Chlamydia pneumoniae, Herpes Simplex Virus Type 1, and Cytomegalovirus and Incident Myocardial Infarction and Coronary Heart Disease Death in Older Adults

The Cardiovascular Health Study

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Background—Whether serological evidence of prior infection with Chlamydia pneumoniae, herpes simplex virus type 1 (HSV-1), and cytomegalovirus (CMV) is associated with myocardial infarction (MI) and coronary heart disease (CHD) death remains a source of controversy.

Methods and Results—We conducted a nested case-control study among participants in the Cardiovascular Health Study, a cohort study of persons aged ≥65 years. Cases experienced an incident MI and CHD death (n=213). Control subjects were matched to cases by age, sex, clinic, year of enrollment, and month of blood draw (n=405). Serum was analyzed for IgG antibodies to C pneumoniae, HSV-1, and CMV. After adjustment for other risk factors, the risk of MI and CHD death was associated with the presence of IgG antibodies to HSV-1 (odds ratio [OR] 2.0, 95% CI 1.1 to 3.6) but was not associated with the presence of IgG antibodies to either C pneumoniae (OR 1.1, 95% CI 0.7 to 1.8) or CMV (OR 1.2, 95% CI 0.7 to 1.9). Although there was little association with low to moderate C pneumoniae antibody titers (≤1:512), high-titer (1:1024) C pneumoniae antibody was associated with an increased risk (OR 2.2, 95% CI 1.1 to 4.4).

Conclusions—Among older adults, the presence of IgG antibodies to HSV-1 was associated with a 2-fold increase in the risk of incident MI and CHD death. For C pneumoniae, only high-titer IgG antibodies were associated with an increased risk of MI and CHD death. The presence of IgG antibodies to CMV was not associated with risk among the elderly. (Circulation. 2000;102:2335-2340.)

Key Words: bacteria ■ viruses ■ myocardial infarction ■ coronary heart disease ■ aging

The role of viruses and other infectious agents, such as Chlamydia pneumoniae, in the development of atherosclerosis and incident myocardial infarction (MI) and coronary heart disease (CHD) death has long been an area of investigative interest.1–22 Animal/experimental, pathological, and cross-sectional seroepidemiological studies conducted among middle-aged populations provide some support for the hypothesis that infections with herpes simplex virus type 1 (HSV-1), cytomegalovirus (CMV), and C pneumoniae are associated with the development of atherosclerosis and CHD.5–16 However, recent reports from several prospective studies failed to demonstrate consistent associations between the presence of IgG antibodies to C pneumoniae, HSV-1, and CMV and incident MI.17–22

Given both the high prevalence of IgG antibodies to these agents and the high incidence of cardiovascular disease among older adults, it is particularly important to determine whether serological evidence of prior infection with these agents, assessed late in life, is associated with incident MI and CHD death. We examined this question in an ancillary study to the Cardiovascular Health Study (CHS), a cohort study of men and women aged ≥65 years.23

Methods

Overview

We conducted a nested case-control study within the CHS cohort.24–31 Cases (n=213) were defined by the occurrence of an incident fatal or nonfatal MI (n=167) or CHD death (n=46) that
occurred from 0.1 to 4.3 years after a blood draw. The median time to event was 2.1 years (interquartile range 1.3 to 3.1 years. Approximately 2 control subjects (n = 405) were individually matched to each case (patient) by age, sex, field center, and date (year and month) of blood draw. Sera from cases and control subjects were tested for IgG antibodies to C pneumoniae, HSV-1, and CMV.

**Setting**

Briefly, CHS is a prospective cohort study of 5888 men and women ≥65 years old who were randomly selected from Medicare eligibility lists in 4 communities: Forsyth County, NC; Sacramento County, Calif; Washington County, Md; and Allegheny County, Pa. In 1989 through 1990, 5201 participants were recruited and examined; in 1992 to 1993, 687 additional black participants were recruited and examined.

