Increased Aortic Intima-Media Thickness in 11-Year-Old Healthy Children With Persistent *Chlamydia pneumoniae* Seropositivity

Iina Volanen, Mikko J. Järvisalo, Raija Vainionpää, Martti Arffman, Katariina Kallio, Susanna Anglé, Tapani Rönneamaa, Jorma Viikari, Jukka Marniemi, Olli T. Raitakari, Olli Simell

**Objective**—The relationship between *Chlamydia pneumoniae* (*Cpn*) infection and arterial measures of preclinical atherosclerosis has remained controversial. Because atherogenesis begins in early life, we examined whether carotid and aortic intima-media thickness (IMT) and brachial artery endothelial function are associated with *Cpn* seropositivity in children.

**Methods and Results**—*Cpn*-specific IgG and IgA antibodies were assessed by enzyme immunoassay in 199 healthy children followed-up annually from 7 to 11 years of age. Carotid (cIMT) and aortic IMT (aIMT), and brachial artery flow-mediated dilatation (FMD) were measured in 137 of the 199 children at the age of 11 years using high-resolution ultrasound. Children with persistent IgG and/or IgA seropositivity to *Cpn* had significantly increased aIMT compared with seronegative children (IgG ≤ 45 and IgA ≤ 12 enzyme immunounits) or children with transient *Cpn* seropositivity (seronegative, 0.496 [0.054]; transient, 0.494 [0.061]; and persistent, 0.532 [0.086] mm; *P*<0.05 for trend). This trend was not explained by traditional atherosclerotic risk factors or pubertal stage. cIMT and FMD were not associated with *Cpn* seropositivity.

**Conclusions**—Eleven-year-old children with persistent *Cpn* seropositivity show increased aIMT but not cIMT, suggesting that *Cpn* may affect the aortic wall, the site where the earliest atherosclerotic lesions are known to occur, in otherwise healthy children. (*Arterioscler Thromb Vasc Biol. 2006;26:000-000.*)

**Key Words:** antibodies ■ atherosclerosis ■ infection ■ pediatrics ■ ultrasound

Early morphological signs of atherosclerosis in the coronary artery intimal layer have been observed already in infants.1 These postmortem intimal changes have been related, in addition to conventional atherosclerotic risk factors,2 also to the presence of systemic infection.3 Recently, increasing evidence has linked inflammation and infection to atherosclerosis and myocardial infarction, and atherosclerosis is generally considered an inflammatory disease.

Research into the infectious hypothesis in the pathogenesis of atherosclerosis has focused, in particular, on *Chlamydia pneumoniae* (*Cpn*), an obligate intracellular human pathogen responsible for a significant portion of atypical pneumonia. The evidence of *Cpn* as a causative agent in the development of atherosclerosis is based on seroepidemiological studies,3 and detection of viable *Cpn* from the atheromas of coronary, carotid and femoral arteries, and of abdominal aortic aneurysms.4–5 However, in the available studies in adults, the relation of *Cpn* infection to endothelial dysfunction and increased intima-media thickness of the common carotid artery (cIMT), 2 measures of subclinical atherosclerosis, has not been conclusively demonstrated.

High-resolution ultrasound is a reliable noninvasive method for detecting early functional and structural atherosclerotic changes in the arterial wall. Flow-mediated dilatation (FMD) of the brachial artery is a marker of endothelial function.6 The carotid artery has been the target in the assessment of early structural vascular changes because it is located superficially on the neck and is thus easily visualized by ultrasound. However, autopsy studies have shown that the earliest morphological alterations in the arterial wall emerge in the abdominal aorta.7 Therefore, intima-media thickness of the abdominal aorta (aIMT) may provide an even better index of preclinical atherosclerosis than cIMT. Consistent with this idea, we have recently shown that children with increased risk factor load are more efficiently identified by measuring aIMT than cIMT, which shows more overlapping with healthy controls.8

Numerous cardiovascular risk factors have been related to early functional and structural vascular wall changes already.
in the first decade of life.\textsuperscript{9, 13} In addition, impaired FMD and increased cIMT have been demonstrated in HIV-infected children,\textsuperscript{14} and in children with elevated C-reactive protein (CRP) concentrations.\textsuperscript{15} Currently, there are no data on the association between exposure to \textit{Cpn} and markers of subclinical atherosclerosis in childhood. Therefore, we assessed whether the thickness of aortic and carotid intima-media complex, and brachial artery reactivity are related to \textit{Cpn} seropositivity in otherwise healthy children.

