Circulating Cell Adhesion Molecules Are Correlated With Ultrasound-Based Assessment of Carotid Atherosclerosis


Abstract—Although cellular adhesion molecules (CAMs) are hypothesized to play an important role in atherogenesis, the relationship between CAMs and systemic atherosclerosis is uncertain. Among 92 outpatients (48 men; mean ± SD age, 65 ± 9 years), we evaluated the association of soluble vascular CAM-1 (sVCAM-1) and intercellular adhesion molecule-1 (sICAM-1) with carotid intimal-medial thickness (IMT), an index of early atherosclerosis. All subjects underwent a 2-dimensional ultrasound examination of both carotid arteries at the distal common carotid arteries and bifurcation. sVCAM-1 and sICAM-1 levels measured by enzyme-linked immunosorbent assay were significantly correlated with mean IMT of the common carotid artery (r = 0.34 and r = 0.30, respectively; P < 0.01) and carotid bifurcation (r = 0.31 and r = 0.26, respectively; P < 0.05), whereas sVCAM-1 was also positively associated with maximal carotid IMT (r = 0.35, P < 0.01). Adjustment for age attenuated the association between sVCAM-1 and common (r = 0.16, P = 0.13) and bifurcation (r = 0.18, P = 0.07) carotid IMT but had minimal effect on the associations between sICAM-1 and carotid measurements (r = 0.32, P < 0.01; r = 0.23, P < 0.05; for common and bifurcation IMT, respectively). Age-adjusted sICAM-1 levels increased in a stepwise fashion across common carotid IMT tertiles (253 ± 27 versus 275 ± 24 versus 384 ± 26 pg/mL for the lowest, intermediate, and highest IMT tertiles, respectively; P < 0.01). A similar trend was also found between sVCAM-1 levels and common carotid IMT tertiles (625 ± 60 versus 650 ± 53 versus 714 ± 58 pg/mL; P < 0.15). These associations were minimally affected in analyses adjusting for hypertension, diabetes, smoking, low and high density lipoprotein cholesterol, lipoprotein(a), and homocysteine, or in a subgroup analysis limited to those with no prior history of atherothrombotic disease. These data demonstrate a positive association between serum CAMs with carotid IMT and further support the hypothesis that systemic inflammation may have a role in atherosclerotic lesion development. (Arterioscler Thromb Vasc Biol. 1998;18:1765-1770.)

Key Words: adhesion molecules ■ atherosclerosis ■ ultrasound ■ inflammation

Cellular adhesion molecules (CAMs) mediate the adhesion and migration of leukocytes, steps that have been hypothesized to play a critical role in early atherogenesis.12 Focal expression of CAMs has been demonstrated in atherosclerotic lesions,1-5 precedes leukocyte infiltration,6,7 and appears to be mediated in part by modified lipoproteins or their constituents.8,9 However, data evaluating circulating CAMs and clinical atherosclerotic disease are limited. Two recent reports measured levels of soluble CAMs in patients with peripheral vascular disease and found contradictory results.9,10 In addition, Caterina et al11 recently demonstrated a significant association between soluble vascular cell adhesion molecule-1 (sVCAM-1) with carotid atherosclerosis in a small group of hypertensive patients. Conversely, Hwang et al12 failed to detect a significant association between sVCAM-1 and carotid and coronary disease in a case-control study.

Quantitative measures of mild vascular lesions and wall irregularities in the carotid system can be explored by ultrasound interrogation.13 Although it is not known why specific areas of thickening eventually progress to fully developed plaques and obstructive lesions, measurements of carotid intimal-medial thickness (IMT) are often used in epidemiological studies as a surrogate for early atherosclerosis.14 Carotid IMT increases with age,15 is correlated with traditional cardiovascular risk factors,16,17 and identifies subjects at increased risk of severe coronary artery disease17-19 and cerebrovascular morbidity.20

To further explore the relationship of inflammatory markers and atherosclerosis, we performed a cross-sectional survey of 92 outpatients and evaluated the association between circulating levels of sVCAM-1 and soluble intercellular adhesion molecule-1 (sICAM-1) with ultrasound-based carotid IMT.

