**Chlamydia pneumoniae but Not Cytomegalovirus Antibodies Are Associated With Future Risk of Stroke and Cardiovascular Disease**

A Prospective Study in Middle-Aged to Elderly Men With Treated Hypertension

Björn Fagerberg, MD, PhD; Judy Gnarpe, PhD; Håkan Gnarpe, MD, PhD; Stefan Agewall, MD, PhD; John Wikstrand, MD, PhD

**Background and Purpose**—Several cross-sectional and prospective studies have indicated that high titers of antibodies to *Chlamydia pneumoniae* and cytomegalovirus (CMV) are associated with coronary heart disease. The aim of the present study was to examine whether elevated titers of antibodies to these pathogens are predictive of not only coronary but also cerebrovascular disease.

**Methods**—Serum titers of antibodies to *C pneumoniae* (IgM, IgG, IgA, IgG immune complex) and CMV (IgG) were determined at baseline (n = 130) and after 3.5 years (n = 111) in a total sample of 152 men. All individuals had treated hypertension and at least 1 additional risk factor for cardiovascular disease (hypercholesterolemia, smoking, or diabetes mellitus) and constituted 93% of a randomly selected subgroup (n = 164) of patients participating in a multiple risk factor intervention study.

**Results**—Elevations of any or both of the IgA or IgG titers to *C pneumoniae* at entry or after 3.5 years were found in 84 cases (55%). Of those with high titers at entry, 97% remained high at the 3.5 year reexamination. After 6.5 years of follow-up, high titers to *C pneumoniae* at entry were associated with an increased risk for future stroke (relative risk [RR], 8.58; \( P = 0.043 \); 95% CI, 1.07 to 68.82) and for any cardiovascular event (RR, 2.69; \( P = 0.042 \); 95% CI, 1.04 to 6.97). A high serum titer of antibodies to CMV was found in 125 cases (85%), and this was not associated with an increased risk of future cardiovascular events.

**Conclusions**—Seropositivity for *C pneumoniae*, but not for CMV, was associated with an increased risk for future cardiovascular disease and, in particular, stroke. (Stroke. 1999;30:299-305.)

**Key Words:** cerebrovascular disorders ■ cardiovascular diseases ■ *Chlamydia pneumoniae* ■ prospective studies

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There is an accumulating amount of data indicating that infections may be linked to atherosclerotic disease. There are also a number of potential underlying mechanisms for such an association that may be of a causative nature. Infection may both augment the atherosclerotic process and contribute to later manifestations of overt clinical disease by facilitating plaque rupture and thrombosis.¹

*Chlamydia pneumoniae* TWAR is an intracellular gram-negative bacterium that commonly causes respiratory infections in all age groups.² The clinical picture varies from asymptomatic infection or unrecognized, mildly symptomatic disease to bronchitis and pneumonia. Specific antibodies to *C pneumoniae* have been found in more than half of the adult population.³ Persistent infection is not uncommon after acute respiratory infection with *C pneumoniae*.⁴ A recent meta-analysis indicated that available data support the hypothesis that *C pneumoniae* may be causative for arterial disease, although further research is needed.¹

The cytomegaloviruses (CMVs) are a subgroup of agents closely related to the herpes group of viruses. The cytomegaloviruses are ubiquitous, and the incidence of infection gradually increases with age: 60% to 90% of adults have experienced infection.⁵ CMV infection may be acquired transplacentally, during birth, or by contact with infected secretions or excretions at any time thereafter. Infections acquired postnatally or later in life are often asymptomatic but may appear as an acute febrile illness, termed cytomegalovirus mononucleosis or hepatitis, depending on the clinical...
picture. A more fulminant severe disease may develop in patients with a compromised immunological system.

CMV is also suggested as an infectious agent of importance for the atherosclerotic disease process. However, only a limited number of patients with classic atherosclerotic heart disease have been examined for CMV.

