Clinical Investigation and Reports

Distribution of Chlamydia pneumoniae in the Human Arterial System and Its Relation to the Local Amount of Atherosclerosis Within the Individual

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Background—Chlamydia pneumoniae has been suggested to play a role in the origin of atherosclerosis. We studied the prevalence of C pneumoniae at multiple locations in the arterial system within the same individual. Studying the association between atherosclerosis and C pneumoniae within the individual excludes confounding by interindividual variability.

Methods and Results—Postmortem, the presence in the intima/plaque and media of C pneumoniae membrane protein was determined by use of a C pneumoniae–specific monoclonal antibody. In 24 individuals, 33 arterial locations were studied (n=738 segments). Area stenosis was determined in adjacent cross sections. In all individuals, immunostaining of C pneumoniae was observed in ≥1 artery. The highest prevalences were observed in the abdominal aorta (67%), internal and common iliac arteries (41%), and coronary arteries (33%). The lowest prevalences were observed in the radial (0%) and cerebral (2%) arteries. Within the individual, area stenosis was larger in cross sections with immunoreactivity compared with cross sections without immunoreactivity (31.0±11.9% versus 14.3±6.1%, respectively; P<0.001). In the individual, immunoreactivity was observed in 15±10% of the arteries (range, 3% to 45%). Between individuals, the percentage of arteries with immunoreactivity to C pneumoniae was associated with the average area stenosis throughout the arterial system (r²=0.56, P<0.001).

Conclusions—C pneumoniae was mostly observed at locations that are related to clinically relevant features. Within the individual, the distribution of C pneumoniae is associated with the distribution of atherosclerosis. The role of the microorganism in atherosclerotic disease remains to be elucidated. (Circulation. 2001;103:1613-1617.)

Key Words: atherosclerosis  ■  Chlamydia pneumoniae  ■  pathology

Chlamydia pneumoniae is a Gram-negative obligate intracellular bacterium that is a common cause of respiratory disease. Infection with C pneumoniae seems to be geographically widespread, and a high population prevalence of antibodies against the microorganism has been found, suggesting that most people are infected. Increasing evidence exists that C pneumoniae might play a role in atherosclerotic disease. An association between the microorganism and atherosclerosis was first demonstrated in seroepidemiological studies. In addition, C pneumoniae has been detected in human atherosclerotic lesions by various techniques like polymerase chain reaction, immunohistochemistry, electron microscopy, and culture. Thus far, the presence of the microorganism has been studied in only 1 or a small number of arterial locations within the same individual. In addition, negative control samples without atherosclerosis were obtained from different individuals who were mostly not age matched. Concern about these controls has been expressed by others. Within the individual, the distribution pattern of C pneumoniae has not been described.

Studying the localization of C pneumoniae within individuals is the most appropriate method to study the relation between local plaque formation and the presence of C pneumoniae, thereby excluding interindividual variability. In 24 individuals, we studied the presence of C pneumoniae at 33 different arterial locations with and without atherosclerotic disease. The aims of the present study were to determine the distribution pattern of C pneumoniae in the human arterial tree and to study whether within the individual this distribution pattern is associated with the distribution of atherosclerosis.

Methods
Postmortem, 24 donated corpses (11 men and 13 women; age, 81.9±9.9 years; history of cardiovascular risk factors unknown) were pressure fixed with 4% formalin in situ (pressure, age+100

Received October 11, 2000; revision received December 1, 2000; accepted December 9, 2000.


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mm Hg). In each corpse, arterial segments were dissected at 33 different locations: left and right (L+R) anterior, medial, and posterior cerebral arteries; basilar artery; common and internal carotid arteries (L+R); brachial arteries (L+R); radial arteries (L+R); ascending and abdominal aorta; pulmonary arteries (L+R); left anterior descending coronary artery; left circumflex coronary artery; right coronary artery; superior mesenteric artery; renal arteries (L+R); common, internal, and external iliac arteries (L+R); and femoral arteries (L+R). For each artery type, the location was standardized.

