The Association of Seropositivity to Helicobacter Pylori, Chlamydia Pneumoniae, and Cytomegalovirus With Risk of Cardiovascular Disease
A Prospective Study

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OBJECTIVES
We sought to determine whether seropositivity to Helicobacter pylori, Chlamydia pneumoniae, and cytomegalovirus (CMV) is an independent predictor of incident cardiovascular disease.

BACKGROUND
Recent reports have suggested that infections may contribute to risk of cardiovascular disease. However, prospective studies of these associations in a free-living population are lacking.

METHODS
We measured serum H. pylori IgG, C. pneumoniae IgG and IgA, and CMV IgG levels in Framingham Heart Study cohort participants. Blood samples were drawn during the 16th biennial examination cycle (1979 to 1982) from 1,187 participants free of cardiovascular disease (mean age 69 years) and stored at −20°C. A pooled primary end point of myocardial infarction, atherothrombotic stroke, and coronary heart disease deaths was studied in relation to serology. Using a Cox model, hazard ratios (HR) and 95% confidence intervals (CI) were calculated, adjusting for age, gender, and established risk factors.

RESULTS
Seropositivity to H. pylori IgG, C. pneumoniae IgG, C. pneumoniae IgA, and CMV IgG was 60%, 45%, 11%, and 69%, respectively. During 10 years of follow-up, incident cardiovascular disease occurred in 199 participants (16.8%). In age- and gender-adjusted models, H. pylori IgG (HR 1.09, 95% CI 0.81 to 1.46), C. pneumoniae IgG (HR 0.91, 95% CI 0.68 to 1.20), C. pneumoniae IgA (HR 0.65, 95% CI 0.39 to 1.07), and CMV IgG (HR 0.84, 95% CI 0.62 to 1.12) were not associated with incident cardiovascular disease. These associations were further attenuated after adjustment for risk factors including body mass index, total and high-density lipoprotein cholesterol, diabetes mellitus, smoking, and hypertension. These estimates did not change for the individual components of cardiovascular disease, and seropositivity to more than one organism did not alter these risk estimates substantially.

CONCLUSIONS
In this elderly cohort, chronic H. pylori, C. pneumoniae, and CMV infections, as evidenced by seropositivity, were not associated with increased risk for cardiovascular disease. Additional studies are needed to determine the relations of chronic infections to cardiovascular disease risk in younger persons.

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Systemic response to chronic infections may play an important role in the pathogenesis of atherosclerosis (1). Recently, there has been a resurgence of interest in examining the role of chronic bacterial and viral infections and accompanying inflammation in the pathogenesis of atherosclerotic cardiovascular diseases (2,3). Several studies have reported an increased risk of myocardial infarction (MI), coronary death, and stroke in association with Helicobacter pylori, Chlamydia pneumoniae, and cytomegalovirus (CMV) infections (2–4), but only few reports (4–6) have measured antibodies to all three agents in the same population. Reports of such associations have raised the possibility that anti-infective treatments might be able to prevent cardiovascular disease (7,8). However, most of the reported positive associations emanate from case-control studies or studies conducted in hospitals, and only a few have reported prospective follow-up for co-infection with more than one organism in a community-based sample (2–4).

Many established risk factors might influence the development of cardiovascular disease. It is therefore important to account for these potential confounders. Also, previously published studies of infection and cardiovascular disease have generally been prone to biases and have lacked adequate sample sizes (2–4). The Framingham Heart Study provides an opportunity to examine the role of chronic infections in the pathogenesis of cardiovascular disease. To this end, we have examined the association of
Abbreviations and Acronyms

CI = confidence interval
CMV = cytomegalovirus
HDL = high-density lipoprotein
HR = hazard ratio
MI = myocardial infarction

seropositivity to *H. pylori*, *C. pneumoniae*, and CMV with the risk of development of cardiovascular disease in the original Framingham Heart Study cohort. This is a carefully described population in which standard risk factors are routinely measured and ascertainment for end points is extensive.

