Relationship Between C-Reactive Protein and Progression of Early Carotid Atherosclerosis in Hypertensive Subjects

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Background and Purpose—Hypertensive outpatients were investigated for C-reactive protein (CRP) and carotid atherosclerosis because the influence of CRP on the progression of subclinical atherosclerosis in hypertensives remains unclear.

Methods—A total of 124 outpatients (aged 40 to 79 years) in treatment for hypertension were enrolled. They underwent repeated ultrasonographic evaluation of the carotid arteries for 35±12 months. Focal intima-media thickening of ≥1.1 mm was defined as plaque, and the plaque number, plaque score, and the sum of all plaque thickness were calculated.

Results—Multivariate linear regression analysis revealed that CRP, pulse pressure, and systolic blood pressure were related to the annual change of plaque number (β=0.34, 0.27, and 0.30; all P<0.01) and plaque score (β=0.38, 0.27, and 0.23; P<0.01, P<0.01, and P<0.05, respectively) independently of other risk factors. In 64 patients taking antihypertensive medications with a blood pressure of <140/90 mm Hg, CRP and the pulse pressure were related to the annual change of plaque number (r=0.40 and 0.26; P<0.01 and P<0.05, respectively) and plaque score (r=0.44 and 0.31; P<0.001 and P<0.05, respectively).

Conclusions—In hypertensive patients being managed by drug therapy or lifestyle modification, CRP is an equivalent or superior independent predictor of the progression of carotid atherosclerosis than the pulse pressure or systolic blood pressure. (Stroke. 2004;35:1625-1630.)

Key Words: atherosclerosis ■ hypertension ■ carotid artery ■ inflammation ■ ultrasonography

Hypertension is 1 of the major traditional risk factors for atherosclerosis. The management of hypertension is very important, but its treatment and that of other traditional risk factors does not completely inhibit the development of atherosclerosis or prevent cardiovascular events. Atherosclerosis is now considered to be partly attributable to an inflammatory response, and there is evidence of a link between atherosclerosis or cardiovascular disease (CVD) and elevated serum levels of C-reactive protein (CRP). With regard to the contribution of CRP to the relationship between hypertension and CVD, a recent study showed that the risk of myocardial infarction was marginal in hypertensives without a simultaneous high CRP level. Several other inflammatory serum proteins are reported to be associated with an increased risk of stroke among men with a high systolic blood pressure (SBP). New guidelines suggest that patients who have an intermediate risk of CVD based on traditional risk factors may benefit from the measurement of high-sensitivity CRP (hs-CRP). However, there have been no reports about the influence of CRP on the progression of subclinical atherosclerosis in patients with hypertension. It is well known that the severity of carotid atherosclerosis is closely related to the presence of CVD and the risk of CVD events. In the present study, we tested hs-CRP and office blood pressure as predictors of the progression of carotid atherosclerosis in hypertensive patients.

Materials and Methods

Patients

Between September 1996 and March 1998, we examined outpatients aged 40 to 79 years who were attending the Department of Internal Medicine and Therapeutics at Osaka University Hospital for carotid atherosclerosis because of the presence of risk factors for CVD. Each patient gave written informed consent to the collection of blood samples and follow-up for at least 2 years to evaluate the development of carotid atherosclerosis. Patients were excluded from the study if they had experienced a cardiovascular event during the previous year (n=2) or if they had advanced carotid atherosclerosis (n=25) or other diseases that could increase the hs-CRP level (18 had aortitis, 2 had collagen diseases, 2 had malignant tumors, and 1 had chronic bronchitis). During the follow-up period, 8 patients experienced a new cardiovascular event, 5 of whom did not undergo follow-up carotid ultrasonography. Another 5 patients developed malignant tumors, and 2 patients were lost to follow-up. A total of 12
patients without follow-up carotid ultrasonography were deleted from the analysis. Ultimately, 179 patients were enrolled in the previous study, of whom 129 patients were hypertensives being treated with drug therapy or lifestyle modification. Information about antihypertensive therapy was missing for 5 patients, so this study presents data on the remaining 124 patients.

