

Baseline IgG Antibody Titers to *Chlamydia pneumoniae*, *Helicobacter pylori*, Herpes Simplex Virus, and Cytomegalovirus and the Risk for Cardiovascular Disease in Women

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Background: Results of cross-sectional and retrospective studies have suggested that chronic infection may be a risk factor for cardiovascular disease. However, prospective data evaluating the relation between baseline antibody titers against various plausible agents and risk for cardiovascular disease are sparse, particularly among women.

Objective: To determine whether previous exposure to *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus, or cytomegalovirus is associated with increased risk for cardiovascular events.

Design: Prospective, nested, case-control study.

Setting: Women's Health Study.

Participants: Apparently healthy postmenopausal women.

Measurements: IgG antibody titers against *C. pneumoniae*, *H. pylori*, herpes simplex virus, and cytomegalovirus were measured in baseline blood samples obtained from 122 study participants who subsequently reported a first cardiovascular event (case-patients) and 244 participants matched for age and smoking status who did not report a cardiovascular event (controls) during 3 years of follow-up.

Results: Little evidence was found of an association between risk for cardiovascular events and baseline IgG seropositivity for antibodies against *C. pneumoniae* (rate ratio, 1.1 [95% CI, 0.7 to 1.8]), *H. pylori* (rate ratio, 0.90 [CI, 0.6 to 1.4]), herpes simplex virus (rate ratio, 1.2 [CI, 0.6 to 2.1]), and cytomegalovirus (rate ratio, 0.9 [CI, 0.6 to 1.5]). In addition, there was little evidence of an association between a participant's total number of infections and subsequent cardiovascular risk ($P > 0.2$).

Conclusion: In apparently healthy postmenopausal women, little evidence was found that previous infection, as measured by IgG antibody titers to *C. pneumoniae*, *H. pylori*, herpes simplex virus, and cytomegalovirus, is associated with subsequent risk for cardiovascular disease.

Data from several studies have suggested that persons with coronary heart disease have an increased prevalence of chronic infection with such agents as *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus, and cytomegalovirus (1). In addition, it has been hypothesized that infection may be a risk factor for acute coronary events (2). However, chronic infection is also more prevalent among smokers, elderly persons, and persons of lower socioeconomic status, and persons with a history of coronary disease may be more susceptible to subsequent infection. Therefore, it is uncertain whether the associations between infection and coronary heart disease that have been observed in retrospective and cross-sectional studies were caused by confounding or represent a result of ischemic heart disease rather than a cause (3).

To resolve these issues, investigators have used prospective, controlled settings—in which exposure status can be ascertained before the onset of thrombosis—to evaluate the theory that previous infection is related to atherosclerosis. The few reported prospective studies of *C. pneumoniae* (4–7), *H. pylori* (8–12), herpes simplex virus (13), and cytomegalovirus (13) have not provided strong evidence of an association; however, none of these studies evaluated multiple infectious exposures simultaneously. This is a potentially important issue because it has been hypothesized that a person's total burden of pathogens may be a critical factor in determining atherogenesis (14). In addition, the available prospective data were derived from studies that predominantly or exclusively evaluated men.

To further investigate the theory that previous infection is related to atherothrombosis, we measured IgG antibody titers against *C. pneumoniae*, *H. pylori*, herpes simplex virus, and cytomegalovirus in baseline blood samples obtained from a large cohort of apparently healthy postmenopausal women who were followed prospectively for the occurrence of first cardiovascular events. We also related these antibody titers to plasma concentrations of high-sensitivity C-reactive protein, a marker of chronic inflammation that has previously been shown to predict coronary risk in this cohort (15).

Ann Intern Med. 1999;131:573-577.

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Methods

We performed a nested case-control study among participants in the Women's Health Study, an ongoing primary prevention trial that enrolled 39 876 postmenopausal female health professionals with no history of myocardial infarction, stroke, or transient ischemic attack (16). Of women enrolled in the study, 28 311 (71%) provided baseline blood samples, which were frozen in liquid nitrogen until analysis.

Case-patients were initially healthy Women's Health Study participants who provided a baseline blood sample and who subsequently reported a first cardiovascular event (myocardial infarction, stroke, cardiovascular death, or coronary revascularization procedure) during follow-up. Reported myocardial infarction was confirmed if review of hospital records met World Health Organization criteria and if the event was associated with characteristic electrocardiographic changes or elevated levels of cardiac enzymes. Reported stroke was confirmed if records showed a new neurologic deficit persisting for more than 24 hours; such records almost always included evidence from computed tomography or magnetic resonance imaging. Reported coronary angioplasty or coronary bypass surgery was confirmed by record review. Cardiovascular death was confirmed by autopsy reports, death certificates, and circumstances at the time of death.