**Baseline Examination**

The baseline examination, which was conducted in 1989 to 1990, consisted of standard questionnaires that were used to assess risk factors and medical history,23-24 and a clinic examination that included seated blood pressure, anthropometric measures, venipuncture, resting ECG, spirometry, ankle-arm systolic blood pressure index, and carotid sonography.25-29 Fasting serum glucose, plasma lipids and lipoproteins, serum fibrinogen, C-reactive protein, and white blood cell count were measured. During an examination in 1992 to 1993, the baseline examination described here was repeated for the initial (1989 to 1990) cohort and was conducted for the first time for the supplemental black (1992 to 1993) cohort.

**Follow-Up and Classification of Events**

Details of the follow-up for events are described elsewhere.31 All participants were contacted every 6 months, and the contacts alternated between a telephone interview and an in-clinic examination, at each 6-month contact, participants were asked about cardiovascular events and all hospitalizations. Discharge summaries and diagnoses were obtained for all hospitalizations. For all potential cardiovascular events, additional information, including cardiac factors and medical history, were obtained for all hospitalizations. The baseline examination, which was conducted in 1989 to 1990, consisted of standard questionnaires that were used to assess risk factors and medical history, and a clinic examination that included seated blood pressure, anthropometric measures, venipuncture, resting ECG, spirometry, ankle-arm systolic blood pressure index, and carotid sonography. Fasting serum glucose, plasma lipids and lipoproteins, serum fibrinogen, C-reactive protein, and white blood cell count were measured. During an examination in 1992 to 1993, the baseline examination described here was repeated for the initial (1989 to 1990) cohort and was conducted for the first time for the supplemental black (1992 to 1993) cohort.

**Selection of Cases and Control Subjects**

The cases and matched control subjects were selected for this ancillary study in 2 phases, on the basis of the first available serum specimen, with each phase defined according to the source of the blood specimen used to test for antibodies. For the first phase, the source of the blood specimen was the baseline CHS examination (1989 to 1990), and we selected all cases of incident MI or CHD death that occurred during the initial 5.5 years of follow-up after the baseline examination. For the second phase, the source of the blood specimen was the 1992 to 1993 clinical examination, and we selected all cases of incident MI or CHD death that occurred during 3.5 years of follow-up among participants in that examination and whose events had not already been selected in the first phase.

**Serological Evidence of Prior Infection**

Sera obtained at baseline or at the 1992 to 1993 clinic examinations were shipped to the University of Washington (Seattle) for testing. The samples were batch-analyzed with case-control pairs retained in random order within the same batch. The laboratories were blinded to case-control status of the paired sera.

IgG antibody to C pneumoniae was measured with a microimmuno- fluorescence antibody test.32 Sera were screened for a positive antibody response at a dilution of 1:8, and those with a positive response were further tested at dilutions of 1:8, 1:16, 1:32, 1:64, and 1:1024. Based on an earlier report,4 an IgG antibody titer of at least 1:8 was considered diagnostic of past C pneumoniae infection a priori. Prior duplicate testing demonstrated that the percent agreement with a cut point of 1:8 as a positive antibody response was 100%.

IgG antibody to HSV-1 was assessed with a Western blot test. Because HSV titers are not associated with reactivation of latent HSV or with clinical disease,33 we focused on the presence or absence of antibody to HSV-1 and did not measure antibody titers. The methods and criteria for assay of the presence of HSV-1 antibody have been described in detail elsewhere.34 The assay discriminates between HSV-1 and HSV-2.

Antibody to CMV was assessed with a passive latex agglutination assay (CMVScan; Becton Dickinson). This test has been shown to be both sensitive and specific compared directly with other test formats for CMV antibody.35,36 Because of variation in end point titers between runs, the result of considerable run-to-run variability in the performance of the test for the antigen, we focused on the presence or absence of antibody to CMV, rather than on CMV antibody titers.