**Methods**

For a more detailed description, please see online data supplement, available at http://atvb.ahajournals.org.

**Study Design and Subjects**

The design and protocol of the ongoing STRIP study (Special Turku Coronary Risk Factor Intervention Project for Children) have been published.\textsuperscript{16} Briefly, 1054 voluntary families with 1062 healthy 6-month-old infants were recruited in 1990 through 1992, and randomized to the intervention group (n = 540) to receive detailed individualized dietary and lifestyle counseling aiming at minimizing intervention children’s exposure to known environmental atherosclerosis risk factors, or to a control group (n = 522). The study protocol was approved by the local ethics committee. Informed consent was obtained from all parents.

As part of the STRIP trial, venous blood samples were drawn annually after an overnight fast. The samples were kept at \(-70^\circ\text{C}\) until analyzed. \textit{Cpn} antibodies were measured between the ages of 7 and 11 years in a random time-restricted subsample of 199 consecutive STRIP children (55% boys, n = 110), born in 1989 to 1990. An equal proportion of the children belonged to the STRIP intervention (n = 99) and control groups (n = 100). The children showed no apparent respiratory symptoms at the time of the blood sampling. Of the 199 children with \textit{Cpn} antibody measurements, 137 (69%) volunteered to participate in the ultrasound study at the age of 11 years. The main reasons for nonparticipation were the child’s unwillingness to participate, lack of time, and difficulties with transportation. aIMT measurements were available in 128, cIMT measurements in 135, and brachial artery measurements in 130 children. All ultrasound studies were performed in the morning after the children had fasted overnight.

**Chlamydia pneumoniae Antibodies**

\textit{Cpn}-specific IgG and IgA antibodies were determined at the ages of 7, 8, 9, 10, and 11 years by a commercial enzyme immunoassay (EIA) technique (IgG-EIA and IgA-EIA; Ani Labsystems, Helsinki, Finland) as described.\textsuperscript{17} Seropositivity was defined by the manufacturer and expressed as enzyme immunoassays (EUI) using an IgG value of \(>45\) EUI and an IgA value of \(>12\) EUI as cutoff points. IgG values \(>130\) EUI and IgA values \(>50\) EUI were considered to be high.

**Ultrasound Measurements**

The abdominal aorta, both carotid arteries, and the left brachial artery (at rest, during reactive hyperemia, and after administration of sublingual nitroglycerin) were scanned according to a predetermined, standardized scanning protocol using an Acuson Sequoia 512 high-resolution ultrasound mainframe (Acuson, Mountain View, Calif) with a 13-MHz linear array transducer.\textsuperscript{8, 13} All the ultrasound scans were performed when the children were 11 years old by the same experienced vascular sonographer unaware of the clinical and laboratory characteristics of the children. Subsequent off-line analysis of the scans was performed by the same blinded reader.

**Statistical Methods**

To study the effect of \textit{Cpn} antibody positivity on the ultrasound measures, children were divided into predetermined three groups. First, children having IgG and IgA antibody values continuously below the cutoffs between the ages of 7 and 11 years served as controls (seronegative). The second group consisted of children with \textit{Cpn} IgG and IgA antibodies, yet without persistent seropositivity for either antibody class over the follow-up (transient). Third, we considered children to be persistently \textit{Cpn}-seropositive if serum samples were positive for IgG or IgA, or for both antibody measurements in at least the last three age points during the follow-up (9, 10, and 11 years) (persistent).

We assessed relationships between the aforementioned 3 groups and background variables (collected at the age of 11 years, i.e., at the same age as the ultrasound measurements) using regression analysis (continuous variables), or Cochran-Armitage test for trend (categorical variables). Variables with a skewed distribution were log-transformed for the analyses. Univariate regression analysis was used to study associations between established risk factors and ultrasound measures.

