Relations of Serum High-Sensitivity C-Reactive Protein and Interleukin-6 Levels With Silent Brain Infarction

Taku Hoshi, MD; Kazuo Kitagawa, MD, PhD; Hiroshi Yamagami, MD, PhD; Shigetaka Furukado, MD; Hidetaka Hougaku, MD, PhD; Masatsugu Hori, MD, PhD

Background and Purpose—Small silent brain infarction (SBI) is often found on magnetic resonance (MR) images of apparently healthy individuals at cardiovascular risk. Particularly, small SBI found in subcortical white matter, basal ganglia, or thalamus is thought to be caused by cerebral small vessel disease. Although several lines of evidence suggest a role of inflammatory processes in atherothrombotic vascular events, their involvement in SBI remains to be determined. This study examines the associations between serum inflammatory markers and SBI as a manifestation of cerebral small vessel disease.

Methods—One hundred ninety-four patients without histories of cardiovascular accidents were prospectively enrolled for this study. All patients underwent brain MR imaging and carotid ultrasonography, and patients with SBI diagnosed underwent further MR angiography. As common inflammatory markers, serum levels of high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) were evaluated.

Results—SBIs were found in 40 patients, and all of those were located in subcortical and infratentorial area, without MR angiographic evidence for obstructive lesions in proximal cerebral arteries. Mean hsCRP and IL-6 levels were higher in patients with SBI than in those without. Also, higher levels of both hsCRP (odds ratio [OR], 1.85 per standard deviation [SD] increase) and IL-6 (OR, 2.00/SD increase) were associated with higher likelihood for SBI. Moreover, the associations were only slightly attenuated when adjusting traditional cardiovascular risk factors and carotid IMT.

Conclusions—Higher levels of hsCRP and IL-6 appear to be associated with small SBI, suggesting a role of inflammatory processes in cerebral small vessel disease.

Key Words: inflammation ■ interleukins ■ magnetic resonance imaging

Silent brain infarction (SBI), often seen on brain magnetic resonance (MR) images of healthy elderly individuals, is associated with increased risk for stroke and cognitive decline.1 In previous studies, >90% of such SBIs were small (<15 mm in diameter) and found in subcortical white matter, basal ganglia, thalamus, or infratentorial region.2–5 Also, autopsy studies have shown that cerebral small vessel disease underlies such asymptomatic brain lesions.6–8 Although several risk factors have been identified for the occurrence of SBI, including age, hypertension, diabetes,5,10 homocysteine,11–13 and carotid intima-media thickness,14,15 whether inflammatory processes are involved in its cause remains to be determined.

Recent studies in vascular biology have shown that chronic inflammation plays a crucial role in the development of atherosclerosis.16 Particularly, several lines of evidence suggest the value of measuring serum levels of inflammatory markers, such as high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6), for predicting stroke and other cardiovascular events.17–20 Additionally, such inflammatory markers have been associated with plaque progression and its instability in large arteries.21,22 However, we are unaware of studies investigating the involvement of inflammation in SBI.

In the current study, we examine the associations of hsCRP and IL-6 with SBI in neurologically asymptomatic patients at cardiovascular risk to explore the relationships between inflammation and cerebral small vessel disease.

Patients and Methods

Patients

The subjects for this study were enrolled from neurologically asymptomatic patients who consecutively visited the Department of Internal Medicine and Therapeutics at Osaka University Hospital between April 2002 and December 2003. The majority of patients had been referred from another hospital or department for the risk assessment and primary prevention of stroke. At the time of referral, comprehensive neurological evaluations were performed by our stroke neurologists, including physical and psychological examinations. When no neurological signs/symptoms were identified, patients were found to be candidates for this study. Thus, patients with histories of stroke or other neurological disease were not included in

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the study sample. Additionally, patients who had ever experienced nonspecific neurological symptoms, such as dizziness, vertigo, headache, tinnitus, and syncope, were included, but only if the symptoms were not present at the time of neurological evaluations. Given the nature of study sample, many of the patients had cardiovascular risk factors, including hypertension, hyperlipidemia, and diabetes.

During the study period, 236 patients were found to be potential candidates for this study. However, patients with ischemic heart disease (n = 24) or peripheral vascular disease (n = 7) were excluded. Additionally, patients with collagen disease (n = 5), malignant disease (n = 2), or acute viral infection (n = 4) were excluded, because such conditions could increase the levels of inflammatory markers, potentially modifying the relationships between inflammatory markers and SBI. Consequently, this study comprised 194 neurologically asymptomatic patients (mean ± standard deviation age, 67.3 ± 7.5 years) who subsequently underwent brain MR imaging.

This study was approved by the Ethics Committee of Osaka University Graduate School of Medicine. All patients gave written informed consent before entry to the current study.

