C-Reactive Protein, Carotid Atherosclerosis, and Cerebral Small-Vessel Disease
Results of the Austrian Stroke Prevention Study

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Background and Purpose—C-reactive protein (CRP) is an inflammatory marker known to be a risk factor for stroke. We examined the associations between CRP, carotid atherosclerosis, white matter lesions, and lacunes as manifestations of cerebral large- and small-vessel disease.

Methods—In the community-based Austrian Stroke Prevention Study, CRP concentrations were measured by a highly sensitive assay in 700 participants at baseline. All underwent carotid duplex scanning, and a subset of 505 subjects underwent brain magnetic resonance imaging. Imaging was repeated after 3 and 6 years. We graded carotid atherosclerosis in both common and internal carotid arteries on a 5-point scale and calculated the sum of scores as an index of the severity of carotid atherosclerosis. The volume of white matter lesions and the number of lacunes were considered small vessel disease–related brain abnormalities.

Results—After adjustment for vascular risk factors, the severity and progression of extracranial carotid atherosclerosis increased with increasing quintiles of CRP. Only study participants in the fourth and fifth quintile (≥2.50 mg/L) had significantly more baseline atherosclerosis and greater progression when we used the first quintile (<0.80 mg/L) as a reference. No interactions were seen between CRP quintiles and vascular risk factors for carotid atherosclerosis. The associations between severity and progression of small vessel disease–related brain abnormalities and CRP were nonsignificant.

Conclusions—We found evidence for differential effects of CRP in different beds of the arterial brain supply. CRP was a marker for active carotid atherosclerosis but not for small vessel disease–related brain abnormalities.

Key Words: carotid atherosclerosis ■ cerebral small-vessel disease ■ lacunes ■ risk factors ■ white matter lesions

C-reactive protein (CRP), a nonspecific marker of inflammation, is known to be a risk factor for coronary heart disease1 and peripheral vascular disease.2 The role for CRP measured by high-sensitivity assays as a risk factor for cerebrovascular disease is less well established. Some studies reported elevated CRP to increase the risk for ischemic strokes,3-5 an association that was found to be independent of atherosclerosis severity.6 So far, it is unclear whether CRP is a risk factor for stroke in general or whether the risk increase can be explained by the associations of CRP with certain stroke subtypes. Based on the fundamental role of inflammation in the pathogenesis of atherosclerosis,7 an association with large-vessel cerebrovascular disease is likely, but only few studies on the relation between CRP and carotid atherosclerosis have been published so far. One study in stroke patients described higher levels of CRP to be related to the progression of carotid artery stenosis within a short follow-up period of 7.5 months.8 Another investigation reported that high CRP levels indicate the presence of unstable carotid plaques.9 Only 1 study explored the role of CRP in cerebral small-vessel disease.10 This investigation used white matter lesions on MRI scans as manifestation of cerebral small-vessel disease and reported significant associations in subjects with levels in the highest compared with the lowest quartile of CRP distribution.10 The current study extends previous work by studying the associations between the presence and progression of carotid...
atherosclerosis and small vessel disease–related brain damage in a given well-defined, community-dwelling cohort of middle-aged and elderly subjects without a history of symptomatic stroke. It is the first study that provides long-term longitudinal data on the role of CRP as a risk factor for cerebral large- and small-vessel disease. The main objective of our investigation was to assess possible differential relations of CRP in different territories of the arterial brain supply.

**Subjects and Methods**

Details of the Austrian Stroke Prevention Study have been published previously. In brief, 2007 subjects aged 50 to 75 years without a history of clinical symptoms of neuropsychiatric disease were recruited. The flow chart (the Figure) demonstrates the recruitment of participants into the current study. In a first study panel between 1991 and 1994, we randomly selected 509 subjects of this cohort to undergo duplex scanning of the extracranial carotid arteries, brain MRI, and cognitive testing. Another 567 individuals of the original study cohort were randomly selected in a second panel between 1999 and 2003 to undergo identical imaging procedures. Participants of the first and second panels were pooled, which resulted in a total of 1076 individuals who were selected to undergo imaging. Originally, we had plasma samples stored from all participants. However, during reconstruction work in our department, several trays including plasma samples from 376 subjects were lost. Therefore, we had blood samples allowing measurement of baseline CRP levels from only 700 participants of the baseline cohort. Measurements were done 1 year after the end of recruitment. There were 376 women and 324 men. Their mean age was 69.9 ± 7.7 years. Hypertension was present in 302 (43.1%), diabetes in 48 (6.9%), and cardiac disease in 241 (34.4%) subjects. Eighty-two (11.7%) were current and 206 (29.4%) former smokers. The mean (SD) body mass index and total cholesterol level of participants were 26.8 (3.9) kg/m² and 226 (39.3) mg/dL, respectively. The definition of risk factors is described elsewhere. All subjects underwent duplex scanning of the carotid arteries, and 505 of them had brain MRI scans. Follow-up studies after 3 and 6 years with identical protocols were done only in those 509 study participants who had undergone duplex scanning and MRI during 1991 and 1994. Duplex scanning was done in 339 and 185 subjects after 3 and 6 years, respectively. MRI of the brain was obtained from 249 and 164 subjects after 3 and 6 years, respectively. The reasons for drop-out are shown in the Figure. Subjects in the follow-up study were younger than participants not entering this study phase, and they had fewer vascular risk factors.