The relations between the arterial measures and \textit{Cpn} antibody positivity were studied using univariate and multivariate regression analyses. Variables showing unequal class level trends or significant linear trends with increasing exposure to \textit{Cpn}, and those established determinants of IMT and FMD that had an independent effect in the univariate model (\(P < 0.10\)) were controlled for using multivariate analyses. Variables showing unequal class level trends or significant potential interaction, and thereby confounding effect, of gender with \textit{Cpn} seropositivity groups in cIMT, aIMT, and FMD. A 2-sample \(t\) test was used to determine differences in the ultrasound variables between the children with different antibody concentrations, and between the children with different serum high-sensitivity CRP (hs-CRP) concentrations. Results are expressed as mean (SD), unless stated otherwise. \(P < 0.05\) were considered statistically significant. The SAS release 9.1.3 program package (SAS Institute, Cary, NC) was used in statistical analyses.

**Results**

The prevalences of \textit{Cpn} antibody positivity of the 199 study children at the ages of 7, 8, 9, 10, and 11 years have been published elsewhere.\textsuperscript{18} In this cohort of 199 children, the characteristics of those belonging to the 3 \textit{Cpn} seropositivity groups (n = 135) are shown in Table 1. Interestingly, the proportion of pubertal children was higher in the groups with \textit{Cpn} antibodies than in the seronegative group (seronegative, 51%: transient, 61%: persistent, 76%; \(P = 0.01\)), whereas the family history of premature cardiovascular disease was more often positive in seronegative than in seropositive subjects (seronegative, 21%: transient, 9%: persistent, 6%; \(P = 0.03\)).

There were no significant differences across the three \textit{Cpn} seropositivity groups regarding gender, proportion of STRIP intervention children, body size measures, blood pressure, serum lipids, hs-CRP concentration, aortic or carotid luminal diameter, brachial artery diameter at rest, increase in blood flow during reactive hyperemia, or parental smoking rates.

The numbers of the children with ultrasound data in the three \textit{Cpn} seropositivity groups were as follows: aIMT: seronegative, 59, transient, 15, and persistent, 22; cIMT: seronegative, 63, transient, 15, and persistent, 23; FMD: seronegative, 58, transient, 14, and persistent, 24. Seronegative children with incomplete data during the follow-up were excluded, and the transient group consisted of children with both IgG and IgA antibodies, to exclude children with either a borderline positive IgG or IgA result at only one age point. Thus, \(\approx 75\%\) of the children with ultrasound data belonged to the aforementioned 3 groups and were included in the following analyses (for details, see the paragraph Relation of \textit{Cpn} seropositivity to aIMT, cIMT, and FMD). Because gender produced no confounding effects on the
association between \textit{Cpn} seropositivity and ultrasound measures (mean aIMT, \( P = 0.35 \) for interaction; maximum aIMT, \( P = 0.17 \) for interaction; mean cIMT, \( P = 0.23 \) for interaction; maximum cIMT [the mean of left and right maximum IMT], \( P = 0.18 \) for interaction; and peak FMD, \( P = 0.24 \) for interaction), both sexes were analyzed combined.

\textbf{Relation of \textit{Cpn} Seropositivity to aIMT, cIMT, and FMD}

Mean aIMT was significantly higher in children with persistent \textit{Cpn} positivity than in seronegative children or those with transient \textit{Cpn} seropositivity (mean aIMT: seronegative, 0.496 [0.054]; transient, 0.494 [0.061]; and persistent, 0.532 [0.086] mm; \( P = 0.039 \) for trend). In contrast, cIMTs were unaffected by the \textit{Cpn} antibody status (mean cIMT: seronegative, 0.444 [0.043]; transient, 0.431 [0.034]; and persistent, 0.439 [0.051] mm; \( P = 0.52 \) for trend) (Figure). The relationships were essentially similar when maximum IMTs were used in the models instead of mean values (maximum aIMT: \( P = 0.045 \) for trend; maximum cIMT: \( P = 0.49 \) for trend).

