Association of Chlamydia pneumoniae DNA in Peripheral Blood Mononuclear Cells and IgA Antibody with Atherosclerotic Diseases

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Original Article

Association of Chlamyphila pneumoniae DNA in Peripheral Blood Mononuclear Cells and IgA Antibody with Atherosclerotic Diseases

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Abstract An association has been demonstrated between Chlamyphila pneumoniae (C. pneumoniae) infection and atherosclerosis, but data on the relationship between C. pneumoniae DNA in peripheral blood mononuclear cells (PBMC) and antibodies to this organism are lacking. We investigated the C. pneumoniae DNA in PBMC by polymerase chain reaction (PCR) and C. pneumoniae IgG and IgA antibodies by enzyme-linked immunosorbent assay of 168 patients with atherosclerotic diseases and 27 controls (healthy control subjects). C. pneumoniae DNA was detected for 48/168 (29%) atherosclerosis patients. IgG for 79 (47%), and IgA for 98 (58%), whereas the corresponding numbers for the controls were 11 (41%), 13 (48%), and 7 (26%). There was no significant difference of the C. pneumoniae DNA positivity rate between the atherosclerosis patients and the controls. However, the C. pneumoniae IgA-positive rate was significantly higher for carotid atherosclerosis patients who had C. pneumoniae DNA in their PBMC than for those without it (74% vs. 18%, P < 0.05). Among the patients with coronary artery disease, the C. pneumoniae IgA antibody positive rate was significantly higher for the patients with DNA than for those without it (68% vs. 18%, P < 0.05). Our results suggest that a high C. pneumoniae IgA antibody titer and C. pneumoniae DNA positivity are associated with an increased risk of atherosclerotic diseases due to endovascular C. pneumoniae infection.

Key words: Chlamyphila pneumoniae: Atherosclerosis: Peripheral blood mononuclear cells: Polymerase chain reaction: Antibody

Introduction

Chlamyphila pneumoniae (C. pneumoniae), an obligatory intracellular pathogen, is a common cause of respiratory tract infection. Several studies have already shown the presence of C. pneumoniae in blood stream of healthy volunteers and have indicated that more than half of the adult population has been exposed to this organism, with infection and reinfection occurring during their lifetime.

Atherosclerosis is a highly prevalent disease, and it is currently the greatest cause of morbidity and mortality in developed societies. Many risk factors have long been identified as contributing to the development of atherosclerosis that manifests as coronary artery disease (CAD) and myocardial infarction (MI). More recently, the possibility has been raised that infectious agents may trigger a cascade of biological and biochemical reactions leading to inflammation, atherosclerosis, and vascular thrombosis. A serological association between C. pneumoniae and CAD was
first demonstrated by Saikku et al. in 1988\textsuperscript{4}. This association has been confirmed by subsequent studies\textsuperscript{5}, although several authors\textsuperscript{6~10} have failed to find any association. We\textsuperscript{11} previously studied a relationship between \textit{C. pneumoniae} infection and the effect of lipid-lowering drugs on the carotid atherosclerosis (CA) of hypercholesterolemic patients.

\textit{C. pneumoniae} infection reduced the effect of lipid-lowering therapy on CA, indicating that this organism may play a role in the progression of atherosclerosis. Moreover, \textit{C. pneumoniae} has been detected in atherosclerotic tissues by polymerase chain reaction (PCR), immunohistochemistry, electron microscopy, culture, and other techniques\textsuperscript{10}.

\textit{C. pneumoniae} infection generally starts in the respiratory tract, and the organisms within alveolar macrophages are probably spread systemically through the bloodstream\textsuperscript{11~12}. The elementary body, the metabolically inactive extracellular stage of the life cycle of chlamydiae, has never been found circulating freely in the blood, but monocytes/macrophages may carry \textit{C. pneumoniae} from the lungs to the arterial walls. In vitro studies have indicated that \textit{C. pneumoniae} can infect and reproduce within human endothelial cells, smooth muscle cells, and macrophages, which are key cell types involved in the process of atherosclerosis\textsuperscript{13~14}. Also, recent studies have detected \textit{C. pneumoniae} DNA in the peripheral blood mononuclear cells (PBMC) of patients with CAD\textsuperscript{15}. Because serology alone cannot diagnose vascular infection, direct detection methods based on examination of peripheral blood components may be more useful as markers of infection.