Methods

Patients

From March 1997 to July 1997, outpatients aged 50 years or older who were referred to the Noninvasive Cardiac Laboratory of the Brigham and Women’s Hospital were eligible for the study. The presence of peripheral vascular disease, coronary artery disease, or cerebrovascular disease was determined by review of medical records. To further exclude subjects with severe atherosclerosis, we excluded 7 patients who had a history of myocardial infarction or stroke within the past 3 years. Patients were referred to the Noninvasive Cardiac Laboratory for clinical assessment of cardiovascular disease. All patients had been referred for routine clinical evaluation and were not participating in any clinical trials. The study was approved by the institutional review board at the Brigham and Women’s Hospital.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
Carotid Imaging
Each patient underwent a detailed ultrasound evaluation of the carotid arteries. These examinations were performed by an experienced ultrasonographer using commercially available equipment (Hewlett-Packard Sonos 2500, Hewlett-Packard Medical Products) and a 5.5/7.0-MHz linear transducer. The carotid image acquisition protocol was adapted from the Atherosclerosis Risk in Communities (ARIC) Study.21 In brief, images were obtained with the patients in the supine position with the neck mildly extended and the head rotated contralaterally to the side. The imaging protocol involved obtaining longitudinal, lateral, and anterior oblique views of the distal 10 mm of the right and left common carotid arteries, the carotid bifurcation, and the internal carotid artery. Because high-quality images in the internal carotid artery were obtained in <50% of the subjects, internal carotid IMTs were not analyzed in this study. The images were digitized and all examinations were evaluated according to standard recommendations26 by a single cardiologist (L.E.R.). In brief, lines were drawn along the lumen-intima and media-adventitial interfaces in the far wall of the common carotid artery and carotid bifurcation, and a mean IMT was computed in each region. For the purpose of statistical analysis, right and left measurements were averaged. Maximal thickness in any particular region was also measured, irrespective of location. The maximal IMT index was created to integrate data concerning the highest levels of thickening or obstruction in any level of the carotid system. Patients were also classified according to the presence of well-defined atherosclerotic plaques. Plaques were defined by the presence of focal, severe wall thickening (IMT >2 mm), wall irregularities, and calcification. Interrater reproducibility of the same carotid IMT measurements was evaluated in a subset of patients (n=20). Correlation coefficients in this blinded assessment exceeded 98%. Ultrasound technicians and reader were unaware of serum analysis results.

Blood Measurements
EDTA-anticoagulated samples were obtained by nontraumatic venipuncture by using a 19-gauge butterfly catheter. Blood was then centrifuged for 20 minutes at 2500 rpm, and aliquots were stored at −70°C. sVCAM-1 and sICAM-1 were measured in duplicate by a sandwich ELISA based on purified proteins and polyclonal antibodies (R&D Systems) according to the manufacturer’s recommendations. Reported sensitivity of the assays for sVCAM-1 and sICAM-1 is <2 and 0.35 ng/mL, respectively. Methods used to measure LDL and HDL cholesterol, lipoprotein(a), and total homocysteine have been described elsewhere.21–23

Statistical Analysis
Unless otherwise noted, data are expressed as mean±SD. Spearman correlation coefficients were calculated to evaluate evidence of the association between carotid and serum measurements. In addition, carotid measurements were categorized into 3 groups according to tertiles of the distribution. The significance of any differences in serum markers on the categorized carotid groups was computed by 1-way ANOVA. To control for multiple comparisons, Tukey’s test was used. To evaluate the association between serum levels and carotid measurements after controlling for other cardiovascular risk factors, adjusted means of serum levels were estimated by using general linear models. Adjusted associations were controlled for

Table 1 shows the baseline characteristics of the 92 outpatients studied (mean±SD age, 65±9 years; 48% female). Approximately half had a history of hypertension or hypercholesterolemia, <10% were current smokers, and 56% were former smokers. Previous myocardial infarction had occurred in 18 (20%) patients, and 7 (8%) had a history of previous neurological events (5 were transient ischemic episodes). Reasons for ordering the echocardiogram included left ventricular functional evaluation (34%), valvular assessment (31%), coronary artery disease (14%), arrhythmia (5%), preoperative evaluation (5%), or others (11%).

Mean levels of sVCAM-1 and sICAM-1 were 664±315 and 305±142 ng/mL, respectively (medians were 572 and 270 ng/mL). The lipid profile and other serum measurements are also described in Table 1. Mean IMT in the carotid bifurcation was greater than in the common carotid artery (1.2±0.3 versus 0.9±0.2 mm, P<0.05). Well-defined plaques were observed in 29 patients (31%) (all with wall thickening >2 mm), although no severe obstruction was identified. Common carotid IMT

### Table 1. Clinical Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>N=92 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean±SD, y</td>
<td>65±9</td>
</tr>
<tr>
<td>Male/female</td>
<td>48 (52/44 (48)</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>50 (54)</td>
</tr>
<tr>
<td>Hypercholesterolemia*</td>
<td>45 (49)</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>17 (18)</td>
</tr>
<tr>
<td>Family history of premature IHD†</td>
<td>21 (23)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Past</td>
<td>52 (56)</td>
</tr>
<tr>
<td>Stable angina</td>
<td>13 (14)</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>18 (20)</td>
</tr>
<tr>
<td>Coronary revascularization</td>
<td>11 (12)</td>
</tr>
<tr>
<td>Previous neurological events</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Echocardiogram ejection fraction, %</td>
<td>62±16</td>
</tr>
<tr>
<td>Serum levels</td>
<td></td>
</tr>
<tr>
<td>sVCAM-1, ng/mL</td>
<td>664±315</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
<td>305±142</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>199±42</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>48±14</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>118±30</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>30±28</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>11±5</td>
</tr>
</tbody>
</table>

IHD indicates ischemic heart disease.

