Papers

Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses

John Danesh, Peter Whincup, Mary Walker, Lucy Lennon, Andrew Thomson, Paul Appleby, J Ruth Gallimore, Mark B Pepys

Abstract

Objective To assess associations between baseline values of four different circulating markers of inflammation and future risk of coronary heart disease, potential triggers of systemic inflammation (such as persistent infection), and other markers of inflammation.

Design Nested case-control comparisons in a prospective, population based cohort.

Setting General practices in 18 towns in Britain.

Participants 506 men who died from coronary heart disease or had a non-fatal myocardial infarction and 1025 men who remained free of such disease until 1996 selected from 5661 men aged 40-59 years who provided blood samples in 1978-1980.

Main outcome measures Plasma concentrations of C reactive protein, serum amyloid A protein, and serum albumin and leucocyte count. Information on fatal and non-fatal coronary heart disease was obtained from medical records and death certificates.

Results Compared with men in the bottom third of baseline measurements of C reactive protein, men in the top third had an odds ratio for coronary heart disease of 2.13 (95% confidence interval 1.38 to 3.28) after age, town, smoking, vascular risk factors, and indicators of socioeconomic status were adjusted for. Similar adjusted odds ratios were 1.65 (1.07 to 2.55) for serum amyloid A protein; 1.12 (0.71 to 1.77) for leucocyte count; and 0.67 (0.43 to 1.04) for albumin. No strong associations were observed of these factors with *Helicobacter pylori* seropositivity, *Chlamydia pneumoniae* IgG titres, or plasma total homocysteine concentrations. Baseline values of the acute phase reactants were significantly associated with one another ($P < 0.0001$), although the association between low serum albumin concentration and leucocyte count was weaker ($P = 0.08$).

Conclusion In the context of results from other relevant studies these findings suggest that some inflammatory processes, unrelated to the chronic infections studied here, are likely to be involved in coronary heart disease.

Introduction

Circulating concentrations of C reactive protein, serum amyloid A protein, and serum albumin and the leuco-cyte count can fluctuate widely during acute responses to tissue damage or infection. Plasma concentrations of C reactive protein and serum amyloid A protein can each rise 10 000-fold; the leucocyte count can increase about threefold; and the concentration of serum albumin can fall by about 20%.[1 2] In recent years these "acute phase reactants" have been studied as potential markers of more subtle and persistent systemic alterations that may be loosely called low grade inflammation. If the sharp short term fluctuations are ignored, then long term circulating concentrations of these factors show a similar year-to-year consistency within individuals to levels of some more extensively studied risk factors such as blood cholesterol concentration and blood pressure.[2 3] Moreover, highly sensitive assays for C reactive protein and serum amyloid A protein are now available that can detect low grade inflammation that would previously have been unnoticed.[4 5]

Several reports have suggested that plasma C reactive protein and other possible markers of low grade inflammation can predict increased risks of coronary heart disease, but it is not known whether the associations are causal.[2 6] A variety of mechanisms by which C reactive protein might directly promote vascular disease have been proposed,[7] but none is proved. These markers of inflammation might, however, be indicators of chronic infective processes possibly correlated with risk of coronary heart disease, such as infection by *Chlamydia pneumoniae* or chronic gastric infection with *Helicobacter pylori*.[8] Alternatively, the markers might be mainly indicators of classic vascular risk factors (such as smoking and obesity) or of the extent of pre-existing disease (since atherosclerosis may be partly an inflammatory lesion[6]). If so, the markers themselves would not be of causal relevance to disease. Long term prospective studies with data collected on many possible confounders or mediators can help to distinguish among these different possibilities since this approach is generally less influenced than retrospective studies by any effect of pre-existing disease itself on the factors being investigated.[9]

We report one of the largest and most prolonged prospective studies of "inflammatory" factors and coronary heart disease so far, with updated meta-analyses of previous relevant studies to put our findings in context. We measured four circulating markers of inflammation (C reactive protein, serum amyloid A

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Tables showing distribution of risk factors in the control population are available on the BMJ's website

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protein, leucocyte count, and albumin[10]), which should help to determine whether there is some underlying process related to inflammation (rather than any one marker by itself) that might be relevant to disease. Most previous studies could not make such assessments because they typically reported on only one marker of inflammation.