The aim of this study is to evaluate the association between \textit{C. pneumoniae} and atherosclerosis to investigate the prevalence of \textit{C. pneumoniae} DNA within PBMC and \textit{C. pneumoniae} antibodies from patients with various atherosclerotic diseases.

\textbf{Methods}

\textbf{Subjects}

Between May 2001 and July 2002, 168 patients with various atherosclerotic diseases (109 men and 59 women, mean age 67 ± 9 years) and 27 controls (6 men and 21 women, mean age 59.0 ± 7.1 years) were enrolled at Kyushu University Hospital.

\textbf{Selection of Subjects}

All of the patients with atherosclerotic diseases admitted to Kyushu University Hospital (Fukuoka, Japan) were considered eligible for the present study. The type of atherosclerotic disease was CA for 68 patients, stable angina (SA) for 29, acute coronary syndrome (ACS) including acute myocardial infarction and unstable angina for 39, old myocardial infarction (OMI) for 32. The patients with CA had no history of CAD, but they all had an abnormal carotid intima–media thickness (IMT) and/or plaque on ultrasonography (defined as a generalized or focal IMT \( \geq 1.1 \) mm, respectively)\textsuperscript{9}. Patients with ACS had ischemic chest pain and typical changes on their electrocardiogram (ECG) and/or increased cardiac enzyme levels. Angina patients without clinical evidence of ischemia within the previous one month were defined as having SA. All of the patients with SA, ACS, or OMI (n = 100) underwent coronary angiography.

Exclusion criteria were acute infection, exacerbation of chronic infectious or inflammatory diseases, and severe liver or renal disease. Informed consent for collection of blood or tissues was obtained from the patients (or their closest relatives). Information on each participant was compiled from the medical records and from a questionnaire about the personal medical history and lifestyle. The design of this study was approved by the Ethics Committee and the Data Protection Committee of Kyushu University Hospital (Fukuoka, Japan).
Selection of Controls

The control subjects were chosen from among, asymptomatic outpatients with hyperlipidemia who had no cardiac or infectious diseases. The absence of atherosclerosis in the controls was assessed as follows: normal 12-lead ECG, normal findings on echocardiography, <25% stenosis of the carotid arteries on Doppler ultrasonography, and normal lower limb arteries on physical examination. A history of cardiac disease meant exclusion from the control group.

Laboratory Tests

Peripheral venous blood specimens and serum samples were obtained from all participants and stored at −80°C until analysis.

C. pneumoniae DNA was isolated from PBMC using the Smitest EX−R&D (Genome Science Laboratories, Fukushima, Japan) in accordance with the manufacturer’s recommendations. PCR for the detection of C. pneumoniae was done using a C. pneumoniae−specific pair of primers (CP1/CP2 and CPC/CPD)

C. pneumoniae IgG and IgA antibodies were measured with enzyme−linked immunosorbent assay (ELISA) kits (Hitazyme C. pneumoniae, Hitachi Chemical Co., Ltd., Tokyo, Japan), as described previously.

The IgG and IgA were positive when indices were ≥ 1.10. When the IgG and IgA antibody detection rates by ELISA were compared with those for the microimmunofluorescence method, sensitivity was 90.4% for IgG and 84.6% for IgA, while specificity was 89.9% for IgG and 86.7% for IgA.

The rate of agreement between ELISA and Western blotting was 80.0% for IgG and 87.5% for IgA. All laboratory tests were done in a blinded fashion.