**METHODS**

The Framingham Heart Study, which began in 1948 with 5,209 participants aged 28 to 62 years, has followed subjects at regular intervals as part of a prospective epidemiologic investigation of cardiovascular disease. Enrollment criteria and study design have been published previously (9). Biennial follow-up visits included a medical history, physical examination, blood pressure measurements, and laboratory tests. Participants who were free of cardiovascular disease at the 16th examination (1979 to 1982) and who had a stored blood sample were eligible for the present investigation.

Methodology for assessing risk factors has been published previously (9,10). Risk factors including age, gender, cigarette smoking, total and high-density lipoprotein (HDL) cholesterol, and blood pressure were assessed at the baseline examination. Body mass index (kg/m²) was used as a measure of obesity. Participants were categorized as smokers if they currently smoked cigarettes regularly or if they had quit within a year before the baseline examination. Diabetes mellitus was defined on the basis of a fasting blood glucose level greater than 140 mg/dl (7.77 mmol/l), or two random nonfasting levels greater than 200 mg/dl (11.10 mmol/l), or the use of insulin or an oral hypoglycemic agent.

The primary end point was the occurrence of cardiovascular disease, which encompassed three outcomes: incident MI, death from coronary heart disease, and fatal or nonfatal atherothrombotic stroke. Criteria for MI have been described previously (10). At each clinic examination a history of interim MI was obtained and evaluated for interim MI. All suspected interim events were evaluated by a panel of three physicians who reviewed relevant Framingham Heart Study clinic notes, hospitalization records, and pathology reports. Myocardial infarction was diagnosed when at least two of the following criteria were fulfilled: 1) symptoms consistent with MI; 2) diagnostic electrocardiographic changes of MI; and 3) diagnostic elevation of biomarkers. Death was attributed to the coronary heart disease if there was evidence that coronary heart disease resulted in death and a noncoronary heart disease cause of death was not suspected. Additional information was obtained from records supplied by the hospital or private physician, pathologist, medical examiner, or family (9). The criteria employed for the diagnosis of stroke have been published previously (10). The stroke entity included atherothrombotic brain infarction. Minimum criteria for a stroke consisted of a focal neurological deficit of abrupt onset lasting more than 24 h without other explanation. Subjects were often examined at the time of hospitalization and subsequently in the Framingham Heart Study clinic by a qualified neurologist assigned to the study. Each new case was verified by a review panel of physicians, including a neurologist (10).

**Laboratory protocol.** Blood samples were collected from the original cohort (n = 1,503) at the baseline examination. Serum was frozen in glass vials and stored at −20°C. Specimens were thawed and aliquotted into cryogenic vials beginning in the summer of 1997. One aliquot was shipped on dry ice by courier to Medical Reference Laboratory (Cypress, California). Laboratory personnel were blinded to the clinical status of the study participants. Commercially available IgG antibody tests for *H. pylori*, *C. pneumoniae*, and CMV were conducted according to the manufacturer’s instructions. Blinded duplicate specimens were included (2%) to assess the reproducibility of the laboratory tests, as measured by between-subject variance as a percentage of total variance. Reproducibility estimates were 98% for *H. pylori* and CMV, 69% for *C. pneumoniae* IgG, and 41% for *C. pneumoniae* IgA.

**HELICOBACTER PYLORI IgG.** Sera were diluted in sample buffer, and 0.1 ml was added to individual microtiter wells (Enteric Products International kit). After 20 min at room temperature, plates were washed three times, and 0.1 ml of horseradish peroxidase–conjugated goat anti-human IgG was added. After 20 min at room temperature the plates were washed. Thrombo broth was added, and after 10 min, 1N sulfuric acid was added to stop the reaction. Absorbance was read at 450 nm. Absorbance values were converted to ELISA values using a 3-point standard curve. As recommended by the manufacturer, an ELISA value ≥2.2 was considered positive.

**CHLAMYDIA PNEUMONIAE IgG AND IgA.** Slides containing *C. pneumoniae* elementary bodies attached to the glass were used. There were 12 wells per slide. Sera were diluted 1:16, 1:64, and 1:256 in sample buffer. For IgG measurements, 0.025 ml of each dilution was added to slide wells. After 1 h at 37°C, the slides were washed and dried and then treated with FITC-conjugated goat anti-human IgG or FITC-conjugated goat anti-human IgA. After 30 min at 37°C, slides were washed and dried as before. Glycerol mounting sodium was added, along with a coverslip. Slides were examined using a fluorescent microscope at 400X. The presence of antibody was indicated by fluorescence of elementary bodies. If the IgA was positive then all three...
dilutions were tested to determine the titer. For IgG, titers of 1:128 or greater and for IgA a titer of 1:32 or higher were considered positive as per laboratory protocol.