Diagnosis of SBI
All MR imaging was performed with 1.5-T Signa Horizon (GE Medical Systems) or 1.5-T Magnetom Vision (Siemens). The whole brain was scanned, and 20 axial images were produced; slice thickness was 5 mm and interslice gap was 2 mm. The imaging protocol was consisted of a T2-weighted spin-echo (repetition time/echo time [TR/TE] = 5000/130 ms), T1-weighted spin-echo (TR/TE = 500/9 ms), and fluid-attenuated inversion-recovery (TR/TE = 8000/155 ms, inversion time = 2000 ms) imaging.

A single trained physician who was blinded to patients’ clinical details evaluated the existence, location, and size of brain infarcts on MR images. Thereby, SBI was defined as an area of focal hyperintensity on T2-weighted images with corresponding low signal intensity on T1-weighted images, which was ≥3 mm in diameter. Also, the diagnosis was made only when such a lesion was surrounded by hyperintense gliotic rim on fluid-attenuated inversion-recovery images to exclude dilated perivascular space.

When patients had SBI diagnosed, they subsequently underwent MR angiography to explore the existence of large vessel disease that could explain the cause of SBI.

Measurement of Serum Inflammatory Markers
After MR examination, blood was drawn with minimally traumatic venipuncture for measurement of serum inflammatory markers. Blood was then centrifuged at 3000 rpm at 4°C for 15 minutes, and aliquots were stored at −70°C. Circulating hsCRP was measured by latex turbidimetric immunoassay with a sensitivity of 0.01 mg/dL. (Shionogi Biomedical Laboratory Inc). Serum IL-6 was measured by enzyme-linked immunosorbent assay (High Sensitivity Quantikine kit; R&D System). The detectable limit for IL-6 was 0.10 pg/mL.

Evaluation of Cardiovascular Risk Factors
Supine blood pressure was measured before the MR imaging examination. Fasting blood glucose, serum total cholesterol, high-density lipoprotein cholesterol, triglycerides, and serum creatinine levels were determined from the blood sample taken for inflammatory marker evaluation. Information on patient medical history and medication use was obtained from the clinical records, with investigators blinded to the MR findings. Hypertension was defined by the diagnosis of raised blood pressure (≥140/90 mm Hg) or by current use of antihypertensive agents. Diabetes mellitus was defined by fasting blood glucose level ≥7.0 mmol/L or by use of glucose-lowering agents. Hyperlipidemia was defined by fasting serum total cholesterol level >5.7 mmol/L, triglycerides level >1.7 mmol/L, or by use of cholesterol-lowering agents. Smoking status was evaluated based on self-reports. Patients were categorized according to their smoking habits as nonsmoker (never smoked) or ever-smoker (smoked habitually at some time in their lives).

Evaluation of Carotid Atherosclerosis
Duplex carotid ultrasonography was performed to evaluate the severity of carotid atherosclerosis. All ultrasound examinations were performed with a Phillips SONOS 5500 equipped with a 7.5-MHz linear-array transducer. The intima-media thickness (IMT), defined as the distance between the intimal-luminal interface and the medial-adventitial interface, was measured as previously described.21 We calculated the mean carotid artery IMT (mean IMT) by averaging the thickness at 12 sites: the near and far walls of both the right and left distal common carotid artery, carotid bifurcation, and internal carotid artery.

Statistical Analyses
Because the distributions of hsCRP and IL-6 levels appeared to be left-skewed, they were normalized by logarithmic transformation. To analyze the associations between inflammatory markers and patient characteristics, we used Pearson correlation analysis and 2-sample t test. To compare prevalence of SBI by the tertile of hsCRP and IL-6, we used χ² test. To analyze the relation between SBI and patient characteristics, we used χ² test for categorical data and 2-sample t test for continuous data. Odds ratio was calculated for the likelihood of SBI by multivariate logistic regression analyses, in which logarithmically transformed values of inflammatory markers were used. Of note, odds ratio was shown per standard deviation in log (inflammatory marker) increase. Probability values were 2-tailed, and values of P < 0.05 were considered significant. All statistical analyses were performed with SPSS 11.5J (SPSS Japan Inc).

Results
Patient Characteristics
Forty patients (21%) were found to have 1 or more SBIs on MR images (26 patients had a single infarct, whereas 14 had from 2 to 8 infarcts). Also, 50% of infarcts were located in the subcortical white matter (corona radiata, centrum semiovale, subcortical frontal, temporal and parietal lobes), and 45% were in the basal ganglia and thalamus (Figure 1). No patients had cortical infarcts in the study sample. Additionally, all infarcts (n = 72) were <15 mm in diameter, and 97% of those were <10 mm. By MR angiographic examinations, 38 of 40 patients had no significant obstructive lesions in the proximal cerebral arteries. Although 2 patients had stenotic lesions (50% to 75%) in the horizontal portion of middle cerebral arteries, they were on the contralateral side to SBI.