Methods

Cases and controls
During 1978-80, 7735 men aged 40-59 (response rate 78%) were randomly selected from general practice registers in each of 24 British towns. Nurses administered questionnaires, made physical measurements, recorded an electrocardiogram, and, for 5661 men in 18 of the towns, collected non-fasting venous blood samples, which were stored at −20°C for subsequent analysis.[11] Additional questionnaires on car ownership and childhood social circumstances (father's social class and childhood household amenities) were posted to participants five years (98% response among survivors) and 12 years (90% response among survivors) after entry.

All men have been monitored subsequently for all cause mortality and for cardiovascular morbidity, with a loss to follow up of less than 1% to date. A total of 507 men with available samples had major coronary heart disease events between the beginning of follow up and December 1995 (223 deaths and 284 non-fatal myocardial infarctions). Deaths from coronary heart disease were ascertained through NHS central registers on the basis of a death certificate with ICD-9 codes 410-414, and the diagnosis of non-fatal myocardial infarction was based on reports from general practitioners, supplemented by regular reviews of general practice records, and diagnosed in accordance with World Health Organization criteria. We selected 1026 controls who were "frequency" matched to cases on town of residence and age in five year bands from among men who had survived to the end of the study period without a myocardial infarction. One potential case and one potential control were found not to have a serum sample available. In previous interim analyses data were reported on albumin concentration and coronary heart disease in only 97 of the 507 cases included here[12] and on leucocyte count and coronary heart disease in only 235 cases.[13]

Laboratory methods
Laboratory workers blind to the case-control status of participants measured concentrations of C reactive protein and serum amyloid A protein using sensitive enzyme immunoassays.[4 5] The C reactive protein assay was standardised on the WHO international reference standard, and the results of the serum amyloid A protein assay were used to establish the WHO international reference standard for this protein.[14] Albumin was measured with bromocresol green, and leucocyte counts were done with Coulter counters at the time of blood collection.[11] The coefficients of variation within each of these assays was 2-4%; the variation between the assays was about 6-8%. Measurements were also made of concentrations of serum IgG antibodies to *Helicobacter pylori* (by enzyme linked immunoassay, Meridian Diagnostics, Cincinatti, Ohio), IgG antibodies to *Chlamydia pneumoniae* (whole organism antigen by time resolved fluorimetry[15]) and serum lipids, plasma homocysteine, glucose, insulin, and markers of renal function (by standard assays[11]).

Statistical methods
Case-control comparisons were made by unmatched stratified logistic regression fitted by unconditional maximum likelihood (Stata Corporation, Texas, USA). Analysis of concentrations of C reactive protein, serum amyloid A, and albumin and of leucocyte count was prespecified to be by thirds of values in the controls. Adjustment was made for age; cigarette smoking (never, former, current); daily cigarette consumption; non-fasting blood concentrations of total cholesterol, high density lipoprotein cholesterol, and triglycerides; current social class (registrar general's 1980 classification with separate category for armed forces[11]); housing tenure (owner, private rent, council rent); marital status; current car ownership; and father's occupation (manual, non-manual), family car ownership, bathroom in house, hot water tap in house, and bedroom sharing. *H pylori* seropositivity, *C pneumoniae* IgG titres, and concentrations of non-fasting blood lipids, plasma homocysteine, and other blood components were investigated as possible correlates of levels of each of the acute phase reactants.

We have previously described the methods for meta-analyses of prospective studies of non-fatal myocardial infarction or death from coronary heart disease with over a year's follow up.[2] The meta-analysis was updated to include all studies identified with data available in March 2000. Most studies related risk of coronary heart disease to baseline measurement of risk factors, even though the levels of each factor can fluctuate within individuals over time. Our comparisons of those in the top third of baseline values with those in the bottom third have therefore been corrected for such regression dilution[16] by use of self-correlation coefficients derived from other studies.[2] Results of studies were combined by using inverse variance weighted averages of log odds ratios. Heterogeneity was assessed by standard χ^2 tests. Odds ratios are given with 95% confidence limits, and two sided probability values are used.

