Chlamydia pneumoniae Strain TWAR
Antibody and Angiographically Demonstrated Coronary Artery Disease

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A recent case-control study from Finland reported a strong association between high antibody titers to Chlamydia pneumoniae, strain TWAR, and both chronic coronary heart disease and acute myocardial infarction. The current case-control study investigated the relation between C. pneumoniae immunoglobulin G antibody titers and angiographically diagnosed coronary artery disease. Cases (n=461) were angiography patients with at least one coronary artery lesion occupying at least 50% of the luminal diameter. Controls (n=95) were angiography patients with no demonstrable coronary artery disease. After standardization for age and gender, the geometric mean antibody titer was higher for cases than for controls (30.0 versus 24.0, p=0.04). The estimated risk of coronary artery disease, adjusted for age and gender, was greater among subjects with high (≥1:64) antibody titers than among subjects with low (<1:8) antibody titers (relative risk, 2.0; 95% confidence interval, 1.0–4.0). The risk associated with a high antibody titer was particularly great for coronary artery disease with five or more lesions (relative risk, 2.8; 95% confidence interval, 1.2–7.0). The results of this cross-sectional study support an association between infection with C. pneumoniae and coronary artery disease.

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Infection has been suggested as a cause of atherosclerosis, based on theoretical considerations,1 animal models,2 laboratory investigations,3,4 and seroepidemiological studies.5,6 Most studies have focused on viral agents, especially herpes viruses. A recent case–control study from Finland7 reported a strong association between high antibody titers to Chlamydia pneumoniae, strain TWAR, a newly identified obligate intracellular parasitic bacterium commonly associated with adult respiratory disease,8,9 and both chronic coronary heart disease and acute myocardial infarction. An association between C. pneumoniae antibody and incident coronary heart disease has also been reported in subjects from the Helsinki Heart Study.10

The current study was intended to test the hypothesis that high C. pneumoniae antibody titers are positively associated with angiographically diagnosed coronary artery disease.

Methods

A case–control study was conducted among patients undergoing coronary angiography at the University of Washington Cardiac Catheterization Laboratory between December 13, 1988, and December 15, 1989. Patients with previous bypass surgery were excluded because it was not possible to reliably evaluate the degree of disease in their native coronary arteries.

Cases were defined as patients with at least one coronary artery lesion occupying at least 50% of the luminal diameter. Controls were defined as patients with no demonstrable lesion on angiography. Patients with one or more lesions but with no lesion occupying more than 49% of the luminal diameter were excluded to minimize misclassification of disease status.

Catheterization reports were abstracted to obtain age, gender, the description of the coronary arteries, and indications for catheterization, including history of myocardial infarction or unstable angina.

Blood was obtained at the time of catheterization, and the serum was tested for immunoglobulin G (IgG) and IgM antibody to C. pneumoniae, strain...
TWAR, using the microimmunofluorescent antibody test previously described. The test is specific for C. pneumoniae. A positive test at a titer of greater than or equal to 1:16 for either IgG or IgM is considered diagnostic of C. pneumoniae antibody. IgM antibody was found in less than 2% of the sera. Analysis was therefore limited to IgG antibody.

The difference in geometric mean titers was evaluated using the Mann–Whitney U test and analysis of covariance. Odds ratios, adjusted for age and gender, were used to estimate the relative risks of disease in subjects with medium antibody titers and those with high antibody titers compared with risks in subjects with low antibody titers. Adjusted odds ratios were calculated by logistic regression rather than by stratification methods to obtain more precise odds ratio estimates.

Blood specimens were available from only 32% of all patients catheterized during the study period because the catheterization laboratory staff did not save blood samples when the laboratory was busy. Of the 706 patients whose blood was available, 40 (6%) were missing catheterization reports and an additional 38 (5%) were excluded because of prior bypass surgery. Seventy-two (11%) of the remaining patients had one or more lesions with no lesion occupying more than 49% of the luminal diameter, leaving 556 subjects for analysis. Medical records of a 10% sample of angiography patients whose blood samples had not been saved but who were otherwise eligible for the study (n=125) were also reviewed for comparison with the 556 subjects in the current study. The mean age of angiography patients who did not have a blood sample saved was virtually identical to the study subjects (61.0 years versus 60.8 years). However, a larger proportion of the patients who did not have a blood sample available were male (72% versus 64%). After adjusting for this difference in the proportion of males, the prevalence of coronary artery disease was not significantly different between the patients without available blood samples and the subjects in the current study (86% versus 83%; p=0.41 by χ² test).

Among the 556 subjects in our study, controls (n=95) were more likely than cases (n=461) to be male (67% versus 51%) and older (age, 62.4±11.1 [mean±SD] versus 53±13.3 years). In addition, the prevalence of high antibody titers in the control group was greater in men than in women and increased slightly with age. For these reasons, we adjusted for age and gender in the analyses.