Relation Between SBI and Inflammatory Markers
By univariate analysis, age, BMI, prevalence of hypertension, and systolic blood pressure were higher in patients with SBI than in those without, and so were hsCRP and IL-6 levels (Table 1). Additionally, prevalence of SBI was higher in the highest tertile of hsCRP level than in the lowest or middle tertile (Figure 2). Also, the prevalence was higher in the middle and highest tertiles of IL-6 level than in the lowest tertile.

Associations between inflammatory markers levels and SBI are summarized in Table 2. In unadjusted analysis, each 1 SD greater log hsCRP and each 1 SD greater log IL-6 were associated with 1.85-fold and 2.00-fold higher likelihood for SBI, respectively. Adjustments for age and sex modified these associations only slightly (model 1). After additional adjustments for traditional cardiovascular risk factor and medication use (model 2), both hsCRP and IL-6 remained to be associated with SBI. After further adjustments for mean IMT (model 3), these associations persisted.

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Discussion

Recently, SBI has attracted much attention because it increases the risk for future stroke\(^1\) and dementia.\(^2\)\(^4\) In the present study, levels of hsCRP and IL-6 were higher in patients with SBI than in those without. Also, higher levels of such markers were associated with higher likelihood for SBI. To the best of our knowledge, this is the first study that demonstrates the associations between inflammatory markers and SBI.

**TABLE 1. Baseline Characteristics of Patients With and Without Silent Brain Infarction**

<table>
<thead>
<tr>
<th>Index</th>
<th>All Patients (n=194)</th>
<th>No (n=154)</th>
<th>Yes (n=40)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>67.3±7.5 (66.5±7.6)</td>
<td>70.6±6.0 (70.6±6.0)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Male sex, no. (%)</td>
<td>93 (48)</td>
<td>69 (45)</td>
<td>24 (60)</td>
<td>0.087</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>23.0±2.7 (22.8±2.6)</td>
<td>23.8±3.3 (23.8±3.3)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>128 (66)</td>
<td>94 (61)</td>
<td>34 (85)</td>
<td>0.004</td>
</tr>
<tr>
<td>Diabetes mellitus, no. (%)</td>
<td>31 (16)</td>
<td>21 (14)</td>
<td>10 (25)</td>
<td>0.081</td>
</tr>
<tr>
<td>Hyperlipidemia, no. (%)</td>
<td>130 (67)</td>
<td>101 (66)</td>
<td>29 (73)</td>
<td>0.4</td>
</tr>
<tr>
<td>Atrial fibrillation, no. (%)</td>
<td>5 (3)</td>
<td>3 (2)</td>
<td>2 (5)</td>
<td>0.6</td>
</tr>
<tr>
<td>Ever smoker, no. (%)</td>
<td>78 (40)</td>
<td>59 (38)</td>
<td>19 (48)</td>
<td>0.3</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>137.3±15.0 (135.3±14.6)</td>
<td>145.1±14.4 (145.1±14.4)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>81.4±10.0 (81.3±10.0)</td>
<td>81.8±9.9 (81.8±9.9)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.50±0.83 (5.54±0.85)</td>
<td>5.41±0.75 (5.41±0.75)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.54±0.41 (1.50±0.39)</td>
<td>1.46±0.41 (1.46±0.41)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.45±0.67 (1.44±0.68)</td>
<td>1.31±0.56 (1.31±0.56)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>5.63±1.02 (5.58±1.02)</td>
<td>5.79±1.03 (5.79±1.03)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.80±0.40 (0.76±0.24)</td>
<td>0.95±0.73 (0.95±0.73)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Mean IMT, mm</td>
<td>0.99±0.24 (0.97±0.23)</td>
<td>1.05±0.26 (1.05±0.26)</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>Medication use, no. (%)</td>
<td>105 (54)</td>
<td>81 (53)</td>
<td>24 (60)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Inflammatory marker**

| High-sensitivity CRP, mg/dL   | 0.13±0.42 (0.05)     | 0.08±1.69 (0.04) | 0.33±0.86 (0.08) | 0.004 |
| Interleukin-6, pg/mL          | 2.08±3.57 (1.32)     | 1.67±1.96 (1.20) | 3.63±6.69 (1.89) | <0.001 |
| Mini-Mental State Examination | 28.1±2.0             | 28.1±2.2         | 28.0±1.6         | 0.6    |

Values are unadjusted mean±SD (median) or no. of patients (percentage).

\(P\) values are \(t\) test for the categorical data, and 2 sample t test for the continuous data.

