The Role of Chlamydia Pneumoniae in Human Aortic Disease—A Hypothesis Revisited

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Background. The role of Chlamydia pneumoniae in the pathogenesis of aortic aneurysm is controversial. We investigated the presence of C. pneumoniae in tissue samples excised from patients and controls.

Methods. Aortic wall specimens were obtained from 17 patients with acute Stanford type A aortic dissection, 25 patients with thoracic aortic aneurysms (TAA) and 23 patients with abdominal aortic aneurysms (AAA). Eighty-three tissue samples of 73 control patients free of aortic disease were obtained either at surgery or autopsy. The presence of Chlamydia subspecies DNA (sequences specific for all known Chlamydiaceae and DNA of C. pneumoniae, C. trachomatis and C. psittaci were assessed by a validated highly sensitive and specific real time polymerase chain reaction (PCR) analysis. Atherosclerotic risk factors were assessed in all patients.

Results. We failed to detect C. pneumoniae and C. psittaci-DNA in any of the 148 vessel specimens. C. trachomatis-DNA was detected in 1/65 patients and in none of 83 controls (P ≥ 0.43). Chlamydia subspecies DNA was found in samples of eight cases and in one control (P ≥ 0.01), however, no significant differences were found between the subgroups aortic dissection (P ≥ 0.09), TAA (P ≥ 0.99) and AAA (P ≥ 0.15) and respective controls.

Conclusions. C. pneumoniae does not play a clinically relevant role in acute and chronic aortic disease. The impact of other organisms of the family Chlamydiaceae needs further evaluation.

Keywords: Chlamydia pneumoniae; Aortic dissection; Aortic aneurysm; Real time PCR.

Introduction

Chlamydia, obligate intracellular Gram-negative bacteria, capable of multiplying in vascular endothelial and mononuclear cells, has been suggested to be related to the development and progression of aortic aneurysms.1–3 The processes underlying aortic aneurysm formation are still unknown, however, it is widely recognized that aortic aneurysms are closely associated with chronic transmural inflammation and destruction of connective tissue proteins within the aortic wall.4,5 Chlamydia infection may initiate and trigger this vascular inflammatory process thus increasing the susceptibility to aortic disease. In particular, Chlamydia lipopolysaccharides and various outer membrane proteins of these bacteria are recognized by the human humoral immune system and immune response may induce pathological processes.6

Seroepidemiological studies have demonstrated an enhanced serological positivity against C. pneumoniae in patients within different vascular pathologies (e.g. coronary artery disease, peripheral artery disease, cerebral vascular disease and abdominal aortic aneurysm (AAA)).7–9 Experimental and clinical intervention studies assessing the possible role of chronic infection with Chlamydia, however, provided controversial results.10–15 Studies employing polymerase chain reaction (PCR), to directly identify chlamydial DNA, have not confirmed the presence of Chlamydia within vascular pathologies.16,17 As a consequence, the role of Chlamydia in the development and progression of aortic aneurysm has remained uncertain.
of aortic aneurysms is questionable, and the detection of Chlamydia in vascular tissue may only be coincidental.

Data on the presence of Chlamydia subspecies in thoracic aneurysm and dissection are rare, and results from abdominal aneurysm studies need validation in independent patient samples. Using a highly sensitive and specific real time PCR we have assessed the presence of Chlamydia subspecies in acute and chronic thoracic and abdominal aortic diseases. We hypothesized that C. pneumoniae and other Chlamydia species, known as human pathogens, might be more frequently found in tissue samples of patients with aortic pathologies compared to disease-free controls.

Methods

Study design

We prospectively studied 65 patients and 73 controls. Aortic wall specimens were obtained from 17 patients with acute Stanford type A aortic dissection, 25 patients with thoracic aortic aneurysms (TAA) and 23 patients with AAA. Tissue samples of controls, free of aortic disease, were obtained from the thoracic aorta in 73 controls. In 63 of them aortic wall specimens were obtained during surgery for valve replacement and in ten cases specimens were obtained at autopsy from thoracic and from the abdominal aorta. The latter procedures were done with special care but not under sterile conditions. There was no clinical evidence of a current infectious disease in any of the patients or controls.

The study was approved by the local ethics committee and performed according to the Declaration of Helsinki.