Statistical Analysis

The mean levels of numerical variables were compared by the Mann−Whitney U test, while categorical variables were compared by the chi−square test or Fisher’s exact test, as was appropriate. A P value < 0.05 was considered to indicate statistical significance.

Results

The characteristics of the participants are summarized in Table 1.

Compared with the controls, the patients with atherosclerosis were significantly older and were more likely to be men, to be current smokers, and to have a history of hypertension. C. pneumoniae DNA was found in the PBMC of 11 controls (41%), while IgG was positive in 13 controls (48%) and IgA was detected in 7 controls (26%).

The DNA detection rates of the patients with atherosclerosis and controls showed no significant difference, but the IgA positive rate was significantly higher in the patients with atherosclerosis. In contrast, the IgG positive rate was not significantly different between the two groups.

C. pneumoniae DNA was detected in the PBMC of 19 patients (28%) with CA, while IgG for C. pneumoniae was found in for 30 patients (44%) and IgA was detected in 32 patients (47%).

C. pneumoniae DNA was detected in the PBMC of 10 patients (35%) with SA, while IgG for C. pneumoniae was found in 14 patients (48%) and IgA was detected in 15 patients (52%).

C. pneumoniae DNA was detected in the PBMC of 9 patients (23%) with ACS, while IgG for C. pneumoniae was found in 16 patients (41%) and IgA was detected in 24 patients (62%).

C. pneumoniae DNA was detected in the PBMC of 9 patients (28%) with OMI, while IgG for C. pneumoniae was found in 14 patients (44%) and IgA was detected in 20 patients (63%). The IgA positive rate of the patients with CAD (SA, ACS, and OMI) was significantly higher than the controls (p < 0.05, p < 0.01, p < 0.01, respectively).

The relationship between the C. pneumoniae DNA and IgG or IgA antibodies of the patients with atherosclerosis is shown in Table 2.

IgG for C. pneumoniae with atherosclerosis was found in 79 patients (42%) and IgA was
detected in 98 patients (58%), while IgG was positive in 13 controls (48%) and IgA was detected in 7 controls (29%). There was no significant different between the two groups. IgA positivity was significantly more common in the patients with atherosclerotic diseases (58%) than in the controls (29%) ($P < 0.05$). Among the patients with atherosclerotic diseases, the *C. pneumoniae* IgA antibody positive rate was significantly higher in the patients with DNA than in those without it (71% vs. 18%, $P < 0.05$). Among the patients with each type of atherosclerotic disease and the controls are shown in Table 3.

The *C. pneumoniae* IgA–positive rate was significantly higher for CA patients who had *C. pneumoniae* DNA in their PBMC than for those without it (74% vs. 18%, $P < 0.05$). In contrast, the *C. pneumoniae* IgG–positive rate was not significantly higher for CA patients who had *C. pneumoniae* DNA than for those without it (47% vs. 64%). Among the patients with CAD (SA + ACS + OMI), the *C. pneumoniae* IgA antibody positive rate was significantly higher for the

### Table 1  Characteristics of 168 patients with atherosclerotic diseases and 27 controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Atherosclerotic diseases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=168)</td>
<td>CA (n=68)</td>
</tr>
<tr>
<td>Age, years</td>
<td>67 ± 9**</td>
<td>65 ± 8**</td>
</tr>
<tr>
<td>Male</td>
<td>109 (65)**</td>
<td>25 (37)</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>65 (36)*</td>
<td>23 (34)*</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>61 (36)</td>
<td>24 (32)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>92 (55)**</td>
<td>35 (52)**</td>
</tr>
<tr>
<td><em>C. pneumoniae</em> DNA in PBMC</td>
<td>48 (29)</td>
<td>19 (28)</td>
</tr>
<tr>
<td><em>C. pneumoniae</em> IgG $\geq 1.10$</td>
<td>79 (47)</td>
<td>30 (44)</td>
</tr>
<tr>
<td><em>C. pneumoniae</em> IgA $\geq 1.10$</td>
<td>98 (58)**</td>
<td>32 (47)</td>
</tr>
</tbody>
</table>

