient quantity of serum remaining; this was omitted along with the two controls, leaving 647 cases and 1294 controls.

The frozen serum samples were retrieved; analyses were performed without knowledge of whether they came from cases or controls. IgG antibodies to *C pneumoniae* were measured with a time resolved fluoroscopic immunoassay, which has been validated against the microimmunofluorescence antibody test.¹² IgA antibodies were similarly measured using 1:1000 α chain specific goat antihuman IgA labelled with biotin (Kierkegaard and Perry, Gaithersburg, MD, USA). Antibody concentrations were expressed as fluorescence counts (arbitrary units) and typically show a bimodal distribution.

The statistical analysis was as reported previously for *H pylori* and ischaemic heart disease.³ We used Cox's proportional hazards models, taking account of both the matching and the survival time.

Results

The established risk factors for ischaemic heart disease were, as expected, associated with ischaemic heart disease in our population,³ and the associations were similar in magnitude to those reported in other large cohort studies. The data are as previously shown,³ except that the average values for serum cholesterol concentration should have been 6.7 and 6.3 mmol/l respectively (not 7.1 and 6.7).

Examination of the association between antibody concentrations to *C pneumoniae* and mortality from heart disease is complicated by the fact that there is no

agreed or validated cut-off point for concentrations of IgG or IgA that identifies individuals who have and have not been infected with *C pneumoniae*. Consequently, there is no accepted indication as to what proportion of the adult population has been infected. We have therefore presented our results as distributions of antibody concentrations in the 647 men who died of heart disease and the 1294 that did not. The distributions of concentrations for IgG and IgA antibodies in cases and controls are shown in figures 1 and 2 respectively. For both antibodies the distributions in cases and controls are similar. Whatever antibody concentration is chosen to define IgG or IgA seropositivity, there is little difference between the proportions of cases and controls who are positive for *C pneumoniae*.

The main risk factors for ischaemic heart disease were similar in people with and without IgA or IgG antibody to *C pneumoniae*, confirming that any confounding is likely to be minimal. Table 1 shows the values of the risk factors according to antibody status when the cut-off point to define positivity is taken as the 85th percentile of antibody concentrations among the controls—that is, a positive rate of 15% among the controls. The only statistically significant association with antibody concentration (at the 1% level because many comparisons were made) was a lower concentration of IgA on average in men whose father had died of ischaemic heart disease. Although this was almost certainly a chance association, it was allowed for in the statistical analysis. Using the antibody cut-off points that define 15% of controls as seropositive, the odds of death from ischaemic heart disease were 1.26 (95% confidence interval, 0.95 to 1.68) for IgG antibody and 1.09 (0.82 to 1.43) for IgA antibody.

Table 2 shows the proportions of cases and controls above a range of cut-off points of antibody concentrations used to define seropositivity, from figures 1 and 2, and the associated odds ratio for death from ischaemic heart disease for each cut-off point. (Although it is usual when considering a possible relation between a risk factor and a disease to assess risk at different levels of exposure in relation to a baseline risk of one in the unexposed category, this procedure was not followed because there is no clear cut-off point for antibody to chlamydia that defines an unexposed

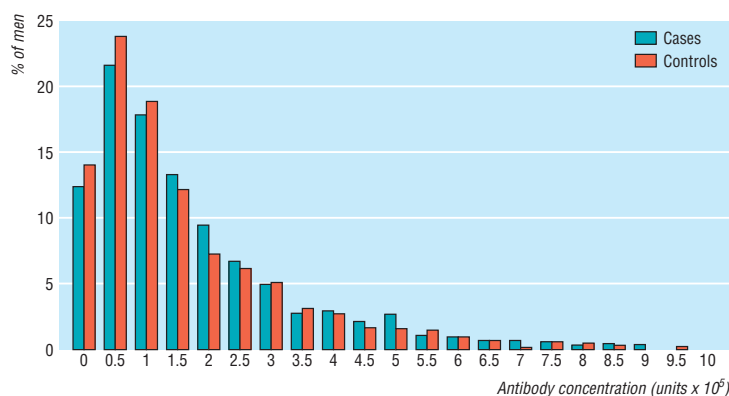


Fig 2 Distribution of serum concentration of IgA antibodies to *Chlamydia pneumoniae*, expressed as fluorescent counts (arbitrary units), in 647 men who died of ischaemic heart disease and 1294 matched controls who did not

Table 1 Risk factors for heart disease according to antibody status to *Chlamydia pneumoniae* in 1294 men who did not die of ischaemic heart disease (controls). Values are means (standard deviations) unless stated otherwise

