Impact of Infectious Burden on Progression of Carotid Atherosclerosis

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Background and Purpose—Recent findings suggest a causative role of infections in the pathogenesis of atherosclerosis. The extent of atherosclerosis and the prognosis of patients with atherosclerosis seem to be increased by the number of infections to which an individual has been exposed. In a prospective study, we evaluated the effect of 8 pathogens and the aggregate pathogen burden on the progression of carotid atherosclerosis.

Methods—In 504 patients (74.9% men; age, 62.9±10 years), we measured intima-media thickness and prevalence of carotid artery stenosis. Follow-up measurements after a mean of 2.5 years were available in 427 patients (85%). Blood samples were taken, and IgG or IgA antibodies to Chlamydia pneumoniae, Helicobacter pylori, Haemophilus influenzae, Mycoplasma pneumoniae, cytomegalovirus, Epstein-Barr virus, and herpes simplex virus types 1 and 2 were measured. Statistical evaluation was performed with logistic regression procedures.

Results—Elevated IgA antibodies against C pneumoniae (P<0.04) and IgG antibodies against Epstein-Barr virus (P<0.01) and herpes simplex virus type 2 (P<0.04) were associated with progression of atherosclerosis (increase of intima-media thickness ≥0.1 mm/y or progression of carotid stenosis) after adjustment for age, sex, cardiovascular risk factors, highly sensitive C-reactive protein, and statin intake. Infectious burden, divided into 0 to 3, 4 to 5, and 6 to 8 seropositivities, was significantly associated with progression of atherosclerosis, with odds ratios of 1.8 (95% confidence interval, 1.1 to 2.9) for 4 to 5 and 3.8 (95% CI, 1.6 to 8.8) for 6 to 8 compared with 0 to 3 seropositivities after adjustment.

Conclusions—Our results support the hypothesis that the number of infectious pathogens to which an individual has been exposed independently contributes to the progression of carotid atherosclerosis. (Stroke. 2002;33:2581-2586.)

Key Words: atherosclerosis • carotid arteries • infections

Injury to the arterial vessel wall and the inflammatory processes associated with this are considered to play an important role in the pathogenesis of atherosclerosis.1–4 Evidence is accumulating that certain infectious agents are candidate triggers of these inflammatory responses.5–6 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.7 Several retrospective and cross-sectional studies have shown an association between previous infections with Chlamydia pneumoniae, herpes simplex virus (HSV), cytomegalovirus (CMV), Helicobacter pylori, and hepatitis A or respiratory tract infection and the presence of atherosclerosis in the coronary, carotid, or peripheral vessels, but others did not.5–10

If infections are causally related to atherosclerosis, it would be unlikely that a single agent plays a unique role. It seems to be more likely that the risk of developing atherosclerosis is related to the number of pathogens to which an individual has been exposed. This concept of pathogen burden was recently introduced by Epstein et al.11 The same group demonstrated that the number of infectious pathogens to which an individual has been exposed is related to the presence of coronary artery disease and the risk for future cardiac events.11–13

In a large prospective data set (the AtheroGene Study), we evaluated the impact of various available and forthcoming biochemical markers on the prognosis of patients with documented atherosclerosis. We previously demonstrated associations of early and advanced carotid atherosclerosis with C pneumoniae, CMV, and HSV type 2 (HSV-2) infection, but associations with each of these pathogens were of borderline significance only, which seems to confirm the concept that multiple pathogens may contribute to atherosclerosis.14 Furthermore, we were able to show that the number of infectious pathogens was related to the extent of atherosclerosis in different vascular regions and to future cardiovascular death.15,16 If infectious burden contributes to the development of atherosclerosis, we would also expect a direct association.
between the echoes arising from the blood-intima interface and the media-adventitia interface was taken as the measure of IMT. If a plaque was localized at the side of IMT measurement, plaque thickness was included in the IMT value in accordance with previous reports.19 When an optimal image was obtained, it was frozen and the actual IMT was measured; then the image was erased. This procedure was repeated 8 times for longitudinal and cross-sectional images for each left and right carotid artery. The multiple longitudinal and cross-sectional measurements of both common carotid arteries were summarized, and the mean carotid IMT was calculated for each individual. The difference between the mean IMT at the inclusion and that at the follow-up measurement was evaluated, and the annual decrease or increase in IMT (millimeters per year) was calculated for each patient.