Results

The mean age at coronary heart disease event among cases was 62 years (mean duration of follow up 9.5 years). There were highly significant differences between cases and controls with respect to known vascular risk factors such as smoking, obesity, blood pressure, and blood lipids concentrations (table 1).

Associations among different acute phase reactants
In controls without evidence of coronary heart disease at baseline, the correlation coefficients of the associations between plasma C reactive protein and serum amyloid A protein, leucocyte count, and albumin were 0.58, 0.33, and −0.19 respectively (P<0.0001 for each), and the correlation coefficients between serum amyloid A protein and leucocyte count and albumin were 0.19 and 0.14 respectively (P<0.0001 for each). These associations among the four acute phase reactants were not adjusted for values

Table 1 Baseline characteristics of men with coronary heart disease and of controls matched for age and town. Values are mean (SD) unless stated otherwise

Characteristic	Cases (n=507)	Controls (n=1026)	P value
Questionnaire			
Age (years)	52.2 (5.3)	52.2 (5.3)	Matched
No (%) current smokers	268 (53)	436 (42)	<0.0001
No (%) with evidence of coronary disease*	177 (35)	204 (20)	<0.0001
No (%) with treated diabetes	12 (2)	15 (1)	NS
No (%) >2 drinks alcohol/day	110 (22)	232 (23)	NS
No (%) in non-manual occupation	112 (22)	280 (27)	0.03
No (%) of home owners†	275 (64)	667 (69)	0.03
Physical measurements			
Body mass index (kg/m^2)	25.8 (3.4)	25.3 (3.3)	0.008
Height (cm)	171 (6.4)	172 (6.5)	0.002
Weight (kg)	76.3 (11.4)	75.8 (11.2)	NS
Systolic blood pressure (mm Hg)	151 (21)	147 (21)	<0.0001
Diastolic blood pressure (mm Hg)	86 (14)	83 (13)	<0.0001
Forced expiratory volume (l)	308 (68)	326 (77)	<0.0001
Blood sample			
Total cholesterol (mmol/l)	6.63 (1.10)	6.20 (0.99)	<0.0001
HDL cholesterol (mmol/l)	1.10 (0.28)	1.15 (0.29)	0.0003
Triglyceride (mmol/l)	2.26 (1.33)	1.93 (1.22)	<0.0001

*Evidence of ischaemia on baseline electrocardiogram or reported history of angina or myocardial infarction.
†Information on home ownership was available for only 431 cases and 964 controls.

of one another to avoid overadjustment since they may reflect a common underlying process (see tables on *BMJ*'s website). Leucocyte count and serum albumin concentration were not strongly related ($R = -0.06$, $P = 0.08$).

Acute phase reactants, classic vascular risk factors, and other characteristics

C reactive protein concentration was strongly associated with cigarette smoking, body mass index, and low forced expiratory volume in one second ($P < 0.0001$ for each), and the associations were little changed by adjustments for age, town, smoking, and indicators of socioeconomic status. Associations of C reactive protein with high density lipoprotein cholesterol, triglyceride, urate, and insulin, however, weakened substantially after such adjustments (table A on *BMJ*'s website). There were strong and highly significant associations of serum amyloid A protein with body mass index and total cholesterol ($P < 0.0001$) and with low forced expiratory volume ($P < 0.001$) which remained significant after adjustment for possible confounders, but such adjustment substantially weakened the association with high density lipoprotein cholesterol (table B on website). Strong adjusted associations were observed between leucocyte count and cigarette smoking and packed cell volume ($P < 0.0001$ for each, table C on website) and between low albumin and age and cigarette smoking ($P < 0.0001$ for each, table D on website) and between albumin and blood pressure, total cholesterol, and packed cell volume ($P < 0.0001$ for each). Possible associations existed between albumin and high density lipoprotein cholesterol, triglycerides, and serum markers of renal function. No consistent associations were observed of these acute phase proteins with *H pylori* seropositivity, *C pneumoniae* IgG titres, plasma total homocysteine concentrations, or a range of childhood or adult indicators of socioeconomic status.