Definitions

Aortic aneurysm was diagnosed and classified according to the guidelines published by the European Society of Cardiology. Diagnosis was aided with the use of computed tomography (n=64), conventional angiography (n=73), magnetic resonance imaging angiography (n=1), or autopsy (n=10). Diabetes mellitus was defined as a HbA1c greater than 6.0% on admission and was assumed to be present in all patients on anti-diabetic medication or insulin therapy. Hyperlipidemia was considered to be present in all patients receiving lipid lowering therapy or in patients with fasting total serum cholesterol greater than 200 mg/dl, LDL cholesterol greater than 130 mg/dl or serum triglycerides greater than 180 mg/dl. Chronic arterial hypertension was defined as a history of hypertension with or without chronic intake of antihypertensive drugs. History of previous vascular intervention upon the arteries of lower extremities, or the presence of an ankle brachial index lower than 0.9 were considered as peripheral artery disease. Chronic renal insufficiency was defined in stable elevation of creatinine greater than 2 mg/dl, assessed in laboratory studies prior to admission. Cerebral vascular disease was defined as a history of ischemic cerebral events (e.g., transitory ischemic attack, prolonged reversible ischemic neurologic deficiency or stroke). Concomitant coronary artery disease was excluded or confirmed via history of angina or myocardial infarction, coronary angiography, past history of coronary revascularisation or identification of hemodynamic significant atherosclerotic lesions at autopsy. Patients, who were smoking more than three cigarettes per day, were considered as active smokers. Patients with diagnosed or suspected Marfan, Turner or Ehler–Danlos syndrome were not included in the study.

Patients

All consecutive patients with chronic stable aortic disease, scheduled for elective aneurysm surgery, as well as patients with acute aortic dissection or rupture admitted within a 3 year—period (March 2000—March 2003) to the Department of Emergency Medicine and Department of Surgery, Division of Cardiothoracic Surgery and Vascular Surgery, of a tertiary care university hospital were eligible for study participation. Control aortic specimens, free of aortic disease, were obtained from patients with non-rheumatic aortic valvular disease, undergoing elective aortic valve replacement, and from 10 individuals without any aortic disease at routine autopsy within the same time interval. In all patients, cardiovascular risk factors and comorbidities were evaluated by medical chart review. Signs of current infectious disease were excluded by medical history, clinical examination, urinary analysis, laboratory studies of inflammatory markers and radiologic investigations or autopsy reports, if applicable.

Aortic wall sample

Aortic wall specimens were obtained from the ascending or the infrarenal abdominal aorta in patients with thoracic or AAA and/or dissection. Control-specimens, obtained during autopsy, were collected simultaneously from the thoracic and
abdominal aorta. Samples of the ascending aorta from patients undergoing aortic valve replacement were obtained either when establishing cardiopulmonary bypass from the access site of the arterial cannulae or in fitting aortic valve prostheses into the ascending aorta.

**PCR-analysis**

Real time PCR was performed as a two-step procedure with the TaqMan sequence detection system (Applied Biosystems, Amersham, CA, USA). Detection of chlamydial DNA was achieved with primers as described elsewhere. Tissue samples were placed in sterile screw capped vials, transferred to the laboratory where they were immediately processed. Samples were cut into pieces of equal weight (25 mg each) using sterile scissors and forceps, and distributed into sterile Eppendorf vials (2 ml size). DNA extraction was performed using QIAGEN tissue kit (QIAGEN GmbH, Munich, Germany) and the extract was used for Real Time PCR (Applied Biosystems, Amersham, USA). For each sample four reaction tubes were used; one positive control, one negative control containing only PCR mix, the tissue sample extract, and the tissue sample extract spiked with chlamydial DNA (reference strains, *C. pneumonia* [EB Wien IV], *C. trachomatis* [CH834], *C. psittaci* [99P0381] were kindly provided by P. Apfalter and V. Brade) in order to exclude contamination and inhibitors. For the detection of all known *Chlamydiaceae* (further *Chlamydia* DNA) a sequence of 130 bp of the 23-rDNA served as target for the detection. For *C. pneumoniae* a 62 bp sequence of the 16 S-rDNA was targeted, for *C. trachomatis* a 85 bp sequence of the cryptic plasmid and for *C. psittaci* an 89 bp sequence of the 16–23 S intergenic spacer region. The lower detection limit was 10 copies per mililiter.

**Statistical methods**

Continuous data are presented as the median and the interquartile range (IQR, range from the 25th to the 75th percentile). Percentages were determined for dichotomous variables. Fisher’s Exact Test was applied for group comparisons. A two-sided *p*-value <0.05 was considered to be statistically significant. All calculations were performed with MS Excel for Windows 2002 and SPSS (Version 10.0.7, SPSS Inc., IL, USA) for Windows.