**CYTOMEGALOVIRUS IgG.** Sera were diluted 1:21 in sample buffer, and 0.1 ml was added to individual microtiter wells (Zeus Scientific kit). After 20 min at room temperature, plates were washed three times, and 0.1 ml of horseradish peroxidase-conjugated goat anti-human IgG was added. After 20 min at room temperature the plates were washed as before, and 1N sulfuric acid was added to stop the reaction. Absorbance was read at 450 nm. An index value was calculated by dividing the patient absorbance value by a cutoff absorbance value (determined by multiplying the low positive control absorbance value by a kit-specific conversion factor). Index values ≥3.52 were considered positive, as recommended by the manufacturer.

**Data analysis.** Prevalence of positive serology for *H. pylori*, *C. pneumoniae*, and CMV antibodies as a function of age, gender, and other demographic features was determined. The association of positive serology with incident cardiovascular events was examined in participants free of cardiovascular disease at the baseline examination. Cox proportional hazards regression models were used to analyze time to end points (11). Hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated after adjusting for age and gender as well as body mass index, total and HDL cholesterol, diabetes mellitus, smoking, and hypertension. The sample size was sufficient to detect an HR of 1.5 or larger with 80% power at the 5% level of significance, for each of the infective agents. Secondary analyses were carried out for incident MI, coronary heart disease death, and stroke.

**RESULTS**

The mean age of study participants was 69 years at the baseline examination. Table 1 shows the demographics and clinical characteristics of the study sample. Seropositivity to *H. pylori*, *C. pneumoniae* IgG and IgA, and CMV was 60%, 45%, 11%, and 69%, respectively. Seropositivity to *H. pylori* and *C. pneumoniae* was higher in men, whereas seropositivity to CMV was higher in women (Table 2). Seropositivity to CMV increased with age in men and women (p = 0.006), whereas no such pattern was observed for *H. pylori* or *C. pneumoniae* (Table 3).

During an average of 10 years (maximum 13 years) of follow-up, 199 (16.8%) of those free of cardiovascular disease at baseline developed an incident cardiovascular event. Table 4 shows the association of seropositivity to each of the agents with new-onset cardiovascular disease. None of the organisms was associated with incident cardiovascular disease in age- and gender-adjusted models, or after adjustment for other risk factors. These estimates did not change for the individual components of cardiovascular disease, and secondary analyses for MI, coronary heart disease death, and stroke revealed no significant association of seropositivity to the organisms with these end points (data not shown).

The presence of co-infections did not impart a greater risk for development of cardiovascular disease, and seropositivity to two or more agents was not associated with incident events (Table 5). Risk estimates did not change substantially when the definition of seropositivity was changed around the predefined levels for each infection (Table 6).

**DISCUSSION**

Our study revealed no significant association between *H. pylori*, *C. pneumoniae*, and CMV infection and incidence of cardiovascular disease. Our results do not support a role of CMV in promoting the risk of cardiovascular disease (HR = 0.90). Age- and gender-adjusted analyses revealed no association of *H. pylori* seropositivity with incident cardiovascular disease (HR = 1.09), and it was further attenuated after adjustment for confounders (HR = 1.02). Our analyses also failed to reveal any association of *C. pneumoniae* IgG (HR = 0.86) and *C. pneumoniae* IgA (HR = 0.77) with incident cardiovascular events.