Acute phase reactants and incident coronary heart disease

The odds ratio for coronary heart disease was 3.46 (95% confidence interval 2.59 to 4.62; $\chi^2 = 71$, df = 1) in men in the top third compared with those in the bottom third of baseline C reactive protein concentration (tertile cut offs, > 2.4 v < 0.9 mg/l). The odds ratio fell to 2.13 (1.38 to 3.28; $\chi^2 = 11.8$, df = 1) after smoking, vascular risk factors, and indicators of socioeconomic status were adjusted for (table 2). Comparisons between the top and bottom thirds of the other factors gave the following adjusted odds ratios for coronary heart disease: 1.65 (1.07 to 2.55) for serum amyloid A protein (> 9 v < 6 mg/l); 1.12 (0.71 to 1.77) for leucocyte count (> 7.8 v 6.4×10^9/l); and 0.67 (0.43 to 1.04) for albumin (> 46 v < 44 g/l). These results were not
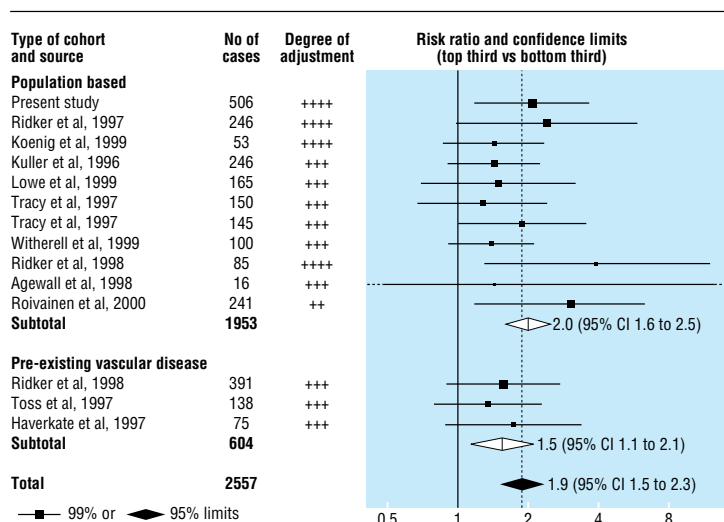
Table 2 Odds of coronary heart disease in men who had values of inflammatory factors in top third of distribution of controls relative to those who had values in bottom third of this distribution

Factor (tertile cut offs)	No of cases			No of controls			Odds ratio (95% CI) adjusted for:			
	Top	Middle	Bottom	Top	Middle	Bottom	Age and town	Age, town, and smoking	Age, town, smoking, and risk factors*	Age, town, smoking, risk factors, socioeconomic status†
All 506 cases and 1025 controls										
C reactive protein (2.4 and 0.9 mg/l)	268	150	88	347	298	380	3.46 (2.59 to 4.62)	3.32 (2.47 to 4.47)	2.92 (2.13 to 4.01)	2.13 (1.38 to 3.28)
Serum amyloid A protein (9 and 6 mg/l)	255	144	107	362	351	312	2.12 (1.60 to 2.80)	2.13 (1.61 to 2.82)	1.66 (1.22 to 2.26)	1.65 (1.07 to 2.55)
Leucocyte count (7.8 and 6.4×10⁹/l)	212	168	110	332	329	330	1.89 (1.43 to 2.51)	1.69 (1.25 to 2.28)	1.46 (1.06 to 2.00)	1.12 (0.71 to 1.77)
Albumin (46 and 44 g/l)	174	141	191	348	347	327	0.83 (0.63 to 1.10)	0.90 (0.68 to 1.20)	0.61 (0.44 to 0.83)	0.67 (0.43 to 1.04)
329 cases and 820 controls without evidence of coronary heart disease at entry										
C reactive protein (2.4 and 0.9 mg/l)	164	95	70	265	234	321	2.93 (2.10 to 4.09)	2.74 (1.95 to 3.85)	2.61 (1.81 to 3.77)	2.31 (1.42 to 3.76)
Serum amyloid A protein (9 and 6 mg/l)	163	87	79	284	288	248	1.90 (1.37 to 2.64)	1.93 (1.38 to 2.68)	1.50 (1.05 to 2.16)	1.67 (1.02 to 2.74)
Leucocyte count (7.8 and 6.4×10⁹/l)	142	104	71	253	261	277	2.19 (1.56 to 3.07)	1.87 (1.30 to 2.68)	1.57 (1.07 to 2.30)	1.34 (0.80 to 2.27)
Albumin (46 and 44 g/l)	115	95	118	282	266	269	0.90 (0.64 to 1.26)	1.0 (0.71 to 1.41)	0.65 (0.45 to 0.95)	0.75 (0.45 to 1.25)