**TABLE 1. Characteristics of Patients With MI/CHD Death and Control Subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=213)</th>
<th>Control Subjects (n=405)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>74.3±6.2</td>
<td>73.6±5.5</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>63.9</td>
<td>63.2</td>
</tr>
<tr>
<td>White, %</td>
<td>85.5</td>
<td>88.6</td>
</tr>
<tr>
<td>Education, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12, %</td>
<td>38.0</td>
<td>29.0</td>
</tr>
<tr>
<td>12-16, %</td>
<td>30.1</td>
<td>35.6</td>
</tr>
<tr>
<td>17+, %</td>
<td>31.9</td>
<td>35.4</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>11.9</td>
<td>11.8</td>
</tr>
<tr>
<td>Former</td>
<td>48.6</td>
<td>44.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.2±4.4</td>
<td>26.7±4.5</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>25.2</td>
<td>14.1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>50.2</td>
<td>40.7</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>142.3±21.9</td>
<td>136.6±21.7</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>116.3±40.0</td>
<td>109.9±32.2</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>143.7±79.4</td>
<td>131.8±65.9</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>51.1±14.8</td>
<td>53.1±14.2</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>123.8±34.7</td>
<td>123.1±36.1</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>331.3±62.2</td>
<td>324.7±67.6</td>
</tr>
<tr>
<td>WBC, ×10³</td>
<td>6.74±1.9</td>
<td>6.20±1.7</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>3.2±3.6</td>
<td>3.5±6.5</td>
</tr>
<tr>
<td>Albumin, mg/dL</td>
<td>4.0±0.3</td>
<td>4.0±0.3</td>
</tr>
<tr>
<td>FEV1</td>
<td>2.05±0.7</td>
<td>2.24±0.7</td>
</tr>
<tr>
<td>Energy expenditure, kcal/wk</td>
<td>1534.8±1918.2</td>
<td>1715.0±2005.9</td>
</tr>
<tr>
<td>Self-assessed health status, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good or excellent</td>
<td>31.0</td>
<td>42.8</td>
</tr>
<tr>
<td>Fair</td>
<td>42.3</td>
<td>38.9</td>
</tr>
<tr>
<td>Aspirin use,* %</td>
<td>34.5</td>
<td>36.0</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; CRP, C-reactive protein.

* = >2 d in 2 wk before blood draw.

Values are mean±SD.
We used conditional logistic regression, and we estimated the odds ratio (OR) and 95% CIs associated with serological evidence of prior infection, as reflected by the presence of IgG antibodies. Separate multivariate models were used to examine the association between each infection and incident MI and CHD death. We also examined the associations with prior infection when the 3 agents were considered simultaneously in the same logistic model. For C pneumoniae, we also examined the associations of antibody titers with incident MI and CHD death. In addition, we explored whether the time to event influenced the risk associated with either the presence (for HSV-1 and CMV) or titer (for C pneumoniae) of IgG antibody.

To determine whether the association between antibodies and incident MI and CHD death was modified by other factors, we estimated the relative risk in both the presence and absence of other risk factors, markers of chronic inflammation, and measures of subclinical atherosclerotic disease. All probability values represent 2-sided tests.

### Results

The cases and control subjects were similar with respect to age (mean 74 years) and sex (63% were men) (Table 1). Diabetes, hypertension, and former cigarette smoking were more common among the cases, and higher educational attainment and good or excellent self-assessed health were more common among the control subjects. Mean systolic blood pressure and serum glucose, triglyceride, and fibrinogen levels were higher, and mean HDL cholesterol, FEV1, and energy expended in leisure-time physical activity were lower among the cases than the control subjects. The mean body mass index (BMI), LDL cholesterol, C-reactive protein, and serum albumin levels were similar among the cases and control subjects.