Of each artery, 1 paraffin section was stained for C pneumoniae–specific membrane protein. Sections were deparaffinized, and endogenous peroxidase was blocked with methanol containing 2% H2O2. Next, sections were pretreated with 0.5% blocking solution (Roche) and incubated with a monoclonal antibody directed against C pneumoniae–specific membrane protein (clone RR-402; Washington Research Foundation) in a concentration of 5 μg/mL. Biotinylated horse anti-mouse polyclonal antibody (Vector) was used as secondary antibody. For detection, sections were incubated with streptavidin–horseradish peroxidase followed by treatment with diaminobenzidine/nickel substrate. The sections were counterstained with Nuclear Fast Red (Merck). Adjacent sections were incubated with an irrelevant primary monoclonal antibody of the same isotype (mouse IgG3, clone G19-143; Pharmingen). HEp2 cells (CCL23; American Type Culture Collection) infected with C pneumoniae strain TW-183 were used as positive control. Mock-infected HEp2 cells were used as negative control.

A cell was considered positive when cellular immunoreactivity was observed. Immunoreactivity in the intima/plaque and media was categorized according to the following 4 grades: 3+, immunoreactivity in >50 cells; 2+, immunoreactivity in 10 to 50 cells; 1+, immunoreactivity in 2 to 9 cells; and 0, no immunoreactivity.

To assess the local amount of atherosclerosis, adjacent sections were stained with Elastin-van Gieson stain. Microscopic images of the cross sections were recorded on VHS videotape with a 3CCD video camera. In each cross section, the lumen area, the area encompassed by the internal elastic lamina (IEL), and the circumference of the IEL were measured. Plaque area was calculated by subtracting the lumen area from the measured IEL area. To avoid any distortion of the IEL area by cutting, the corrected IEL area was calculated as follows: IEL area corrected = (circumference IEL)2/4π. Area stenosis is a measure of the amount of plaque in a cross section corrected for arterial size and was calculated as follows: (plaque area/IEL area corrected)×100%.

Under physiological conditions, the pulmonary artery is exposed to a lower pressure than the other artery types. Therefore, it is unknown whether the mechanisms underlying the development of atherosclerosis are the same for the pulmonary artery and the other artery types. Consequently, the pulmonary artery was not included in the analysis in which cross sections of multiple locations were pooled.

Statistical Analysis

Student’s t test was used to compare area stenosis of cross sections with and without immunoreactivity. A paired t test was used to compare area stenosis of cross sections with and without immunoreactivity within individuals. Linear regression analysis was used to correlate the percentage of arteries with immunoreactivity with the average area stenosis of the individual. Values are presented as mean±SD. A value of P<0.05 was considered statistically significant.

Results

From 24 individuals, 766 arterial segments were harvested. Appropriate staining was available of 738 cross sections (30.8±1.7 per individual). The staining of 28 cross sections could not be interpreted because of background staining. In all 24 individuals, immunoreactivity to C pneumoniae–specific antigen was observed in ≥1 artery. Immunoreactivity to C pneumoniae–specific antigen (Figure 1) was observed in 111 of 738 arterial segments (15%). In 64 of these 111 cross sections (58%), immunoreactivity was observed in <10 cells (the Table). In 77 cross sections, immunoreactivity was found in the plaque only; in 9 cross sections, in the media only; and in 25 cross sections, in both the plaque and media. Mock-infected HEp2 cells and sections incubated with the isotype antibody consistently showed no staining.

The Table shows the number of arteries with immunoreactivity to C pneumoniae–specific antigen per artery type. The highest prevalences of immunoreactivity were found in the abdominal aorta, common and internal iliac arteries, and coronary arteries. Immunoreactivity was not observed in the radial artery and medial cerebral artery. Immunoreactivity was predominantly observed in artery types with the highest area stenosis (the Table). Pooling all cross sections of all individuals showed that area stenosis in cross sections with immunoreactivity was larger than in cross sections without immunoreactivity (Figure 2). Within the individual, area stenosis was larger in cross sections in which immunoreactivity was observed compared with cross sections without immunoreactivity (Figure 3A). Within the same individual,
Furthermore, area stenosis was associated with the presence of immunoreactivity within the same artery type, e.g., the coronary arteries. In 15 individuals, coronary immunoreactivity to C pneumoniae was observed in 1 or 2 of the 3 coronary arteries. In these individuals, area stenoses of C pneumoniae-positive and −negative coronary cross sections were compared. Comparison of the coronary arteries of the same individual revealed that area stenosis of cross sections with immunoreactivity was larger than that of nonstaining cross sections (Figure 3B).

In the individual, immunoreactivity was observed in 15 ± 10% of the studied arteries (range, 3% to 45%). Figure 4 shows a histogram of the percentage of arteries with immunoreactivity per individual. The histogram indicates that there is wide variation between different individuals in the number of arteries in which C pneumoniae was detected. A significant correlation between the average area stenosis of all studied arteries of the individual and the percentage of arteries with immunoreactivity in that individual is shown in Figure 5 ($r^2=0.56, P<0.001$).