The maximal endothelium-dependent (flow-mediated) dilatation did not differ significantly across the three \textit{Cpn} seropositivity groups (peak FMD: seronegative, 7.9 [4.1]; transient, 8.0 [4.0]; and persistent, 8.6 [3.1]%; \( P = 0.48 \) for trend), as did not the endothelium-independent (nitrate-mediated) dilatation (NMD: seronegative, 12.2 [5.8]; transient, 10.9 [5.2]; and persistent, 10.2 [5.5]%; \( P = 0.09 \) for trend).

In the children excluded from the analyses, mean aIMT, mean cIMT, and peak FMD were 0.49 (0.07) mm, 0.44 (0.04) mm, and 7.78 (3.41)\%, respectively, corresponding closely to the values of the children in the seronegative group.

To evaluate the relation between \textit{Cpn} and IMT more closely, we investigated whether the concentration, in addition to the persistence, of antibodies is related to IMT. Compared with children having continuously low IgG and IgA antibody concentrations (ie, the seronegative children, IgG\( \leq 45 \) and IgA\( \leq 12 \) EIU), children possessing high IgG and/or IgA values (IgG\( > 130 \) and/or IgA\( > 50 \) EIU) and being persistently seropositive thereafter had greater aIMT (maximum aIMT: 0.532 [0.061] versus 0.566 [0.090] mm, \( P = 0.048 \); mean aIMT: 0.496 [0.054] versus 0.525 [0.083] mm, \( P = 0.061 \)), whereas there were no significant differences in cIMT between these 2 groups (maximum

### TABLE 1. Characteristics of Study Children Stratified by \textit{Chlamydia pneumoniae} Seropositivity

<table>
<thead>
<tr>
<th></th>
<th>Seronegative†</th>
<th>Transient‡</th>
<th>Persistent§</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>77</td>
<td>24</td>
<td>34</td>
<td>—</td>
</tr>
<tr>
<td>Proportion of boys</td>
<td>55</td>
<td>46</td>
<td>59</td>
<td>0.80</td>
</tr>
<tr>
<td>In STRIP intervention group, %</td>
<td>52</td>
<td>42</td>
<td>47</td>
<td>0.55</td>
</tr>
<tr>
<td>Exact age, y</td>
<td>11.1 (0.1)</td>
<td>11.1 (0.1)</td>
<td>11.1 (0.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Height, cm</td>
<td>147 (7)</td>
<td>150 (7)</td>
<td>148 (7)</td>
<td>0.59</td>
</tr>
<tr>
<td>Weight*, kg</td>
<td>39.0 (8.6)</td>
<td>40.4 (7.1)</td>
<td>41.7 (10.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>106 (12)</td>
<td>109 (10)</td>
<td>106 (13)</td>
<td>0.77</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>59 (8)</td>
<td>59 (7)</td>
<td>57 (7)</td>
<td>0.15</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.5 (0.7)</td>
<td>4.4 (0.6)</td>
<td>4.4 (0.7)</td>
<td>0.24</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.9 (0.6)</td>
<td>2.8 (0.6)</td>
<td>2.8 (0.6)</td>
<td>0.50</td>
</tr>
<tr>
<td>HDL cholesterol*, mmol/L</td>
<td>1.29 (0.29)</td>
<td>1.29 (0.34)</td>
<td>1.24 (0.24)</td>
<td>0.35</td>
</tr>
<tr>
<td>Triglycerides*, mmol/L</td>
<td>0.87 (0.67)</td>
<td>0.79 (0.35)</td>
<td>0.81 (0.35)</td>
<td>0.45</td>
</tr>
<tr>
<td>hs-CRP*, mg/L, geometric mean (95% CI)</td>
<td>0.3 (0.02-3.95)</td>
<td>0.3 (0.02-3.86)</td>
<td>0.2 (0.02-1.70)</td>
<td>0.15</td>
</tr>
<tr>
<td>Abdominal aortic diameter, mm</td>
<td>9.60 (1.57)</td>
<td>9.78 (1.11)</td>
<td>9.70 (1.46)</td>
<td>0.75</td>
</tr>
<tr>
<td>Carotid artery diameter, mm</td>
<td>5.33 (0.42)</td>
<td>5.19 (0.31)</td>
<td>5.30 (0.58)</td>
<td>0.61</td>
</tr>
<tr>
<td>Brachial artery baseline diameter, mm</td>
<td>3.04 (0.34)</td>
<td>3.13 (0.37)</td>
<td>2.98 (0.25)</td>
<td>0.58</td>
</tr>
<tr>
<td>Increase in blood flow, %</td>
<td>349 (159)</td>
<td>274 (133)</td>
<td>299 (156)</td>
<td>0.12</td>
</tr>
<tr>
<td>Pubertal¶, %</td>
<td>51</td>
<td>61</td>
<td>76</td>
<td>0.01</td>
</tr>
<tr>
<td>Positive cardiovascular family history</td>
<td></td>
<td>, %</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Both parents non-smokers, %</td>
<td>79</td>
<td>83</td>
<td>83</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Values are mean (SD), unless otherwise indicated. *P value after logarithmic transformation. †Seronegative = IgG<45 and IgA<12 enzyme immunounits throughout the follow-up (7–11 years). ‡Transient = IgG and IgA seropositivity without persistence between ages 7 and 11 years. §Persistent = IgG and/or IgA seropositivity at least at 9, 10, and 11 years of age. ¶Testicle size or breast development, and/or pubic hair 1 in Tanner stage. ||Myocardial infarction in mother/grandmother before age 65 y and/or in father/grandfather before age 55 y. Numbers of children vary between various variables because of missing data. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein.
cIMT: 0.485 [0.049] versus 0.482 [0.053] mm, P = 0.76; mean cIMT: 0.444 [0.043] versus 0.439 [0.051] mm, P = 0.70). We also examined the relation between Cpn and IMT depending on IgA seropositivity alone (seronegative: IgA≤12 EIU throughout the follow-up; transient: IgA>12 EIU during the follow-up, yet without persistence; and persistent: IgA>12 EIU at least at the ages of 9, 10, and 11 years), and found no significant association with aIMT or cIMT (data not shown). Of note, there were only 12 children with available IMT data and IgA antibodies persistently elevated. Finally, children with persistently elevated IgG and/or IgA values and hs-CRP concentration above the median (>0.15 mg/L) had no significant differences either in aIMT (P = 0.33) or cIMT (P = 0.60) compared with children with persistent Cpn positivity and hs-CRP concentration below the median.