Although most studies have been focused on coronary heart disease, there have also been reports on associations between infection and cerebrovascular disease. Most of the published reports have been based on cross-sectional studies, and there is a need for more prospective studies.

The Risk Factor Intervention study was a prospective, randomized study that examined whether a dedicated program aimed at lowering lipids and cessation of smoking, in addition to antihypertensive treatment, would improve the prognosis compared with usual care. All patients were men with treated hypertension and at least 1 of the risk factors hypercholesterolemia, smoking, or diabetes mellitus. The follow-up period was >6 years. Frozen serum samples from this study were used to test the hypothesis that high titers of antibodies to *C. pneumoniae* and CMV were associated with future cardiovascular disease.

**Subjects and Methods**

**Study Outline and Patients**

Five hundred eight male patients with treated primary hypertension were included in a risk factor intervention study. The inclusion criteria for the intervention study were, apart from treated hypertension and male sex, 1 or more of the following: hypercholesterolemia (serum cholesterol of ≥6.5 mmol/L), tobacco smoking (≥1 cigarettes/d), or diabetes mellitus (fasting blood glucose of >7 mmol/L). The patients were representative of high-risk hypertensives in Gothenburg, since the majority (>90%) were recruited by random sampling one third of all men in the respective age groups in Gothenburg. In the aforementioned intervention study, the patients had been randomized either to a multiple risk-factor treatment program or to conventional treatment. The intervention program was based on a nonpharmacological and, if necessary, pharmacological regimen aimed at lowering hypercholesterolemia and cessation of smoking.

From this group of 508 men, one third of the patients were randomly selected to take part in substudies on the development of atherosclerosis as assessed by ultrasound examinations of the carotid arteries, and blood was drawn for a number of biochemical assessments. In this group of 164 patients, serum samples for serological analysis were available for 130 patients at entry. After 3.5 years of follow-up (median) in the intervention study, sera from 111 patients were available for analyses. In total, 152 patients were included in the present study, and serological analyses were performed at entry as well as after 3.5 years in 89 patients (Figure 1).

All men gave informed consent after written and oral information, and the study was approved by the ethics committee of the Faculty of Medicine, Göteborg University, Göteborg, Sweden.

**Measurements**

Resting blood pressure was measured phonographically (Korotkoff sounds recorded on ECG paper) in the right arm after supine rest in connection with the ultrasound examination, as described previously. Blood pressure was calculated to the nearest 1 mm Hg, and the mean of 2 recordings was used. Body weight and body mass index were measured according to established principles. Smoking was assessed with a questionnaire. Cigarette-years were calculated as the number of years as a smoker times the average number of smoked cigarettes. Manifest cardiovascular disease was defined as the presence of one or more of the following diagnoses: stroke, myocardial infarction, angina pectoris, or intermittent claudication.

**Serological Analyses**

The analysis of the antibodies to *C. pneumoniae* was performed with use of a modified microimmunofluorescence technique. Sera were diluted 1:32 in PBS, pH 7.4, and tested for IgG, IgA, and IgM antibodies on 21-well antigen slides containing elementary body preparations of *Chlamydia psittaci*, *C. pneumoniae*, and *Chlamydia trachomatis* in each well (Laboratory Systems Oy). Sera that were positive in screening tests for IgG were rediluted and tested in doubling dilutions. Sera positive in screening tests for IgA and/or IgM were absorbed with Gullsorb (Gull Laboratories) at a dilution of 1:16 to remove all IgG,14 then titrated in doubling dilutions with PBS. Serum dilutions were incubated with antigen slides for 14 to 16 hours at 4°C to 8°C, after which slides were gently agitated in three 5-minute changes of PBS and air dried. Fluorescein isothiocyanate conjugated rabbit anti-human IgG, IgA or IgM (Dakopatts) was applied to appropriate wells and incubation was done for 30 minutes at 37°C. After a renewed washing procedure with three 5-minute changes of PBS, slides were immersed in H2O for 2 minutes and air dried. Coverslips were mounted with buffered glycerol, and slides were read in a Zeiss UV microscope with a ×40 oil immersion lens and a ×10 ocular lens (total magnification, ×400). All slides were read by the same investigator, who was unaware of the case-reference status of the sera or any clinical characteristics of the patients. Control sera routinely used in the laboratory was included in every test run, and tests were accepted only if the control sera titers were within 1 titer step of the earlier calculated mean. The last dilution step to give specific fluorescence was reported as the reciprocal titer. Based on Grayston’s suggestions and on earlier experiences, a reciprocal IgG titer of ≥512 and/or an IgA titer of ≥64 were used as lower limits for positive serology.