Values are represented as the mean ± SD or number (%).
CA, carotid atherosclerosis; SA, stable angina; ACS, acute coronary syndrome; OMI, old myocardial infarction
*Chlamydophila pneumoniae*: *C. pneumoniae*

*P < 0.05 vs. controls

**P < 0.01 vs. controls

### Table 2  Relationship between *C. pneumoniae* DNA and IgG or IgA seropositivity; atherosclerosis patients versus controls

<table>
<thead>
<tr>
<th><em>C. pneumoniae</em> antibody</th>
<th>Atherosclerotic disease patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=168)</td>
<td>DNA + (n=47)</td>
</tr>
<tr>
<td><em>C. pneumoniae</em> IgG $\geq 1.10$</td>
<td>79 (42)</td>
<td>27 (59)</td>
</tr>
<tr>
<td><em>C. pneumoniae</em> IgA $\geq 1.10$</td>
<td>98 (58)^*</td>
<td>33 (71)^*</td>
</tr>
</tbody>
</table>

Values are represented as numbers (%).
DNA +, DNA positive; DNA−, DNA negative

*P < 0.05 vs controls

### Table 3  Relationship between *C. pneumoniae* DNA and seropositivity by type of atherosclerosis

<table>
<thead>
<tr>
<th><em>C. pneumoniae</em> antibody</th>
<th>CA</th>
<th>CAD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNA + (n=19)</td>
<td>DNA − (n=49)</td>
<td>DNA + (n=28)</td>
</tr>
<tr>
<td><em>C. pneumoniae</em> IgG $\geq 1.10$</td>
<td>9 (47)</td>
<td>21 (43)</td>
<td>18 (64)</td>
</tr>
<tr>
<td><em>C. pneumoniae</em> IgA $\geq 1.10$</td>
<td>14 (74)^*</td>
<td>18 (37)</td>
<td>19 (68)*</td>
</tr>
</tbody>
</table>

Values are represented as numbers (%).
DNA +, DNA positive; DNA−, DNA negative

*P < 0.05 vs controls
patients with DNA than for those without it (68% vs. 18%, $P < 0.05$). However, the *C. pneumoniae* IgG positive rate was not significantly higher for the patients who had *C. pneumoniae* DNA than for those without it (64% vs. 64%).

**Discussion**

The present case–control study adds new information to the growing pool of data regarding the association between atherosclerosis and *C. pneumoniae* infection. We demonstrated that a high *C. pneumoniae* IgA antibody titer and *C. pneumoniae* DNA positivity are associated with an increased risk of various atherosclerotic diseases. When we investigated whether or not detection of *C. pneumoniae* DNA (in PBMC and atherosclerotic lesions) or antibodies was associated with atherosclerosis, we found a strong association between *C. pneumoniae* DNA in PBMC and advanced atherosclerosis. In fact, both *C. pneumoniae* DNA and the *C. pneumoniae* IgA positive rate were significantly higher for patients with CAD than for controls and the IgA seropositive rate was significantly higher for patients with advanced atherosclerosis than for the controls.

Previous studies$^{20-23}$ have found *C. pneumoniae* DNA in PBMC at significantly higher rates in patients with atherosclerosis than in controls, but these reports have also shown that the prevalence of circulating *C. pneumoniae* DNA varies widely, being 8.8–59.4% of patients with atherosclerosis and 0.0–46.1% of controls. This wide variation of the *C. pneumoniae* positive rate was found both by using different methods and when independent investigators used similar methods. *C. pneumoniae* generally infects the respiratory tract initially, and may then be disseminated systemically by infected macrophages$^{11,12}$. A recent interesting study$^{24}$ clearly reported that *C. pneumoniae* could not survive in macrophages for long term. Other group also demonstrated that lymphocytes have an important role as host cells for *C. pneumoniae*.$^{25}$ Our results indicate that detection of chlamydiae in the bloodstream may be a general phenomenon among the adult population rather than being related to the stage of atherosclerosis, so the detection of *C. pneumoniae* DNA may not be a valid marker of current infection.