**TABLE 2. Univariate Regression Analysis for the Determinants of aIMT, cIMT, and FMD**

| Explanatory variable | Mean aIMT |  | Mean cIMT |  | Peak FMD |  |
|----------------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|-----------|
|                      | Regression Coefficient β‡ | P     | Regression Coefficient β‡ | P     | Regression Coefficient β‡ | P     |
| Cpn group variable*  | 0.016     | 0.039           | −0.003    | 0.52            | 0.327    | 0.48            |
| Weight               | −0.00007  | 0.93            | 0.0006    | 0.22            | −0.0147  | 0.76            |
| Systolic blood pressure | 0.0004  | 0.59            | 0.0011    | 0.04            | 0.0067   | 0.89            |
| Diastolic blood pressure | −0.0018 | 0.13            | 0.0002    | 0.86            | 0.0044   | 0.95            |
| Total cholesterol    | −0.008    | 0.44            | 0.008     | 0.23            | −0.8870  | 0.11            |
| LDL cholesterol      | −0.004    | 0.71            | 0.004     | 0.59            | −0.9668  | 0.15            |
| HDL cholesterol      | −0.007    | 0.75            | 0.025     | 0.08            | −2.0735  | 0.11            |
| Triglycerides        | −0.015    | 0.29            | 0.0002    | 0.98            | 0.9855   | 0.30            |
| Brachial diameter at rest | −3.0336 | 0.01          |

aIMT indicates abdominal aortic intima-media thickness; cIMT, carotid artery IMT; and FMD, flow-mediated dilatation.

*Cpn group variable = seronegative, transient, persistent.

‡β-values indicate the change in the response variable for a 1-unit change in explanatory variable.
In the present study, no increases were observed in the carotid artery wall-thickness in connection with Cpn seropositivity. This finding may be in line with previous postmortem data showing that atherosclerosis first affects the abdominal aorta. Thus, the preferential association between Cpn seropositivity and aIMT may not be a peculiarity of the aorta but reflect the higher variability of aIMT, corresponding to an earlier manifestation of atherosclerosis at this site.

