Impact of Infectious Burden on Extent and Long-Term Prognosis of Atherosclerosis

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Background—Recent findings suggest a causative role of infections in the pathogenesis of atherosclerosis. In hypothesizing an association between infectious agents and the development of atherosclerosis, we would expect a correlation to the extent of atherosclerosis. Moreover, this effect could be multiplied by the number of pathogens to which an individual had been exposed.

Methods and Results—In 572 patients, IgG or IgA antibodies to herpes simplex virus 1 and 2, cytomegalovirus, Epstein-Barr virus, Hemophilus influenzae, Chlamydia pneumoniae, Mycoplasma pneumoniae, and Helicobacter pylori were measured. The extent of atherosclerosis was determined by coronary angiography, carotid duplex sonography, and evaluation of the ankle-arm index. Elevated IgA antibodies against C pneumoniae (P<0.04) and IgG antibodies against H pylori (P<0.02), cytomegalovirus (P<0.05), and herpes simplex virus 2 (P<0.01) were associated with advanced atherosclerosis (≥2 vascular regions), adjusted for age, sex, cardiovascular risk factors, and highly sensitive C-reactive protein. Infectious burden divided into 0 to 3, 4 to 5, and 6 to 8 seropositivities was significantly associated with advanced atherosclerosis, with an odds ratio (95% CI) of 1.8 (1.2 to 2.6) for 4 to 5 (P<0.01) and 2.5 (1.2 to 5.1) for 6 to 8 seropositivities (P<0.02) (adjusted). After a mean follow-up of 3.2 years, cardiovascular mortality rate was 7.0% in patients with advanced atherosclerosis and seropositive for 0 to 3 pathogens compared with 20.0% in those seropositive for 6 to 8 pathogens.

Conclusions—Our results support the hypothesis that infectious agents are involved in the development of atherosclerosis. We showed a significant association between infectious burden and the extent of atherosclerosis. Moreover, the risk for future death was increased by the number of infectious pathogens, especially in patients with advanced atherosclerosis. 

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Key Words: infection ■ atherosclerosis ■ carotid arteries ■ coronary disease ■ peripheral vascular disease

Inflammation in the arterial vessel wall is considered to play an important role in the pathogenesis of atherosclerosis. Pathological studies have demonstrated the presence of inflammatory cells such as macrophages and T lymphocytes in every stage of the atherosclerotic process.1 Recent prospective studies have shown that several markers of systemic inflammation, such as C-reactive protein or fibrinogen, are associated with stable and unstable angina pectoris, peripheral arterial disease, and carotid artery stenosis.2,3

See p 2

An association of viral infection with atherosclerosis was first reported in the 1970s, when experimental infection of germ-free chickens with an avian herpesvirus was found to produce arterial disease.4 Currently there is also evidence that other Herpesviridae such as cytomegalovirus (CMV) and herpes simplex virus (HSV) may contribute to the pathogenesis of atherosclerosis.5,6

Associations of human atherosclerosis with bacteria such as Chlamydia pneumoniae and Helicobacter pylori also have been reported.7,8 It has been hypothesized that the number of different pathogens to which an individual has been exposed might promote a synergistic inflammatory response that could exacerbate atherosclerosis.9–11

Most investigators have focused on the association of different pathogens with advanced atherosclerosis in one vascular bed, such as coronary artery disease, carotid artery stenosis, or early carotid atherosclerosis. There is little information available about the influence of any bacterial or viral infection on the extent of atherosclerosis including more than one vascular area in the arterial vessel tree.12–15

It is known that patients with atherosclerosis in multiple vascular regions have a higher cardiovascular mortality rate than patients with limited disease,16 but until now there are no data available as to whether this risk could be influenced by...
an infection to various infectious pathogens. We therefore undertook the present study in 572 patients undergoing coronary angiography and additionally performed carotid duplex sonography and Doppler sonography in the peripheral arteries to address the following questions:

1. Is there a correlation between the extent of atherosclerosis and persistent infections with different bacterial or viral pathogens?

2. Is there a relation between the extent of atherosclerosis and the number of infectious pathogens to which an individual has been exposed?