*Blood pressure, total cholesterol, high density lipoprotein cholesterol, triglycerides, body mass index
†Occupation, housing tenure, marital status, car ownership, and childhood socioeconomic factors (father's occupation, family car ownership, bathroom in house, hot water tap in house, bedroom sharing, height).
Analyses of acute phase reactants and coronary heart disease were not adjusted for values of other inflammatory markers to avoid possible overadjustment (see results).

Type of cohort and source	No of cases	Degree of adjustment	Risk ratio and confidence limits (top third vs bottom third)
Population based			
Present study	506	++++	
Ridker et al, 1997	246	++++	
Koenig et al, 1999	53	++++	
Kuller et al, 1996	246	+++	
Lowe et al, 1999	165	+++	
Tracy et al, 1997	150	+++	
Tracy et al, 1997	145	+++	
Witherell et al, 1999	100	+++	
Ridker et al, 1998	85	++++	
Agewall et al, 1998	16	+++	
Roivainen et al, 2000	241	++	
Subtotal	**1953**		2.0 (95% CI 1.6 to 2.5)
Pre-existing vascular disease			
Ridker et al, 1998	391	+++	
Toss et al, 1997	138	+++	
Haverkate et al, 1997	75	+++	
Subtotal	**604**		1.5 (95% CI 1.1 to 2.1)
Total	**2557**		1.9 (95% CI 1.5 to 2.3)

—■— 99% or ◆ 95% limits   0.5 1 2 4 8

Fig 1 Prospective studies of C reactive protein and coronary heart disease. Risk ratios compare top and bottom thirds of baseline measurements. Black squares indicate the risk ratio in each study, with the size of square proportional to number of cases. The combined risk ratio and its 95% confidence interval are indicated by unshaded diamonds for subtotals and shaded diamond for total. All studies adjusted for age, sex, smoking, and some other standard vascular risk factors; the first three studies also adjusted for markers of socioeconomic status

materially changed in analyses restricted to the 329 cases and 820 controls with no evidence of coronary heart disease at baseline (table 2) or in analyses restricted to the 227 cases and 779 controls who had complete information on childhood socioeconomic status. The findings were also unaffected by varying the prespecified cut-off levels for analysis of each factor.

Discussion

Our prospective study in the general British male population shows that baseline values of four acute phase reactants are associated with one another as well as with future risk of coronary heart disease. These data support the idea that there are some underlying processes related to inflammation that are relevant to coronary heart disease. To determine what factor(s) might be triggering such low grade inflammation years in advance of disease, we studied classic risk factors, plasma homocysteine concentration, serological evidence of chronic infection with *C pneumoniae* or *H pylori*, and a range of possible other triggers. None, however, was strongly related to inflammatory markers, suggesting that these factors cannot fully account for the associations observed with coronary heart disease. Even though the strength of the association of coronary heart disease with baseline concentrations of C reactive protein and serum amyloid A protein seems comparable with that for some more extensively studied risk factors (such as blood fibrinogen concentration[2]), several uncertainties remain. We discuss below the strengths and limitations of the available evidence on coronary heart disease and each of these factors in the context of updated meta-analyses of other relevant studies.