Several previous studies in adults have associated Cpn IgG seropositivity with increased cIMT in study populations with more pronounced atherosclerosis, whereas no such association has been found in younger asymptomatic subjects without known cardiovascular disease or significant carotid lesions. These observations indicate that Cpn may accelerate atherogenesis in individuals with preexisting intimal injury rather than trigger early stages of disease progression. Consistent with this idea, Cpn is able to grow in various vascular cells present in atherosclerotic plaques and viable Cpn has been found in atherosclerotic lesions but not in healthy vessel walls. However, our findings suggest that, at least in the most lesion-prone site of the arterial tree, i.e., the abdominal aorta, Cpn might also contribute to the development of atherosclerotic vascular changes. Because of the relatively small number of children in the subgroups, however, these results should be considered preliminary and interpreted with some caution.

The increased aIMT in our study children with persistently elevated Cpn antibodies probably reflects increased fatty streak formation, as fatty streaks may be prevalent in the aortas of children as young as 3 years of age, whereas advanced lesions, arising from fatty streaks, are mainly found after the age 20. Fatty streaks in the proximal half of the abdominal aorta rarely are converted to raised lesions, whereas those on the dorsal surface of the distal abdominal aorta, corresponding the site scanned in the present study, are susceptible to progression in the presence of established risk factors. Of interest, Cpn has been previously found in aortic fatty streaks of young adults. Furthermore, strengthening the probability of causality, inoculation of rabbits with Cpn alone, as well as in combination with cholesterol-supplemented diet has resulted in aortic intimal thickening.

Animal studies have suggested that acute Cpn infection might impair arterial vasodilatory function. Moreover, acute childhood infections, defined by clinical symptoms, have been recently associated with transient and recoverable functional, followed by later structural arterial wall abnormalities. In the present study, Cpn seropositivity was not associated with decreased brachial artery endothelial function. Thus, our findings complement the observations of earlier studies by demonstrating that persistent Cpn seropositivity in healthy children may predispose to structural atherosclerotic changes but is not associated with permanent endothelial dysfunction. The lack of relationship between Cpn seropositivity and endothelial function in healthy population has also been confirmed by two previous studies in young men without risk factors for coronary artery disease.

In this pediatric study, other known causes of vascular damage presumably did not affect the findings, as the groups predominately did not differ in terms of traditional atherosclerotic risk factors. Some lifestyle factors such as smoking, which selectively augments atherosclerosis in the abdominal aorta, could not have confounded the results. Another strength of this study is its prospective design in defining exposure to Cpn.

We also acknowledge a number of limitations to the present study. Fatty streaks in the aortas of children are usually found in the most distal part of the abdominal aorta and downstream of the arterial branches. Accordingly, there may be considerable local variation in the distribution of atherosclerotic lesions. The ultrasound method has limitations, because it allows measurement of wall thickness only in the dorsal arterial wall of the most distal abdominal aorta. The relatively small number of children included in the subgroups might have influenced the possibility to establish significant differences in cIMT and FMD between the groups. With the number of subjects enrolled, this study had >80% power to detect a 2.4% unit difference in FMD and 0.035 mm difference in cIMT between seronegative and persistently seropositive children at the P < 0.05 level. Therefore, our study excludes a major effect of Cpn on cIMT and brachial artery FMD, although the study may have been underpowered to detect more subtle effects on carotid artery structure and brachial artery function. Furthermore, the present study examined the relationships between Cpn and arterial properties in a cross-sectional fashion. A longitudinal study, enabling the perception of potential vascular changes and the investigation of their association with Cpn seropositivity, would be a more ideal approach.

Assessment of a chronicity of an infection remains an unresolved issue. IgG antibodies to Cpn are generally thought to mark previous exposure to the pathogen, whereas persistent presence of IgA antibodies, with IgA antibodies having a half-life of approximately only a week, presumably reflects chronicity. It has been suggested that the combination of Cpn antibodies and an inflammatory marker, especially CRP, an emerging risk factor for cardiovascular disease, could also be a reliable marker of chronicity. Although the definite stimulus for CRP is unknown, higher CRP values might arise from the chronic inflammatory state evident in persistent infections.