3. Does a previous infection with multiple pathogens have any influence on adverse outcome in patients with extended atherosclerosis in various vascular regions?

Methods

Study Population

Between November 1996 and July 1998, a total of 1168 consecutive patients had been admitted to the 2nd Medical Department of the University Clinic Mainz, Germany, for diagnostic heart catheterization. Five hundred seventy-two patients had been randomly chosen as the subgroup, and additional examinations of the carotid and leg arteries were performed. The coronary syndrome was stable in 320 (56%) patients and unstable in 103 patients (18%); 68 patients (12%) had acute myocardial infarction and 81 patients (14%) had come to the hospital for treatment of other diseases (e.g., hypertensive heart disease). Cardiovascular risk factors such as family history of cardiovascular disease, smoking, diabetes mellitus, hypertension, and hyperlipidemia were defined according to a previous publication.11

A total of 570 of 572 patients (99.6%) were followed up during an mean of 3.2 years (minimum, 1.9; maximum, 4.1 years). Patients either presented at our clinic (87.2%) or were interviewed by telephone by trained medical staff. Follow-up information was obtained about death from cardiovascular causes (n=15, 2.6%) and/or leg artery stenosis were defined as having advanced atherosclerosis.18

Patients were classified into three groups according to the extent of atherosclerosis: In 61 patients, examinations of coronary, carotid, and leg arteries were normal (control, 11%). In 265 patients, at least one coronary artery stenosis was detected; however, carotid duplex sonography and Doppler examination of the leg arteries was normal (limited disease, 46%). Patients with CAD and additional carotid and/or leg artery stenosis were defined as having advanced atherosclerosis (n=246, 43%).

Labroratory Methods

Blood samples were drawn from each subject after an overnight fasting period. Serum was centrifuged at 4000g for 10 minutes, immediately divided into aliquots, and frozen at −80°C until analysis. Each study subject’s serum was tested for specific IgG class antibodies against CMV, Epstein-Barr virus (EBV), HSV-1, HSV-2, C pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, and H pylori as well as IgA class antibodies against C pneumoniae, M pneumoniae, H pylori, and EBV through the use of quantitative in vitro ELISAs or indirect immunofluorescence (C pneumoniae and H influenzae) (EUROIMMUN). In the IgG ELISA a value of ≥20 relative units per mL and in the IgA ELISA a ratio of >1 was considered positive according to the manufacturer’s instructions. Concerning indirect immunofluorescence, the starting dilution was 1:100 and specific fluorescence patterns at or above these dilutions were considered positive. The anti-chlamydia antibody test is based on broad-reactive chlamyidal inclusions. C-reactive protein (CRP) was determined by a highly sensitive, latex particle–enhanced immunoassay (detection range, 0 to 20 mg/dL, Roche Diagnostics).

Statistical Analysis

Differences between groups were tested by χ² test for categorical variables and by Kruskal-Wallis test for continuous variables. Logistic regression analysis was performed, including the number of seropositivitites in categories (0 to 3, 4 to 5, and 6 to 8) or titers of each pathogen as continuous variables for the end point advanced atherosclerosis. Confidence intervals at the 95% level were calculated for the odds ratios. Odds ratios were described for the increase of 1 SD for pathogens evaluated by ELISA and for seropositivity for pathogens evaluated by indirect immunofluorescence assay. Survival was assessed by Cox regression analysis and risk was described by hazard ratios (HRs) and the corresponding 95% CI. Logistic regression analysis and Cox regression analysis were performed adjusted for age, sex, cardiovascular risk factors (smoking, hyperlipidemia, arterial hypertension, diabetes mellitus, and family history of cardiovascular disease), and CRP in a multivariate model. A value of P≤0.05 was considered locally significant. Computations were carried out with SPSS version 10.0.

Results

Associations Between Each Pathogen and the Extent of Atherosclerosis

Baseline characteristics according to the extent of atherosclerosis are shown in Table 1. Table 2 demonstrates the correlation of baseline pathogen IgG and IgA antibodies with advanced atherosclerosis in the study population. After adjustment for age, sex, risk factors, and CRP, only IgA seropositivity to C pneumoniae (P<0.04) and elevated IgG antibodies to H pylori (P<0.02), CMV, and HSV-2 (P<0.01) revealed an independent association with advanced atherosclerosis.