C reactive protein
A previous meta-analysis of seven prospective studies of C reactive protein, with a total of 1053 cases, indicated a risk ratio for coronary heart disease of 1.7 (95% confidence interval 1.4 to 2.1) in individuals in the top third compared with those in the bottom third of baseline measurements.[2] Since the publication of that review, six additional prospective studies of C reactive protein have been identified, including an additional 994 cases of non-fatal myocardial infarction or death from coronary heart disease.[17-22] All used sensitive assays and adjusted for smoking and some standard vascular risk factors. In aggregate, the 14 available prospective reports (including the present study of 506 cases) include 2557 cases with a weighted mean age at entry of 58 and weighted mean follow up of eight years.[17-28] There was no significant heterogeneity among them ($\chi^2 = 6.9$, df $= 13$; P > 0.1), and a comparison of individuals with C reactive protein concentrations in the top third with those in the bottom third at baseline gave a combined risk ratio of 1.9 for coronary heart disease (1.5 to 2.3; fig 1) with a similar risk ratio of 2.0 (1.6 to 2.5) in the 11 studies of the general population (test for heterogeneity, $\chi^2 = 5.8$, df $= 10$; P > 0.1). The estimated mean usual log C reactive protein values in the top third and bottom thirds were 0.38 and 0.02 mg/l in the general population, which correspond to mean estimated usual values of 2.4 and 1.0 mg/l. Hence, this updated meta-analysis indicates a similar risk ratio to the previous review, but the scope for the play of chance is now substantially reduced because there are more than twice as many cases reported.

For two main reasons, however, it remains uncertain whether C reactive protein is an independent risk factor for coronary heart disease. Firstly, the odds ratio in our study was reduced from 3.46 to 2.13 after baseline confounding factors were adjusted for. This substantial reduction suggests that more exact adjustment for the usual long term concentrations of these and other factors might produce a greater reduction—for example, we did not measure blood fibrinogen concentration. Secondly, although experimental studies suggest that C reactive protein might directly contribute to vascular damage (such as its frequent detection in atherosclerotic plaques,[29] its ability to stimulate tissue factor production by macrophages,[30] and its enhancement of complement activation after binding partly degraded, non-oxidised low density lipoprotein cholesterol[31 32]), no direct evidence exists for such involvement.[7] Additional studies are also needed to determine the relevance of apparently sustained falls in C reactive protein concentrations after the use of lipid lowering statin drugs[3] as well as apparently sustained increases after postmenopausal hormone replacement therapy.[33]

Serum amyloid A protein
Only three previous studies have reported on serum amyloid A protein and incident coronary heart disease,[22 28 34] including two cohorts defined on the basis of pre-existing coronary heart disease. They comprised 551 cases in total (fig 2). Our 506 cases in the general population almost double the available evidence. In aggregate, these four studies include 1057 cases, with a weighted mean age at entry of 56 and weighted mean follow up of 10 years. There was no significant heterogeneity among them ($\chi^2 = 0.3$, df $= 3$; P > 0.1), and a comparison of individuals with values in the top third with those in the bottom third at baseline

gave a combined risk ratio of 1.6 for coronary heart disease (1.1 to 2.2; fig 2). The estimated mean usual log values in these groups were 1.00 and 0.68 mg/l, which correspond to mean values of 10.0 and 4.7 mg/l. Further studies are needed to determine whether this association is independent of possible confounders.

Leucocyte count and serum albumin

The adjusted odds ratio for coronary heart disease in our study was 1.12 (0.71 to 1.77) in men with leucocyte counts in the top third compared with those in the bottom third of baseline measurements. Although this odds ratio is not significant, it is consistent with a combined risk ratio for coronary heart disease of 1.4 (1.3 to 1.5) reported in a previous meta-analysis of 19 prospective studies of leucocyte count including a total of 7229 cases, with top and bottom thirds corresponding to mean usual leucocyte counts of 8.4 and 5.6×10^9/l, respectively.[2] We also observed an adjusted odds ratio for coronary heart disease of 0.67 (0.43 to 1.04) in men in the top third compared with those in the bottom third of baseline albumin measurements. Again, despite a lack of conventional significance, this odds ratio is compatible with a combined risk ratio of 0.7 (0.6 to 0.8) reported in a meta-analysis of eight prospective studies including a total of 3770 cases, with top and bottom thirds corresponding to mean usual albumin concentrations of 42 and 38 g/l, respectively.[2] The strongly positive associations of serum albumin values with blood pressure and serum lipid concentrations indicate that these variables cannot account for the association between low serum albumin and coronary heart disease—rather the reverse in fact.