The reader estimated the percent diameter stenosis for each internal and external carotid artery using both color duplex imaging and Doppler peak systolic flow velocities. In accordance with previous reports, a peak systolic velocity of \( >1.4 \text{ m/s} \) and a ratio of peak velocity of the common carotid artery to peak velocity of the internal carotid artery of \( \geq 2 \) were assumed to indicate \( \geq 60\% \) diameter lumen stenosis.14,20 The development of a new \( \geq 60\% \) stenosis or an increase in a previous stenosis (increase in peak velocity ratio of at least 0.5) was defined as progression of stenosis (new stenosis, \( n = 32 \); increase in previous stenosis, \( n = 26 \); total progression of stenosis, \( n = 58 \); 13.5\%). Follow-up IMT and progression of stenosis were assessed in all 427 patients with completed follow-up. Patients were divided into quartiles with regard to changes in carotid atherosclerosis: first quartile (\( n = 104 \), 24.3\%), regression of IMT or no change; second quartile (\( n = 104 \), 24.3\%), progression of IMT \( >0 \) to 0.04 mm/y; third quartile (\( n = 103 \), 24.1\%), progression of IMT \( \geq 0.04 \) to 0.10 mm/y; and fourth quartile (\( n = 116 \), 27.1\%), progression of IMT \( \geq 0.10 \) mm/y or progress of stenosis. Patients with progression of IMT of at least 0.1 mm/y or progression of a stenosis were defined as suffering from progression of atherosclerosis (fourth quartile).

Statistical Analysis
Serological assays were performed by individuals blinded to clinical details and the results of ultrasound examinations. For IMT measurements, the intraclass correlation coefficient for interobserver variability (\( n = 40 \); 2 investigators) was 0.994 (95\% CI, 0.991 to 0.998) and for intraobserver variability (\( n = 40 \)) was 0.995 (95\% CI, 0.991 to 0.998). Differences between the 2 groups were tested by the \( t \)-test for categorical variables and by the Kruskal-Wallis test for continuous variables. Logistic regression analysis was performed, including the number of seropositivities in the categories of 0 to 3, 4 to 5, and 6 to 8 or titers of each pathogen as continuous variables for the end-point progression of atherosclerosis. CIs at the 95\% level were calculated for the odds ratios (ORs). ORs were described for the increase of 1 SD for pathogens evaluated by ELISA and for seropositivity for pathogens evaluated by indirect immunofluorescence assay. Logistic regression analysis was performed after adjustment for age, sex, risk factors (smoking, hyperlipidemia, arterial hypertension, diabetes mellitus, and family history of cardiovascular diseases), use of statin medication, and CRP (continuous) in a multivariate model. A value of \( P \leq 0.05 \) was considered locally significant. Computations were carried out with SPSS version 11.0.

Results
Baseline Characteristic
Table 1 demonstrates patient characteristics, comparing patients with and without progression of atherosclerosis. The mean age of the entire study population was 63±9 years, and 75\% of the patients were male. Patients with progression of carotid atherosclerosis were similar in age, sex, and most cardiovascular risk factors compared with those without progression. CRP tended to be higher in the group of patients with progression of atherosclerosis, but the
difference was not significant. No relevant difference in statin intake was noted between the 2 patient groups.

Pathogens and Progression of Atherosclerosis

Table 2 demonstrates the correlation between baseline pathogen IgG and IgA antibodies and the progression of atherosclerosis in the study population. ORs for all except *M. pneumoniae* IgG point in the same direction. After adjustment for age, sex, cardiovascular risk factors, CRP, and use of statin medication, IgA seropositivity to *C. pneumoniae* (*P*=0.04) and to EBV (*P*=0.01) and elevated IgG antibodies to HSV-2 (*P*=0.04) were independent predictors of atherosclerosis progression. We also found an association between elevated IgA antibodies to *M. pneumoniae* and progression of carotid atherosclerosis in the univariate analysis, but this association did not persist after adjustment.

**Infectious Burden and Progression of IMT**

For analysis of the association between atherosclerosis and the aggregate number of anti-pathogen antibodies, we used IgG seropositivities to HSV-1 and -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 or very high pathogen burden, we stratified patients into groups with 0 to 3 (n=211), 4 to 5 (n=188), and 6 to 8 (n=28) seropositivities. Table 3 demonstrates the association between infectious burden and early carotid atherosclerosis. At the time of inclusion, carotid IMT was significantly higher in patients with a high pathogen burden than in those with a low pathogen burden (*P*=0.046). After the 2.5-year follow-up period, this difference between the 3 infectious burden groups had increased. Progression of IMT was 0.03 mm/y in patients seropositive to 0 to 3 pathogens compared...
Infectious Burden and Progression of Atherosclerosis

In total, 116 patients (27%) showed progression of carotid atherosclerosis. Twenty percent of the patients with the lowest pathogen burden demonstrated progression of carotid atherosclerosis compared with 31% of those seropositive to 4 to 5 pathogens and 50% of those with the highest pathogen burden (P=0.0001; Figure 1). We found an association between increasing numbers of infectious pathogens to which an individual has been exposed and the progression of atherosclerosis, with ORs of 1.8 (95% CI, 1.1 to 2.9) for patients seropositive for 4 to 5 pathogens (P=0.01) and 3.8 (95% CI, 1.6 to 8.8) for patients seropositive for 6 to 8 pathogens (P=0.002) compared with patients seropositive for 0 to 3 pathogens after adjustment (Table 4).