Risk Factors
Blood pressure was measured in the right arm with the patient in the seated position after a 5-minute rest, following recommendations of the American Heart Association. The average of 2 consecutive blood pressure measurements was calculated. Hypertension was defined as an SBP of ≥140 mm Hg, a diastolic blood pressure (DBP) of ≥90 mm Hg, or current use of antihypertensive medications. The other traditional risk factors for CVD were classified as follows. Hypercholesterolemia was defined as a total cholesterol level of ≥220 mg/dL (5.69 mmol/L) or current cholesterol-lowering therapy. Diabetes mellitus was defined as a glycated hemoglobin A1c concentration of >6.5% or current use of oral hypoglycemic agents. Body mass index was the weight in kilograms divided by the square of the height in meters. 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 calculated for each patient as a measure of cumulative smoking exposure. Patients were categorized as having CVD if there was a history of cerebrovascular disease, ischemic heart disease, aortic aneurysm, or peripheral vascular disease.

Evaluation of Carotid Atherosclerosis
To evaluate the progression of carotid atherosclerosis, high-resolution B-mode ultrasonography using a 7.5-MHz duplex probe (EUB-525, Hitachi) was performed repeatedly over a period of ≥2 years. Baseline and follow-up ultrasound images were recorded on VHS videotape, and the changes of each plaque were evaluated in a blinded manner. The method was similar to that used by us in another prospective study. On the basis of our previous findings, the upper limit of normal for the intima-media thickness (IMT) was set at 1.0 mm, and areas with an IMT of ≥1.1 mm were defined as atheromatous plaques. The plaque score was calculated by summing the thickness of all plaques measured in both carotid arteries (Figure 1), and we used the number of plaques and the plaque score to estimate the severity of carotid atherosclerosis. The progression of atherosclerosis was estimated by inserting each parameter into the following formula:

\[ \Delta \text{value/year} = \left( \text{final value} - \text{baseline value} \right) / \text{years of follow-up} \]

Advanced carotid atherosclerosis was defined as a plaque score of >10, and such patients were not enrolled in this study.

Measurement of the 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 using an automatic immunonephelometer with a sensitivity of 0.02 mg/dL (Behring NA latex CRP; Behring Institute).

Statistical Analysis
Natural log transformation of the hs-CRP data achieved a normal distribution, so log-transformed hs-CRP values were used. All hs-CRP concentrations below the detection limit were assigned a log-transformed value of −4.605 (ie, an hs-CRP value of 0.01 mg/dL). The relationship between measured risk factors, including log-transformed CRP values, and the parameters of carotid atherosclerosis was evaluated by calculation of Pearson correlation coefficients. Spearman rank correlation coefficients were used for the skewed distribution of cigarette pack years. Student’s t test was used to evaluate the difference between the parameters in relation to the presence and absence of categorized traditional risk factors, including treatment with statins, aspirin, or angiotensin-converting enzyme (ACE) inhibitors. Multiple linear regression analyses were performed to assess the contribution of CRP to the prediction of annual changes of each parameter compared with the contribution of hypertension and other traditional risk factors. Two-way ANOVA with Newman–Keuls test was used to estimate between-group differences of parameters of carotid atherosclerosis in relation to hs-CRP and blood pressure. Probability values (2-tailed) of <0.05 were considered significant. For 2-way ANOVA test, Statistica for Windows R 5.5 (StatSoft) was used. The other statistical analyses were performed with SPSS for Windows version 9.0J.

Results
The baseline characteristics of the 124 subjects are summarized in Table 1. The follow-up period was 35±10 months. With regard to the relationships between hs-CRP and traditional risk factors, there was a significant association of the hs-CRP level with age (r=0.22; P<0.05), fasting blood glucose (r=0.19; P<0.05), and high-density lipoprotein cholesterol (r=−0.19; P<0.05). The relationship of CRP with cigarette pack years (r=0.17; P=0.059), pulse pressure (r=0.17; P=0.060), and SBP (r=0.15; P=0.086) was also positive but showed no statistical significance. There was no significant relationship between the hs-CRP level and the other traditional risk factors. Sex and the presence or absence of risk factors and treatment with ACE inhibitors, statins, or aspirin had no significant influence on the hs-CRP levels.