Conclusions

We found that baseline levels of four circulating markers of low grade inflammation were associated with one another and with future risk of coronary heart disease. The markers, however, were not associated with some chronic infective processes possibly related to

Type of cohort and source	No of cases	Degree of adjustment	Risk ratio and confidence limits (top third vs bottom third)
Population based			
Present study	506	++++	
Ridker et al, 2000	85	++++	
Pre-existing vascular disease			
Ridker et al, 1998	391	+++	
Haverkate et al, 1997	75	+++	
Total	1057		1.6 (95% CI 1.1 to 2.2)

—■— 99% or ◆ 95% limits   0.5   1   2   4   8

Fig 2 Prospective studies of serum amyloid A protein concentration and coronary heart disease. Risk ratios compare top and bottom thirds of baseline measurements. Black squares indicate the risk ratio in each study, with the size of square proportional to number of cases. All studies adjusted for age, sex, smoking, and some other standard vascular risk factors; our study also adjusted for markers of socioeconomic status

coronary heart disease. These findings—in the context of results from other relevant studies—suggest that low grade inflammatory processes may be relevant to coronary heart disease.

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What is already known on this topic

Plasma concentrations of C reactive protein and other sensitive markers of systemic inflammation may be correlated with future risk of coronary heart disease in the general population
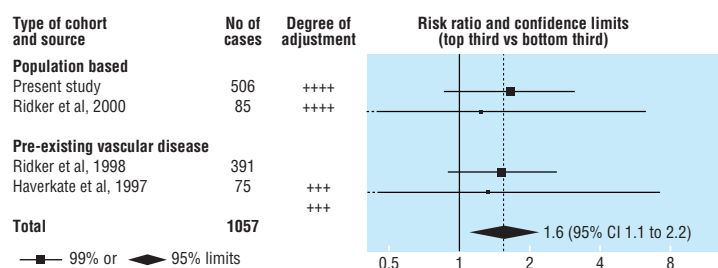
It is not known whether these associations are causal or merely due to confounding by classic risk factors, chronic infective processes, or early disease

What this study adds

Baseline values of C reactive protein, serum amyloid A protein, leucocyte count, and serum albumin were associated with one another as well as with future risk of coronary heart disease

Values of these factors were not associated with markers of chronic infective processes

These findings suggest that low grade inflammatory processes may be relevant to coronary heart disease

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1 Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive and related proteins (pentaxins) and serum amyloid A protein. *Adv Immun* 1983;34:141-212.
2 Danesh J, Collins R, Appleby P, Peto R. Fibrinogen, C-reactive protein, albumin or white cell count: meta-analyses of prospective studies of coronary heart disease. *JAMA* 1998;279:1477-82.
3 Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. *Circulation* 1999;100:230-5.
4 Wilkins J, Gallimore JR, Moore EG, Pepys MB. Rapid automated high sensitivity enzyme immunoassay of C-reactive protein. *Clin Chem* 1998;44:1358-61.
5 Wilkins J, Gallimore JR, Tennent GA, Hawkins PN, Limburg PC, van Rijswijk MH, et al. Rapid automated enzyme immunoassay of serum amyloid A. *Clin Chem* 1994;40:1284-90.
6 Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115-21.
7 Lagrand WK, Visser CA, Hermens W, Niessen WH, Verheugt FW, Wolbink GJ, et al. C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation* 1999;100:96-102.
8 Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? *Lancet* 1997;350:430-6.
9 Danesh J. Smouldering arteries? Low-grade inflammation and coronary heart disease. *JAMA* 1999;282:2169-71.
10 Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448-55.
11 Shaper AG, Pocock SJ, Walker M, Cohen NM, Wale CJ, Thomson AG. British regional heart study: cardiovascular risk factors in middle-aged men in 24 towns. *BMJ* 1981;283:179-86.
12 Phillips A, Shaper AG, Whincup PH. Association between serum albumin and mortality from cardiovascular disease, cancer, and other causes. *Lancet* 1989;ii:1434-6.
13 Phillips AN, Neaton JD, Cook DG, Grimm RH, Shaper AG. Leukocyte count and risk of coronary heart disease. *Am J Epidemiol* 1992;136:59-70.
14 Poole S, Walker D, Gaines Das RE, Pepys MB. The first international standard for serum amyloid A protein (SAA). Evaluation in an international collaborative study. *J Immunol Methods* 1998;214:1-10.
15 Wong Y-K, Sueur JM, Fall CHD, Orfila J, Ward ME. The species specificity of the microimmunofluorescence antibody test and comparisons with a time resolved fluoroscopic immunoassay for measuring antibodies against Chlamydia pneumoniae. *J Clin Pathol* 1999;2:99-103.
16 Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, et al. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. *Am J Epidemiol* 1999;150:341-53.