**Carotid Duplex Scanning**

Color-coded equipment (Diasonics, VirMed CFM 750) was used to determine atherosclerotic vessel-wall abnormalities of the carotid arteries at baseline and after 3 and 6 years. All B-mode and Doppler data were transferred to a Macintosh personal computer for processing and storage on optical disk. The imaging protocol involved scanning of both common carotid arteries (CCAs) and internal carotid arteries (ICAs) in multiple longitudinal and transverse planes. The examinations were performed by 3 readers without knowledge of the clinical data of the individuals. Image quality was assessed and graded as good (CCA and ICA clearly visible and ICA detectable for a distance of >2 cm), fair (CCA and ICA sufficiently visible and
ICA detectable for a distance of at least 2 cm), and poor (CCA or ICA insufficiently visible or ICA detectable for a distance of <2 cm). There was no poor-quality study at the baseline and follow-up examinations. At baseline and follow-up, the extent of atherosclerosis was graded in each vessel according to the most severe visible changes as 0, normal; 1, vessel wall thickening >1 mm; 2, minimal plaque (<2 mm); 3, moderate plaque (2 to 3 mm); 4, severe plaque (>3 mm); and 5, lumen completely obstructed. The interrater variability for grading the extent of sonographic changes was independently assessed in 200 vessels in 50 subjects. The κ values for interrater agreement for the sonographic score among the 3 sonographers ranged from 0.89 to 0.95.12 The carotid atherosclerosis index was the sum of scores of the 4 vessels (range, 0 to 20) and was used as a measure of the severity of extracranial carotid atherosclerosis. The difference in the sum of scores in the carotid arteries between baseline and the 3- and 6-year follow-up was determined to define regression or progression of carotid atherosclerosis. Assessment of atherosclerosis in the CCAs and ICAs in a dose-dependent manner was performed to detect changes in atherosclerosis during follow-up in all visible sections of the carotid arteries.

MRI
At baseline and at each follow-up visit, 1.5-T scanners from the same manufacturer (Philips Medical Systems) and identical protocols were used as previously described.11,13 The scans were evaluated for small vessel disease–related abnormalities. As has been shown by numerous histopathological correlations, these changes are white matter hyperintensities and lacunar lesions.12

White Matter Hyperintensities
The scans of each study participant were analyzed by a single experienced investigator (C.E.). He first graded white matter lesions according to our scheme.14 Caps and a pencil-thin lining of hyperintensity were disregarded, as they represent normal anatomic variants. C.E. then marked and outlined each white matter lesion on a transparency that was overlaid on the proton density scans. He was blinded to the clinical data of study participants. Blinding of the reader for the date of the examinations was impossible, as the format of hard copies had changed from baseline to follow-up. Follow-up scans were compared with the baseline scan, and the lesions were drawn as before. Scan series were reviewed by C.E. and R.S., and consensus was reached for equivocal abnormalities.

Lesion load measurements were done on proton density–weighted images on an UltraSPARC workstation (Sun Microsystems) independently of this visual analysis by 1 trained operator (K.P.) using DISPImage16 without knowledge of the temporal sequence of the scans or other clinical and MRI data. Repeated determinations of the operator’s performance showed a maximum intrarater coefficient of variation of 6.4%. Using a hard copy with all lesions outlined as a reference, a trained technician outlined all lesions on the computer image with use of a semiautomated segmentation algorithm provided by DISPImage program. The total lesion volume (in cm³) was calculated by multiplying the total lesion area by slice thickness. The reproducibility of the volumetric assessments of white matter lesions has been described previously.13 The limits of possible measurement error are between −1.59 and 1.81 cm³.