Infectious Burden and Extent of Atherosclerosis

For analysis of the association between atherosclerosis and the aggregate number of antipathogen antibodies, we used IgG seropositivities to HSV-1 and HSV-2, CMV, and...
H influenzae as well as IgA seropositivities to H pylori, M pneumoniae, C pneumoniae, and EBV. Because of the limited number of subjects with very low and very high pathogen burden, we stratified patients into groups with 0 to 3, 4 to 5, and 6 to 8 seropositivities. Patients were divided into those with high (>0.5 mg/dL, n=273, 48%) or low (≤0.5 mg/dL, n=299, 52%) CRP level.

An increasing pathogen burden was significantly associated with the extent of atherosclerosis (Figure 1) in patients with high and low CRP levels. We found an association between the extent of atherosclerosis and increasing numbers of infectious pathogens to which an individual has been exposed, with an OR (95% CI) of 1.8 (1.2 to 2.6) for patients seropositive for 4 to 5 pathogens (P=0.002) and 2.5 (1.2 to 5.1) for patients seropositive for 6 to 8 pathogens (P<0.02) compared with patients seropositive for up to 3 pathogens (adjusted) (Table 3). In contrast, analyses performed for viral burden did not reveal an association with the extent of atherosclerosis (Figure 3 and Table 3).

**Infectious Burden and Cardiovascular Death**

Cardiovascular death was associated with the extent of atherosclerosis (Figure 4). There were no deaths in the control group, compared with a mortality rate of 3.5% in patients with limited and 13.9% in patients with advanced disease.
The HR (95% CI) for cardiovascular death was 3.0 (1.4 to 6.3) for patients with advanced disease compared with those with limited disease (adjusted) \((P<0.005\), Table 4). We also found a correlation between cardiovascular death and the number of seropositivities. Mortality rate was 3.1% in patients seropositive for up to 3 pathogens, 9.8% in patients seropositive for 4 to 5 pathogens, and 15.0% for those seropositive for 6 to 8 pathogens \((P<0.001\). The HR for future cardiovascular death was 2.5 (1.2 to 5.4) in patients seropositive for 4 to 5 pathogens \((P<0.02\) and 2.9 (1.2 to 9.6) for patients seropositive for 6 to 8 pathogens \((P<0.02\) compared with those seropositive for up to 3 pathogens (adjusted).

Further analyses were performed to evaluate mortality rates for each group of atherosclerosis according to the number of seropositivities. In the group of patients with limited disease, cardiovascular mortality rate was 1.4% in patients seropositive for 0 to 3 pathogens compared with 7.7% in patients seropositive for 6 to 8 pathogens. For patients with advanced disease, mortality rate was 7.0% in patients seropositive for 0 to 3 pathogens compared with 20.0% in those seropositive for 6 to 8 pathogens. We found an HR (95% CI) of 10.8 (2.0 to 58.5) for patients with advanced atherosclerosis and highest infectious burden (6 to 8 pathogens) compared with those with limited disease and lowest infectious burden (0 to 3 pathogens, adjusted, \(P=0.005\)).

**Discussion**

Inflammatory mechanisms have been implicated in the pathogenesis of atherosclerosis, and previous investigators have reported an association between elevated inflammatory markers and the development of atherosclerosis.\(^2,3\) Many studies

### Table 3. Logistic Regression Analysis for the End Point Advanced Atherosclerosis According to Numbers of Seropositivities Unadjusted and Adjusted for Age, Sex, Cardiovascular Risk Factors, and CRP