	IgG antibody		IgA antibody	
	Negative (n=1100)	Positive (n=194)	Negative (n=1100)	Positive (n=194)
Age (years)	53.4 (7.1)	54.0 (7.0)	53.4 (7.1)	54.1 (6.7)
Body mass index (kg/m ²)	25.4 (2.7)	25.4 (2.6)	25.3 (2.7)	25.6 (2.8)
Total cholesterol concentration (mmol/l)	6.3 (1.1)	6.4 (1.2)	6.3 (1.1)	6.3 (1.1)
Triglyceride concentration (mmol/l)	1.50 (1.67)*	1.53 (1.64)*	1.51 (1.67)*	1.51 (1.65)*
Systolic blood pressure (mm Hg)	135 (19)	135 (20)	135 (19)	135 (20)
Diastolic blood pressure (mm Hg)	84 (12)	84 (11)	84 (12)	85 (13)
No (%) current cigarette smokers	412 (37)	81 (42)	421 (38)	73 (38)
No (%) whose father died of heart disease	158 (14)	23 (12)	164 (15)	17 (9)
No (%) whose mother died of heart disease	69 (6)	18 (9)	74 (7)	13 (7)

*Geometric mean (standard deviation expressed as multiple of geometric mean).

Table 2 Odds of death from ischaemic heart disease according to antibody level in men who were positive for *Chlamydia pneumoniae* compared with men who were negative. Results are adjusted for IgA antibody for father dying of ischaemic heart disease

Antibody concentration* (units × 10 ⁵)	Cases (n=647)†	Controls (n=1294)†	Odds ratio (95% CI)
IgG antibody			
≥11	84	84	1.01 (0.78 to 1.31)
≥14	78	77	1.07 (0.85 to 1.35)
≥17	68	66	1.15 (0.92 to 1.43)
≥20	48	44	1.24 (0.99 to 1.54)
≥23	15	13	1.27 (0.94 to 1.71)
≥26	1	1	0.87 (0.24 to 3.11)
IgA antibody			
≥0.75	78	74	1.26 (1.00 to 1.58)
≥1.5	48	43	1.27 (1.04 to 1.55)
≥2.25	29	27	1.17 (0.93 to 1.47)
≥3.0	19	18	1.10 (0.85 to 1.43)
≥3.75	12	11	1.14 (0.84 to 1.56)
≥4.5	9	7	1.22 (0.85 to 1.74)

*Concentration used to define men positive for *C pneumoniae*.

†Proportion positive.

group.) Table 2 shows that whether a small or large proportion of controls are considered seropositive, there is no strong relation between infection with *C pneumoniae* and mortality from heart disease. The data provide no convincing evidence of an association, but they do not rule out a weak association with IgG antibody.

Discussion

Our study is the largest published prospective study on *C pneumoniae* and ischaemic heart disease. It also

reduces confounding by socioeconomic status because the study population was socially homogeneous. The prospective design of our study, and the fact that the men with known ischaemic heart disease were excluded, avoids the possibility of spurious associations arising because antibodies to heart muscle proteins developing after myocardial infarction may be indistinguishable from antibodies to *C pneumoniae*.¹ The laboratory staff did not know which samples were from cases and which were from controls, and the assays involved no subjective interpretation because they were read by machine, problems that may affect other studies. The results were similar irrespective of the cut-off point chosen to define positivity. No method of detecting antibody to *C pneumoniae* is entirely specific: we chose the delayed fluorescence assay because of its objectivity and suitability to large scale testing, but it has been validated against the classic microimmunofluorescence test for IgG antibody to chlamydia.¹² Confirmation of the expected increasing prevalence of chlamydia infection with age also served as a means of validating the assay.

Table 3 summarises the results of six published cohort studies of infection with *C pneumoniae* and the incidence of ischaemic heart disease. The US physicians study has the most cases after the BUPA study and, like it, was able to minimise confounding through differences in social class because of the socio-economic homogeneity of the cohort; this study showed no suggestion of an association between concentrations of IgG antibodies and ischaemic heart disease. All the studies except the Caerphilly one⁷ suggest no material association between concentration of IgA antibody and ischaemic heart disease. Even in the Caerphilly study,⁷ no association was seen in relation to

Table 3 Results of published cohort studies of *Chlamydia pneumoniae* and incidence of ischaemic heart disease

