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Prospective Study of *Chlamydia pneumoniae* IgG Seropositivity and Risks of Future Myocardial Infarction

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Background—*Chlamydia pneumoniae* has been hypothesized to play a role in atherothrombosis. However, prospective data relating exposure to *Chlamydia pneumoniae* and risks of future myocardial infarction (MI) are sparse.

Methods and Results—In a prospective cohort of nearly 15 000 healthy men, we measured IgG antibodies directed against *Chlamydia pneumoniae* in blood samples collected at baseline from 343 study participants who subsequently reported a first MI and from an equal number of age- and smoking-matched control subjects who did not report vascular disease during a 12-year follow-up period. The proportion of study subjects with IgG antibodies directed against *Chlamydia pneumoniae* increased with age and cigarette consumption. However, prevalence rates of *Chlamydia pneumoniae* IgG seropositivity were virtually identical at baseline among men who subsequently reported first MI compared with age- and smoking-matched control subjects. Specifically, the relative risks of future MI associated with *Chlamydia pneumoniae* IgG titers $\geq 1:16$, 1:32, 1:64, 1:128, and 1:256 were 1.1, 1.0, 1.1, 1.0, and 0.8, respectively (all probability values not significant). There was no association in analyses adjusted for other risk factors, evaluating early as compared with late events, or among nonsmokers. Further, there was no association between seropositivity and concentration of C-reactive protein, a marker of inflammation that predicts MI risk in this cohort.

Conclusions—In a large-scale study of socioeconomically homogeneous men that controlled for age, smoking, and other cardiovascular risk factors, we found no evidence of association between *Chlamydia pneumoniae* IgG seropositivity and risks of future MI. (*Circulation*. 1999;99:1161-1164.)

Key Words: antibodies ■ myocardial infarction ■ proteins ■ risk factors ■ atherosclerosis

Retrospective and cross-sectional studies have tended to show positive associations between *Chlamydia pneumoniae* and prevalent coronary heart disease.¹⁻⁶ Further, histopathological evidence of *Chlamydia pneumoniae* has been detected in atherosclerotic plaque.⁷⁻¹¹ Thus it has been hypothesized that infection with this agent may play a causative role in atherothrombosis.^{12,13} The observation that inflammation is associated with increased risk of future myocardial infarction (MI) is also compatible with this hypothesis.¹⁴

Despite these observations, prospective epidemiological data demonstrating evidence of *Chlamydia pneumoniae* exposure before the onset of cardiovascular disease are sparse. For example, data from the Helsinki Heart Study are consistent with a 40% increase in risk among those with baseline IgG titers $\geq 1:128$.¹⁵ However, this observation was limited to an analysis of 87 case-control pairs and was not statistically significant (OR=1.4, 95% CI 0.5 to 4.1). In a second study from Finland, no association between elevated *Chlamydia pneumoniae* titers and coronary risk was reported overall, although a nonsignificant increase in risk was observed in the subgroup of nondiabetic men (RR=1.8, 95% CI 0.9 to 3.7).¹⁶ Thus the

hypothesis that prior exposure to *Chlamydia pneumoniae* is a risk factor for future MI requires more data deriving from large prospective studies.^{17,18}

To directly address this issue, we determined IgG antibody titers directed against *Chlamydia pneumoniae* in a large cohort of apparently healthy men participating in the Physicians' Health Study (PHS) who were followed prospectively over a 12-year period for the occurrence of first MI.

Methods

We used a prospective nested case-control study design within the PHS, a randomized trial of aspirin and beta-carotene among 22 071 apparently healthy US physicians 40 to 84 years of age at the time of randomization.¹⁹ Study subjects were free of prior MI, stroke, transient ischemic attack, or cancer at study entry. Potentially eligible participants were asked to provide baseline blood samples, which have been stored at -80°C . Of 22 071 physicians randomized, 14 916 returned a baseline sample (68%).

For this analysis, potential cases were those PHS participants who were free of vascular disease at baseline when blood was collected and subsequently reported MI during follow-up. Potential control subjects were PHS participants who provided baseline blood and remained free of cardiovascular disease during follow-up; individually matched control subjects were selected at random from partic-

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ipants who met the matching criteria of age (± 1 year), time since randomization, and smoking status. Following this design, 343 case-control pairs were constructed.