Controls were randomly selected from the pool of initially healthy Women's Health Study participants who remained free of cardiovascular disease during study follow-up and also provided a baseline blood sample. Two controls, matched for age (± 1 year) and smoking status (never, past, or current), were selected for each case-patient.

Baseline plasma samples from case-patients and

controls were thawed and assayed for IgG antibody titers against *C. pneumoniae* by using microimmunofluorescence techniques (6, 17). Similarly, enzyme-linked immunosorbent assays were used to qualitatively detect IgG antibodies to herpes simplex virus (Wampole Laboratories, Cranbury, New Jersey), cytomegalovirus (Gull Laboratories, Salt Lake City, Utah), and *H. pylori* (Wampole Laboratories) in baseline plasma samples. For each variable, samples from a case-patient and two matched controls were assayed as a group; samples from the case-patient were positioned randomly within the group to reduce interassay variability and to avoid systematic bias. All laboratory investigators were unaware of case-patient or control status at the time of IgG analysis. C-reactive protein levels were evaluated by using a high-sensitivity assay (Abbott Laboratories, Abbott Park, Illinois), as described elsewhere (15).

We used conditional logistic regression analyses to test for evidence of an association between the presence of IgG antibodies at baseline and subsequent risk for cardiovascular events. A priori, we chose to evaluate the association between *C. pneumoniae* and subsequent risk across a series of IgG antibody titers (range, $>1:16$ to $>1:128$). The presence or absence of IgG seropositivity for herpes simplex virus, cytomegalovirus, and *H. pylori* was determined according to cut-points established by the assay manufacturers. Adjusted estimates of risk were computed after we controlled for baseline differences between case-patients and controls.

To evaluate the theory that total infectious burden rather than any single IgG titer may be associated with risk, we further classified study participants as having zero, one, two, three, or four positive antibody titers. Because the number of study participants with zero positive titers at baseline was small ($n = 15$), data from these participants and from participants with one positive titer were combined for analysis. Where applicable, tests for trend were used to evaluate evidence of increasing risk across increasing antibody titers (18). We also compared the distribution of C-reactive protein values for participants with two or more positive IgG antibody titers with the distribution of values for participants with zero or one positive IgG antibody titer. All *P* values are two-tailed.

Results

Among 122 case-patients, 85 myocardial infarctions or strokes occurred and 37 coronary revascularizations were performed. As is expected in a study of incident coronary events, case-patients were more likely than controls to have a history of hyperlipidemia (45.9% compared with 28.3%; *P* =

Table 1. Rate Ratios for Future Cardiovascular Events among Apparently Healthy Women, according to Baseline IgG Antibody Status*

Titer	Case-Patients	Controls	Crude Rate Ratio (95% CI)†	Adjusted Rate Ratio (95% CI)†‡
	%			
<i>Chlamydia pneumoniae</i>				
>1:16	38.0	35.3	1.1 (0.7–1.8)	0.9 (0.6–1.6)
>1:32	20.7	19.3	1.1 (0.6–1.9)	0.8 (0.5–1.6)
>1:64	14.1	13.5	1.1 (0.6–2.0)	0.9 (0.4–1.8)
>1:128	4.1	4.1	1.0 (0.4–3.2)	0.9 (0.3–3.1)
Herpes simplex virus	84.4	82.0	1.2 (0.6–2.1)	0.9 (0.5–1.7)
Cytomegalovirus	65.6	66.4	0.9 (0.6–1.5)	1.0 (0.6–1.7)
<i>Helicobacter pylori</i>	34.4	36.9	0.9 (0.6–1.4)	0.9 (0.5–1.5)

* In all analyses, case-patients and controls were matched for age and smoking status (past, current, or never).

† *P* > 0.2 overall.

‡ Controlled for body mass index (kg/m^2), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), exercise frequency (times per week), diabetes (yes or no), and family history of coronary artery disease before 60 years of age (yes or no).

0.001), hypertension (55.5% compared with 31.3%; $P = 0.001$), and diabetes (9.8% compared with 2.1%; $P = 0.001$) and to have a family history of premature coronary disease (21.3% compared with 12.7%; $P = 0.04$). Case-patients also had a greater mean body mass index than controls (27.1 compared with 26.0 kg/m²; $P = 0.05$). Because of matching, mean age (59.3 ± 8.3 years) and smoking status (27.9% of participants currently smoked, 42.6% had never smoked, and 29.5% had smoked in the past) were identical in the case-patient and control groups.