Several studies have reported associations between chronic infection with *H. pylori*, *C. pneumoniae*, or CMV and risk for coronary heart disease (2,3). It is unclear whether infection with any of these agents is really associated with coronary heart disease because some of these studies were prone to selection biases, limited by small

**Table 2. Prevalence of Seropositivity by Gender**

<table>
<thead>
<tr>
<th></th>
<th>Men (%)</th>
<th>Women (%)</th>
<th>p Value</th>
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<tbody>
<tr>
<td>Helicobacter pylori IgG (≥2.2)</td>
<td>66.5</td>
<td>56.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chlamydia pneumoniae IgG (≥1:128)</td>
<td>58.1</td>
<td>36.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chlamydia pneumoniae IgA (≥1:32)</td>
<td>16.7</td>
<td>8.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cytomegalovirus IgG (≥3.52)</td>
<td>62.5</td>
<td>72.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 3. Prevalence of Cytomegalovirus Seropositivity by Age and Gender**

<table>
<thead>
<tr>
<th></th>
<th>&lt;70 Years (%)</th>
<th>70–79 Years (%)</th>
<th>&gt;80 Years (%)</th>
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<tbody>
<tr>
<td>Men</td>
<td>59.3</td>
<td>65.9</td>
<td>73.8</td>
</tr>
<tr>
<td>Women</td>
<td>69.6</td>
<td>74.9</td>
<td>82.3</td>
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*Age group differences: chi-square = 10.18, 2 df, p = 0.006, accounting for gender difference. Gender difference: chi-square = 12.39, 1 df, p < 0.001, accounting for age group differences.*
Table 4. Seropositivity and Risk of Cardiovascular Disease

<table>
<thead>
<tr>
<th><strong>Age and gender adjusted</strong></th>
<th><strong>Hazard Ratio (95% Confidence Intervals)</strong></th>
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<tbody>
<tr>
<td><em>Helicobacter pylori</em> IgG (≥2.2)</td>
<td>1.09 (0.81–1.46)</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em> IgG (≥1:128)</td>
<td>0.91 (0.68–1.20)</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em> IgA (≥1:32)</td>
<td>0.65 (0.39–1.07)</td>
</tr>
<tr>
<td>Cytomegalovirus IgG (≥1:32)</td>
<td>0.84 (0.62–1.12)</td>
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Age, gender, and risk factor adjusted* |
*Helicobacter pylori* IgG (≥2.2) | 1.02 (0.75–1.40) |
*Chlamydia pneumoniae* IgG (≥1:128) | 0.86 (0.64–1.16) |
*Chlamydia pneumoniae* IgA (≥1:32) | 0.77 (0.47–1.28) |
Cytomegalovirus IgG (≥1:32) | 0.90 (0.66–1.24) |

*Adjusted for age, gender, body mass index, total and high-density lipoprotein cholesterol, diabetes mellitus, smoking, and hypertension.

Table 5. Co-Infection and Risk of Cardiovascular Disease

<table>
<thead>
<tr>
<th><strong>Seropositivity to any one organism</strong></th>
<th><strong>Hazard Ratio (95% Confidence Intervals)</strong></th>
</tr>
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<tbody>
<tr>
<td>Seropositivity to any one organism</td>
<td>0.88 (0.50–1.56)</td>
</tr>
<tr>
<td>Seropositivity to any two organisms</td>
<td>0.78 (0.45–1.37)</td>
</tr>
<tr>
<td>Seropositivity to any three organisms</td>
<td>0.77 (0.44–1.35)</td>
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*Adjusted for age, gender, body mass index, total and high-density lipoprotein cholesterol, diabetes mellitus, smoking, and hypertension.

Comparison with previous studies. This population-based, prospective study of a large cohort was adjusted for established cardiovascular disease risk factors. It confirmed results reported by larger studies. For *H. pylori*, the adjusted HR of 1.02 is similar to previous reports in the elderly (14), in participants of the ARIC study (15), in two British studies (12,16), and a review of prospective reports (2). Thus, although previous cross-sectional studies have suggested an association and despite potential pathogenetic mechanisms (17–19), our study and more recent studies do not support a strong association of *H. pylori* with risk of cardiovascular disease. Because of confounding by socioeconomic status, associations between *H. pylori* and coronary heart disease are difficult to interpret even in population-based studies (20). However, our study, which comprised a large, relatively homogeneous sample, confirms previous observations of a lack of association of this organism with cardiovascular disease (5,12,14–16).