Results

After standardization for age and gender, the geometric mean titers of IgG antibody to C. pneumoniae for cases and controls were 30.0 and 24.0, respectively. This difference was significant at p=0.04 by both the Mann–Whitney U test and analysis of covariance.

The overall association between antibody to C. pneumoniae, dichotomized into low or absent antibody (≤1:8) and antibody definitely present (≥1:16), and coronary artery disease was evaluated using logistic regression. After adjustment for age and gender, the estimated relative risk of coronary artery disease for subjects with definite antibody was 1.6 (95% confidence interval [CI], 1.0–2.7) compared with that for subjects with low or absent antibody. Table 1 presents the distribution of C. pneumoniae IgG antibody titers among cases and controls. Antibody titers are grouped into low (≤1:8), medium (1:16 or 1:32), and high (≥1:64) categories to provide more stable estimates for the relative risk in each group. Subjects with coronary artery disease had a higher prevalence of high and medium antibody titers compared with those of the control group.

The association between antibody and coronary artery disease is reported as the age- and gender-adjusted relative risk of disease in subjects with medium and high antibody titers compared with subjects with low or absent antibody. The estimated relative risks for the presence of coronary artery disease were 1.5 among persons with medium antibody titer (95% CI, 0.9–2.6) and 2.0 among those with high antibody titer (95% CI, 1.0–4.0).

Table 2 presents the relative risk of coronary artery disease associated with each titer group for men and women. The relative risk estimates are similar for men and women examined separately. The wider CIs reflect the smaller numbers in each group compared with the analysis combining men and women.

Table 3 presents the relative risk of coronary artery disease associated with each antibody titer in subjects less than 60 years of age and subjects 60 years and older (men and women combined). There is a suggestion of a greater relative risk in the younger subjects. The relative risk of disease in younger subjects with high antibody titers was 2.3 (95% CI, 0.9–5.9) compared with 1.5 (95% CI, 0.5–4.5) in the older subjects. Again, CIs are wider than in the combined analysis.

Additional analyses were performed to examine the association between antibody titer and severe coronary artery disease, defined as five or more lesions occupying at least 50% of the luminal diameter (n=94). Subjects with high antibody titers had a 2.8-fold increased risk for severe coronary artery disease.
TABLE 2. Chlamydia pneumoniae Antibody Titer in Men With (n=309) and Without (n=48) Coronary Artery Disease and in Women With (n=152) and Without (n=47) Coronary Artery Disease

<table>
<thead>
<tr>
<th></th>
<th>IgG antibody titer*</th>
<th>With CAD*</th>
<th>Without CAD</th>
<th>Estimated relative risk†</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Percent</td>
<td>No. Percent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>Low ≤(1:8):</td>
<td>117 38 27</td>
<td>56</td>
<td>1.5 0.7-3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium (1:16, 1:32)</td>
<td>115 37 14</td>
<td>20</td>
<td>1.5 0.7-3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High ≥(1:64)</td>
<td>77 25 10</td>
<td>15</td>
<td>2.1 0.8-5.3</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>Low ≤(1:8):</td>
<td>77 51 28</td>
<td>60</td>
<td>1.5 0.7-3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium (1:16, 1:32)</td>
<td>52 34 14</td>
<td>30</td>
<td>1.5 0.7-3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High ≥(1:64)</td>
<td>23 15 5 11</td>
<td>1.7</td>
<td>0.6-5.2</td>
<td></td>
</tr>
</tbody>
</table>

*Defined as at least one lesion occupying 50% or more of the luminal diameter.
†Estimated relative risk adjusted for age and gender.

Discussion

The current study has several limitations. The study was cross-sectional and thus could not determine if high antibody titers preceded or followed the development of coronary artery disease. Smoking, hypertension, and lipid status were not measured. However, since the two studies by Saikku et al. reported no association between these characteristics and C. pneumoniae antibody, it is not likely that they introduced an important degree of confounding in the current study. The relation of C. pneumoniae antibody titers to the recency or chronicity of infection is not known. High titers may reflect a recent infection, recurrent infections, chronic infection, or unusual persistence of antibody from a past infection. Low titers or undetectable antibody may reflect loss of antibody rather than a lack of past infection. Finally, the current study was limited to the subset of patients with coronary artery disease who underwent angiography and who had blood samples saved, and thus it may not be generalizable to patients with coronary artery disease in the general population.

The association reported in the current study is consistent with that reported by Saikku et al. The study of Saikku et al compared the prevalence of antibody to C. pneumoniae among 40 patients with an acute myocardial infarction, 30 patients with chronic coronary heart disease and no infarction for at least 6 months, and 41 population controls. The study was restricted to men aged 50 years or younger. The estimated relative risks for IgG antibody titers greater than or equal to 1:128, calculated from the data reported, were 5.8 (95% CI, 1.8-19.6) for acute myocardial infarction and 5.1 (95% CI, 1.5-18.5) for chronic coronary heart disease.