Lacunes
Lacunes were focal cerebrospinal fluid–containing lesions that involved the basal ganglia, the internal capsule, the thalamus, or brain stem not, exceeding a maximum diameter of 10 mm. The number of lacunes was assessed at baseline. At follow-up, all newly occurring lacunes were recorded.

High-Sensitivity CRP Measurements
Nonfasting blood samples were taken from the study participants at the baseline examination and centrifuged at 3000g for 10 minutes, and plasma was separated and stored at −70°C. The CRP concentration was measured with a particle-enhanced immunoturbidimetric assay (Tina-quant CRP latex ultrasensitive assay; Roche Diagnostics) performed on a Hitachi 717 automated analyzer. The detection limit of this assay is 0.1 mg/L, and the extended measuring range (with reruns) is 0.1 to 240 mg/L. The between-assay coefficient of variation was 2.6% at 4.65 mg/L CRP.17 The technicians were blinded to the clinical status of study participants.

Data Analysis
There were 48 subjects in the entire cohort with CRP levels >10.0 mg/L. The value increased at all acute-phase response. Removal of these participants from the analyses did not change the results. Therefore, all presented results refer to the whole study group. For analyses, we created quintiles of CRP based on the distribution of CRP concentrations in the entire study cohort. We also used CRP concentration as a continuous variable. CRP was skewed to the left and therefore logarithmically transformed. We also distinguished between low (<1 mg/L), intermediate (1 to 3 mg/L), and high (>3 mg/L) CRP risk based on previous reports on the utility of CRP in risk stratification in cardiovascular disease.18 The association of CRP to demographics, vascular risk factors, and imaging finding was investigated by ANOVA for continuous variables and a χ² test for categorical variables. To assess the relations between CRP levels and baseline extracranial atherosclerosis, white matter lesion volume, and the presence of lacunes, multivariable linear-regression analysis was used. The effects of CRP on the progression of vascular abnormalities were assessed by repeated-measures regression, implementing the generalized estimating equation (GEE) approach to longitudinal studies.19 The GEE model treated the cumulative change in the sum score of the 4 carotid vessels, in white matter lesion volume, and in the number of lacunes as independent variables. Analyses were adjusted for sex, age, and vascular risk factors including hypertension, diabetes, cardiac disease, body mass index, smoking status, and total cholesterol. Correlation between successive measures in each patient was accommodated with an unstructured correlation structure. Possible interactions between CRP levels and age, sex, hypertension, diabetes, cardiac disease, and smoking were explored by calculation of respective interaction terms in the adjusted GEE models. For a multiple linear-regression model that already included covariates with a squared multiple correlation R² of 0.43, a sample size of 505 would have had 85% power to detect at α=0.05 an increase in R² of 0.01 owing to the inclusion of 1 additional covariate. To assess the relative importance of CRP in relation to other acute-phase proteins, models were also analyzed that included both CRP and fibrinogen quintiles. All statistical analyses were performed with STATA 6.0 for Windows.20 A probability value of <0.05 was considered statistically significant. No corrections for multiple comparisons were made owing to the exploratory nature of the study.

Results
At baseline, the CRP levels of study participants ranged from 0.1 to 47.0 mg/L with a median of 1.9 mg/L (interquartile range, 0.9 to 3.9 mg/L). Duplex scanning showed changes in the atherosclerosis index in 467 (66.7%) individuals. After 3 and 6 years, carotid atherosclerosis had progressed in 104 (30.7%) and 99 (53.5%) study participants, respectively; regression was seen in 8 (2.4%) and 5 (2.7%) individuals, respectively. A total of 408 (80.8%) subjects had white matter lesions on MRI scans at baseline. Progression of white matter damage beyond possible measurement error (1.81 cm³) occurred in 25 (10.0%) participants after 3 years and in 25 (15.2%) individuals after 6 years. Decrease in white matter lesion volume beyond measurement error (−1.59 cm³) was not seen. The ranges, medians, and interquartile ranges of the carotid atherosclerosis score; the volume of white matter abnormalities at baseline; and the changes of these measures at follow-up are shown in Table 1. Thirty-five (6.9%) participants had lacunar lesions on baseline MRI scans,
ranging in number from 1 to 97 lesions. Only 5 subjects showed new lacunes after 3 and 6 years. As shown in Table 2, there were significant differences in body mass index, frequency of diabetes, and the baseline atherosclerosis score across quintiles of CRP. No differences were seen for any MRI findings. After controlling for possible confounders, we found that the baseline severity of extracranial carotid atherosclerosis increased with an increase of the quintile of CRP (P for trend=0.03). The same association existed for progression of atherosclerosis (P for trend=0.04). Compared with the first quintile of CRP concentration (<0.80 mg/L), only study participants in the fourth and fifth quintiles (>2.50 mg/L) had significantly more baseline atherosclerosis and greater progression of carotid disease during follow-up. The associations between CRP quintiles and baseline white matter