**Results**

One hundred and forty-eight tissue samples from 138 individuals were included in this study. Cardiovascular comorbidities and atherothrombotic risk factors were found in a minority of patients suffering from acute thoracic aortic dissection and aneurysm, and were frequently observed in patients with AAA (Table 1).

**Chlamydia and aortic disease**

We analyzed samples of all patients with aortic disease (*n* = 65) and controls (*n* = 83) with respect to the presence of *Chlamydia* DNA. *C. pneumoniae* DNA was not detectable in any aortic tissue sample (0 of 148), *C. trachomatis* DNA was detected in one patient with aortic disease, but none of the controls (*P* = 0.43). DNA sequences of *C. psittaci* were not detected in any sample. *Chlamydia* DNA was found in eight patients suffering from aortic disease and in one control (*P* = 0.01) (Table 2).

**Acute thoracic aortic dissection**

We failed to detect *C. pneumoniae, C. psittaci* and *C. trachomatis* DNA in all samples of patients suffering from acute type A aortic dissection (*n* = 17) and control samples (*n* = 73). *Chlamydia* DNA was found in two of 17 patient samples versus one of 83 samples from the control group (*P* = 0.09) (Table 2).

**Thoracic aortic aneurysms**

DNA of *C. pneumoniae, C. trachomatis, C. psittaci* and *Chlamydia* subspecies was not detectable in specimens from patients with TAA (*n* = 25), but DNA of *Chlamydia* subspecies was found in one specimen of the control group (*P* = 0.99) (Table 2).

**Abdominal aortic aneurysm**

In all samples from patients suffering from AAA (*n* = 23) and 10 controls *C. pneumoniae* DNA was not detectable. *C. trachomatis* DNA, however, was present in one of the 23 patients (*P* = 0.99) and *Chlamydia* DNA in six of 23 patients (*P* = 0.15), but in none of the controls (Table 2).

**Discussion**

In our analysis *C. pneumoniae* DNA could not be detected in any aortic tissue sample of 65 patients with thoracic or abdominal aortic disease and 83 control tissue samples using a high-sensitive and specific,
validated Real time PCR method. In a minority of specimens Chlamydial DNA was detected. This was significantly more often in samples from patients than in controls, although certain atherosclerotic risk factors were more frequently found in the latter. These findings suggest that *Chlamydia* does not play a significant role in aortic disease.

*C. pneumoniae*, a gram-negative and obligate intracellular bacterium, has been detected in various pathologies of the vascular system.\(^1\),\(^2\),\(^{20}\)--\(^22\) Primary reports suggested a chronic infection with *C. pneumoniae* as a possible trigger mechanism for atherosclerosis, and experimental investigations supported this hypothesis.\(^10\) Pharmacological interventions with specific antibiotic treatment, however, have shown no beneficial effects on the progression of atherosclerosis. In addition, several recent studies reported failure to detect *C. pneumoniae* in atherosclerotic vascular tissue.\(^14\),\(^16\) This paradox may be explained by the fact that early studies were based on the

Table 1. Demographics of patients with acute and chronic aortic disease compared to controls

<table>
<thead>
<tr>
<th></th>
<th>Aortic dissection</th>
<th>Thoracic aneurysm</th>
<th>Abdominal aneurysm</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Diseased n=17</td>
<td>Control n=73</td>
<td>Diseased n=25</td>
</tr>
<tr>
<td>Median age (IQR)</td>
<td>59 (54–68)</td>
<td>72 (65–76)</td>
<td>58 (47–66)</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>8 (47)</td>
<td>46 (63)</td>
<td>19 (76)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>1 (6)</td>
<td>25 (34)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>13 (76)</td>
<td>49 (67)</td>
<td>20 (80)</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>6 (35)</td>
<td>51 (69)</td>
<td>8 (32)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>4 (23)</td>
<td>11 (15)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>5 (29)</td>
<td>41 (56)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Cerebrovascular disease (%)</td>
<td>1 (6)</td>
<td>11 (15)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Peripheral artery disease (%)</td>
<td>1 (6)</td>
<td>15 (20)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Chronic renal insufficiency (%)</td>
<td>2 (12)</td>
<td>7 (10)</td>
<td>4 (16)</td>
</tr>
</tbody>
</table>

Continuous data are presented as the median and the interquartile range (IQR, range from the 25th to the 75th percentile). Percentages are determined for dichotomous variables.