The current study presents additional evidence of an association between C. pneumoniae infection and coronary heart disease in a different study population and with a different disease definition. The association was stronger for high antibody titers than for medium antibody titers. This pattern was consistent between men and women and between younger and older subjects. There was a suggestion that the association between high antibody titer and coronary artery disease may be stronger for younger subjects than older subjects. The best estimate of the association between antibody and disease, from the analysis of all subjects, is that subjects with high antibody titers are twice as likely to have coronary artery disease as are subjects with low or absent antibody.

The relative risk for coronary artery disease in subjects with high titers of IgG antibody to C. pneumoniae reported in the current study is less than that found by Saikku et al. This difference between our study and the study by Saikku et al may be due to chance, or it may reflect differences in study design. The current study relied on angiographically negative controls, whereas the study by Saikku et al used population controls. Angiographically negative controls may be more like angiographically diagnosed cases than are population controls with regard to C. pneumoniae antibody status, resulting in a weaker association than would be found with population controls. Also, the current study used a lower threshold to define high antibody titer (≥1:64 versus ≥1:128) because there were too few subjects with titers greater than or equal to 1:128 to provide a meaningful analysis. Despite these differences, both studies support an association between C. pneumoniae antibody and coronary atherosclerosis.

Several lines of evidence point to infection as a possible contributing cause of atherosclerosis. Infection is compatible with the "response-to-injury" model of
atherogenesis. Cell death, cell damage, or initiation of an immune-mediated inflammatory response are possible mechanisms whereby infection could cause epithelial injury, thus initiating or promoting atherogenesis. Infection with Marek's disease virus, an avian herpes virus, has been shown to induce atherosclerosis in chickens.\textsuperscript{2} The resulting atherosclerotic plaques are reportedly similar to those of human atherosclerosis in histological appearance and anatomic distribution.\textsuperscript{2,16} Studies have also found that human endothelial cells infected with herpes simplex virus type 1 demonstrate excessive thrombin formation,\textsuperscript{17} increased adherence to platelets\textsuperscript{17} and granulocytes,\textsuperscript{18} and decreased cell-to-cell adherence.\textsuperscript{19} Endothelial cells infected with cyto-

megalovirus, another human herpes virus, have an increased adherence to poly morphonuclear leukocytes.\textsuperscript{20} These in vitro changes are similar to changes believed to occur in the development of atherosclerosis.\textsuperscript{15}

Seroprevalence studies have found a higher prevalence of antibody to cytoplamalovirus among several groups of patients with atherosclerotic heart disease compared with controls.\textsuperscript{5,6} Two studies of cardiac transplant patients have reported a significant association between posttransplant infection with cytoplamalovirus and the development of coronary atherosclerosis.\textsuperscript{21,22}

\textit{C. pneumoniae} has several epidemiological and microbiological characteristics that suggest it could play a role in atherogenesis. IgG antibody to \textit{C. pneumoniae} is uncommon among children in developed countries but rises quickly during adolescence and early adulthood to a prevalence of 50–60% in the North American and European populations tested.\textsuperscript{9} The prevalence is usually about 25% higher in men than in women.\textsuperscript{23} Since antibody to \textit{C. pneumoniae} is generally lost over a period of several years,\textsuperscript{24} this high prevalence suggests that most adults are infected and reinfected with \textit{C. pneumoniae}. While most \textit{C. pneumoniae} infections produce only a mild respiratory illness or are asymptomatic,\textsuperscript{25} \textit{C. pneumoniae} has been shown to cause 6–12% of community-acquired pneumonias and 3–5% of bronchitis.\textsuperscript{8,9,26–28} Although \textit{C. pneumoniae} is primarily a respiratory pathogen, it has also been associated with endocarditis.\textsuperscript{29} Like other \textit{Chlamydia} organisms, \textit{C. pneumoniae} is an obligate intracellular parasite that is potentially capable of causing a chronic infection and immune-mediated cellular damage.\textsuperscript{30–32}

The current study provides preliminary evidence for an association between high \textit{C. pneumoniae} IgG antibody titer and coronary artery disease in individuals undergoing diagnostic coronary angiography. A prospective study is clearly needed to evaluate the temporal relation between high antibody titers and the development and progression of atherosclerosis. Such a study should also aim to distinguish an association between antibody and atherosclerosis from an association between antibody and acute atherosclerotic-related events, such as myocardial infarction or unstable angina. In addition to epidemiological studies, laboratory investigations should be undertaken to search for chlamydial proteins and DNA in normal endothelium and atherosclerotic plaques.

The public health and clinical implications of a causal relation between \textit{C. pneumoniae} infection and coronary heart disease are substantial. The current findings should provide a further impetus for additional studies that examine the association between antibody titers to \textit{C. pneumoniae} and coronary heart disease.

\textbf{Acknowledgment}

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