C-Reactive Protein Is an Independent Predictor of the Rate of Increase in Early Carotid Atherosclerosis

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Background—An elevated plasma concentration of high-sensitivity C-reactive protein (hs-CRP) is a strong predictor of cardiovascular events. However, there have been no longitudinal studies of the relations between development of atherosclerotic lesions and hs-CRP concentrations. Furthermore, it remains unknown whether increased hs-CRP concentrations result in the development of atherosclerosis.

Methods and Results—The study included 179 outpatients 40 to 79 years of age who were treated at our institute for traditional risk factors for cardiovascular disease. The patients had no evidence of advanced carotid atherosclerosis at the time of baseline examination. Patients underwent repeated ultrasonographic evaluation of the carotid arteries for 35±10 months. Blood samples were collected for hs-CRP measurements. Based on focal intima-media thickening ≥1.1 mm representing plaque, plaque number (PN) and plaque score (PS; the sum of all plaque thicknesses) were calculated. The development of atherosclerosis was estimated by the formula Δvalue/year=(last value−baseline value)/number of follow-up years. Multivariate linear regression analysis revealed that the log-transformed value for hs-CRP concentration was not related to baseline PN or PS but was related to ΔPN/year and ΔPS/year (β=0.29 and 0.30; P<0.001 for both) independently of the effect of traditional risk factors.

Conclusions—During the early stages of carotid atherosclerosis, the hs-CRP concentration is a marker of carotid atherosclerotic activity rather than extent of atherosclerosis. (Circulation. 2001;104:63-67.)

Key Words: C-reactive protein ■ atherosclerosis ■ inflammation ■ ultrasononics ■ carotid arteries

The development of atherosclerosis is now considered to be due in part to an inflammatory response.1 Several investigators have examined a variety of circulating markers of inflammation as potential predictors of the presence of cardiovascular disease (CVD) and the risk of future CVD events. Of the variety of circulating markers, high-sensitivity C-reactive protein (hs-CRP) has the most consistent relations to the risk of CVD events in a variety of clinical settings. hs-CRP predicts CVD risk in healthy men2 and women,3 in selected high-risk patients with traditional risk factors,4 and among patients with CVD.5 The ability of hs-CRP to predict CVD risk is independent of the effects of traditional risk factors.2,6 Furthermore, the combination of hs-CRP and the total/HDL cholesterol ratio more strongly predicts CVD events than either factor alone.8 The association of hs-CRP with future CVD events extends not only to 1-year follow-up in older women with subclinical CVD7 but also to 6 to 7 years of follow-up in middle-aged men at high risk for CVD4 and to 6 years or more in apparently healthy middle-aged and older men.5 However, there have been no longitudinal studies to determine the relations between the development of atherosclerotic lesions and hs-CRP concentrations. Furthermore, there is no clinical evidence that elevated hs-CRP concentrations result in the development of atherosclerosis.

It is well known that the severity of carotid atherosclerosis is closely related to the presence of CVD and the risk of CVD events.7–10 Several indices, such as intima-media thickness (IMT) and scores that sum plaque thickness, have proved valuable to estimate the relations between carotid atherosclerosis and CVD. We have previously established the plaque score (PS) as an index of the severity of carotid atherosclerosis and reported the relationship of PS with traditional factors for CVD11 and future ischemic cerebrovascular disease.12 In the present study, we determined the relations between hs-CRP concentrations and the development of carotid atherosclerosis, as an index of generalized atherosclerosis, after adjusting for the effects of traditional risk factors.

Methods

Patients

Between September 1996 and March 1998, 191 outpatients 40 to 79 years of age were examined in the Department of Internal Medicine of Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail brain@medone.med.osaka-u.ac.jp

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and Therapeutics, Osaka University Hospital for carotid atherosclerotic involvement because of the presence of risk factors for CVD. The patients gave written informed consent to provide blood samples and undergo follow-up examinations for at least 2 years to evaluate the development of carotid atherosclerosis. Patients were excluded from the study if they had experienced a clinical CVD event in the previous year or if they had advanced carotid atherosclerosis or other diseases that could elevate the hs-CRP concentration. In the follow-up period, 8 patients suffered a new CVD event, and 5 of them experienced no follow-up carotid survey. Another 5 patients suffered malignant disease, and 2 patients were lost to follow-up. A total of 12 patients experienced no follow-up carotid survey and were removed from the analyses. No patients were being treated with antioxidant vitamin supplements, estrogen therapy, or steroid therapy.

