Soluble Intercellular Adhesion Molecule-1, Soluble Vascular Adhesion Molecule-1, and the Development of Symptomatic Peripheral Arterial Disease in Men

Aruna D. Pradhan, MD, MPH; Nader Rifai, PhD; Paul M. Ridker, MD, MPH

Background—Elevated levels of soluble cellular adhesion molecules have been linked to the development of occlusive coronary events in otherwise healthy individuals. It is not certain, however, whether similar relationships exist for the development of early systemic atherosclerosis.

Methods and Results—In a prospective, nested case-control study conducted among 14,916 middle-aged men, we evaluated the relationship between baseline levels of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and the subsequent development of symptomatic peripheral arterial disease (PAD) during a 9-year follow-up period. Median levels of sICAM-1 but not sVCAM-1 were significantly higher at baseline among men who developed PAD than among those who did not (285.2 versus 267.8 ng/mL [\(P = 0.005\)] for sICAM-1 and 701.0 versus 709.3 ng/mL [\(P = 0.8\)] for sVCAM-1). In analyses adjusted for age and smoking, the odds ratio in the highest compared with the lowest quartile of sICAM-1 was 3.9 (95% CI 1.7 to 8.6; \(P_{\text{trend}} = 0.001\)). After additional adjustment for lipid and nonlipid risk factors, including C-reactive protein, elevated sICAM-1 remained significantly associated with subsequent PAD (OR 3.5, 95% CI 1.4 to 8.5, \(P_{\text{trend}} = 0.008\)). Whereas a monotonic dose-response relationship was evident over the full spectrum of ICAM-1 levels, elevated sVCAM-1 was not associated with future PAD in either age- and smoking-adjusted or fully adjusted models.

Conclusions—Elevated levels of sICAM-1 are independently associated with the development of accelerated atherosclerosis among otherwise healthy men even in the absence of acute coronary occlusion. (Circulation. 2002;106:820-825.)

Key Words: cell adhesion molecules ■ peripheral vascular disease ■ men
at −80°C until analysis. Participants were monitored over an average follow-up period of 9 years for the occurrence of incident health events, including the development of intermittent claudication and hospitalizations for peripheral arterial revascularization procedures. Case subjects were those who subsequently developed either of these PAD end points. Control subjects were selected at random from the remaining study participants who were free of reported cardiovascular disease. Controls were matched with cases on the basis of age, smoking status, and year of follow-up. No participant had a baseline history of intermittent claudication or prior lower-extremity revascularization procedures. Because the study design focused on symptomatic lower-extremity PAD, participants who underwent revascularization of either the renal or carotid arteries were not considered.

**Measurements of Biochemical Parameters**

Baseline plasma samples from case and control subjects were thawed and assayed for sICAM-1 and sVCAM-1 by ELISA with commercially available analytic systems (R&D Systems). C-reactive protein (CRP) and lipid levels were measured as described previously.15 Samples were analyzed in randomly ordered case-control pairs to reduce systematic bias and interassay variation.

**Statistical Analysis**

We used the Student *t* test and the *χ*² statistic to evaluate differences in means and proportions between cases and controls. Because the distributions of sICAM-1 and sVCAM-1 were skewed, differences in medians were assessed by the Wilcoxon rank sum test. To evaluate the relationship between these biomarkers and subsequent PAD risk, the study population was divided into quartiles based on control values for each parameter. We then used logistic regression, adjusting for matching factors, to estimate the odds ratio (OR) for future PAD associated with increasing quartiles of each adhesion molecule. Multivariable ORs were obtained that, in addition to the matching variables of age and smoking, were adjusted for total cholesterol to HDL cholesterol ratio (TC:HDL), hypertension, body mass index, family history of coronary artery disease, diabetes, and exercise frequency. Tests for trend were computed to evaluate for a linear increase in ORs across quartiles. In secondary analyses, we adjusted for baseline concentrations of CRP to assess the residual predictive role of sICAM-1. In addition, to evaluate a potential joint role of CRP and sICAM-1 in predicting PAD risk, we computed ORs among 4 groups defined by the median cutpoint of each biomarker. The dose-response relationships between plasma concentration of sICAM-1, sVCAM-1, and the adjusted OR for PAD were estimated by generalized additive logistic regression.16 To evaluate time-dependent effects, we performed stratified analyses in which the OR associated with sICAM-1 levels above the 75th percentile for controls was calculated for individuals diagnosed during the first 2 years, years 2 through 4, and >4 years of follow-up.

