Relation Between Direct Detection of *Chlamydia pneumoniae* DNA in Human Coronary Arteries at Postmortem Examination and Histological Severity (Stary Grading) of Associated Atherosclerotic Plaque

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**Background**—Numerous studies have suggested a link between *Chlamydia pneumoniae* infection, atherosclerosis, and coronary artery disease. However, it is still unclear whether *C pneumoniae* plays a causal role in the pathogenesis of these conditions. Accordingly, we have performed a systematic dissection of the 3 coronary arteries on 33 postmortem subjects and studied the relationship in individual artery segments between the presence of *C pneumoniae* DNA and the severity of associated atherosclerosis.

**Methods and Results**—The prevalence of *C pneumoniae* DNA in arterial segments was determined by polymerase chain reaction (PCR) after controlling for the presence of PCR inhibitors. Atherosclerosis in each arterial segment was graded histologically with the Stary classification. *C pneumoniae* was detected by PCR in 78.8% of subjects, but there was no association between the presence of this DNA and cause of death or grade of atherosclerosis. When paired mild and severe atherosclerotic lesions within subjects were compared, mild lesions were as likely to be positive for *C pneumoniae* as severe lesions.

**Conclusions**—This study demonstrates that *C pneumoniae* can frequently be detected in atheromatous plaques in coronary arteries. However, its distribution did not correlate with severity or extent of disease. *(Circulation. 1999;99:2733-2736.)*

**Key Words:** *Chlamydia pneumoniae* ◆ atherosclerosis ◆ coronary disease ◆ infection

Known risk factors account inadequately for the incidence of coronary artery disease (CAD),1 suggesting that other factors, such as infection, may be important.2 *Chlamydia pneumoniae* infection has been strongly implicated as a possible cause of atherosclerosis; most seroepidemiological and pathological studies have reported a positive association between prior or current infection with the organism and CAD.3 Furthermore, in 3 studies, viable *C pneumoniae* have been cultured from atherosclerotic tissue.4–6 However, there are problems with these data. First, many studies relied on the immunohistological detection of *C pneumoniae* antigen in atheroma. This method generally gives the highest prevalence of chlamydial infection but correlates poorly with detection of *C pneumoniae* DNA by polymerase chain reaction (PCR).5,8–9 Second, PCR-based studies have not been adequately controlled for the differential effect of PCR inhibitors. Third, the ubiquitous occurrence of atheroma from early age10 makes it difficult to select appropriately age-matched control vessels at similar risk of infection. Indeed, a number of studies either have not compared diseased with control vessels or have compared material from subjects of widely different ages.4,11 We have overcome this problem by determining the relationship between *C pneumoniae* infection and the severity of associated CAD within individuals within whom there can be no variation in exposure to blood-borne infection. The effect of tissue inhibitors on PCR sensitivity has been determined by use of internal amplification controls.

**Methods**

**Specimen Collection**
All 3 coronary arteries plus segments of the lung and myocardium were collected from 33 consecutive adult subjects at autopsy. Twenty-one subjects (63.6%) had died of causes related to CAD, and 12 had died of noncardiac causes. Postmortem examinations were performed within 48 hours of death, and the heart was aseptically removed before removal of other organs. In a laminar flow cabinet, each coronary artery was inspected, and 1-cm segments of the most and least diseased regions were sampled. Half of each segment was frozen in liquid nitrogen before detection of *C pneumoniae* DNA by PCR; the remainder was processed for histology.
Polymerase Chain Reaction
DNA was extracted from coronary arteries by conventional proteinase K digestion and phenol-chloroform extraction; PCR was performed in triplicate as previously described. To check for PCR inhibitors in the extracted DNA, samples were spiked with phage λ DNA and subjected to PCR with specific primers. If inhibition was present, samples were diluted 10-fold. A nested PCR was used to detect C. pneumoniae, and all positive products were confirmed by Southern hybridization. Mock extraction controls and PCR-negative controls were interspersed every 5 to 6 samples. A PCR-positive control of DNA equivalent to 1 to 10 elementary bodies was used for every PCR experiment.

Histology
Sections of coronary arteries adjacent to those studied by PCR were stained with hematoxylin and eosin. Most sections were also stained with Schmorl’s stain for lipofuscin and with alizarin for calcium. The severity of atherosclerosis was graded with the Stary classification. This classification ranges from grade 0 (normal) to grade 6 (plaque rupture, fissure, or hemorrhage); grade 4 represents the earliest lesions visible macroscopically.

Results
Characteristics of the 33 recruited subjects are shown in Table 1. Three coronary arteries were obtained from 30 subjects and 2 coronary arteries were from 3 subjects, for a total of 96 arteries. Two segments were available from all but 5 of these arteries, resulting in 187 segments. Segments with atherosclerosis of Stary grade 3 or less were considered to have mild disease; those of grade 4 or greater were considered to have moderate or severe disease. To assess the reproducibility of Stary grading, 48 segments were recoded and then regraded by the same specialist cardiac pathologist. Thirty-four segments were graded identically on both occasions, with the remaining segments differing by only 1 grade (κ test, $P=0.78$). On classifying into mild or severe groups, only 3 of 187 segments was Stary grade 0. Thirty-one subjects had mild atherosclerosis throughout, whereas in another subject there was atherosclerotic lesion in each artery of Stary grade 3 or less.