Medication use means the percentage of patients who are using at least one anti-inflammation agent; Aspirin, HMG-CoA reductase inhibitors, angiotensin converting enzyme-inhibitors and angiotensin receptor blockers.

*Statistical tests performed in logarithmically transformed variables.
In previous studies, prevalence of SBI is reported to be 10.6% to 24.8% in apparently healthy individuals. In the current study, 21% (40 out of 194) patients were found to have SBI, which is not surprising when the nature of our study sample is taken into account. Also, 95% of SBIs were located in the subcortical white matter, basal ganglia, or thalamus, and 97% of those were <10 mm (Figure 1). These findings are consistent with the commonly known features of SBI. Moreover, MR angiographic examinations revealed no significant obstructive lesions that could explain the occurrence of such SBI. Taken together, SBI found in this study is likely to be the manifestation of cerebral small vessel disease.

Previous studies have shown associations of SBI with age, hypertension, and diabetes. In line with such studies, age and prevalence of hypertension were higher in patients with SBI than in those without (Table 1). Also, hsCRP and IL-6 levels were higher in SBI patients than in those without (Table 1), suggesting an enhanced level of chronic inflammation in SBI patients. Moreover, prevalence of SBI increased in a stepwise fashion across the tertiles of hsCRP and IL-6 levels (Figure 2). Additionally, increases in such inflammatory markers were associated with higher likelihood for SBI (Table 2, model 1), and the associations persisted when traditional cardiovascular risk factors were adjusted (Table 2, model 2). These findings suggest link between inflammation and SBI. Of note, recent studies have shown associations of SBI with age, hypertension, and diabetes. Given such studies, we have performed additional analysis with carotid IMT taken into account. When such parameters were included in the model (Table 2, model 3), the association between inflammatory markers and SBI were only slightly modified, further supporting their linkages.

Our findings suggest that inflammation is related to pathologic changes in cerebral small vessels that cause lacunar infarction. By measuring soluble plasma markers (eg, soluble intercellular adhesion molecule-1, soluble endothelial leukocyte adhesion molecule, and thrombomodulin), previous studies have shown inflammatory endothelial activation and endothelial dysfunction in patients with cerebral small vessel disease. Also, autopsy examinations revealed migration of foamy macrophages in the vessel walls of cerebral arterioles together with hyaline thickening and ectasia of parenchymal arteries in 15 out of 20 patients with ischemic vascular dementia. These clinical and autopsy studies may support our result. In other words, although whether inflammatory endothelial activation induces arteriosclerosis and lipohyalinosis is not established, it is likely that chronic inflammatory response in cerebral small vessels is involved in the pathology of this microangiopathy.

This study has some limitations. First, because the current study is cross-sectionally designed, we cannot refer to the causal relationships between inflammatory markers and SBI. Second, recent studies have reported that elevated level of plasma homocysteine is associated with the risk factor for SBI, which could potentially impact on the relationships between inflammatory markers and SBI. Third, although SBI found in this study is likely to represent cerebral small vessel disease, possibility of cardiogenic or arteriogenic emboli cannot be completely denied. Taken together, extensive prospective studies are necessary to establish the link between inflammation and cerebral small vessel disease.

In conclusion, this study demonstrates that levels of circulating hsCRP and IL-6 are associated with SBI independent of traditional cardiovascular risk factors, suggesting an involvement of inflammation in cerebral small vessel disease.

### Table 2. OR (95% CI) for The Prevalence of SBI According to Levels of Inflammatory Markers

<table>
<thead>
<tr>
<th>inflammatory marker</th>
<th>Unadjusted OR (95% CI)</th>
<th>Model 1 OR (95% CI)</th>
<th>Model 2 OR (95% CI)</th>
<th>Model 3 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP, mg/dL per SD in log (hsCRP) increase</td>
<td>1.85 (1.29–2.63)</td>
<td>1.73 (1.20–2.51)</td>
<td>1.49 (1.00–2.22)</td>
<td>1.50 (1.00–2.24)</td>
</tr>
<tr>
<td>IL-6, pg/mL per SD in log (IL-6) increase</td>
<td>2.00 (1.39–2.88)</td>
<td>1.87 (1.29–2.71)</td>
<td>1.85 (1.24–2.78)</td>
<td>1.85 (1.24–2.78)</td>
</tr>
</tbody>
</table>

Model 1, adjusted for age and sex.
Model 2, adjusted for age, sex, BMI, smoking, hypertension, diabetes mellitus, hyperlipidemia and medication use.
Model 3, adjusted for age, sex, BMI, smoking, hypertension, diabetes mellitus, hyperlipidemia, medication use and mean IMT.
SD for log (hsCRP)=0.55; SD for log (IL-6)=0.30.
Acknowledgments
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References
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