**Results**

As expected, men who subsequently developed PAD were more likely than controls to have traditional coronary risk factors at baseline (Table 1). However, in this study of healthy male physicians with no prior history of cardiovas-
cular disease, the overall prevalence of atherosclerotic risk factors was low: 11.9% with a family history of premature coronary artery disease; 24.0%, 10.7%, and 3.9% with a history of hypertension, hypercholesterolemia, and diabetes, respectively; and 20.9% with a history of current smoking. There were no differences in mean age or smoking status, because these risk factors were matching variables. Physical activity levels were virtually identical at baseline among the case and control groups.

Median sICAM-1 levels were higher at baseline among men who subsequently developed PAD than among controls (285.2 versus 267.8 ng/mL, \( P < 0.005 \); Table 1). By contrast, there was no significant difference in median sVCAM-1 levels (701.0 versus 709.3 ng/mL, \( P = 0.8 \)). Total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride levels, and TC:HDL were all significantly higher among cases than among controls. Spearman age-adjusted partial correlation coefficients showed moderate associations between sICAM-1 and HDL \((r = -0.22, P < 0.001)\), triglycerides \((0.18, P = 0.002)\), and TC:HDL \((0.23, P < 0.001)\). These associations were not present for sVCAM-1 (all \( P > 0.1 \)). The correlation coefficient between sICAM-1 and sVCAM-1 was 0.16 \( (P < 0.007) \). In analyses that controlled for matching variables, increasing levels of sICAM-1 were associated with increasing relative odds for PAD (Table 2). Additional adjustment for TC:HDL and other nonlipid risk factors minimally attenuated this association. In fully adjusted models, the OR associated with plasma sICAM-1 levels in the highest compared with the lowest quartile was 3.2 (95% CI, 1.4 to 7.4; \( P_{\text{trend}} = 0.01 \)). This result was unaffected by additional control for baseline

### TABLE 2. OR of Developing Future PAD According to Baseline Levels of sICAM-1 and sVCAM-1

<table>
<thead>
<tr>
<th>Quartile of Biomarker</th>
<th>Median, mg/mL</th>
<th>Range, ng/mL</th>
<th>OR (Adjusted)</th>
<th>95% CI</th>
<th>( P_{\text{trend}} )</th>
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<tr>
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<td></td>
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<td></td>
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</tr>
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<td>1</td>
<td>195.7</td>
<td>&lt;217.5</td>
<td>1.0</td>
<td>1.0</td>
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<td>2</td>
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<td>(1.2–5.9)</td>
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<tr>
<td>3</td>
<td>267.2</td>
<td>267.7–323.5</td>
<td>3.6</td>
<td>(1.6–7.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>382.0</td>
<td>&gt;323.5</td>
<td>3.9</td>
<td>(1.7–8.6)</td>
<td>0.001</td>
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</table>

*Adjusted for matching variables.*

<table>
<thead>
<tr>
<th>sICAM-1</th>
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<th></th>
<th>95% CI</th>
<th>( P_{\text{trend}} )</th>
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<td>0.8</td>
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<tr>
<td>95% CI</td>
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<td>( P )</td>
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<td>0.01</td>
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<th>95% CI</th>
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<td>1.0</td>
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</tr>
<tr>
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<tr>
<td>3</td>
<td>769.2</td>
<td>709.4–833.1</td>
<td>0.8</td>
<td>(0.4–1.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>927.9</td>
<td>&gt;833.1</td>
<td>1.3</td>
<td>(0.6–2.5)</td>
<td>0.001</td>
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*Adjusted for matching variables.*

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<th></th>
<th>95% CI</th>
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<td>1.0</td>
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<td>(0.6–2.2)</td>
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*Adjusted for matching variables.*

†Adjusted for age, smoking status, and TC:HDL.

‡Adjusted for age and smoking status, TC:HDL, body mass index, family history of premature coronary disease, hypertension, diabetes, and exercise frequency.
triglyceride levels (3.2 [95% CI, 1.3 to 7.5]; \(P_{\text{trend}} = 0.01\)). No evidence of association was observed for sVCAM-1 in either quartile-specific estimates or linear-trend analysis. Adjustment for randomized treatment assignment to aspirin or beta-carotene did not materially alter these results; specifically, the ORs for PAD in the highest versus lowest quartile were 3.5 (95% CI, 1.5 to 8.2; \(P_{\text{trend}} = 0.006\)) and 1.2 (95% CI, 0.5 to 2.5; \(P_{\text{trend}} = 0.94\)) for sICAM-1 and sVCAM-1, respectively.