Table 2 shows the prevalence of IgG antibodies to HSV-1, CMV, and C pneumoniae among cases and control subjects and the adjusted odds ratios. As expected, the prevalence of IgG antibodies was 82% to HSV-1, 84% to CMV, and 83% to C pneumoniae among control subjects. After adjustment for the matching factors (age, sex, clinic, month, and year of blood draw) and for cigarette smoking, diabetes, hypertension, HDL cholesterol, BMI (continuous), kilocalories expended in physical activity per week (continuous), and years of education (<12, 12–16, 17+), the presence of IgG antibodies to HSV-1 was associated with an increased risk of MI and CHD death (for HSV-1, OR 2.0, 95% CI 1.1–3.2, for CMV, OR 1.2, 95% CI 0.7–1.9). The findings were altered only slightly both after inclusion of the 3 agents in a single conditional logistic regression model and after exclusion of CHD deaths.

For C pneumoniae, there was little evidence of an association between IgG antibody titers of ≤1:512, but the prevalence of an IgG antibody titer of 1:1024 was higher among cases and control subjects. Table 3 shows the prevalence of IgG antibodies to C pneumoniae and the adjusted relative risks. As expected, the prevalence of IgG antibodies was 82% to 1:512, 84% to 1:8, and 83% to 1:64 of C pneumoniae among control subjects. After adjustment for the matching factors (age, sex, clinic, month, and year of blood draw) and for cigarette smoking, diabetes, hypertension, HDL cholesterol, BMI (continuous), kilocalories expended in physical activity per week (continuous), and years of education (<12, 12–16, 17+), the presence of IgG antibodies to C pneumoniae was associated with an increased risk of MI and CHD death (for C pneumoniae, OR 2.0, 95% CI 1.1–3.2, for CMV, OR 1.2, 95% CI 0.7–1.9). The findings were altered only slightly both after inclusion of the 3 agents in a single conditional logistic regression model and after exclusion of CHD deaths.

For C pneumoniae, there was little evidence of an association between IgG antibody titers of ≤1:512, but the prevalence of an IgG antibody titer of 1:1024 was higher among
TABLE 4. Risk of MI/CHD* Death Associated With IgG Antibodies, According to Time to Event

<table>
<thead>
<tr>
<th>Time to Event</th>
<th>Positive Serology or Titer, %</th>
<th>OR† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.1 y</td>
<td>C pneumoniae IgG+ (n=212)</td>
<td>1.6 (0.8–3.3)</td>
</tr>
<tr>
<td></td>
<td>C pneumoniae titer=1024 (n=212)</td>
<td>0.8 (0.4–1.6)</td>
</tr>
<tr>
<td></td>
<td>HSV-1 IgG+ (n=211)</td>
<td>4.2 (1.7–10.7)</td>
</tr>
<tr>
<td></td>
<td>CMV IgG+ (n=212)</td>
<td>1.3 (0.7–2.7)</td>
</tr>
<tr>
<td></td>
<td>Positive titer, %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C pneumoniae IgG 1:1024</td>
<td>2.1† (0.9–4.8)</td>
</tr>
<tr>
<td></td>
<td>HSV-1 IgG 1:1024</td>
<td>1.7 (0.7–3.9)</td>
</tr>
<tr>
<td></td>
<td>CMV IgG</td>
<td>1.1‡ (4.6–2.1)</td>
</tr>
</tbody>
</table>

*Associated with C pneumoniae titer (+/- and 1024 vs <1024), HSV-1 (+/-), and CMV (+/-).
†Adjusted for matching factors and cigarette smoking, diabetes, hypertension, HDL cholesterol, BMI, energy expenditure, and years of education.
‡P for interaction with time to event: for C pneumoniae IgG, 0.045; for HSV-1 IgG+, 0.183; for C pneumoniae titer (1024), 0.045; for HSV-1 IgG+, 0.733; for CMV IgG+, 0.688.

Discussion

Among older adults, the presence of antibodies to HSV-1, measured late in life, was associated with a 2-fold increase in the risk of MI and CHD death overall. In contrast, the presence of IgG antibodies to C pneumoniae and CMV was not associated with incident MI or CHD death. However, there was some evidence that high-titer C pneumoniae antibody was associated with an increased risk, and the association of high titer with risk was particularly large for events that occurred within the first 2.1 years after the blood draw. Multivariate adjustment for other factors that might bias the associations and potential mediators altered the findings only slightly.