We found no correlation between the *C. pneumoniae* DNA positivity and seropositivity or antibody titers, as was previously observed in other studies$^{22,23}$, even though IgA-positive patients were significantly more likely to have *C. pneumoniae* DNA in their PBMC than were IgA-negative patients. Most of the previous studies$^{22,23}$, as well as our study, have shown a higher prevalence of *C. pneumoniae* antibodies than *C. pneumoniae* antigen. Therefore, current infection with *C. pneumoniae* may be indicated by the presence of antibodies in an antigen-positive patient. From our study, measurement of *C. pneumoniae* antibody seemed to be a more sensitive diagnostic method for infection than the detection of *C. pneumoniae* DNA by PCR. The presence of *C. pneumoniae* in atherosclerotic tissues is beyond doubt, but it is difficult to determine whether it is a primary cause of disease or a secondary invader, as well as whether it behaves innocently or aggressively in the latter case. The present results suggested that *C. pneumoniae* may be a secondary invader of atherosclerotic lesions and not the primary cause of atherosclerosis.

Our previous study$^{9}$ showed that *C. pneumoniae* infection reduced the effect of lipid–lowering therapy on carotid atherosclerosis and that this organism may play a role in the progression of the atherosclerosis of hypercholesterolemic patients with advanced CA, although no association was found between *C. pneumoniae* seropositivity and mild atherosclerosis (such as early CA) in the general population$^{8}$. In the present study, we also found a significantly greater prevalence of IgA seropositivity in patients with more advanced atherosclerosis than in the controls, indicating that *C. pneumoniae* IgA positivity may be
associated with advanced atherosclerosis. Future research should take into account the fact that the lesions of atherosclerosis are not sterile, and studies such as clinical antibiotic intervention trials will be necessary.

The main limitation of the present study was its case-control design. Although care was taken to avoid potential biases, it is well known that retrospective studies are often unable to reproduce the associations detected in case-control studies, so further prospective studies are needed to confirm our findings.

In conclusion, a high C. pneumoniae IgA antibody titer and C. pneumoniae DNA positivity are associated with atherosclerosis but further studies are required to confirm whether or not chronic C. pneumoniae infection is actually an independent risk factor for atherosclerosis.

Acknowledgements

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PBMC中のChlamyophila pneumoniae DNAとChlamyophila pneumoniae抗体との関連

Chlamyophila pneumoniae（C. pneumoniae）感染は動脈硬化に関与しているといわれているが、末梢血中のC. pneumoniae DNAとC. pneumoniae抗体との関連を示した報告は少ない。私共は168例の動脈硬化性疾患群と27例のコントロール群に対して、ELISA法によるC. pneumoniae抗体測定と同時に、PCR法を用いて末梢血中のC. pneumoniaeDNAを測定し比較検討した。動脈硬化性疾患群において、C. pneumoniaeのDNAの検出率は48/168（29%）、IgG抗体陽性率は79/168（47%）、IgA抗体陽性率は98/168（58%）であった。一方、コントロール群では、DNAの検出率は11/27（41%）、IgG抗体陽性率は13/27（48%）、IgA抗体陽性率は7/27（26%）であった。DNAの検出率、両群に有意差は認めなかったが、末梢血中のDNA陽性例におけるIgA抗体陽性率、動脈硬化性疾患群（74%）では、コントロール（18%）と比較して有意に高く（p<0.05）、その中の冠動脈疾患（急性冠症候群、不安定狭心症、不安定狭心症）においても、コントロールと比較して有意に高かった（p<0.05）。これらの結果よりC. pneumoniaeにおけるIgA抗体高値かつDNA陽性の場合は、血管内C. pneumoniae感染による動脈硬化性疾患発症の危険因子に深く関与している可能性が示唆された。