### TABLE 1. Atherosclerosis Score and White Matter Lesion Volume at Baseline and Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Atherosclerosis Score</th>
<th>White Matter Lesion Volume, cm³</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>700</td>
<td>505</td>
</tr>
<tr>
<td>Range</td>
<td>0 to 14</td>
<td>0 to 34.5</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2.0 (0.0–2.0)</td>
<td>0.7 (0.1–2.6)</td>
</tr>
<tr>
<td>3-year change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>339</td>
<td>249</td>
</tr>
<tr>
<td>Range</td>
<td>−5 to 6</td>
<td>−1.9 to 13.9</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.0 (0.0–1.0)</td>
<td>0.0 (0.0–0.4)</td>
</tr>
<tr>
<td>6-year change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>185</td>
<td>164</td>
</tr>
<tr>
<td>Range</td>
<td>−1 to 10</td>
<td>1.2 to 31.4</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.0 (0.0–2.0)</td>
<td>0.2 (0.0–0.7)</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range

### TABLE 2. Demographics, Risk Factors, Duplex Scan and MRI Findings, and Quintiles of CRP

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Quintile of CRP, Mean (SD)</th>
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<tr>
<td></td>
<td>1 (n=164)</td>
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<tr>
<td>Age, y*</td>
<td>63.7 (8.0)</td>
</tr>
<tr>
<td>Body mass index*</td>
<td>25.3 (3.2)</td>
</tr>
<tr>
<td>Sex, male, %†</td>
<td>50.6</td>
</tr>
<tr>
<td>Hypertension, %†</td>
<td>35.4</td>
</tr>
<tr>
<td>Diabetes, %†</td>
<td>3.0</td>
</tr>
<tr>
<td>Cardiac disease, %†</td>
<td>32.9</td>
</tr>
<tr>
<td>Smoking status, %†</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>65.9</td>
</tr>
<tr>
<td>Former</td>
<td>25.6</td>
</tr>
<tr>
<td>Current</td>
<td>8.5</td>
</tr>
<tr>
<td>Cholesterol, mg/dL*</td>
<td>221.8 (37.9)</td>
</tr>
<tr>
<td>Carotid atherosclerosis index*‡</td>
<td>2.7 (2.9)</td>
</tr>
<tr>
<td>White matter lesion volume, cm³*</td>
<td>2.2 (4.6)</td>
</tr>
<tr>
<td>No. of lacunes*</td>
<td>0.4 (1.5)</td>
</tr>
</tbody>
</table>

*One-way ANOVA; †χ²; §P<0.0001; ‡P<0.01; ¶P<0.05; #Sum of duplex scores (0, normal, to 5, complete obstruction) in the CCAs and ICAs (range, 0–14; median, 3; interquartile range, 0–5).
lesion volume. The associations with number of lacunes were also not significant. CRP was unrelated to progression of MRI abnormalities (Table 3). This also applied when white matter lesion progression was considered to be a change in white matter lesion score. The associations with cerebral small vessel disease–related brain abnormalities remained nonsignificant when CRP was entered as a continuous variable. The tests of interaction between CRP quintiles and age, sex, and major vascular risk factors were nonsignificant for carotid atherosclerosis and for white matter lesion volume.

**Discussion**

These prospective data from a large random sample of middle-aged and elderly, asymptomatic, community-dwelling subjects demonstrate a significant relation between baseline CRP levels and both severity and progression of carotid atherosclerosis during an observational period of 6 years.

Compared with the lowest quintile of CRP concentration, only study participants in the fourth and fifth quintiles (CRP >2.50 mg/L) showed significantly more severe and progressive carotid atherosclerosis, whereas the risk increase in subjects belonging to the second and third quintiles was not significant. The relation between CRP and carotid atherosclerosis was seen after adjustment for age, sex, and vascular risk factors. However, caution is advised, because there were differences in risk factors between subjects belonging to different quintiles of CRP. Statistical adjustment may not fully correct for all of these differences. CRP was significantly related to progression of carotid atherosclerosis in subjects with but also in those without major vascular risk factors. We did not find associations between CRP levels and severity or progression of cerebral small-vessel disease as reflected by the volume of white matter hyperintensities and number of lacunes on brain MRI scans of our study participants.