Exposure rates among controls were similar to those reported in previous studies; for example, 60% of controls had *C. pneumoniae* titers greater than 1:8 (1, 2). However, as shown in **Table 1**, the proportion of study participants with positive IgG titers was similar regardless of case-patient or control status. For example, the crude rate ratios for future cardiovascular events in women with *C. pneumoniae* titers greater than or equal to 1:16, 1:32, 1:64, and 1:128 were 1.1, 1.1, 1.1, and 1.0, respectively ($P > 0.2$ overall) (**Table 1**). Similarly, the crude rate ratios for future cardiovascular events associated with baseline seropositivity to *H. pylori*, herpes simplex virus, and cytomegalovirus were 0.9, 1.2, and 0.9, respectively ($P > 0.2$ overall). As shown in **Figure 1**, these point estimates did not change after adjustment for baseline differences in hyperlipidemia, hypertension, exercise frequency, body mass index, diabetes, and family history of premature coronary artery disease.

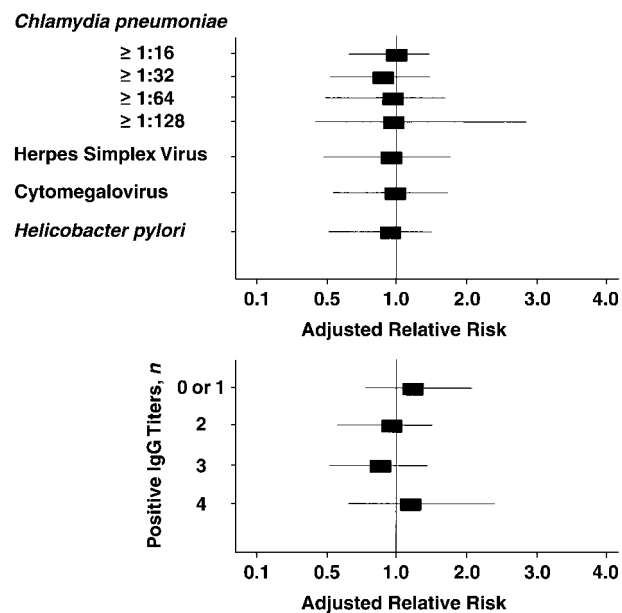


Figure 1. Adjusted rate ratios for future cardiovascular events among apparently healthy women, according to the presence of baseline IgG antibody titers against *Chlamydia pneumoniae*, herpes simplex virus, cytomegalovirus, and *Helicobacter pylori* (top) and the number of positive baseline IgG titers present (bottom). Error bars represent 95% CIs.

Table 2. Rate Ratios for Future Cardiovascular Events among Apparently Healthy Women, according to the Total Number of IgG Antibody Titers Present in a Given Study Participant*

Total Infections	Case-Patients	Controls	Crude Rate Ratio (95% CI)†	Adjusted Rate Ratio (95% CI)†‡
<i>n</i>	%			
0 or 1	24.6	25.8	0.9 (0.6–1.6)	1.2 (0.7–2.2)
2	35.3	34.8	1.1 (0.7–1.7)	0.9 (0.6–1.6)
3	28.7	28.7	1.0 (0.6–1.6)	0.8 (0.5–1.4)
4	11.5	10.7	1.0 (0.5–2.1)	1.2 (0.6–2.7)

* In all analyses, case-patients and controls were matched for age and smoking status (past, current, or never).

† $P > 0.2$ overall.

‡ Controlled for body mass index (kg/m²), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), exercise frequency (times per week), diabetes (yes or no), and family history of coronary artery disease before 60 years of age (yes or no).

To evaluate the theory that total pathogen burden might be associated with increased risk, we stratified patients into four groups according to the total number of positive titers observed in a given case-patient or control. As shown in **Table 2**, the rate ratios for future cardiovascular events in women with zero or one, two, three, or four positive IgG titers were 0.9, 1.1, 1.0, and 1.0, respectively ($P > 0.2$ overall). Similarly, in analyses that evaluated evidence of trend across these four groups, little evidence supported an association ($P > 0.2$ for trend). As shown in **Figure 1**, adjustment for baseline differences between case-patients and controls had little or no effect on these results.

Previously obtained data from this cohort indicate that median C-reactive protein levels are significantly higher in participants who subsequently reported coronary events than in controls (6.45 compared with 3.75 mg/L; $P < 0.001$); this suggests that chronic low-grade inflammation is a marker of risk in this group of women (15). However, when we compared participants with and those without positive IgG antibody titers, the distribution of C-reactive protein levels was similar in isolated analyses limited to each pathogen, in analyses evaluating total pathogen burden (**Figure 2**), and in analyses stratified by case-patient or control status ($P > 0.2$ overall).

Discussion

We found little evidence of an association between baseline IgG antibody titers against *C. pneumoniae*, *H. pylori*, herpes simplex virus, and cytomegalovirus and risk for cardiovascular events. Furthermore, there was little evidence of an association between a participant's total number of pathogens and subsequent cardiovascular risk. Serologic evidence of previous infection was not associated

with statistically significant increases in levels of high-sensitivity C-reactive protein, a marker of chronic inflammation that has been reported to predict cardiovascular risk in this cohort (15).