*Defined by clinical history.
†Defined as myocardial infarction or sudden death before age of 55 for the patient’s father (or other male first-degree relative), and before the age of 65 for the patient’s mother (or other female first-degree relative).
tertiles were defined as follows: lowest (IMT ≤ 0.7 mm), intermediate (0.7 < IMT ≤ 0.9 mm), and highest (IMT > 0.9 mm). Carotid bifurcation IMT tertiles were also defined as lowest (IMT ≤ 0.95 mm), intermediate (0.95 < IMT ≤ 1.25 mm), and highest (IMT > 1.25 mm), as were maximal IMT tertiles: lowest (IMT ≤ 1.0 mm), intermediate (1.0 < IMT ≤ 1.6 mm), and highest (IMT > 1.6 mm).

Unadjusted Association Between Carotid Parameters and Serum Measurements

The correlation coefficients between sVCAM-1 and sICAM-1 and carotid parameters are presented in Table 2. sVCAM-1 and sICAM-1 levels were correlated significantly with mean IMT of the common carotid artery (r=0.34 and r=0.30, respectively; P<0.01) and carotid bifurcation (r=0.31 and r=0.26, respectively; P<0.01). sVCAM-1 was also positively associated with maximal carotid IMT (r=0.35, P<0.001). Subgroup analysis of patients without prior manifestations of atherothrombotic vascular disease (no history of angina, myocardial infarction, revascularization, or previous neurological events) also demonstrated a statistically significant association between CAMs and most of the carotid variables evaluated (Table 2). No other serum marker was significantly associated with IMT measurements, except for a trend for a negative association between HDL levels and common carotid, bifurcation, and maximal IMT.

Risk Factor–Adjusted sVCAM-1 and sICAM-1 Levels

Tables 3 and 4 present estimated sVCAM-1 and sICAM-1 levels according to carotid tertiles after individual adjustment for age, hypertension, diabetes, current smoking, lipids, and homocysteine. Estimated sVCAM-1 levels were significantly correlated with carotid parameters after adjustment for these risk factors, except for age-adjusted level in common carotid artery IMT tertiles. Estimated sICAM-1 values also remained positively associated with common carotid IMT after adjustment. As noted, most of the risk factors evaluated had minimal impact on the association between sVCAM-1 or sICAM-1 and carotid IMT.
We further evaluated the impact of other risk factors on CAM levels. sVCAM-1 was significantly associated with age only ($r=0.41$, $P<0.001$), whereas sICAM-1 levels were negatively associated with HDL levels ($r=-0.27$, $P<0.01$). In multivariate analysis after adjustment for age, history of hypertension, hypercholesterolemia, diabetes, family history of ischemic heart disease, lipids, and homocysteine levels, sVCAM-1 ($P<0.01$) was the only variable that remained significantly associated with maximal IMT, whereas sICAM-1 ($P<0.05$) and age ($P<0.01$) were the only independent correlates for both common carotid and bifurcation IMTs.

**Discussion**

These data demonstrate a direct association between plasma concentrations of CAMs (sVCAM-1 and sICAM-1) and ultrasound-based assessment of carotid IMT. Furthermore, this association appears to be minimally influenced by a large number of lipid-related and non–lipid-related risk factors. These findings are in agreement with previous reports on the role of CAMs in atherogenesis and add potentially important information on the early pathophysiological events that lead to development of atherosclerotic plaques.