![Figure 2](image-url)  
**Figure 2.** Area stenosis (AS, %) of all pooled cross sections with (Cmp+) and without (Cmp−) immunoreactivity to C pneumoniae–specific membrane protein.

<table>
<thead>
<tr>
<th>Artery</th>
<th>Cmp Positive, n (%)</th>
<th>1+/2+/3+, n</th>
<th>IEL Area, mm²</th>
<th>Plaque Area, mm²</th>
<th>AS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal aorta</td>
<td>12/18 (67)</td>
<td>8/3/1</td>
<td>241.6±63.9</td>
<td>48.9±40.0</td>
<td>18.5±9.3</td>
</tr>
<tr>
<td>LAD</td>
<td>11/24 (46)</td>
<td>8/2/1</td>
<td>9.9±4.6</td>
<td>4.6±2.3</td>
<td>47.1±19.5</td>
</tr>
<tr>
<td>Internal iliac</td>
<td>20/47 (43)</td>
<td>8/12/0</td>
<td>42.2±16.3</td>
<td>13.1±8.2</td>
<td>30.7±16.6</td>
</tr>
<tr>
<td>Common iliac</td>
<td>18/45 (40)</td>
<td>10/7/1</td>
<td>104.9±43.6</td>
<td>22.9±16.6</td>
<td>21.7±14.7</td>
</tr>
<tr>
<td>RCA</td>
<td>8/24 (33)</td>
<td>5/3/0</td>
<td>10.9±5.4</td>
<td>4.8±3.5</td>
<td>42.4±19.8</td>
</tr>
<tr>
<td>LCx</td>
<td>5/24 (21)</td>
<td>3/2/0</td>
<td>6.4±2.8</td>
<td>2.3±1.6</td>
<td>32.9±15.1</td>
</tr>
<tr>
<td>Common carotid</td>
<td>7/48 (15)</td>
<td>3/3/1</td>
<td>39.3±16.3</td>
<td>7.2±5.9</td>
<td>17.3±11.6</td>
</tr>
<tr>
<td>Femoral</td>
<td>4/34 (12)</td>
<td>3/1/0</td>
<td>31.0±13.3</td>
<td>7.6±8.7</td>
<td>22.1±22.9</td>
</tr>
<tr>
<td>Brachial</td>
<td>5/48 (10)</td>
<td>2/3/0</td>
<td>13.5±7.8</td>
<td>2.2±3.6</td>
<td>12.4±12.4</td>
</tr>
<tr>
<td>Renal</td>
<td>4/44 (9)</td>
<td>3/1/0</td>
<td>16.5±17.4</td>
<td>3.5±4.5</td>
<td>16.6±17.2</td>
</tr>
<tr>
<td>Superior mesenteric</td>
<td>2/23 (9)</td>
<td>2/0/0</td>
<td>27.4±13.3</td>
<td>4.0±5.3</td>
<td>11.2±11.5</td>
</tr>
<tr>
<td>External iliac</td>
<td>4/48 (8)</td>
<td>2/2/0</td>
<td>50.2±15.6</td>
<td>7.4±7.3</td>
<td>14.7±15.1</td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>1/15 (7)</td>
<td>1/0/0</td>
<td>437.3±81.8</td>
<td>43.0±35.2</td>
<td>9.4±6.4</td>
</tr>
<tr>
<td>Basilar</td>
<td>1/21 (5)</td>
<td>1/0/0</td>
<td>8.5±6.5</td>
<td>0.8±1.3</td>
<td>8.2±8.2</td>
</tr>
<tr>
<td>Internal carotid</td>
<td>2/46 (4)</td>
<td>1/1/0</td>
<td>14.9±6.9</td>
<td>1.2±2.5</td>
<td>6.4±11.2</td>
</tr>
<tr>
<td>Cerebral anterior</td>
<td>2/47 (4)</td>
<td>2/0/0</td>
<td>3.8±1.1</td>
<td>0.4±0.9</td>
<td>9.0±15.0</td>
</tr>
<tr>
<td>Cerebral posterior</td>
<td>1/48 (2)</td>
<td>1/0/0</td>
<td>4.1±2.2</td>
<td>0.3±0.7</td>
<td>4.0±7.6</td>
</tr>
<tr>
<td>Radial</td>
<td>0/47 (0)</td>
<td>0/0/0</td>
<td>3.7±1.6</td>
<td>0.5±0.5</td>
<td>15.1±12.8</td>
</tr>
<tr>
<td>Cerebral media</td>
<td>0/48 (0)</td>
<td>0/0/0</td>
<td>6.3±2.6</td>
<td>0.8±1.1</td>
<td>10.4±11.7</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>4/39 (10)</td>
<td>1/3/0</td>
<td>313.6±112.2</td>
<td>2.1±8.5</td>
<td>0.6±2.2</td>
</tr>
</tbody>
</table>