Association Between CRP Level, Infectious Burden, and Progression of Atherosclerosis

To evaluate a possible interaction between pathogen burden, CRP, and progression of carotid atherosclerosis, we repeated the analyses with respect to elevated (>0.5 mg/dL; n=183; 43%) or normal (≤0.5 mg/dL; n=244; 57%) CRP levels. Figure 2 demonstrates the association of total pathogen burden with progression of atherosclerosis, which was independently signif-

TABLE 3. Carotid IMT and Pathogen Burden

<table>
<thead>
<tr>
<th>Seropositivities</th>
<th>0–3 Pathogens (n=211)</th>
<th>4–5 Pathogens (n=188)</th>
<th>6–8 Pathogens (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
</tr>
<tr>
<td>IMT at inclusion, mm</td>
<td>0.70 0.50–1.00</td>
<td>0.72 0.50–1.11</td>
<td>0.78 0.51–1.22</td>
</tr>
<tr>
<td>IMT at follow-up, mm</td>
<td>0.77 0.51–1.18</td>
<td>0.83 0.51–1.35</td>
<td>0.91 0.67–1.45</td>
</tr>
<tr>
<td>Change in IMT, mm</td>
<td>0.07 0.18–0.34</td>
<td>0.11 0.12–0.45</td>
<td>0.17 0.02–0.42</td>
</tr>
<tr>
<td>Annual change in IMT, mm/y</td>
<td>0.03 0.08–0.14</td>
<td>0.04 0.05–0.19</td>
<td>0.07 0.01–0.19</td>
</tr>
<tr>
<td>Change in IMT, %</td>
<td>11.8 22.2–46.2</td>
<td>15.6 18.8–75.9</td>
<td>24.8 6.9–66.9</td>
</tr>
<tr>
<td>Annual change in IMT, %/y</td>
<td>4.9 8.8–21.1</td>
<td>6.4 8.3–29.4</td>
<td>9.9 3.3–25.1</td>
</tr>
</tbody>
</table>

with 0.04 mm/y in patients seropositive to 4 to 5 pathogens and 0.07 mm/y in those seropositive to 6 to 8 pathogens (P=0.004).

Patients with a low pathogen burden (0 to 3 seropositivities) showed a 12% increase in carotid IMT compared with 25% in those with a high pathogen burden (6 to 8 seropositivities). Table 4 shows the results of logistic regression analysis. For the end-point progression of IMT of ≥0.1 mm/y, the OR was 2.9 (95% CI, 1.1 to 7.3) for patients with a high pathogen burden (6 to 8 seropositivities) compared with patients with a low pathogen burden (0 to 3 seropositivities), adjusted for age, sex, cardiac risk factors, CRP, and use of statin medication.

Infectious Burden and Progression of Stenosis

Fifty-eight patients (14%) showed progression of stenosis. Ten percent of the patients seropositive to 0 to 3 pathogens demonstrated progression of stenosis compared with 17% of the patients seropositive to 4 to 5 pathogens and 25% of the patients seropositive to 6 to 8 pathogens (P=0.006; Figure 1). As demonstrated in Table 4, the risk of stenosis progression increased with increasing numbers of seropositivities. In the adjusted model, patients seropositive to 6 to 8 pathogens revealed a 3.0 (95% CI, 1.1 to 8.3) increased risk for stenosis progression compared with patients seropositive to 0 to 3 pathogens (P=0.03).

TABLE 4. Logistic Regression Analysis for the Different End Points According to Infectious Burden Unadjusted and Adjusted for Age, Sex, Cardiac Risk Factors, Statin Intake, and Highly Sensitive CRP

<table>
<thead>
<tr>
<th>Seropositivities</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Progression of IMT (≥0.1 mm/y))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3*</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>4–5</td>
<td>1.19 (0.68–2.07)</td>
<td>0.53</td>
</tr>
<tr>
<td>6–8</td>
<td>2.97 (1.23–7.20)</td>
<td>0.015</td>
</tr>
<tr>
<td>Progression of stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3*</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>4–5</td>
<td>1.88 (1.03–3.44)</td>
<td>0.04</td>
</tr>
<tr>
<td>6–8</td>
<td>3.18 (1.20–8.41)</td>
<td>0.02</td>
</tr>
<tr>
<td>Progression of atherosclerosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3*</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>4–5</td>
<td>1.79 (1.13–2.81)</td>
<td>0.01</td>
</tr>
<tr>
<td>6–8</td>
<td>3.91 (1.73–8.81)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Patients in this group were used as the reference group.
icant in both subgroups, regardless of CRP level. We found stronger associations among patients with lower CRP values, although a significant trend was present in both CRP strata levels. These results provide further evidence suggesting that pathogen burden significantly predicts the end-point progression of atherosclerosis.