All serum specimens were investigated for complex-bound IgG antibodies to *C. pneumoniae* (IC) after treatment of the serum sample with 7% PEG 6000 (Janssen Chimica). Equal parts of sera and PEG
were mixed, left overnight at 4°C, and centrifuged the next day. Pellets obtained were resuspended and washed twice with 3.5% PEG, then finally resuspended in PBS, pH 7.2, to the same volume as the original serum aliquot. The resuspended specimens were then diluted 1:2 with PBS and tested for C pneumoniae IgG antibodies with use of the species-specific microimmunofluorescence technique. All antibody tests were read with a Zeiss UV microscope with a plane achromatic oil immersion lens at a final magnification of ×400. High titers of circulating immune complexes were arbitrarily defined as a reciprocal titer of ≥8.

IgG antibodies to cytomegalovirus were determined by use of a microparticle enzyme immunoassay technique (AxSYM CMV IgG, Abbott Laboratories). Sera were loaded into cups, which were placed in the AxSYM unit and processed according to manufacturer’s instructions. All sera with particulate matter or cells were first centrifuged to remove debris. Reciprocal IgG antibody titers are expressed as arbitrary units (AU) per milliliter, where ≥15.0 AU/mL is considered a positive result. Sera with titers of ≥250 AU/mL, the limit of the system, were not diluted and retested.

Statistical Analyses
Defined end points in the study were cardiovascular death, fatal and nonfatal myocardial infarction, fatal and nonfatal stroke, and other cardiovascular disease, as previously defined. All first cardiovascular events were also used as a combined end point.

Poisson models were used in a hazard function for outcome that was

$$\exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n)$$

where $\beta_n$ are calculated parameters and $x_n$ are variables. The analyses were focused on the risk for a future end point associated with elevated titers of antibodies to C pneumoniae and CMV (for definitions, see above). The presence or absence of elevated titers were used as time-dependent covariates; ie, up to the time point at which a new measurement was performed, the previous value (high or low titer) was used in the equation. After that, the new value replaced the previous value. The calculation of the RR and the associated 95% CI were adjusted for smoking and the presence of previous cardiovascular disease and for group allocation in the underlying multiple risk factor intervention study.

The model was also used to calculate the probability of stroke or any cardiovascular event occurring within 5 years, provided that the individual patient was followed up during the observation period or up to the time of the event. The examples represent patients who were not current smokers, who had no previous cardiovascular disease, and who had been randomized to the control group in the underlying risk factor intervention study.

$P<0.001$), and a high or low titer of IgG antibody was in general accompanied by a similar level in the concentration of IgA antibody (Table 3). The IgG antibodies demonstrated a close correlation to the immune complex level both at entry ($r=0.77, P<0.001$) and after 3.5 years ($r=0.73, P<0.001$). The IgM antibody titer was elevated in only 1 patient, both at the first and second examinations. No cross-reactions were seen between C pneumoniae and the other Chlamydia species.

As shown in Table 4, high titers of IgG or IgA antibodies to C pneumoniae at entry was associated with titers remaining high after 3.5 years. Among the 89 patients with measurements available at both entry and reexamination, 38 patients had high IgA and/or IgG titer at entry. High titers, according to this definition, remained high in 37 (97%) of these 38 men.