The presence of MI among case subjects was ascertained through review of hospital records, death certificates, and autopsy reports with the use of standardized criteria. The diagnosis of MI was confirmed by documentation of symptoms plus either elevations of cardiac enzymes or diagnostic changes on ECGs or by autopsy reports. Silent MIs were excluded because they could not be dated accurately.

For each case and control subject, plasma collected and frozen at study entry was thawed and assayed for IgG antibody titers directed against *Chlamydia pneumoniae* with the use of microimmunofluorescence techniques.²⁰ All assays were performed and interpreted by a single investigator unaware of case or control status. Matched specimens were analyzed in pairs with the position of the case varied within pairs to avoid systematic bias and reduce interassay variability. Pairs were handled identically throughout processing. In pilot data, 18 of 20 antibody titers performed in blinded split samples were either identical or within 1 dilution of each other; the remaining 2 split samples were within 2 dilutions of each other and were all in excess of 1:256, a range in which increased variability is expected. On study completion, an additional 25 samples were randomly selected for repeat analysis; in this group, all samples with titers $< 1:256$ were again either identical or within 1 dilution.

Prior investigations of *Chlamydia pneumoniae* IgG titers and vascular risk have been inconsistent in the definition of seropositivity, and there is disagreement as to whether sequentially higher titers imply greater burdens of infection. Thus rather than choosing a single cut-point to define seropositivity, we chose on an a priori basis to evaluate the association between *Chlamydia pneumoniae* and subsequent risk in a series of analyses defining seropositivity as IgG titers $\geq 1:16$, 1:32, 1:64, 1:128, and 1:256. Conditional logistic regression was used to compute relative risks of future MI for individuals with titers above and below each of these prespecified cut-points; adjusted estimates were computed in models which, in addition to the matching criteria, controlled for body mass index, hypertension, hypercholesterolemia, diabetes, and family history. Stratified analyses were performed by smoking status and length of follow-up.

Results

Table 1 displays clinical characteristics of the 343 case-control pairs. As expected, case subjects had a higher prevalence of diabetes, hypertension, hyperlipidemia, and obesity.

Prevalence of *Chlamydia* seropositivity (IgG titers $\geq 1:16$) was 68%, a figure comparable to that found in a large cross-sectional survey of middle-aged American men.²¹ Seropositivity was directly associated with age and cigarette consumption (Figure).

Table 2 displays crude and adjusted risks of future MI according to baseline IgG titer. As shown, the number of case and control subjects with positive baseline IgG titers was virtually identical regardless of cut-point used to define seropositivity. For example, 69.4% of cases and 67.4% of the matched control subjects had titers $\geq 1:16$ such that the relative risk of future MI associated with evidence of prior exposure to *Chlamydia pneumoniae* at this cut-point was 1.1 (95% CI 0.8 to 1.5, $P=0.6$). Similarly, the relative risks associated with antibody titers $\geq 1:32$, 1:64, 1:128, and 1:256 were 1.0, 1.1, 1.0, and 0.8, respectively (all probability values not significant). These null values were not altered in analyses adjusting for baseline differences in coronary risk factors.

Table 3 displays the risks of developing MI in analyses stratified by smoking status. Among nonsmokers, the relative risks of future MI associated with IgG antibody titers $\geq 1:16$,

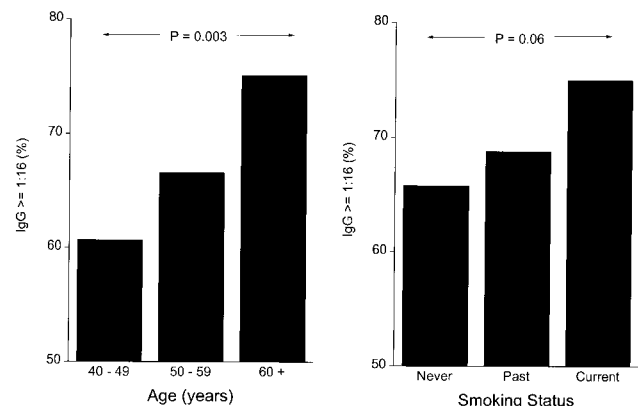
TABLE 1. Baseline Clinical Characteristics of 343 Apparently Healthy Study Subjects Who Subsequently Developed MI (Case Subjects) Compared With 343 Apparently Healthy Study Subjects Who Remained Free of Vascular Disease During Follow-up (Control Subjects)