**Table 1.** Number of Arteries With Immunoreactivity to C pneumoniae Membrane Protein and Average Area Stenosis per Artery Type. Cmp Positive indicates immunoreactivity to C pneumoniae–specific membrane protein; 1+, positive immunoreactivity in 2 to 9 cells; 2+, immunoreactivity in 10 to 50 cells; 3+, immunoreactivity in >50 cells; AS, area stenosis; LAD, left anterior descending coronary artery; RCA, right coronary artery; and LCx, left circumflex coronary artery. Values are mean±SD.

![Figure 3](image-url)  
**Figure 3.** A, Average area stenosis (AS, %) of cross sections with (Cmp+) and without (Cmp−) immunoreactivity to C pneumoniae–specific membrane protein for each individual. In each individual, average area stenoses in cross sections with and without immunoreactivity are connected by line. B, Same analysis in subgroup of 15 individuals in whom immunoreactivity to C pneumoniae was observed in 1 or 2 of 3 coronary arteries. In each individual, (average) area stenoses in cross sections with immunoreactivity are wider than that of nonstaining cross sections (Figure 3B).
The respiratory pathogen \textit{C. pneumoniae} is assumed to disseminate from the lungs to vascular tissue.\textsuperscript{8,9} Evidence that the presence of \textit{C. pneumoniae} in human atherosclerotic arteries was systematically studied on only 1 or a limited number of locations within a single individual. In most of these studies, negative controls without atherosclerotic disease were obtained from another group of individuals. As previously reported,\textsuperscript{6} most controls that were used were younger, raising the question of whether the difference in prevalence was due to the age of the subjects rather than the condition of the vessels. Also, other interindividual variables, like smoking status,\textsuperscript{11,12} merit careful consideration. In addition, only a selection of artery types, like the coronary arteries, aorta, and carotid artery, was studied in adequate numbers to obtain representative results for these artery types. This is the first study in which the presence of \textit{C. pneumoniae} in human atherosclerotic arteries was systematically studied throughout the arterial system and related to atherosclerotic disease within the same individual. Therefore, interindividual variation in factors that influence infection and dissemination of \textit{C. pneumoniae} is controlled for, because both atherosclerotic and nonatherosclerotic arteries are studied within the same individual.

**Distribution in Arterial System**

Our results indicate that within the individual \textit{C. pneumoniae} is widely disseminated in the arterial tree. To the best of our knowledge, this study adds the renal, superior mesenteric, basilar, and anterior and posterior cerebral arteries to the list of artery types in which the microorganism has been reported. Although the microorganism was widespread, it was not randomly distributed in the human arterial tree. Artery types in which the microorganism was highly prevalent included the abdominal aorta, coronary arteries, and common and internal iliac arteries, which are all known for their relation to clinically relevant atherosclerosis. Detection of \textit{C. pneumoniae} in these artery types is in accordance with previous findings.\textsuperscript{13-17} \textit{C. pneumoniae} seemed to be preferentially located in atherosclerotic arteries, because even within the arterial system of one individual, area stenosis was found to be associated with the presence of immunoreactivity to \textit{C. pneumoniae} (Figure 3). Moreover, within the same individual, area stenosis in the 3 coronary arteries was found to be positively associated with immunoreactivity for \textit{C. pneumoniae}, indicating that the association between atherosclerosis and the presence of the microorganism persists within the same artery type. In previous studies, \textit{C. pneumoniae} was preferentially found in atherosclerotic arteries compared with arteries without atherosclerotic disease obtained from different individuals.\textsuperscript{13,14,16} The results of the present study demonstrate that within a single individual, \textit{C. pneumoniae} was preferentially located in atherosclerotic arteries.