Discussion

There is consistent evidence that inflammation plays a crucial role in the pathogenesis of atherosclerosis. It is hypothesized that infectious agents exert their effects by inducing a local or systemic inflammatory response and/or by infection-induced autoimmune response involving molecular mimicry.

In the present study, we selected a panel of 8 pathogens because they shared 2 main characteristics: they were obligate intracellular pathogens (except H influenzae and M pneumoniae), and all established persistent antibodies targeted to the pathogen. Furthermore, 6 pathogens produce a lifelong latent infection (Herpesviridae) or persistent infection, whereas H influenzae and M pneumoniae are not known to provoke a persistent infection, although they establish lifelong persistence of antibodies. The results of this study confirm and extend previous findings. Of 8 infectious pathogens, 3 were identified as being independently predictive of the progression of carotid atherosclerosis. After a 2.5-year follow-up, the progression of atherosclerosis was associated with elevated antibodies against C pneumoniae, HSV-2, and EBV. Sander et al. also showed an enhanced progression of early carotid atherosclerosis in patients with previous C pneumoniae infection, but other investigators could not find any correlation between C pneumoniae and atherosclerosis. Results for other pathogens such as Herpesviridae or H pylori are contradictory, also.

According to the hypothesis of Epstein et al. and Zhu et al., it seems unlikely that 1 specific pathogen causes atherosclerosis. It has been hypothesized that infectious pathogens have direct effects at the vessel wall by inducing macrophage foam cell formation and indirect effects by inducing an immune response, called infection-induced molecular mimicry. It is possible that such an effect is multiplied if multiple pathogens are involved in the atherosclerotic process. Epstein et al. and Zhu et al. reported a positive association between infectious burden and the prevalence of coronary artery disease and cardiovascular events. Results from Rupprecht et al. support this hypothesis, showing increased cardiovascular mortality in patients with documented coronary artery disease and high infectious burden. We also showed increased cardiovascular mortality according to the extent of atherosclerosis and the number of infections to which a patient has been exposed.

This hypothesis is further supported by the results of this study showing a significant relationship between the number of infectious pathogens to which an individual has been exposed and progression of carotid atherosclerosis. We found the same independent association with regard to progression of both early and advanced carotid atherosclerosis. Similar results have been reported by Kiechl et al., who showed an association between chronic infections of the respiratory dental systems and urinary tract and changes in carotid atherosclerosis.

Previous investigations showed an association between the number of seropositivities and increased CRP levels. An elevated CRP level, believed to reflect underlying inflammation, is predictive of cardiovascular events. However, in the stratified analysis between pathogen burden and progression of atherosclerosis the basis of low or high CRP value, we found stronger associations among those with lower values, although results were significant in both groups. Moreover, the significant association between infectious burden and progression of atherosclerosis persisted after adjustment for highly sensitive CRP. These results provide further evidence that the pathogen burden significantly predicts progression of atherosclerosis independently of an underlying inflammatory process.
Study Limitations

The presence of periodontal disease and antibodies against hepatitis viridae has not been evaluated, although several investigators have found associations between these infections and atherosclerosis.12,25,26–29 It might be possible that there are interactions between some pathogens; other pathogens like HSV-1 and -2 might generate cross-reactive antibodies. Socioeconomic status is a potential confounding factor that has not been evaluated that represents an important limitation of this study. Previous investigators showed an increase in carotid atherosclerosis in patients with low socioeconomic status.30 Others suggested an association between chronic infections, low social status, and increase in early carotid atherosclerosis.25 It is possible that individuals with greater infectious burden may seem to be at increased vascular risk only because they have less access to care or lower socioeconomic status.31

Conclusions

In conclusion, our investigation provides further evidence that infection plays an important role in the pathogenesis of atherosclerosis. Previous investigators showed an association between increased pathogen burden and cardiovascular events, and in a recent investigation, we found a significant association between pathogen burden and the extent of atherosclerosis in different vascular regions. In this trial, we could show an independent association between increasing infectious burden and progression of carotid atherosclerosis. Our results are compatible with the concept that infections are involved in the development and progression of atherosclerosis and that persistent infections with multiple pathogens may augment the risk conveyed by 1 pathogen.

Appendix

The AtheroGene Group

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Stroke. 2002;33:2581-2586
doi: 10.1161/01.STR.0000034789.82859.A4
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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