Among categorized risk factors, men had further progression than women (0.76±1.18 versus 0.43±1.00 in annual change of plaque score; P<0.05). The relationships between hs-CRP, pulse pressure, SBP, DBP, and the parameters of carotid atherosclerosis are shown in Table 2. Pulse pressure, SBP, and hs-CRP were correlated with the annual changes of plaque number and plaque score in simple regression analysis, and the correlations remained significant after adjusting for the effect of other traditional risk factors and for the baseline severity of carotid atherosclerosis. No other traditional risk factors (including DBP) were significantly correlated with the parameters of carotid atherosclerosis in simple regression analysis. When analysis was limited to the patients without hypercholesterolemia, diabetes mellitus, or current smoking, the results were similar to those in the total patient population, except that there was no significant association with SBP in the nonhypercholesterolemic or nondiabetic subgroups (Table 2). The progression of carotid atherosclerosis in relation to pulse pressure/SBP and hs-CRP is shown in Figures 2 and 3, respectively. Patients were divided into 2
groups at the median pulse pressure (53 mm Hg), an SBP of 140 mm Hg, and an hs-CRP value of 0.12 mg/dL. We reported previously that annual rate of increase in carotid atherosclerosis was accelerated in patients with an hs-CRP value of $\geq$0.12 mg/dL. Patients with higher hs-CRP levels had greater progression of atherosclerosis than those with lower hs-CRP levels in both the lower and higher pulse pressure groups and even in patients with an SBP of $<140$ mm Hg on antihypertensive therapy. When analysis was limited to 16 patients with blood pressure of $<140/90$ mm Hg on antihypertensive therapy, the relationship between hs-CRP and carotid atherosclerosis was stronger than that for pulse pressure. There were no significant relationships between the other traditional risk factors (including SBP and DBP) and the annual changes of plaque number or plaque score, except for body mass index (Table 3).

**Discussion**

This is the first study to demonstrate that evaluation of CRP could be equal or superior for predicting the development of carotid atherosclerosis to measurement of the pulse pressure or SBP in hypertensives and that its predictive value is independent of blood pressure. With respect to the association between blood pressure and carotid atherosclerosis, to the best of our knowledge, there have been few longitudinal studies focused on the middle-aged and elderly population. These studies have emphasized an elevated pulse pressure and SBP as risk factors for atherosclerosis. Similar to the results of such studies, our findings suggested that pulse pressure and SBP are related to the progression of carotid atherosclerosis. It is thought that an elevated pulse pressure causes greater stretching of the arteries, which induces fatigue and fracture of the elastic elements and thus is likely to hasten the development of intimal damage that leads to atherosclerosis. The Framingham study demonstrated a link between cardiovascular mortality and pulse

### Table 1. Baseline Characteristics of the Patients (n=124)

| Age, yr | 62.7±8.7 |
| Male   | 66 (53)  |
| Antihypertensives medication | 102 (82) |
| ACE/CCB/β-blocker | 33 (27)/74 (60)/37 (30) |
| α-blocker/diuretics | 11 (9)/5 (4) |
| SBP/DBP, mm Hg | 139±16/83±11 |
| Pulse pressure, mm Hg | 56±15 |
| Hypercholesterolemia/statin medication | 48 (39)/26 (21) |

The age, blood pressure, cholesterol, fasting blood glucose, hemoglobin A1c, and body mass index are shown as mean±SD. Data on the blood pressure, cholesterol, fasting blood glucose, and hemoglobin A1c are shown for all 124 patients. Cigarette pack years, CRP, plaque no., and plaque score are shown as the median and interquartile range. The mean values of cigarette pack years for past and current smokers and the mean plaque no. and plaque score for the patients with carotid atherosclerosis are shown in square brackets. Other values are the no. of patients, along with the proportion in parentheses.