Distribution of C. pneumoniae in Coronary Arteries
Subjects were as likely to have C. pneumoniae DNA in 1 or 3 (9 of 26) coronary arteries. Considering the individual arteries, 41 of 96 had both mild and moderate or severely diseased segments. In these arteries, the mild segments were just as likely to be positive for C. pneumoniae as the severe segments (McNemar test, $P=1.0$). Similarly, arteries that had mild disease only were just as likely to contain C. pneumoniae DNA as arteries that had moderate or severe disease only (9 of 14 versus 20 of 36; $\chi^2$ test, $P=0.77$). When all arterial segments were considered as a group, no correlation was observed between Stary classification and the presence of C. pneumoniae DNA (Table 3; $\chi^2$ test, $P=0.57$). Thus, in one subject with severe atherosclerosis throughout, C. pneumoniae was found at only 1 site, whereas in another subject who had only mild disease, every arterial segment was positive for C. pneumoniae.

Effect of PCR Inhibitors
As anticipated, DNA samples extracted from coronary artery segments with moderate or severe disease were more likely to contain PCR inhibitors than samples extracted from mildly diseased segments (40 of 114 versus 15 of 73; odds ratio [OR], 2.1; 95% CI, 1.1 to 4.1). Inhibition was associated with the presence of lipid (44 of 128 versus 10 of 54; OR, 2.3; CI, 1.1 to 5.0) and especially calcium (42 of 103 versus 12 of 79; OR, 3.8; CI, 1.9 to 8.0) and was eliminated in all but 1 case by 10-fold dilution. Sixteen of 55 inhibited samples (29.1%) were positive for C. pneumoniae compared with 52 of 132 uninhibited samples (39.4%; $\chi^2$ test, $P=0.17$). We tested each coronary artery segment on 3 separate occasions, but repeated PCR did not always give a consistent result. Nine inhibited samples were positive 1 time; 7 samples, 2 times; and 0 samples, 3 times. For uninhibited samples, 34 were positive 1 time; 8 sample, 2 times; and 10 samples, 3 times.

Discussion
A feature of this study was the careful control of PCR inhibition. DNA extracts of coronary arteries caused signifi-

### Table 1. Characteristics of Patients With and Without C. pneumoniae in Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>Subjects With C. pneumoniae in Coronary Arteries (n=26)</th>
<th>Subjects Without C. pneumoniae in Coronary Arteries (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>14 (53.8)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>Age, y</td>
<td>72.4±9.7</td>
<td>78.3±7</td>
</tr>
<tr>
<td>Coronary deaths, n (%)</td>
<td>17 (65.4)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>Noncoronary deaths, n (%)</td>
<td>9 (34.6)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Presence of plaque rupture, hemorrhage, or acute thrombosis, n (%)</td>
<td>12 (46.1)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>C. pneumoniae in lungs, n (%)</td>
<td>5 (19.2)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>C. pneumoniae in myocardium, n (%)</td>
<td>6 (23.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2. Prevalence of C. pneumoniae DNA in Paired Mild and Severe Segments From 41 Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>Severe Lesions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C. pneumoniae</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>C. pneumoniae</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Mild lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. pneumoniae positive</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>C. pneumoniae negative</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>
TABLE 3. Prevalence of *C* pneumoniae in Atherosclerotic Coronary Artery Segments According to Severity of Disease (Stary Classification)

<table>
<thead>
<tr>
<th>Coronary artery segments positive for <em>C</em> pneumoniae, n (%)</th>
<th>Mild Lesions</th>
<th>Severe Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18/41 (43.9)</td>
<td>9/32 (28.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17/45 (37.8)</td>
<td>18/56 (32.1)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (46.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*C* pneumoniae was more prevalent in severely diseased than mildly diseased arterial segments. In 1 study, 15% of carotid endarterectomy samples were found to be positive for *C* pneumoniae by PCR, whereas the organism could not be detected in macroscopically normal segments adjacent to the diseased areas. Similarly, in an autopsy study of young persons in which samples were age and sex matched, *C* pneumoniae could not be found in normal segments but was found in 2 of 11 segments with intimal thickening and in 6 of 7 samples with atheroma.

In this study, the finding that *C* pneumoniae vascular infection is focal and not associated with the extent or severity of atherosclerosis does not disprove a role for the organism in CAD. After all, if *C* pneumoniae causes atherosclerosis, its presence would be expected to precede that of disease. Results from rabbit models indicate that intranasal inoculation with *C* pneumoniae either may result in aortic changes consistent with early atherosclerosis or may accelerate its development. However, if *C* pneumoniae merely colonizes diseased tissue and has no pathological role in CAD, its distribution would also be consistent with that observed here. The ability of *C* pneumoniae to induce or exacerbate atheroma in a population probably depends on more complex interactions with other factors than is generally appreciated. It is likely that the question of whether *C* pneumoniae causes or exacerbates CAD can be answered only by well-controlled animal studies and by large-scale antibiotic intervention trials.

**Acknowledgment**

This work was supported by a grant from the British Heart Foundation and the Wessex Heartbeat Trust.

**References**


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Circulation. 1999;99:2733-2736
doi: 10.1161/01.CIR.99.21.2733

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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