Definition of Traditional Risk Factors for CVD
Hypertension was defined as a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg or the current use of antihypertensive medications. Hypercholesterolemia was defined as a total cholesterol concentration ≥220 mg/dL or the current use of cholesterol-lowering agents. Diabetes mellitus was defined as a glycosylated hemoglobin A1c concentration >5.8% or current use of oral hypoglycemic agents. Patients were categorized as smokers if they were current smokers or had stopped smoking <1 month before entry into the study. Cigarette pack-years were noted for each patient to measure cumulative smoking exposure. Patients were categorized as having CVD if they had a prior history of ischemic heart disease, cerebrovascular disease, aortic aneurysm, or peripheral vascular disease.

Evaluation of the Development of Carotid Atherosclerosis
To evaluate the development of carotid atherosclerosis, high-resolution B-mode ultrasonography with a 7.5-MHz duplex-type probe (EUB-525; Hitachi, Inc) was performed repeatedly over a period of at least 2 years. Baseline and follow-up ultrasound images were recorded on Super VHS videotape, and the progression of each individual plaque was evaluated in a blinded manner. The method used was similar to the method we reported in another prospective study. Based on our previous study, the upper limit of normal for IMT is 1.0 mm, and lesions with a focal IMT ≥1.1 mm were defined as atheromatous plaques. PS was calculated by summing all plaque thickness measurements in both carotid arteries. In the present study, we used both plaque number (PN) and PS to estimate the severity of carotid atherosclerosis. The development of atherosclerosis was estimated by the following formula for each parameter: Δvalue/year=last value−baseline value/number of follow-up years. Advanced carotid atherosclerosis was defined as a PS >10.0 based on our grading system, and patients with such lesions were not enrolled in the present study.

Measurement of Circulating hs-CRP Concentration
Blood samples were collected in tubes containing citric acid and stored at −80°C after centrifugation. The stored serum for each patient was thawed in April 1998 for hs-CRP measurement by an automatic immunonephelometer with a sensitivity of 0.02 mg/dL (Behring NA latex CRP; Behring Institute).

Statistical Analysis
All statistical analyses were performed with SPSS/Windows System, version 9.0J (SPSS Japan Inc). Relations between hs-CRP values and parameters of carotid atherosclerosis were evaluated by Spearman rank order correlation. For ease of interpretation, hs-CRP concentrations were grouped in tertiles, and the differences in values were evaluated by 1-way ANOVA with Bonferroni correction. The Mann-Whitney U test was used to evaluate the difference between hs-CRP levels in the presence and absence of preexisting carotid atherosclerosis. The Mann-Whitney U test was also used to evaluate the difference between parameters in the presence and absence of traditional risk factors and statin use, and Spearman rank order correlation was used to evaluate the association between these parameters and measured risk factors. Multiple linear regression analyses were performed to assess the contribution of hs-CRP concentration to the prediction value of each parameter compared with the contribution of traditional risk factors. A natural log transformation achieved normality, and therefore log-transformed hs-CRP values were used in the model. hs-CRP concentrations below the detection level were assigned a log-transformed value of −4.605 (hs-CRP value of 0.01 mg/dL). Probability values were 2-tailed and were considered significant if <0.05.

Results
Baseline characteristics of the 179 enrolled patients are summarized in Table 1. Age, systolic blood pressure, fasting blood glucose, hemoglobin A1c, cigarette pack-years, and hs-CRP correlated significantly with the severity of baseline carotid atherosclerosis, and carotid atherosclerosis had progressed further in men (Table 2). The follow-up period was 35±10 months. hs-CRP concentrations in 8 patients with a new CVD event showed a median value of 0.32 mg/dL, with a range of 0.12 to 0.79 mg/dL, and were within the highest tertile.