Chlamydial lipopolysaccharide, a typical constituent of the cell wall of all Gram-negative bacteria, is a potent inducer of cytokine production. By promoting production of cytokines and further acute-phase reactants such as CRP, Cpn could provoke induction of a chronic immune activation from afar. Alternatively, Cpn might induce persistent inflammation by its presence in the cells of the vascular wall, after being carried into the circulation inside monocytes. The induction of systemic inflammation could indeed be one of the pathophysiological mechanisms underlying the association between chronic infection and atherogenesis. In line with this, in adults, the risk of coronary events in connection with persistent Cpn infection has increased considerably in the presence of an elevated CRP concentration, and a combination of Cpn antibodies and elevated CRP values has
resulted in more pronounced cIMT progression than Cpn seropositivity alone.\(^2\) In this study, however, there was no significant trend in CRP values across the three Cpn groups, and within the persistent group, IMTs were not associated with, in general very low, hs-CRP concentrations. Likewise, in a recent study in adults, in which circulating Cpn DNA was associated with asymptomatic carotid atherosclerosis, hs-CRP concentrations did not differ significantly between subjects positive for Cpn and those negative for Cpn.\(^4\) Therefore, mechanisms other than the induction of systemic inflammation probably also contribute to the association between Cpn and IMT. Given the fact that Cpn has been detected in aortic fatty streaks in humans,\(^2\) and in the media of the aorta in a rabbit model,\(^4\) possible pathogenetic mechanisms may include the ability of chlamydial lipopolysaccharide to induce human macrophage foam cell formation,\(^4\) which is a key event in early atheroma development.

In conclusion, our data suggest that Cpn infection may affect the arteries of otherwise healthy children by promoting IMT of the abdominal aorta, and thereby have an important role in the pathogenesis of early atherosclerosis. Whether the observed increases in aIMTs gradually convert into more advanced lesions with continuous exposure to Cpn remains to be resolved.

**Acknowledgments**

This study was financially supported by grants from the Academy of Finland (grants 73582, 34316, and 53392), the Finnish Cardiac Research Foundation, the Juho Vainio Foundation, the Emil Aaltonen Foundation, the Research grant from Turku University Central Hospital, the Finnish Cultural Foundation, and its Regional Fund ofVarsinais-Suomi, the Paavikivi and Sakari Sohlberg Foundation, and the Paulo Foundation, Finland. The nitroglycerin preparation used in this study was a gift from Pohl-Boskamp, Hohenlockstedt, Germany.

**References**


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Arterioscler Thromb Vasc Biol. published online January 5, 2006;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Methods

Measurements of cIMT and aIMT

For the cIMT measurements, the far wall of the distal common carotid arteries on both sides was scanned, as previously described in detail. Briefly, the proximal part of the carotid bulb was identified, and the segment of the common carotid artery 1 to 2 cm proximal to the bulb was scanned. The image was focused on the posterior (far) wall, and the resolution box function was used to magnify the arterial far wall. Several images of the common carotid artery segment from 10 to 20 mm proximal to the carotid bulb (a far wall segment of 10 mm in width) were acquired. Two angles were used in each case: anterior oblique and lateral. All scans were digitally stored on the ultrasound system internal hard disk for subsequent off-line analysis. Two end-diastolic frames from both interrogation angles on both sides were selected and analyzed for mean and maximum IMT. The average of these measurements was used as mean cIMT, and maximum cIMT was defined as the mean of left and right maximum IMT in the analyses. In our laboratory, the interobserver coefficient of variation (CV) of cIMT measurements (of the same image data) was 3.0%, and the between-visits variation (CV) of cIMT measurement was 3.9%. 