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Unadjusted Odds Ratio (95% CI)</th>
<th>(P)</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total infectious burden</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 Seroposivities(^*)</td>
<td>286</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>4–5 Seroposivities</td>
<td>246</td>
<td>1.80 (1.27–2.55)</td>
<td>0.001</td>
<td>1.78 (1.23–2.59)</td>
<td>0.002</td>
</tr>
<tr>
<td>6–8 Seroposivities</td>
<td>40</td>
<td>3.10 (1.56–6.14)</td>
<td>0.001</td>
<td>2.45 (1.18–5.10)</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Bacterial pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1 Seroposivities(^*)</td>
<td>147</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2 Seroposivities</td>
<td>308</td>
<td>1.67 (1.25–2.24)</td>
<td>0.0006</td>
<td>1.60 (1.06–2.40)</td>
<td>0.020</td>
</tr>
<tr>
<td>3–4 Seroposivities</td>
<td>117</td>
<td>1.56 (1.25–1.95)</td>
<td>0.0001</td>
<td>2.12 (1.32–3.41)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Herpesviridae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1 Seroposivities(^*)</td>
<td>267</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2 Seroposivities</td>
<td>189</td>
<td>1.03 (0.69–1.53)</td>
<td>0.89</td>
<td>1.01 (0.66–1.55)</td>
<td>0.96</td>
</tr>
<tr>
<td>3–4 Seroposivities</td>
<td>116</td>
<td>1.05 (0.82–1.34)</td>
<td>0.71</td>
<td>0.99 (0.58–1.69)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

\(^*\)Patients in this group were used as reference group.
have shown associations between atherosclerosis and different pathogens, and early investigations showed the presence of infectious pathogens in the whole arterial vessel tree.\textsuperscript{1,5–8} In the present study, we selected eight pathogens because of two main characteristics: They are obligate intracellular pathogens except for \textit{H influenzae} and \textit{M pneumoniae}, and they all develop persistent antibodies targeted to the pathogen. Furthermore, six pathogens produce a life-long latent (Herpetoviridae) or persistent infection, whereas \textit{H influenzae} and \textit{M pneumoniae} are not known to provoke a persistent infection although they establish life-long persistence of antibodies. In all pathogens we determined anti-IgG antibodies; in some pathogens IgA antibodies also were measured because of the nearly 100% prevalence of IgG (EBV) and because of reports suggesting that IgA antibodies might reflect more recent and/or repeated infectious episodes (\textit{C pneumoniae}, \textit{H pylori}, \textit{M pneumoniae}).\textsuperscript{7} 

Although there is controversy regarding the role of each infectious pathogen in different vascular regions, according to the literature we found associations between elevated antibodies against 5 of the 8 evaluated pathogens and the extent of atherosclerosis. The antibody titters for \textit{C pneumoniae}, \textit{H pylori}, \textit{H influenzae}, CMV, and HSV-2 were related to the extent of atherosclerosis. After adjustment for age, sex, classic cardiovascular risk factors, and CRP, the relation persisted for all except \textit{H influenzae}.

According to the hypothesis from Zhu et al\textsuperscript{9} and Epstein et al,\textsuperscript{10} it seems unlikely that one specific pathogen causes atherosclerosis. This is supported by our findings that show a significant relation between the number of infectious pathogens to which an individual has been exposed and the extent of atherosclerosis. It is known that some pathogens, such as \textit{C pneumoniae}, can induce macrophage foam cell formation and

![Figure 2](image_url)

**Figure 2.** Number of bacterial pathogens to which an individual had been exposed with respect to extent of atherosclerosis and CRP levels.

![Figure 3](image_url)

**Figure 3.** Number of viral pathogens to which an individual had been exposed with respect to extent of atherosclerosis and CRP levels.
that this effect might be increased if an individual has been infected by multiple pathogens. It has been hypothesized previously that the infectious pathogen contains proteins homologous to parts of the host proteins, resulting in an immune response called infection-induced molecular mimicry. It is possible that such an effect could be multiplied if multiple pathogens are involved in the atherosclerotic process.

The association between pathogen burden and extent of atherosclerosis was mainly driven by seropositivities to bacterial infections, and we could not find an influence of seropositivities to Herpesviridae. However, in a previous publi-

### Table 4. Cox Regression Analysis for Future Death From Cardiovascular Causes According to Numbers of Seropositivities and Extent of Atherosclerosis Unadjusted and Adjusted for Age, Sex, Cardiovascular Risk Factors, and CRP