The relations between hs-CRP concentrations and parameters associated with carotid atherosclerosis are shown in Table 3. At the time of baseline examination, hs-CRP concentration was correlated with PN and PS on the basis of simple regression analysis. However, on the basis of a multiple linear regression model, hs-CRP concentration did not significantly correlate with PN or PS values after adjustment for the effect of traditional risk factors, ie, age, sex, cigarette pack-years, and the presence or absence of hypertension, hypercholesterolemia, and diabetes mellitus (Table 3). The relations between hs-CRP concentrations and the annual increases in PN (ΔPN/year) and PS (ΔPS/year) are shown in Figures 1 and 2, respectively. For ease of interpretation, ΔPN/year and ΔPS/year are shown in Figure 3 in relation to hs-CRP concentration tertiles. ΔPN/year and
Relations between baseline hs-CRP concentration and number of carotid plaques developing over time (ΔPN/year). Average (±SD) of ΔPN/year was 0.35±0.60, and median was 0.26. Thirteen patients had hs-CRP concentration below level of detection and were assigned a value of 0.01 mg/dL. Correlation was \( r=0.223, P=0.003 \).

ΔPS/year values in the highest tertile were significantly higher than values in the middle or lowest tertile. hs-CRP concentrations correlated with higher than values in the middle or lowest tertile. hs-CRP concentrations correlated with ΔPS/year and ΔPS/year per simple regression analysis, and the correlations remained significant after adjustment for the effect of traditional risk factors and the baseline severity of carotid atherosclerosis (Table 3). In a multivariate analysis fitted with statin medication and HDL cholesterol as independent variables instead of hypercholesterolemia, the statistical values were significantly higher than those in middle or lowest tertiles (ΔPS/year were again found (Table 3). In the present study, the correlation between hs-CRP concentration and the annual increase in individual plaque thicknesses was significant \( r=0.201, P=0.03 \) on the basis of simple regression. However, on the basis of multivariate regression analysis, the correlation was not significant \( (\beta=0.113, P=0.269) \) after adjustment for the effect of traditional risk factors.

### TABLE 2. Association Between Risk Factors for Atherosclerosis Including Statin Medication and Baseline PS and ΔPS/Year

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Baseline PS</th>
<th>ΔPS/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.28*</td>
<td>0.04</td>
</tr>
<tr>
<td>Men/women</td>
<td>3.1±3.0</td>
<td>0.75±1.12/0.39±0.92*</td>
</tr>
<tr>
<td></td>
<td>2.0±2.1†</td>
<td>0.14</td>
</tr>
<tr>
<td>Hypertension, yes/no</td>
<td>2.7±2.6/2.2±2.6</td>
<td>0.61±1.12/0.46±0.81</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.192†</td>
<td>0.14</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.006</td>
<td>-0.066</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.105</td>
<td>0.21</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.085</td>
<td>-0.007</td>
</tr>
<tr>
<td>Statin medication, yes/no</td>
<td>2.7±2.4/2.5±2.7</td>
<td>0.50±0.95/0.60±1.06</td>
</tr>
<tr>
<td>Diabetes mellitus, yes/no</td>
<td>3.8±2.5/2.3±2.6</td>
<td>0.53±1.17/0.57±1.01</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>0.227*</td>
<td>0.18†</td>
</tr>
<tr>
<td>Hemoglobin (A_{hi} )</td>
<td>0.256*</td>
<td>0.084</td>
</tr>
<tr>
<td>Current smoking, yes/no</td>
<td>2.8±2.6/2.5±2.6</td>
<td>1.04±1.52/0.52±0.94</td>
</tr>
<tr>
<td>Cigarette pack-years</td>
<td>0.204*</td>
<td>0.108</td>
</tr>
<tr>
<td>History of CVD, yes/no</td>
<td>3.2±3.1/2.3±2.4</td>
<td>0.69±1.19/0.53±0.96</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.227*</td>
<td>0.268*</td>
</tr>
</tbody>
</table>

Values shown are correlation coefficients for measured risk factors and mean±SD for categorized risk factors.

\*\( P<0.01 \), †\( P<0.05 \).
TABLE 3. Association Between hs-CRP Concentration and Parameters Associated With Carotid Atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>Simple Regression</th>
<th>Multivariate Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
</tr>
<tr>
<td>In total patients, n=179</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN</td>
<td>0.217</td>
<td>0.003</td>
</tr>
<tr>
<td>PS</td>
<td>0.227</td>
<td>0.002</td>
</tr>
<tr>
<td>( \Delta PN/\text{year} )</td>
<td>0.223</td>
<td>0.003</td>
</tr>
<tr>
<td>( \Delta PS/\text{year} )</td>
<td>0.268</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \Delta PS/\text{year per subgroup} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men, n=89</td>
<td>0.381</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women, n=90</td>
<td>0.223</td>
<td>0.036</td>
</tr>
<tr>
<td>Noncurrent smoker, n=158</td>
<td>0.185</td>
<td>0.022</td>
</tr>
<tr>
<td>Patients without statin, n=136</td>
<td>0.278</td>
<td>0.001</td>
</tr>
<tr>
<td>Patients without CVD history, n=128</td>
<td>0.210</td>
<td>0.019</td>
</tr>
<tr>
<td>In patients with preexisting carotid plaque, n=123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta PN/\text{year} )</td>
<td>0.324</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \Delta PS/\text{year} )</td>
<td>0.368</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion

With respect to the association between CRP concentration and the development of carotid atherosclerosis, there have been only a few cross-sectional studies and no longitudinal studies reported. A previous cross-sectional study that used hs-CRP found no association between CRP concentrations and the severity of carotid atherosclerosis. Cross-sectional analysis of baseline values in the present study showed no significant association independent of the effects of traditional risk factors. However, we demonstrated that hs-CRP concentration is an independent predictor of the development of early carotid atherosclerosis. hs-CRP appears to be a marker of inflammation related to the rate of plaque development rather than the extent or severity of plaque burden. Development accelerated with rising hs-CRP levels even when the values were within the normal range. The present finding clinically emphasizes the tight linkage of CRP to atherosclerotic processes and may support a hypothesis that CRP directly interacts with atherosclerotic vessels by activation of the complement system, thereby promoting inflammation and thrombosis. Furthermore, evidence is now accumulating to suggest that CRP may contribute to monocyte recruitment in atherogenesis and to monocyte induction of tissue factor, which is potentiated by interferon-γ and lipopolysaccharide.

Several studies have reported that IMT below certain values may not reflect atherosclerosis but may merely represent an adaptive intimal thickening to physiological variations in shear and tensile forces along the length of the artery. In the present study, atherosclerotic plaque was defined as an IMT ≥1.1 mm based on our previous study. The Rotterdam study suggests that an IMT in the common carotid artery <1.1 mm might not represent local atherosclerosis but might reflect an adaptive response to altered flow, luminal diameter, shear stress, or pressure. The results of the Atherosclerosis Risk In Communities (ARIC) study suggests that the hazard ratio for CVD events for an IMT >1 mm is 5.07 for women and 1.85 for men. Based on such a definition, the present study demonstrates that a higher hs-CRP concentration correlates with an increase in PN and with an increase in PS that sums up individual plaque thickness. When patients with preexisting carotid plaques were analyzed separately, the correlations were a little stronger, possibly because such patients had already been involved in the developmental stage of atherosclerosis. This idea is supported by the Bruneck study, which shows a closer correlation between another novel inflammatory marker and the progression of carotid atherosclerosis in patients with preexisting plaque compared with patients without preexisting plaque. In the Bruneck study, CRP, assessed by standard procedure, showed a tendency to be higher in patients with new incidence of plaques than in those without.

The present study focused on the early stage of carotid atherosclerosis because it reflects generalized atherosclerosis, and a slight progression of carotid atherosclerosis significantly increases the prevalence of CVD and the risk of a CVD event. The Rotterdam study demonstrated that the absolute risk of CVD rises from a range of 8.8% to 15.8% to a range of 14.3% to 19.8% with the presence of plaques in the common carotid artery or bifurcation. According to our previous follow-up study, the annual event rate accelerates with increasing PS, and patients with PS >10 had a 9-fold higher hazard ratio for an annual event than did patients with PS =0; such patients were therefore excluded from the present study.

The management of traditional risk factors for atherosclerosis is very important, but these measures do not completely inhibit the development of atherosclerosis or prevent CVD events. For example, in the United States, up to half of all myocardial infarctions occur in individuals with moderate to low risk of CVD based on assessment of total and HDL cholesterol concentrations. The present study showed that...
hs-CRP concentration predicts the development of carotid atherosclerosis independently of the effects of traditional risk factors. These findings support the notion that a therapeutic strategy that decreases CRP concentration and inhibits inflammatory responses may prevent the progression of atherosclerosis and subsequently prevent CVD events. Ultrasonographic carotid survey has been used to estimate the antiatherosclerotic effect of medication in a wide variety of studies. The combined evaluation of hs-CRP measurement and carotid plaques may be used to estimate the effect of anti-inflammatory treatment for progression of atherosclerosis and future CVD events.

Acknowledgments
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References
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