Table 2. Presence of Chlamydial DNA in aortic specimens; comparison between patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Aortic Dissection</th>
<th>Thoracic Aneurysm</th>
<th>Abdominal Aneurysm</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Diseased n=65</td>
<td>Control n=83</td>
<td>Diseased n=73</td>
<td>Control n=73</td>
</tr>
<tr>
<td>Chlamydia DNA</td>
<td>8 1 0.01</td>
<td>2 1 0.09</td>
<td>0 1 0.99</td>
<td>6 0 0.15</td>
</tr>
<tr>
<td>Chlamydia pneumonia*</td>
<td>0 0 0.99</td>
<td>0 0 0.99</td>
<td>0 0 0.99</td>
<td>0 0 0.99</td>
</tr>
<tr>
<td>Chlamydia trachomatis*</td>
<td>1 0 0.43</td>
<td>0 0 0.99</td>
<td>0 0 0.99</td>
<td>1 0 0.99</td>
</tr>
<tr>
<td>Chlamydia psittaci*</td>
<td>0 0 0.99</td>
<td>0 0 0.99</td>
<td>0 0 0.99</td>
<td>0 0 0.99</td>
</tr>
</tbody>
</table>

Detection of chlamydial DNA in 148 aortic wall samples of 138 patients. Whereas *Chlamydia pneumonia* could not be observed in any patient, *Chlamydia* subspecies DNA was found in samples of eight cases and in one control (P=0.01), however, no significant differences were found between the subgroups aortic dissection (P=0.09), TAA (P=0.99) and AAA (P=0.15) and respective controls. In 10 patients of those aortic wall specimens were obtained at autopsy, samples of the thoracic and the abdominal site of aorta were analyzed.

*Sequences specific for all known *Chlamydiaceae*.
prevalence of anti-chlamydial antibodies, however, recent studies demonstrated that there is no correlation between antibody levels and the detection of \textit{C. pneumoniae} DNA in abdominal aortic tissue. In a recent study we found no association between serological signs of chlamydial infection and TAAs. In contrast we observed in patients with AAA elevated levels of immunoglobulin against \textit{Chlamydia} lipopolysaccharides, reflecting an unspecific \textit{Chlamydia} immunopathogenicity.

Reports on the prevalence of \textit{Chlamydia} infection in thoracic aneurysm and aortic dissection are rare. Nyssström-Rosander and colleagues found an infection rate of 12\% among 32 aortic tissue samples of chronic TAA. Additionally, a high prevalence of serum IgA antibodies to \textit{C. pneumoniae} was observed. In the present study we found no evidence of \textit{C. pneumoniae}-infection in acute and chronic aortic disease, independent of the vascular location. A lack of sensitivity of the applied Real time PCR cannot be presumed. The extraction procedure, the sensitivity and specificity of the detection method has been validated in controlled and multicenter studies. Lindholt and colleagues speculated that a variation in the prevalence of \textit{C. pneumoniae} infection among populations studied may explain the controversial results. No such evidence, however, currently exists.

Atherosclerosis, aortic aneurysm and aortic dissection, although associated, do not seem to be identical diseases: the detection of \textit{C. pneumoniae} in atherosclerotic vascular tissue may be rather coincidental and may neither reflect the basis of the underlying process of atherosclerosis nor be the cause of formation of aneurysms or dissections. Nevertheless, although we could not detect \textit{C. pneumoniae}, the presence of \textit{Chlamydia} subspecies was observed in a rather small percentage of patients. A role for chronic \textit{Chlamydia} infection in aortic aneurysm formation, therefore, cannot be excluded with certainty, and the impact of \textit{Chlamydia} subspecies has to be further investigated.

**Limitations**

We are aware of several limitations of the present study. Most importantly, we were unable to obtain serological results and tissue samples simultaneously from all patients, and the issue of correlation between anti-Chlamydia anti-bodies and positive DNA findings cannot be addressed with this data. Nevertheless, our recent findings are in line with a previous observation from our group with serological data, suggesting that particularly thoracic aortic disease is not associated with \textit{Chlamydia} infection or immunopathogenicity. We cannot exclude observation bias because of the study design chosen. We obtained tissue pieces from the most atherosclerotic site of the aorta, where we expected \textit{Chlamydia} to be present. This method of selection has been evaluated in various previous studies, dealing with the issue of \textit{Chlamydia} and atherosclerosis. Since, we analyzed a large number of samples and failed to detect \textit{C. pneumoniae}, this form of selection bias might be of minor importance. However, we cannot exclude with certainty that if other regions of the tissue would have been analyzed the results might have been different.

**Conclusion**

In respect to our findings, \textit{C. pneumoniae} plays no clinically relevant role in acute and chronic aortic disease. The impact of other organisms of the family \textit{Chlamydiaceae} is unclear and should be further assessed.

**Acknowledgements**

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**References**


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