<table>
<thead>
<tr>
<th>No. of Seropositivities</th>
<th>Extent of Disease</th>
<th>Unadjusted Hazard Ratio (95% CI)</th>
<th>P</th>
<th>Adjusted Hazard Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>No events</td>
<td></td>
<td>No events</td>
<td></td>
</tr>
<tr>
<td>Limited disease</td>
<td></td>
<td>263</td>
<td>1.0</td>
<td>3.87 (1.84–8.13)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Advanced disease</td>
<td></td>
<td>246</td>
<td>1.0</td>
<td>2.96 (1.39–6.30)</td>
<td>0.005</td>
</tr>
<tr>
<td>0–3 Seropositivities</td>
<td></td>
<td>285</td>
<td>1.0</td>
<td>2.87 (1.37–5.99)</td>
<td>0.005</td>
</tr>
<tr>
<td>4–5 Seropositivities</td>
<td></td>
<td>245</td>
<td>1.0</td>
<td>2.54 (1.19–5.40)</td>
<td>0.015</td>
</tr>
<tr>
<td>6–8 Seropositivities</td>
<td></td>
<td>40</td>
<td>1.0</td>
<td>2.87 (1.21–9.65)</td>
<td>0.020</td>
</tr>
<tr>
<td>Combination of (1) and (2)</td>
<td></td>
<td>148</td>
<td>1.0</td>
<td>4.48 (0.90–22.19)</td>
<td>0.08</td>
</tr>
<tr>
<td>Limited disease and</td>
<td></td>
<td>102</td>
<td>1.0</td>
<td>4.19 (0.84–21.00)</td>
<td>0.08</td>
</tr>
<tr>
<td>Advanced disease</td>
<td></td>
<td>13</td>
<td>1.0</td>
<td>3.03 (0.26–35.14)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*Patients in this group were used as reference group.
cation, we showed a higher influence of Herpesviridae on adverse outcome compared with bacterial pathogens in patients with CAD. Over that, we found a stronger association between bacterial burden and extent of disease in patients with high CRP level, possibly indicating higher inflammation processes at the arterial wall. It might be possible that bacterial pathogens are involved in the development and long-term progression of atherosclerosis, but Herpesviridae are responsible for plaque instability, acceleration of atherosclerosis, and development of cardiovascular events.

Zhu et al and Epstein et al reported a positive association between infectious burden and the prevalence of CAD and cardiovascular events. Results from Rupprecht et al support this hypothesis, showing an increased cardiovascular mortality rate in patients with documented CAD and high infectious burden. Our results showed an increased cardiovascular mortality rate according to the extent of atherosclerosis, and our results implicate an additional increase of mortality rate according to the number of infections to which a patient has been exposed.

Our data consistently suggest that an increasing number of seropositivities to infectious pathogens is associated with the extent of atherosclerosis and with cardiovascular death. However, several prospective studies revealed disparate results that may derive from several causes. In contrast to our study, each of these studies was restricted to a homogeneous population without proven CAD or even more, to a highly selected population. Although such highly selected populations offer several advantages (e.g., elimination of various confounders), these populations may not represent other populations (e.g., those with high incidence of risk factors). Furthermore, most of these studies determined only IgG antibodies and did not take into account possible interactions with markers of inflammation or immune response.

Limitations
Several limitations of our study should be considered. Antibodies are weak predictors in prospective studies on advanced atherosclerosis and cardiovascular events, but in cross-sectional studies, elevated antibody titers can reflect the activation of a chronic infection or a reinfection. Because this is a cross-sectional study, we cannot be sure that infection precedes the development of atherosclerosis. Although this is a cross-sectional study concerning the association between the extent of atherosclerosis and infectious burden, we included a prospective approach with regard to clinical events. The epidemiology of atherosclerosis is different in coronary, carotid, and leg arteries; furthermore, the abdominal aorta, which is also an important vascular area, has not been evaluated. We evaluated a patient cohort with a high prevalence of CAD and a mean age of 63 years because we included only patients scheduled for coronary angiography, which is the gold standard for evaluating CAD.

Conclusions
We demonstrated that increasing numbers of infectious pathogens were significantly related to the extent of atherosclerosis and to adverse long-term outcome. Our results are compatible with the concept that infections are involved in the development of atherosclerosis and that infections with multiple pathogens may augment the risk conveyed by one pathogen.

References
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