Results
The patients with high titers to C pneumoniae did not show any characteristics that were statistically different from those of patients with no antibodies or low titers, either at entry or at the examination after 3.5 years (Tables 1 and 2). Higher titers of circulating immune complex were found in the groups with high antibody titers.

C pneumoniae Serology
High titers of IgA and/or IgG antibodies to C pneumoniae at both or either examinations at entry or after 3.5 years were found in 84 cases (55%). The titers of IgA and IgG antibodies to C pneumoniae were interrelated both at entry ($r_c=0.67, P<0.001$) and at the 3.5-year examination ($r_c=0.59, P<0.001$), and then renewed the previous value. The calculation of the RR and the associated 95% CI were adjusted for smoking and the presence of previous cardiovascular disease and for group allocation in the underlying multiple risk factor intervention study.

$P<0.001$), and a high or low titer of IgG antibody was in general accompanied by a similar level in the concentration of IgA antibody (Table 3). The IgG antibodies demonstrated a close correlation to the immune complex level both at entry ($r=0.77, P<0.001$) and after 3.5 years ($r=0.73, P<0.001$). The IgM antibody titer was elevated in only 1 patient, both at the first and second examinations. No cross-reactions were seen between C pneumoniae and the other Chlamydia species.

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CMV Serology
Seropositivity to CMV at both or either examinations, at entry or after 3.5 years, was found in 125 cases (85%). There was no association between high titers of CMV antibodies and high titers of C pneumoniae antibodies (data not shown).

Clinical Outcome
The clinical outcome during 6.5 years of follow-up in relation to C pneumoniae antibody levels is presented in Table 5. In the group with low antibody titers, there was 1 case of hemorrhagic stroke and 1 case of ischemic stroke, whereas the corresponding numbers in the high titer group were 2 and 8, respectively. In addition, there was 1 patient with no available CT brain scan after the event in the high-titer group.
### TABLE 1. Characteristics of Patients With Positive and Negative Serology for Chlamydia pneumoniae, Respectively, at the Baseline Examination

<table>
<thead>
<tr>
<th>Positive Serology, n=61</th>
<th>Negative Serology, n=69</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65.9±4.6</td>
</tr>
<tr>
<td>BMI</td>
<td>26.6±3.3</td>
</tr>
<tr>
<td>Previous myocardial infarction, n (%)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Previous stroke, n (%)</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Angina pectoris, n (%)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Intermittent claudication, n (%)</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Smoking, current, n (%)</td>
<td>23 (38)</td>
</tr>
<tr>
<td>Smoking, cigarette-years</td>
<td>375±41</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>6.8±0.9</td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.30±0.35</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.77±0.92</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>146±21</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81±11</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>63±10</td>
</tr>
<tr>
<td>Assignment to Intervention Group in RIS Study, n (%)</td>
<td>29 (48)</td>
</tr>
<tr>
<td>C pneumoniae IC titer ≥8, n (%)</td>
<td>30 (49)</td>
</tr>
<tr>
<td>CMV titer ≥15 AU/mL, n (%)</td>
<td>52 (85)</td>
</tr>
</tbody>
</table>

Values are mean±SD unless otherwise indicated. *P<0.001.

As shown in Table 6 and Figure 1, high titers of antibodies to C pneumoniae were associated with significantly increased risks for future stroke or the occurrence of any cardiovascular event (RRs of 8.58 and 2.69, respectively) after adjustment for previous cardiovascular disease, for smoking, and for group allocation in the underlying multiple risk factor intervention study. Similarly, a high titer of circulating immune complex was associated with a borderline significant RR (3.61) of future stroke (P=0.053; 95% CI, 0.98 to 13.27), whereas the corresponding risk for any cardiovascular event was 2.09 (P=0.10; 95% CI, 0.88 to 4.98).