	Case Subjects (n=343)	Control Subjects (n=343)	P
Age, y	56.9	56.7	...
Sex, % men	100	100	...
Smoking status, %			
Never	47.1	47.1	...
Past	38.9	38.9	...
Current	14.0	14.0	...
Body mass index, kg/m ²	25.4	24.9	0.02
Blood pressure, mm Hg			
Systolic	128.5	125.5	0.001
Diastolic	79.9	79.0	0.1
History of hypertension, %	23.8	17.3	0.04
History of diabetes, %	6.2	1.5	0.001
Total cholesterol, mg/dL	226.7	213.7	0.001
HDL cholesterol, mg/dL	46.1	50.1	0.005
Triglycerides, mg/dL	206.1	152.4	0.001
Family history of coronary artery disease, %	18.2	12.5	0.04

Case subjects and control subjects were matched for age, sex, and smoking status.

1:32, 1:64, 1:128, and 1:256 were 1.1, 1.1, 1.1, 1.0, and 0.9, respectively (all probability values not significant). Similarly, among past or current smokers, the relative risks associated with each of these titers were 1.1, 0.9, 1.1, 1.0, and 0.7 (all probability values not significant).

To evaluate the possibility that effects of *Chlamydia pneumoniae* on risk of future MI might have a long latency period, we stratified our analysis by time to event. No evidence of increased risk was found at any titer for individuals who went on to have MI during the first 6 years of study follow-up or between years 7 and 12. For example, the relative risk of MI during years 0 through 6 for those with antibody titers $\geq 1:32$ at baseline was 0.8 ($P=0.5$), whereas the relative risk of MI during years 7 through 12 was 1.1 ($P=0.6$).



Proportion of study participants with evidence of baseline IgG titers $\geq 1:16$ directed against *Chlamydia pneumoniae*, according to age and smoking status.

TABLE 2. Relative Risks of Developing MI Among Apparently Healthy Men According to Baseline Antibody Titer Directed Against *Chlamydia pneumoniae*

IgG Titer	Case Subjects n (%)	Control Subjects n (%)	RR*	95% CI	P	RR†	95% CI	P
≥1:16	238 (69.4)	231 (67.4)	1.1	0.8–1.5	0.6	1.1	0.8–1.6	0.5
≥1:32	203 (59.2)	202 (58.9)	1.0	0.7–1.4	0.9	1.0	0.7–1.4	0.9
≥1:64	143 (41.7)	136 (39.7)	1.1	0.8–1.5	0.6	1.1	0.8–1.5	0.6
≥1:128	73 (21.3)	74 (21.6)	1.0	0.7–1.4	0.9	1.0	0.7–1.5	0.9
≥1:256	33 (9.6)	40 (11.7)	0.8	0.5–1.3	0.4	0.8	0.4–1.3	0.3

*Crude relative risk, matched on age and smoking status and controlled for randomized treatment assignment.

†Adjusted relative risk, matched on age and smoking status, controlled for randomized treatment assignment, body mass index, history of hypertension, history of hypercholesterolemia, diabetes, and a family history of coronary artery disease.

Prior data from this cohort demonstrate that C-reactive protein (CRP) levels are significantly higher among those who subsequently develop MI, and it had been hypothesized that elevations of this inflammatory marker might result from *Chlamydial* infection.¹⁴ However, we found no evidence of association between CRP and IgG seropositivity regardless of titer evaluated (Table 4).

Discussion

In prospective data from a large cohort of socioeconomically homogeneous middle-aged men, we found no association between IgG seropositivity to *Chlamydia pneumoniae* and risk of future MI. Indeed, the distribution of baseline IgG antibody titers directed against *Chlamydia pneumoniae* among those who subsequently reported a first MI was virtually identical to the distribution among those who remained free of disease during a 12-year follow-up. Further, we found no significant relation of *Chlamydia* seropositivity with plasma level of CRP, an inflammatory marker previously reported to predict risk of future MI in this cohort.¹⁴ Thus in these data, serological evidence of prior exposure to *Chlamydia pneumoniae* does not appear to be a major cause of chronic low-grade inflammation as measured by CRP or a marker for future MI.