Several studies have previously reported an association of *C. pneumoniae* infection with risk for coronary heart disease (21–25). Most of these studies measured IgG, but only three prospective studies measured IgA (26–28). Larger population-based studies have not supported an association of *C. pneumoniae* infection with cardiovascular disease (5,29) or reported only a weakly positive association (28). The relative risk (RR) estimates of the present study do not suggest an association of *C. pneumoniae* IgG (HR 0.86; 95% CI 0.64 to 1.16) or *C. pneumoniae* IgA (HR 0.77; 95% CI 0.47 to 1.28) with cardiovascular disease. Therefore, the hypothesis that chronic infection or past *C. pneumoniae* exposure is associated with cardiovascular disease risk is not supported by our study. It is to be noted that evidences of chronic infection such as the presence of *C. pneumoniae* within the atherosclerotic plaque and its detection within peripheral blood mononuclear cells do not correlate well with serology (2). Therefore, whether or not serologic titers to *C. pneumoniae* correlate with chronic infection and whether the absence of seropositivity to *C. pneumoniae* correlates with the absence of previous exposure remain to be demonstrated.

Earlier studies have mostly reported association of CMV infection with coronary atherosclerosis in transplanted hearts, following coronary angioplasty, or in carotid arteries (2,3). In a previous investigation from the Framingham Heart Study, no association between clinically apparent herpes virus infection and coronary heart disease was documented (30). Recent prospective studies have also failed to demonstrate an association of CMV infection with risk of cardiovascular disease (5,6,13,31). Our study confirms these observations from population-based studies (5,6,13,31), and it does not indicate a strong association between CMV infection and risk of cardiovascular disease. Although it has been suggested that co-infection with more than one organism may cause greater inflammatory burden, we were unable to detect increased risk of cardiovascular disease in association with infection with more than one agent (Table 5).

Study implications. This report, in context with the previously reported larger studies, shows the pathogens described in this study are unlikely to be strong predictors of risk of cardiovascular disease. Because these infections are...
potential, a reliable assessment of even a small increase in risk would be epidemiologically significant, particularly if interventions might be able to prevent some of the sequelae (4,5). Additional studies are therefore needed to explore the relation of chronic infections with cardiovascular disease risk in younger persons. Such studies may be particularly informative in association with virulent strains, such as Cag A-bearing *H. pylori* (32) and in susceptible subjects due to genetic predisposition (33). Though some studies have suggested a beneficial role of a short course of antibiotics to treat patients with acute coronary syndromes (7,8,34), our study does not support eradication of *H. pylori*, *C. pneumoniae*, and CMV infection to prevent cardiovascular disease. This is in agreement with recently reported large antibiotic trials that failed to show benefit in postmyocardial infarction or unstable angina patients with elevated *C. pneumoniae* titers (35). At present, the evidence is insufficient to designate infections as a causal risk factor for cardiovascular disease.

**Study strengths and limitations.** The Framingham Heart Study is a large population-based sample in which risk factors are routinely assessed and the follow-up is extensive. The study sample comprises both men and women, thus providing a cohort inherently suitable for examining the association of chronic infections with cardiovascular disease (36). Our study sample was overwhelmingly Caucasian; thus, our findings may not apply to other racial and ethnic groups in whom the prevalence of traditional risk factors and chronic infections may be higher. However, the prevalence of infections and the risk estimates in the present study were similar to the results of other population-based studies that examined associations of chronic infections with risk of cardiovascular disease (5,13,29). To our knowledge this is first report that has prospectively examined the association of seropositivity to more than one agent with cardiovascular disease in the elderly. The mean age of the participants was 69 years at the baseline examination, and it is possible that those most susceptible to atherosclerosis in response to chronic infection with these organisms may have been missed. Although it is certainly desirable to repeat this study in a younger cohort, our observations are in conformity with population-based studies that have failed to show strong associations of these infections with cardiovascular disease (29,31,37). Further, this study may be limited by regression-dilution bias (2) and was not designed to examine histological or DNA evidence of infection with the organisms studied, accompanying systemic inflammatory response and risk of cardiovascular disease.

**SUMMARY**

In this elderly cohort, chronic *H. pylori*, *C. pneumoniae*, and CMV infections, as evidenced by seropositivity, were not associated with increased risk for cardiovascular disease. Additional studies are needed to determine the relation of chronic infections to cardiovascular disease risk in younger persons.

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