Because all point estimates for rate ratios are close to 1.0, our prospective data do not confirm observations about infection and atherosclerosis that were made in previous cross-sectional or retrospective studies (1, 2). However, because the 95% CIs in several of our analyses are wide, our data do not exclude the possibility of small to moderate associations.

We believe that our study has several strengths. First, confounding by socioeconomic status seems unlikely because case-patients and controls were chosen from a relatively homogeneous cohort of female health professionals. Similarly, we minimized the potential for confounding by age and smoking status by matching for these factors.

Second, we believe that selection bias was greatly reduced by the prospective design of our study, in which case-patient status was determined solely by the subsequent development of disease. In addition, traditional risk factors (such as hyperlipidemia, hypertension, diabetes, and obesity) and nontraditional markers (such as high-sensitivity C-reactive protein level [15] and total plasma homocysteine concentration [19]) are significant predictors of vascular risk in this group of women. Therefore, the observed event rate is more than adequate to detect the effect of various risk factors.

Finally, in contrast to cross-sectional and retrospective studies that focused on surviving case-patients, our data included fatal and nonfatal events, thereby reducing the likelihood of survival bias.

Our data are consistent with those of the few published prospective studies of infection and

atherothrombosis. Recent data from the prospective British United Provident Association, the Atherosclerosis Risk in Communities Study, the Caerphilly Prospective Heart Disease Study, and the British Regional Heart Study (8–12) also show no statistically significant evidence of an association between *H. pylori* and subsequent cardiovascular risk. Similarly, data from the Physicians' Health Study showed little evidence of an association between herpes simplex virus or cytomegalovirus and cardiovascular risk in men (13); in addition, the Physicians' Health Study and three other studies (4–7) reported little statistically significant evidence of an association between *C. pneumoniae* and coronary risk.

Despite these data, it is important to recognize that none of these studies addressed acute or subacute infection immediately preceding thrombosis. In addition, because experimental evidence suggests that infection with several agents can lead to accelerated atherosclerosis without thrombosis (2), prospective epidemiologic studies focusing on individual infections and thrombotic end points may in theory fail to detect clinically important effects. However, we found little evidence of an association between IgG titers and high-sensitivity C-reactive protein levels; therefore, it seems less likely that any such effect would be mediated by a chronic inflammatory process. A similar lack of an association between C-reactive protein levels and IgG-based evidence of exposure to *C. pneumoniae*, herpes simplex virus, or cytomegalovirus has been reported in men (6, 13).

One potential limitation of our study is that we evaluated baseline IgG titers rather than IgA or IgM titers. We do not believe this to be a major issue for several reasons. First, almost all previous retrospective studies that reported positive associations relied on IgG titers to determine exposure status. Second, it was recently reported that IgG titers (but not IgM or IgA titers) are correlated with the ability to detect infectious particles within human atheroma, at least for *C. pneumoniae* (20); therefore, IgG titers seem to be a better marker of long-term previous exposure than IgA or IgM titers.

Conflicting results from retrospective and prospective epidemiologic studies highlight the controversy over the theory that previous infection is related to atherosclerosis (1–3). Despite our data, we believe that this theory is potentially important to public health, and we support ongoing trials designed to determine whether antibiotic treatment can reduce the risk for cardiovascular disease.

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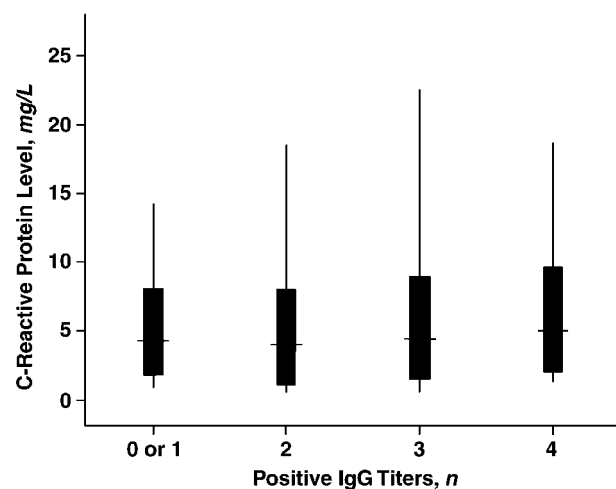


Figure 2. Distribution of C-reactive protein level, according to the number of positive baseline IgG antibody titers present. Error bars represent 95% CIs.

Grant Support: By the National Heart, Lung, and Blood Institute (HL58755) and by an Established Investigator Award from the American Heart Association (Dr. Ridker).

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