### Table 2. Association Between hs-CRP, Blood Pressure, and Parameters Associated With the Development of Carotid Atherosclerosis

<table>
<thead>
<tr>
<th>In total patients (n=124)</th>
<th>Simple Regression</th>
<th>Multivariate Regression* (Standardized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPN/yr hs-CRP</td>
<td>0.314</td>
<td>0.001</td>
</tr>
<tr>
<td>ΔPS/yr hs-CRP</td>
<td>0.328</td>
<td>0.001</td>
</tr>
<tr>
<td>In patients without hypercholesterolemia (n=76)</td>
<td>0.343</td>
<td>0.002</td>
</tr>
<tr>
<td>ΔPN/yr</td>
<td>0.281</td>
<td>0.002</td>
</tr>
<tr>
<td>ΔPS/yr</td>
<td>0.290</td>
<td>0.001</td>
</tr>
<tr>
<td>In patients without diabetes mellitus (n=103)</td>
<td>0.210</td>
<td>0.068</td>
</tr>
<tr>
<td>ΔPN/yr</td>
<td>0.238</td>
<td>0.004</td>
</tr>
<tr>
<td>ΔPS/yr</td>
<td>0.256</td>
<td>0.009</td>
</tr>
<tr>
<td>In patients without any traditional risk factors (n=110)</td>
<td>0.180</td>
<td>0.070</td>
</tr>
<tr>
<td>ΔPN/yr</td>
<td>0.258</td>
<td>0.006</td>
</tr>
<tr>
<td>ΔPS/yr</td>
<td>0.316</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ΔPN/yr indicates annual change of plaque number; ΔPS/yr, annual change of plaque score.

* Each parameter of blood pressure, together with hs-CRP and other traditional risk factors, was used as an independent variable in each multivariate regression model. The standardized β and P values of hs-CRP in Table 2 are adjusted for pulse pressure, age, sex, total cholesterol, hemoglobin A1c, cigarette pack years, body mass index, the severity of carotid atherosclerosis, and use of ACE inhibitor, statin, and aspirin. When SBP or DBP was used instead of pulse pressure as a parameter of blood pressure, the standardized β and P values of hs-CRP were similar to those in Table 2. hs-CRP indicates high sensitivity C-reaction protein; PN/yr, annual change of plaque number; PS/yr, annual change of plaque score.
pressure by longitudinal follow-up of persons >50 years old.16 Another large-scale study revealed similar results in male subjects aged 40 to 69 years,17 whereas the age of the present study population was similar. We found that there was no significant relationship between the other traditional risk factors (including DBP) and the progression of carotid atherosclerosis. The lack of an association with these risk factors in the present study can be partly explained by the influence of drug therapy and lifestyle modification or the low statistical power of our analysis. The age of the present study population may also help to explain the lack of an association between DBP and carotid atherosclerosis.

Multivariate analysis revealed that CRP was one of the independent predictors of the progression of carotid atherosclerosis. Subset analysis excluding each traditional risk factor showed a similar result. One possible reason that a high CRP level is associated with carotid atherosclerosis independently of other risk factors is that CRP is a marker of inflammation. Inflammation is known to promote the development of atherosclerosis, and CRP is involved in the inflammatory process. The association between CRP and carotid atherosclerosis may be due to the fact that CRP is a marker of inflammation and is involved in the inflammatory process.

**Figure 2.** The annual changes of plaque number (top) and plaque score (bottom) in relation to pulse pressure and hs-CRP. ■ hs-CRP ≥0.12 mg/dL, □ hs-CRP < 0.12 mg/dL. Bars represent mean values and lines represent the SEM. *P<0.001; †P<0.01; ‡P<0.05.

**Figure 3.** The annual changes of plaque number (top) and plaque score (bottom) in relation to SBP and hs-CRP. ■ hs-CRP ≥0.12 mg/dL, □ hs-CRP < 0.12 mg/dL. Bars represent mean values and lines represent the SEM. *P<0.001; †P<0.01; ‡P<0.05.