Several limitations must be considered in interpretation of these findings. We used the presence of IgG antibodies (and for C pneumoniae, IgG titer) measured on a single occasion late in life to characterize prior infection. Other clinical characteristics not considered in our analyses might have biased the observed associations, and we may have had inadequate statistical power to detect an 80% to 90% increase in the risk of MI or CHD death associated with the presence of prior infection with C pneumoniae and CMV. In addition, the findings from this study may not be generalizable to young and middle-aged adults.

To our knowledge, this is the first prospective report of an association of recent, high-titer C pneumoniae antibodies with the risk of incident MI and CHD death among elderly persons. These preliminary findings suggest that high-titer C pneumoniae antibodies were associated with an increased risk, particularly for events that occurred during the initial 2.1 years after the blood draw. Of note, in a nested case-control study of incident CHD among the middle-aged persons followed for 3 years in the Atherosclerotic Risk in Communities Study, the prevalence of high-titer antibodies (both 1:512 and 1:1024) was higher among patients with CHD than among control subjects. Whether recently measured high-titer antibodies to C pneumoniae are more likely to reflect the etiologically relevant aspect of exposure to this agent, such as chronic infection or reinfestation, rather than merely past infection remains unknown.

The data suggest a possible association between HSV-1 seropositivity and incident MI and CHD death among older adults. Although a similar association of HSV-1 antibodies with incident MI and CHD death was reported recently from the Helsinki Heart Study,20 an association between HSV-1 antibodies and incident MI and stroke was not observed in several prospective studies.21,22 Whether differences in study design or in the populations studied, such as the prevalence of other risk factors, account for the differences in findings remains unknown. In contrast, the absence of an association with CMV antibodies in this report is consistent with the findings from other prospective studies.20–22

This epidemiological study does not provide information about the potential mechanisms for the association of HSV-1 and acute cardiac events. One hypothesis relates to the potential reactivation of HSV-1 in autonomic nerves that innervate the coronary arteries, subsequent endothelial injury, and the initiation of an acute thrombotic event. HSV-1 reactivation is more frequent and more severe among persons who are immunocompromised; older persons are more likely to be immunocompromised; endothelial cells can be infected with HSV-1; and infection results in cell death and a
leukocytoclastic vasculitis-like picture, including the conversion of the endothelial surface from antithrombotic to prothrombotic. 37–40 Furthermore, mural thrombi have been described in the coronary arteries of chickens infected with Marek’s disease virus. 5 Given the role of plaque disruption and the formation of occlusive thrombi in the occurrence of MI and CHD death, it is possible that reactivation of HSV-1 may result in endothelial injury that may increase the risk of thrombosis in the setting of atherosclerotic coronary disease. Unfortunately, because HSV-1 reactivation is not associated with consistent alterations in IgM, IgA, or IgG subclass antibody responses or in quantitative changes in HSV-1-specific antibodies, in this epidemiological study, we could not directly address this hypothesis.

This report is the first to examine the associations of IgG antibodies to C pneumoniae, HSV-1, and CMV, measured late in life, with incident MI and CHD death among older adults. The finding of a possible association of recent high-titer IgG antibodies to HSV-1, assessed latter in life, with incident MI and CHD among older adults is of potential importance, given the high incidence of CHD and the high prevalence of IgG antibody to HSV-1 among older adults. In addition, the association of recent high-titer IgG antibodies to C pneumoniae with the risk of acute cardiac events among older adults must be confirmed in other studies. Several large-scale secondary prevention trials that examine the effect of macroside antibiotic therapy for C pneumoniae among patients with known coronary heart disease are now in progress, but these trials have not targeted older persons with high-titer IgG antibodies. Additional clinical trials should be considered to determine more fully the potential clinical and public health implications of the associations reported herein.

Appendix

Participating Institutions and Principal Staff


Acknowledgments

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References

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