Potential limitations of our study must be considered. For example, if the assay used to detect *Chlamydia* titers was insensitive or nonspecific, then systematic error might have led to a spurious null result. We believe this unlikely for several reasons. First, the microimmunofluorescence assay used is widely considered the gold standard for determining *Chlamydia* titers, and assays were performed in a laboratory with >25 years of experience. Second, in analyses performed before, during, and

after this study, high levels of reproducibility were consistently found between blinded split samples repeated on different occasions. Third, because all assays were performed and interpreted by a single laboratory investigator, there is no possibility of interobserver variation. Fourth, to ensure that no systematic errors in titer classification occurred, we sent blinded split samples from our laboratory to that of Drs J. Thomas Grayston and S.P. Wang (University of Washington) and again found titers to be highly correlated. Finally, the overall IgG seropositivity observed in our study (68%) is virtually identical to that previously reported for men of similar age in the United States (65% to 70%).²¹

Given this situation, alternative explanations for the null results observed in this large-scale, prospective study must be considered. Chance seems to be an unlikely explanation because our sample size is substantial, and the 95% confidence intervals in our data are narrow. In this regard, power in our data to detect relative risks as small as 1.5 ranged between 84% and 98%, depending on the IgG cut-point used.

With respect to bias, we believe a strength of our prospective nested case-control design is that it greatly reduces selection bias. Specifically, in our analysis, all study subjects derive from a homogeneous population in which case and control status is determined by the subsequent development of disease rather than by any selection process initiated by the investigators or study participants.

With respect to confounding, we were able in our analysis to tightly match case and control subjects on the basis of age and

TABLE 3. Relative Risks of Developing MI Among Apparently Healthy Men According to Baseline Antibody Titer Directed Against *Chlamydia pneumoniae* Stratified by Smoking Status

IgG Titer	Never-smokers (n=322)			Past/Current Smokers (n=362)		
	RR	95% CI	P	RR	95% CI	P
≥1:16	1.1	0.7–1.8	0.6	1.1	0.7–1.7	0.7
≥1:32	1.1	0.7–1.7	0.7	0.9	0.6–1.4	0.7
≥1:64	1.1	0.7–1.7	0.7	1.1	0.7–1.6	0.7
≥1:128	1.0	0.6–1.7	0.9	1.0	0.6–1.6	0.9
≥1:256	0.9	0.4–2.0	0.8	0.7	0.4–1.4	0.3

TABLE 4. Median Levels of CRP for Study Participants According to Baseline IgG Antibody Titer Directed Against *Chlamydia pneumoniae*

IgG Titer	CRP, mg/L	P
≥1:16	1.18	0.4
<1:16	1.49	
≥1:32	1.16	0.3
<1:32	1.41	
≥1:64	1.18	0.7
<1:64	1.24	
≥1:128	1.09	0.8
<1:128	1.31	
≥1:256	0.80	0.8
>1:256	1.24	

smoking status, both of which are positively related to *Chlamydia* titers. Further, as our study population was composed entirely of physicians, we were able to greatly reduce the potential for confounding on the basis of socioeconomic status. On the other hand, despite the large number of cases evaluated in our study, a potential limitation of our study population is that the absolute event rate is low and as such might limit our ability to detect the effect of various risk factors. We believe this possibility unlikely, however, because all established risk factors have been demonstrated to be major determinants of risk in the PHS,^{19,22} as have a series of nontraditional risk factors including tissue plasminogen activator antigen,²³ homocysteine,²⁴ D-dimer,²⁵ CRP,^{14,26} and soluble intercellular adhesion molecule type-1 (sICAM-1).²⁷

In conclusion, these prospective data do not support the hypothesis that IgG seropositivity against *Chlamydia pneumoniae* is a marker of risk for future MI. Although we did not measure IgA or IgM titers in our study, we do not believe this to be a major limitation. First, the great majority of cross-sectional and retrospective studies performed that suggest a positive association for *Chlamydia pneumoniae* have relied primarily or exclusively on IgG serology to determine exposure status.¹⁷ Second, completed as well as ongoing clinical trials of antibiotic therapy to reduce cardiovascular risk have used IgG titers as a critical determinant of eligibility.^{28,29} Finally, it has recently been demonstrated that IgG titers but not IgA or IgM titers correlate with the ability to directly detect *Chlamydia* within human coronary arteries obtained at autopsy.³⁰

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