A high titer of antibodies to CMV was not associated with future stroke or any cardiovascular event, and the relative risk of cardiovascular death was reduced (Table 7).

### Discussion

The results from this prospective study of treated hypertensive men indicate that high titers of circulating antibodies to C pneumoniae, but not CMV, are associated with increased risks for future stroke or the combined end point of cardiovascular events such as fatal or nonfatal stroke or myocardial infarction (time to first event). However, this is an observation based on serological analyses of frozen serum samples obtained within the frame of a previous multiple risk factor intervention study, and there are a number of methodological issues to address.

It may be difficult to obtain relevant information on the persistence of an infectious agent by use of serological tests.

Various combinations of antibody fractions and various cut-off titers to define C pneumoniae seropositivity have been used in previous studies. We used predefined cut-off limits based on results from several studies including studies that have related seropositivity to other evidence of active chronic C pneumoniae infection. Both IgA and IgG antibodies were used because they confer complementary information on the host–C pneumoniae interaction: some patients do not respond to C pneumoniae infection with

### TABLE 3. Comparison of Prevalence of Elevated IgA (High, ≥64; Low, <64) and IgG Antibody Titers (High, ≥512; Low, <512) to Chlamydia pneumoniae at Entry and Corresponding Comparison at the 3.5-Year Examination

<table>
<thead>
<tr>
<th>IgG Titer at Entry</th>
<th>IgA Titer at Entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>34 (67)</td>
</tr>
<tr>
<td>Low</td>
<td>17 (33)</td>
</tr>
<tr>
<td>Total</td>
<td>51 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IgG Titer After 3.5 Years</th>
<th>IgA Titer After 3.5 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>30 (63)</td>
</tr>
<tr>
<td>Low</td>
<td>18 (37)</td>
</tr>
<tr>
<td>Total</td>
<td>48 (100)</td>
</tr>
</tbody>
</table>

Values are n; values in parentheses are percentages.
specific antibodies of both the IgG and IgA classes.\textsuperscript{19–21} The reason for this is not known. The results demonstrated that both elevated IgA and IgG titers remained high after a median follow-up period of several years. By analyzing duplicate serum samples obtained with an interval of several years, we also had the opportunity to examine whether the suggested decision limits were associated with evidence of a persistent infection, as indicated by stable high titers. This was the case, because 97\% of the patients who had either or both of the IgA or IgG antibody titers elevated at entry had persistent high levels at the reexamination. In addition, the patients with high titers also had high levels of circulating immune complexes, containing \textit{C pneumoniae} protein antigen, indicating the presence of the bacterium. High titers of circulating immune complex were also associated with an RR of 3.61 (95\% CI, 0.98 to 13.27) for future stroke. Only 1 patient had raised IgM titers as evidence of recent infection. Thus, it seems reasonable to assume that patients with high titers of either or both IgA and IgG antibodies suffered from a chronic \textit{C pneumoniae} infection.

Our results may have been biased by uncontrolled effects of other risk factors. However, the patients with high and low antibody titers to \textit{C pneumoniae} did not differ in any obvious aspect, and the analysis of final outcome was adjusted for smoking status, previous cardiovascular disease, and group assignment in the underlying multiple risk intervention study. In terms of the association between seropositivity for \textit{C pneumoniae} and risk factors for cardiovascular disease, only smoking has shown a covariability.\textsuperscript{22–24} Varying results have been reported for hypertension and dyslipidemia.\textsuperscript{4,25–27}

Is it possible to generalize our results? The study population consisted of middle-aged to elderly men with treated hypertension and the presence of at least 1 additional risk factor for cardiovascular disease, resulting in a high morbidity and mortality risk.\textsuperscript{11} As previously described, the patients were originally recruited from a randomly selected population sample and may therefore be representative of elderly hypertensive men at high risk.\textsuperscript{10–13}

A chronic \textit{C pneumoniae} infection was found in more than half of the patients investigated, and infection was associated with a substantially increased risk of future cardiovascular disease, stroke in particular. The limited sample size makes the CIs very wide, but RR = 8.58 for stroke and RR = 2.69 for any cardiovascular event support the conclusion of a recent meta-analysis\textsuperscript{3} that \textit{C pneumoniae} may be a causative factor in arterial disease, although this is still unproved.