This is the first study to relate CRP and carotid atherosclerosis in subjects without a history of symptomatic stroke, suggesting that CRP levels can be used to predict progression of carotid disease during subsequent years. Our results are in line with a single study in stroke patients, in which changes of inflammatory markers, including CRP, were linked to rapid progression of carotid stenosis.8

The lack of an association with cerebral small-vessel disease contrasts a previous report of the Rotterdam Scan Study. Therein the authors described a relation not only between CRP and severity but also between CRP and 3-year progression of white matter lesions.10 Several differences between the Rotterdam Scan Study and the Austrian Stroke Prevention Study exist, despite the fact that the median CRP concentrations were identical. The follow-up in our study was longer, and the methodology used to measure the extent and progression of white matter changes was different. We assessed the volume of white matter damage, whereas in the Rotterdam Scan Study, white matter disease severity and progression were rated on ordinal scales. Nonetheless, it is unlikely that this was responsible for the contrasting results, because it was shown by the authors of the Rotterdam Scan Study that their scales were highly correlated with the results of volumetric assessments.21 Differences in the composition of study cohorts are more likely to have been important. Our participants were younger and apparently healthier than those of the Rotterdam cohort. We did not include patients with a history of symptomatic strokes or transient ischemic attacks or evidence of dementia. We also had fewer subjects with hypertension and fewer smokers in our cohort. Consequently, it is likely that we studied earlier stages of cerebral small-vessel disease. Unlike in large-vessel atherosclerosis, in which CRP seems to be involved in very early disease stages,22 the situation in cerebral small-vessel disease may be different. Increases in CRP could rather be the epiphenomenon of brain damage attributable to small-vessel disease rather than being related to the development of arteriolosclerosis per se. In this context, it is of note that inflammatory cellular infiltration is not seen in cerebral small-vessel disease, whereas fatty streak formation, consisting of lipid-laden monocytes and macrophages (foam cells) together with T lymphocytes, represents an important early step in the evolution of large-vessel atherosclerosis.7 The only indication for inflammatory processes playing a role in cerebral small-vessel disease comes from studies reporting increased endothelial inflammation markers in subjects with white matter disease or lacunes.24,25 In our cohort, we have recently shown an association between white matter lesion progression and intercellular adhesion molecule levels in plasma, but this relation was independent of the CRP levels of participants.25
Our study has several strengths. It is the first longitudinal investigation that simultaneously assessed the associations between CRP and both cerebral large- and small-vessel disease. This allowed us to assess the effects of CRP in different arterial beds in the same subjects. At 6 years, the follow-up period in our study was longer than in previous investigations, and we provided repeated carotid duplex and brain MRI scanning at 3-year intervals. There are also weaknesses. Only subjects who agreed to participate were selected. It is likely that responders were more worried about their health than nonresponders. Subjects who participated in the follow-up were younger and had fewer risk factors for stroke than did those who did not participate in the follow-up examinations. This might have resulted in lower progression rates of large- and small-vessel disease than in the general population and most likely has reduced the statistical power of the respective analyses. Treatment of study participants is left fully to the treating general practitioners, and thus, we are unable to provide reliable treatment data. It cannot be excluded that treatment might have had an effect on study results.

We have no intima-media thickness measurements because this was not yet an established measure of preclinical atherosclerosis when this study was in its planning phase. Therefore, the only measure related to intima-media thickness included in our assessment was vessel wall thickening >1 mm, the cutoff that was thought to be abnormal during the time of study planning and still is currently. Although assessment of the carotid atherosclerosis score at follow-up was done without knowledge of baseline findings, we were unable to blind the MRI reader for the date of the examination because of software upgrades resulting in a format change of hard copies. This might have resulted in an overestimation of white matter lesion progression. The lack of repeated CRP measurements is another limitation, which the study shares with most previous longitudinal cardiovascular investigations. However, there are studies that show that a single measurement of CRP is highly stable over repeated measurements, and measures taken 5 years apart are highly correlated.

We conclude that CRP is unrelated to small vessel disease–related brain damage, but in the light of conflicting data from another large-scale, population-based study, this finding needs to be further explored. However, increased levels of CRP are independently related to future progression of carotid atherosclerosis. Progression of carotid stenosis as detected by duplex ultrasound investigations translates clinically into a substantially increased risk for ipsilateral strokes. Thus, our observation suggests that subjects with carotid atherosclerosis and high levels of CRP require close ultrasonic surveillance and aggressive treatment of risk factors.

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Disclosures
None.

References


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