Several studies suggest a role for CAMs in atherogenesis. Focal expression of VCAM-1 has been demonstrated in human atherosclerotic plaques.3,4 This expression occurs particularly in the neovascular endothelium and smooth muscle cells and importantly, is correlated with the macrophage content in human coronary lesions.7 After an atherogenic diet, VCAM-1 expression in endothelial cells rapidly increases and appears to precede leukocyte accumulation.5,6 Studies with endothelial cells in culture8,24–27 show that constituents of modified lipoproteins and other lipid-derived products can regulate expression of VCAM-1. Recently, 2 reports28,29 demonstrated in mice that a deficiency of several CAMs...
(ICAM-1, P-selectin, and CD18) reduces the development of atherosclerotic fatty streaks after a high-fat, high-cholesterol diet. Finally, prospective data from a large cohort of apparently healthy men indicate that elevated baseline levels of sICAM-1 are associated with increased future myocardial infarction.30

Circulating levels of VCAM-1 have been associated with the extent of clinical atherosclerosis as measured angiographically in patients with known peripheral arterial disease.10 Our data agree with this report, as circulating sVCAM-1 was significantly higher in patients with atherosclerotic carotid plaques. Enders et al11 also demonstrated expression of VCAM-1 in advanced atherosclerotic plaques by immunohistochemistry, whereas ICAM-1 expression was identified in normal carotid arteries. Interestingly, Blann and McCollum10 failed to demonstrate increased levels of sVCAM-1 in patients with clinically defined atherosclerosis when compared with apparently healthy control subjects. These apparently conflicting results may be explained in part by the presence of asymptomatic disease in the controls, by differences in sample size, by variability in the assays used, and by residual confounding. Our data also reinforce and extend the findings of Caterina et al.11 In their study, a striking association between sVCAM-1 levels and carotid IMT was demonstrated in a small population of hypertensive patients. Our results were similar in a more heterogeneous population, even after excluding patients with previous atherothrombosis and after adjustment for clinical and serum (lipid and nonlipid) risk factors for atherosclerosis. A recent sub study from the Atherosclerosis Risk in Communities (ARIC) study12 failed to demonstrate differences in sVCAM-1 levels among patients with carotid atherosclerosis, incident coronary heart disease and control subjects. The reasons for these discrepancies are unclear but can be partially explained by differences in population characteristics. Mean sVCAM-1 levels in our patients were higher than in all 3 groups of patients evaluated in the ARIC substudy (664±315 ng/mL in our sample versus 458±150 ng/mL in their carotid atherosclerosis group, for example). Soluble fractions of different CAMs may not be operative in populations in which the atherosclerotic burden is less accentuated.

The association of sVCAM-1 and sICAM-1 with carotid IMT emphasizes the role of these proteins in early phases of atherogenesis. Ultrasound-based IMT evaluation of carotid arteries is a reliable, reproducible, and safe noninvasive method to study vascular disease.13,32 Carotid IMT measurements have been used in epidemiological studies as a surrogate for preclinical atherosclerosis, and several reports have shown a significant association with traditional cardiovascular risk factors.15-17 Recently, cytomegalovirus titers33 and plasma homocysteine levels34 have also been associated with ultrasonography-based carotid measurements. Our data support the concept that circulating CAMs could potentially be used as serum markers for preclinical atherosclerosis35 and further strengthen the link between inflammation and arterial pathology in humans. The independence of the associations between sVCAM-1 or sICAM-1 and carotid IMT of a large number of lipid-related and non–lipid-related risk factors reinforces this hypothesis.

CAMs are known to be present in a variety of cell types, including endothelium, macrophages, lymphocytes, and certain tumor cell lines. The mechanism for generation of soluble forms of adhesion molecules is uncertain but could involve proteolytic cleavage from the cell surface. The plasma concentration of circulating CAMs observed in our study may reflect total body atherosclerotic load, as such molecules can be expressed nonspecifically throughout the vascular system. As such, the current data support the hypothesis that the plasma concentration of soluble CAMs may provide a molecular marker for total atherosclerosis.30

Limitations of our study design merit consideration. Carotid thickening measured by 2-dimensional ultrasound is not a perfect surrogate for preclinical atherosclerosis.36 However, the natural history of atherosclerotic structural changes is well documented and known to progress through the stage of diffuse, intimal thickening.37 Furthermore, as outlined above, carotid thickening has been effectively used in several epidemiological studies. The patients’ use of drugs could have modified lipid measurements and CAM levels. Nevertheless, there were no differences in sVCAM-1 and sICAM-1 levels when patients using lipid-lowering drugs were compared with those who were not (data not shown). The positive associations observed in this study should be considered in the context of the study population characteristics. Because our study evaluated patients referred to a noninvasive cardiac laboratory, the generalizability of our data may be limited. Mean common carotid and bifurcation IMT values in our sample, for example, were higher than carotid IMT levels previously described for younger and healthier subjects.19

In conclusion, sVCAM-1 and sICAM-1 are associated with carotid IMT, an index of early atherosclerosis. This association appears to be unaffected by other known cardiovascular risk factors. Our findings concur with evidence suggesting that inflammation plays a critical role in several atherosclerotic syndromes.10,30,36 Further large-scale prospective studies are thus needed to determine the prognostic value of these inexpensive and easily assessable markers.

Acknowledgments

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