Previous studies have dealt mainly with coronary heart disease and have in most cases been based on cross-sectional designs.\textsuperscript{1} There are only a few prospective studies, but

\begin{table}
\centering
\caption{Comparison Between the Prevalence of Elevated IgG Antibody Titer After 3.5 Years and Corresponding Comparison of the Prevalence of Elevated IgA Titer (High, $\geq$64; Low, <64; Lower Panel)}
\begin{tabular}{l|ll}
\hline
& IgG Titer After 3.5 Years & IgA Titer at Entry \\
\hline
High & 27 (96) & 31 (97) \\
Low & 1 (4) & 1 (3) \\
Total & 28 (100) & 32 (100) \\
\hline
& IgG Titer After 3.5 Years & IgA Titer at Entry \\
\hline
High & 6 (21) & 7 (12) \\
Low & 57 (93) & 50 (88) \\
Total & 63 (100) & 57 (100) \\
\hline
\end{tabular}
\begin{tablenotes}
\item Values are n; values in parentheses are percentages.
\end{tablenotes}
\end{table}

\begin{table}
\centering
\caption{Event Rates During 6.5-Year Follow-Up in Patients With Elevated Antibodies Against \textit{Chlamydia pneumoniae} at Entry and/or at 3.5-Year Examination Versus Patients Without Elevated Antibody Titers}
\begin{tabular}{l|ll}
\hline
& Patients With High vs Low Titers of Antibodies to \textit{C pneumoniae} \\
\hline
Type of Event & n=84 & n=68 \\
\hline
\textbf{Fatal events} & & \\
Fatal acute myocardial infarction & 4 (5) & 4 (6) \\
Sudden death, n (%) & 4 (5) & 0 \\
Fatal stroke, n (%) & 2 (2) & 0 \\
Other fatal cardiovascular disease & 1 (1) & 2 (3) \\
Noncardiovascular cause of death & 3 (4) & 5 (7) \\
\hline
\textbf{Nonfatal events} & & \\
Nonfatal acute myocardial infarction & 18 (21) & 10 (15) \\
Nonfatal stroke & 11 (13) & 2 (3) \\
All first cardiovascular events & 29 (35) & 12 (18) \\
\hline
\end{tabular}
\begin{tablenotes}
\item Elevated antibody titers to \textit{C pneumoniae} was defined as IgA $\geq$64 and/or IgG $\geq$512.
\end{tablenotes}
\end{table}

\begin{table}
\centering
\caption{Relative Risks of High Titers of Antibodies to \textit{Chlamydia pneumoniae} for the Occurrence of Cardiovascular Events}
\begin{tabular}{l|llllll}
\hline
Variable & $\beta$ Coefficient & SD & $P$ & RR & 95\% CI (RR) \\
\hline
Cardiovascular death & 0.14 & 0.84 & $>0.30$ & 1.15 & 0.22–5.99 \\
Myocardial infarction & 0.42 & 0.59 & $>0.30$ & 1.52 & 0.48–4.78 \\
Stroke & 2.15 & 1.06 & 0.043 & 8.58 & 1.07–68.82 \\
Any cardiovascular event & 0.99 & 0.49 & 0.042 & 2.69 & 1.04–6.97 \\
\hline
\end{tabular}
\begin{tablenotes}
\item Adjustment has been made for smoking, presence of previous cardiovascular disease, and group allocation in the underlying multiple risk factor intervention study.
\end{tablenotes}
\end{table}
these have indicated an association between seropositivity to C pneumoniae and coronary heart disease. A few cross-sectional studies showing a relationship between carotid atherosclerosis, carotid stenosis, stroke, TIA, and seropositivity to C pneumoniae have been published. To our knowledge, there is no previous prospective study demonstrating that chronic C pneumoniae infection may be associated with an increased risk of stroke.