**Variation Between Individuals**

Little is known about interindividual differences in distribution of \textit{C. pneumoniae} in the arterial tree. Among individuals, a wide range (3\% to 45\%) in the percentage of arteries with immunoreactivity to \textit{C. pneumoniae} was observed. Figure 4 suggests that dissemination of \textit{C. pneumoniae} is not an “all or nothing” phenomenon, because then all arteries would show \textit{C. pneumoniae} immunoreactivity in one individual, whereas in other individuals, immunoreactivity to \textit{C. pneumoniae} would be absent. Multiple factors could influence the distribution of the microorganism in the arterial tree and explain the observed variation between individuals. The “atherosclerotic status” of the individual seemed to be associated with the number of arteries in which \textit{C. pneumoniae} was observed, because the percentage of arteries with the microorganism present in an individual was found to be associated with the average area stenosis throughout the arterial system of the individual. Thus, the more atherosclerosis there was within the individual, the more arteries in which \textit{C. pneumoniae} was prevalent were seen.

**Role in Atherosclerosis**

Because of the cross-sectional design of this study, we are unable to determine whether \textit{C. pneumoniae} colonizes atherosclerotic plaque when it already exists or plays a role in the initiation of the plaque. Thus, although the present study adds further evidence on the tight junction between \textit{C. pneumoniae} and atherosclerosis, it does not answer the basic question of whether \textit{C. pneumoniae} plays a causative role in atherosclerosis. Within the coronary arteries of the same individual, however, we found a correlation between the amount of atherosclerotic disease and the presence of \textit{C. pneumoniae}. This is consistent with the results of a recent autopsy study\textsuperscript{18} in which samples were obtained from 60 individuals. \textit{C. pneumoniae} immunoperoxidase staining was detected in 80\% of

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**Figure 4.** Histogram of number of individuals vs percentage of arteries with immunoreactivity to \textit{C. pneumoniae} membrane protein (Cpmp+) in each individual.

**Figure 5.** Association between average area stenosis (AS) of all studied arteries within individual and percentage of arteries positive for \textit{C. pneumoniae} membrane protein (Cpmp+) within individual. Plot of percentage of arteries positive for \textit{C. pneumoniae} membrane protein (Cpmp+) within the individual vs average area stenosis with regression line.
individuals with severe atherosclerosis and in 38% of individuals with mild atherosclerosis. In another recent study, no association was found between the Stary grading of the atherosclerotic lesion and the presence of C. pneumoniae in the vessel wall, which seems to contradict our results. A difference in the techniques used to detect the microorganism might explain the different results. We detected a membrane protein of C. pneumoniae by immunohistochemistry, whereas in the above-mentioned study, C. pneumoniae DNA was detected by polymerase chain reaction. If C. pneumoniae would only initiate atherosclerotic disease, an equal prevalence could be expected in plaques of all sizes. It might be possible that C. pneumoniae also has a role in the progression of the atherosclerotic process. Progression of the atherosclerotic lesion by C. pneumoniae infection has been shown in rabbit and murine models.

**Study Limitations**

Only a 4-μm-thick slice of each artery was studied. This single sample likely gives an underestimation of the presence of the microorganism in the total artery. We used immunohistochemistry to detect C. pneumoniae in arterial tissue. The monoclonal antibody used is directed against a membrane protein of C. pneumoniae. Presence of the membrane protein does not necessarily reflect the presence of viable C. pneumoniae. The postmortem material used was obtained from elderly individuals. Therefore, any reference to onset of atherosclerotic disease is unreliable. Because of this advanced age, one may also doubt reference to progression of atherosclerotic disease. However, a previous postmortem study revealed that plaques obtained from elderly individuals (>80 years) showed a comparable staining for inflammatory cells as plaques obtained from younger individuals.

**Conclusions**

C. pneumoniae was preferentially located in artery types that are associated with clinically relevant atherosclerosis. Within a single individual, the distribution of C. pneumoniae was associated with the distribution of atherosclerosis. Between individuals, a wide variation in the presence of C. pneumoniae in the arterial system was observed. However, it should be emphasized that although these results add further evidence of the association between C. pneumoniae and atherosclerosis, they do not prove a causative or pathogenic role for the microorganism in atherosclerosis.

**Acknowledgments**

This study was supported by the Sorbo Foundation. Dr. Pasterkamp is a fellow of the Catharine Foundation, Utrecht. We gratefully thank W.J.A. van Wouwelen and S. Plomp, Department of Functional Anatomy, University Medical Center Utrecht, for technical assistance.

**References**

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Circulation. 2001;103:1613-1617
doi: 10.1161/01.CIR.103.12.1613

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