**Table 3.** Association Between hs-CRP Concentration, Blood Pressure, and Parameters Associated With the Development of Carotid Atherosclerosis in 64 Hypertensive Patients With Blood Pressure of <140/90 mm Hg on Antihypertensive Therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ΔPN/yr r</th>
<th>ΔPN/yr P</th>
<th>ΔPS/yr r</th>
<th>ΔPS/yr P</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>0.404 (0.23, 0.91)</td>
<td>0.002</td>
<td>0.436 (0.48, 1.80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>0.264 (0.015, −0.34)</td>
<td>0.044</td>
<td>0.310 (0.05, −1.8)</td>
<td>0.016</td>
</tr>
<tr>
<td>SBP</td>
<td>0.225</td>
<td>0.086</td>
<td>0.237</td>
<td>0.069</td>
</tr>
<tr>
<td>DBP</td>
<td>−0.007</td>
<td>0.958</td>
<td>−0.040</td>
<td>0.761</td>
</tr>
<tr>
<td>Age</td>
<td>−0.198</td>
<td>0.134</td>
<td>−0.183</td>
<td>0.162</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.078</td>
<td>0.558</td>
<td>0.011</td>
<td>0.931</td>
</tr>
<tr>
<td>Hemoglobin A1c</td>
<td>−0.021</td>
<td>0.882</td>
<td>−0.016</td>
<td>0.907</td>
</tr>
<tr>
<td>Cigarette pack years</td>
<td>0.004</td>
<td>−0.977</td>
<td>0.012</td>
<td>0.930</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.334 (0.048, −0.73)</td>
<td>0.010</td>
<td>0.272 (0.12, −2.36)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

ΔPN/yr indicates annual change of plaque number; ΔPS/yr, annual change of plaque score. The values in parentheses show the regression coefficient and intercept.
dently of blood pressure and other traditional risk factors may be the tight linkage of CRP with atherosclerotic processes. For example, CRP may contribute to monocyte recruitment in atherogenesis and to induction of tissue factor release by monocytes, which is potentiated by interferon-γ and lipopolysaccharide. CRP has a direct influence on atherosclerotic vessels by activation of the complement system, thereby promoting inflammation and thrombosis. A recent clinical study showed that CRP was significantly correlated with the calculated 10-year Framingham coronary heart disease risk (FCHDR) but was weakly correlated with most individual components of the FCHDR score. This suggested that CRP may capture different components than the traditional components of coronary risk reflected in the FCHDR score. Thus, monitoring of the blood pressure is important but not enough to predict the development of atherosclerosis in hypertensives. The average of 2 consecutive office blood pressure measurements at 1 time point was representative of the blood pressure value in the present study. A recent study suggested that circadian SBP variability is the best independent predictor of the development of carotid atherosclerosis, whereas a cross-sectional study revealed that target organ damage caused by hypertension is more closely related to the home blood pressure than the office blood pressure. The serum level of CRP may partly reflect the circadian blood pressure pattern or home blood pressure, or may be an indicator of a step in the process of atherosclerosis itself, making it equal or superior to office blood pressure measurement for the prediction of atherosclerosis.

Chronic inflammation may induce endothelial dysfunction, which is followed by further elevation of blood pressure (pulse pressure and SBP) and the onset of cardiovascular disease. Several studies have shown that CRP is an independent risk factor for hypertension, so CRP, inflammation, and hypertension appear to be linked in the process of atherosclerosis. A recent study suggested that inflammation is important for accelerated progression of atherosclerosis, particularly in hypertensives. Although the relationship of CRP with pulse pressure and SBP was positive in the present study, it did not reach statistical significance. This lack of a significant association might be attributable to the low statistical power of our analysis or use of antihypertensive medication by the subjects, or it may indicate that the actual association is weak.

It could be argued that our results were influenced by a selection bias of the patient population because most of them were on antihypertensive therapy and some had other traditional risk factors. However, the relationship of pulse pressure, SBP, and CRP with carotid atherosclerosis remained significant after adjusting for antihypertensive therapy and other traditional risk factors, and stratified analysis showed similar results. Recent guidelines have proposed that the entire adult population should not be screened for CRP measurement for purposes of cardiovascular risk assessment but that the measurement may be useful in selected patients, such as those estimated to have a moderate risk on the basis of the 10-year FCHDR. The risk management in the present study population was similar to the FCHDR concept of moderate risk, and we demonstrated that CRP was equal or superior to the office blood pressure for predicting the progression of carotid atherosclerosis, with these parameters being independent of each other. In conclusion, measurement of CRP may be valuable for predicting the progression of carotid atherosclerosis in selected hypertensive patients who are already being treated by drug therapy or lifestyle modification.

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References


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