17 Lowe GDO, Rumely A, Sweetnam PM, Yarnell JWG. Fibrin D-dimer, C-reactive protein and risk of ischaemic heart disease: the Speedwell study. *Blood Coagul Fibrin* 1999;10 (suppl 1):S92-3.
18 Agewall S, Wikstrand J, Fagerberg B. Prothrombin fragment 1 + 2 is a risk factor for myocardial infarction in treated hypertensive men. *J Hypertension* 1998;16:537-41.
19 Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998;98:731-3.
20 Witherell HL, Smith KL, Ley C, Freidman GD, Orentreich N, Vogelman JH. Helicobacter pylori infection, C-reactive protein, and risk for myocardial infarction: a prospective study. *Gastroenterology* 1999;116:A355.
21 Roivainen M, Viik-Kajarnder M, Palosuo T, Sacks F Braunwald E. Infections, inflammation, and the risk of coronary heart disease. *Circulation* 2000;101:257.
22 Ridker PM, Rifai N, Pfeffer MA, Toivanen P, Leinonen M, Saikku P, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. *Circulation* 1998;98:839-44.
23 Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *New Engl J Med* 1997;336:973-9.
24 Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA-Augsburg cohort study, 1984-1992. *Circulation* 1999;99:237-42.
25 Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *Am J Epidemiol* 1996;144:537-47.
26 Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, et al. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results from the cardiovascular health study and the rural health promotion project. *Arterioscler Thromb Vasc Biol* 1997;17:1121-7.
27 Toss H, Lindahl B, Siegbahn A, Wallentin L. Prognostic influence of increased fibrinogen and C-reactive protein levels in unstable coronary artery disease. *Circulation* 1997;96:4204-10.
28 Haverkate F, Thompson SG, Pyke SDM, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet* 1997;349:462-6.
29 Zhang YX, Cliff WJ, Schoefl GI, Higgins G. Coronary C-reactive protein distribution: its relation to development of atherosclerosis. *Atherosclerosis* 1999;145:375-9.
30 Cermak JC, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood* 1993;82:513-20.
31 de Beer FC, Soutar AK, Baltz ML, Trayner I, Feinstein A, Pepys MB. Low density and very low density lipoproteins are selectively bound by aggregated C-reactive protein. *J Exp Med* 1982;156:230-42.
32 Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement activation and atherogenesis. Binding of CRP to degraded, nonoxidised LDL enhances complement activation. *Arterioscler Thromb Vasc Biol* 1999;19:2348-54.
33 Cushman M, Legault C, Barrett-Connor EL, Stefanick ML, Kessler C, Judd HL, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins: the postmenopausal estrogen/ progestin interventions (PEPI) study. *Circulation* 1999;100:717-22.
34 Ridker PM, Hennekens CH, Buring JE, Rifai N. C reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836-43.

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

N J Wald, M R Law, J K Morris, X Zhou, Y Wong, M E Ward

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BUPA Epidemiological Research Group, Wolfson Institute of Preventive Medicine, St Bartholomew's and the Royal London School of Medicine and Dentistry, London EC1M 6BQ
N J Wald
professor
M R Law
reader
J K Morris
statistician

Molecular Microbiology Group, Southampton General Hospital, Southampton SO16 6YD
X Zhou
research assistant
Y Wong
research registrar
M E Ward
professor

Correspondence to:
N J Wald
n.j.wald@mds.qmw.ac.uk

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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 *Chlamydophila 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