Although the incidence of coronary heart disease was higher than the incidence of stroke in the present study, seropositivity for C pneumoniae was not associated with a significantly increased risk for cardiovascular death (RR = 1.15; 95% CI, 0.22 to 5.99) or myocardial infarction (RR = 1.52; 95% CI, 0.48 to 4.78), whereas it emerged as a significant predictor for stroke or any cardiovascular event. However, the CIs for the relative risks of cardiovascular mortality and myocardial infarction found in this study seem to agree with the corresponding results reported in previous studies. Our observation that seropositivity for C pneumoniae may be a better risk marker for stroke than for coronary heart disease cannot be compared with previous experiences, because no similar studies have been performed.

Given the high frequency of positive C pneumoniae serology, the results from the present and previous studies indicate that a persistent C pneumoniae infection might be a common and very powerful risk factor for vascular disease, comparable in magnitude with the classic risk factors. The underlying pathophysiological mechanisms are largely unknown, but both effects on plaque growth and plaque rupture with subsequent thrombosis formation have been suggested. There are a number of observations giving support to these hypotheses. Thus, experimental C pneumoniae infection has been shown to induce vascular infection and contribute to the development of the atherosclerotic process. In 13 studies of cardiovascular tissues, local C pneumoniae infection was found in 52% of all atherosclerotic lesions but in only 5% of control samples of arterial tissue. C pneumoniae lipopolysaccharides have been found in circulating immune complexes observed in chronically infected patients. These are known for their deleterious effects on the blood coagulation system and on vascular endothelium. Persistent C pneumoniae infection is associated with increased fibrinogen levels, indicating inflammation and a procoagulant state. Finally, 2 preliminary intervention studies have indicated that treatment with antibiotics might improve prognosis for patients with coronary heart disease.

In contrast to C pneumoniae, high titers to CMV were not associated with any increase in morbidity or mortality. On the contrary, the RR for cardiovascular death had a 95% CI of 0.02 to 0.93. This is an intriguing finding, considering that several previous studies have indicated an association between CMV seropositivity and atherosclerosis. However, as summarized in the cited overview, it should be kept in mind that more than 1200 of the 1600 reviewed cases were defined on the basis of coronary restenosis after atherectomy, or the development of lesions in transplanted hearts or in arteries outside the coronary circulation. Thus, as summarized in this review, even if CMV does cause such lesions, the infection may not be relevant to native overt cardiovascular disease. A recently published study in approximately 900 patients did not find any association between CMV seropositivity and angiographically demonstrated coronary artery atherosclerosis. Against this background, it should be emphasized that the present study is the first to have examined prospectively the relationship between CMV titers and hard end points, such as cardiovascular mortality.

The question of whether our observation that CMV seropositivity was associated with a lower risk for future cardiovascular death was a random finding or may be explained by selection bias cannot be solved until other prospective studies on clinical events have been performed. The study was based on conventional epidemiological methods to select patients from population-representative samples of hypertensive men, and the studied subgroup was randomly selected.

The observation that 85% of the patients showed seropositivity to CMV is in accordance with the expected prevalence. From a principal viewpoint, it is difficult to elucidate the impact of a risk factor that is found in all or almost all patients.

The conclusions which can be derived from this study are that the methods used to analyze and define chronic C pneumoniae infection seemed to be valid, and that seropositivity according to this approach was associated with an increased risk of future cardiovascular disease, stroke in particular. Larger studies are urgently needed to clarify whether a chronic C pneumoniae infection is a risk factor for stroke.

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References
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