Study	Cases	No of cases	No of controls	IgG antibody		IgA antibody	
				Positive controls (%)	Odds ratio (95% CI)	Positive controls (%)	Odds ratio (95% CI)
Helsinki ⁴	Death from ischaemic heart disease, non-fatal infarct	87	87	22	2.1 (0.9 to 5.2)	14	1.1 (0.5 to 2.7)
Zutphen (Netherlands) ⁵	Death from ischaemic heart disease, non-fatal infarct, angina	54	108	34	2.76 (1.31 to 5.81)*	20	0.78 (0.33 to 1.84)*
US physicians ⁶	Death from ischaemic heart disease, non-fatal infarct	343	343	22	1.0 (0.7 to 1.5)	NM	
Caerphilly (United Kingdom) ⁷	Death from ischaemic heart disease	144	1057	21	1.05 (0.70 to 1.59)*	20	1.83 (1.17 to 2.85)
Caerphilly (United Kingdom) ⁷	Non-fatal infarct	134	1057	21	0.76 (0.48 to 1.22)*	20	0.70 (0.43 to 1.14)*
Finland ⁸	Death from ischaemic heart disease, non-fatal infarct	311	1682	†	†	16†	1.19 (0.71 to 2.00)*
Framingham (United States) ⁹	Death from ischaemic heart disease, non-fatal infarct, stroke	188	1315	47	0.89 (0.66 to 1.19)	NM	
Present	Death from ischaemic heart disease	647	1294	15	1.26 (0.95 to 1.68)	15	1.09 (0.82 to 1.43)

NM=not measured.

*Calculated by us from published data, unadjusted for confounding factors.

†20% were IgA positive, but only those who were also IgG positive were considered positive for *C pneumoniae*.

What is already known on this topic

Several reports have linked ischaemic heart disease with various infections, such as with *Chlamydia pneumoniae*

What this study adds

The distribution of concentrations of IgG and IgA antibodies to *C pneumoniae* were similar in men who did and did not die of ischaemic heart disease

The study is the largest to report on the association, with 647 deaths from ischaemic heart disease, and minimises the likelihood of an indirect association arising through differences in social class because the men were of similar socioeconomic status

No important relation was found between infection with *C pneumoniae* and ischaemic heart disease

non-fatal myocardial infarction, and the association in relation to fatal cases relied on not measuring concentrations of IgA antibodies in serum specimens with low concentrations of IgG antibodies (assuming they were all negative for IgA antibody). In an additional cohort study¹⁰ there was "no significant association" between mortality from ischaemic heart disease (67 deaths) and concentration of IgG or IgA antibody. Another study recording a mixture of incident (76) and prevalent (212) cases showed no association with *C pneumoniae* IgG seropositivity.¹¹ In a cohort of people enrolled with a health maintenance organisation, use of antibiotics active against *C pneumoniae* did not reduce the incidence of first myocardial infarction during the next five years.¹³ Taken together, the evidence does not favour any important relation between ischaemic heart disease and infection with *C pneumoniae*.

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Contributors: NJW collected the BUPA data. NJW and MRL designed the study and analysed the results with JKM. NJW, MRL, and JKM drafted the manuscript. MEW, YW, and XZ performed the serological analyses and redrafted the manuscript. NJW will act as guarantor for the paper.

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A memorable patient

The danger of a high C

While about to take blood, I noticed a thin scar running along the left elbow of my elderly patient in exactly the place I would have liked to venesect him. I asked him how he got the scar and was bemused by the reply: "That is from when I broke my neck playing the 'Tiger Rag'." I questioned him further. The events occurred in 1945, when he was posted to Burma. Shortly before the war ended, he was travelling in a jeep that came off the road and rolled over. Apart from a deep cut in his left arm, he recovered quickly and was discharged from the field hospital after a week of convalescence.

While he was on leave awaiting transport back to Britain, he heard the sound of music from a dance hall, and when he went inside he came across a rather morose dance band in rehearsal. The trumpet player, they explained gloomily, had been killed on duty—they still had his trumpet—but they were due to do a concert and were lost without the trumpeter. My patient explained that he had a passion for the trumpet, and was actually rather good at playing by ear. The band recruited him with glee, and all went well until they came to rehearse the opening number, the "Tiger Rag."

At a certain point the trumpet player has to hit a deafeningly high C. When this point came, he played the note, and the next thing he remembered was waking up in hospital.

Unbeknown to him, the original jeep accident had caused him to fracture two vertebrae, which remained relatively stable until the extreme pressure of the "Tiger Rag" caused them to destabilise. He was in a body plaster for four months, and it was a while before he returned to trumpet playing.

Georgia Ingram *surgical house officer, Norwich*

We welcome articles of up to 600 words on topics such as *A memorable patient*, *A paper that changed my practice*, *My most unfortunate mistake*, or any other piece conveying instruction, pathos, or humour. If possible the article should be supplied on a disk. Permission is needed from the patient or a relative if an identifiable patient is referred to. We also welcome contributions for "Endpieces," consisting of quotations of up to 80 words (but most are considerably shorter) from any source, ancient or modern, which have appealed to the reader.