The aIMT was studied as described. Briefly, the image was focused on the far wall (dorsal arterial wall of the most distal 15 mm of the abdominal aorta), and gain settings were used to optimize image quality. Images 15 mm in width were magnified using a resolution box function. A scanning frequency of 13 MHz was preferred but, when necessary (to reach sufficient tissue penetration), scanning frequencies of 11.5 and 10 MHz were used. Several images of the most distal 15 mm aortic far wall were captured in every case, and the images
were digitally stored for subsequent off-line analysis. All images were taken at end-diastole, incident with the R-wave on a continuously recorded ECG. Two images of the best quality were chosen for analysis in each study subject. IMT was measured using ultrasonic calipers. At least 4 to 6 measurements covering the entire far wall segment of interest were taken for each image. Maximum aIMT, and the average of these measurements as mean aIMT were used in the analyses. In our laboratory, the interobserver variation (CV) of aIMT measurements (of the same image data) was 3.9%, and the between-visits variation (CV) of aIMT measurement was 4.9%.1

The carotid and aortic luminal diameters were measured from M-mode images, acquired at the sites corresponding the IMT measurements at end-diastole, incident to the ECG R-wave.

**Brachial artery measurements**

Left brachial artery diameter was measured from B-mode ultrasound images at rest, during reactive hyperemia, and after administration of sublingual nitroglycerin, as described earlier.2 Briefly, a resting scan was performed, and arterial flow velocity was measured using a Doppler signal. Increased flow was then induced by inflation of a blood pressure cuff placed around the forearm (distal to the scanned part of the artery) to a pressure of 250 mmHg for 4.5 minutes, followed by release. Subsequent scans were taken continuously between 30 and 180 seconds after cuff deflation, including a repeated flow velocity measurement during the first 15 seconds after the cuff release. Vessel diameter was measured off-line at a fixed distance from an anatomic marker (e.g., a fascial plane) using ultrasonic calipers at end-diastole, incident with the R-wave on a continuously recorded ECG. Measurements were taken every 10 seconds between 30 and 180 seconds after cuff deflation, to ensure detection of peak FMD. The maximal proportional dilatation from baseline (peak FMD, %) was assessed.
Nitrate-mediated (endothelium-independent) dilatation (NMD) capacity was tested by administration of a 250 µg sublingual dose of glyceryl trinitrate. Maximum diameter 5 minutes after nitrate administration was used to calculate the proportional increase in diameter from the baseline value (NMD, %). In our laboratory, the interobserver variation (CV) of FMD measurements (of the same image data) was 8.6%, and the between-visits CV in FMD measurements was 9.3%.³

**Background data**

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride values were determined by standard enzymatic methods as described.⁴,⁵ None of the children had serum triglycerides exceeding 4 mmol/L. Low-density lipoprotein (LDL) cholesterol concentrations were calculated using the Friedewald equation.⁶

Serum high-sensitivity CRP (hs-CRP) concentrations were assayed using an immunoturbidimetric method (Olympus Diagnostica, Lismeehan, Ireland) in an automatic clinical chemistry analyzer (Olympus AU400). The sensitivity of the method was 0.02 mg/L, with the intra-assay CV of 0.95%, and the interassay CV of 1.07% at the mean CRP level of 1.66 mg/L.

At the STRIP visit, each child’s pubertal stage was determined according to Tanner staging. The children were classified as either prepubertal or pubertal, as none of them had completed puberty. A child was considered to be prepubertal if testicle size or breast development, and pubic hair were in Tanner stage 1 of development. Blood pressure was measured after 10 minutes’ rest using an automated sphygmomanometer (Omron M4, Omron Matsusaka Co., Ltd., Japan) from the right arm three times, and the readings were averaged for the statistical analyses. Height to the nearest 0.1 cm was measured with a Harpenden stadiometer (Holtain, Crymych, United Kingdom), and weight to the nearest 0.1 kg was
measured using an electronic scale (Soehnle S10, Soehnle, Murrhardt, Germany) while the child was wearing underwear.

Families were classified into two categories according to parents’ smoking habits (non-smoking parents versus either or both parents smoking), enquired by a questionnaire during the family’s STRIP visit. All study children were non-smokers. A child was considered having a positive family history of premature cardiovascular disease if child’s mother or either one of the grandmothers had suffered myocardial infarction before age 65 years, or if child’s father or either one of the grandfathers had suffered myocardial infarction before age 55 years.

All background data utilized in the study were collected at the age of 11 years, i.e., at the same age as the ultrasound measurements.

References