Because CRP was correlated with sICAM-1 (Spearman age-adjusted partial correlation coefficient, 0.34; \(P < 0.001\)) and is a known predictor of PAD risk in this population,\textsuperscript{15,17} we additionally adjusted for CRP to minimize potential confounding effects of elevations in sICAM-1 that could more broadly relate to systemic subclinical inflammation. In this analysis that controlled for CRP modeled in quartiles, the fully adjusted ORs across quartiles of sICAM-1 were 1.0, 2.8, 4.3, and 3.5 (\(P_{\text{trend}} = 0.008\)). Almost identical effects were observed in analyses that controlled for CRP as a continuous variable.

To evaluate the potential joint role of CRP and sICAM-1 in predicting PAD risk, we stratified the population into 4 groups based on the median cutpoint of each biomarker. As shown in Figure 1, the OR for men with baseline elevations in both biomarkers appears greater than for those with low levels of both CRP and sICAM-1 or with elevations of either marker alone.

In models that assessed for dose-response relationships, the adjusted OR for future PAD was monotonically related to baseline sICAM-1 but not sVCAM-1 (Figure 2). In addition, we evaluated for time-dependent effects in analyses stratified by duration of follow-up at time of diagnosis (Table 3). The OR for PAD associated with baseline sICAM-1 levels >323.5 ng/mL, the 75th percentile cutpoint for control subjects, appeared to be greatest in the first 2 years of follow-up (OR 3.1, 95% CI 1.0 to 9.9, \(P = 0.05\)) and lower thereafter. Compared with control subjects at baseline, median levels of sICAM-1 were significantly higher among case subjects who developed an event during the first 2 years of follow-up (312.4 versus 283.1 ng/mL, \(P = 0.04\)) and remained higher for case events that occurred during years 2 through 4 and >4 years. However, at these later time points, case-control differences were diminished, and at >4 years of follow-up, they were no longer statistically significant.

Discussion

In this prospective evaluation, baseline levels of sICAM-1 but not sVCAM-1 were independently associated with the future development of symptomatic PAD. The relationship between sICAM-1 and the OR for PAD appeared to be positively graded throughout the range of clinical values and was additive to that of CRP. In addition, we found modest evidence for a time-dependent effect, which suggests that the association between sICAM-1 and symptomatic PAD may primarily be a late phenomenon in disease progression.

Atherosclerosis is a chronic process that involves cellular and humoral inflammatory responses. Leukocyte recruitment is an early event in the atherogenesis and continues during plaque maturation. ICAM-1 and VCAM-1 facilitate these processes by coordinating leukocyte adhesion and subsequent transendothelial migration.\textsuperscript{18,19} Both adhesion molecules are transmembrane glycoproteins that bind \(\beta\)-integrins on white
cells. ICAM-1 interacts with β2-integrins present on all white cells and is constitutively expressed by endothelial cells. ICAM-1 is strongly upregulated by inflammatory cytokines in many cell types, including endothelial cells, fibroblasts, epithelial cells, and multiple cells of hematopoietic lineage. In the absence of generalized inflammatory conditions, ICAM-1 is expressed on few cells, and ICAM-1 induction may be an important means of regulating diverse intercellular interactions that participate in the host immune response. VCAM-1, in contrast, is mononuclear cell selective, serving as a counterligand for β1-integrins present on lymphocytes and monocytes. Although VCAM-1 expression is also modulated by several cytokines with similar kinetics as observed for ICAM-1, the vascular distribution of VCAM-1 is more anotomically restricted to activated endothelial cells in lesion-prone areas and is more prominent on intimal neovascularature than on the arterial luminal surface of complex lesions. ICAM-1 and VCAM-1 expression may also differ in that perturbations in laminar blood flow selectively upregulate ICAM-1 but not VCAM-1, at least in vitro. Thus, although ICAM-1 and VCAM-1 share structural and functional similarities, characteristic differences in tissue distribution, counterreceptor specificity, and response to hemodynamic forces may critically influence discernible associations in clinical studies of systemic atherosclerosis.

The present findings, which establish a relationship between elevated levels of sICAM-1 and future risk of symptomatic PAD, have several important implications. First, our results extend previous observations from this and other cohorts in which elevated sICAM-1 levels were predictive of first myocardial infarction and stroke, but sVCAM-1 levels were not. Endothelial activation and inflammation appear to be important precursors to systemic atherosclerosis initiation and progression. It is also possible, however, that the observed increase in plasma sICAM-1 may be indicative of generalized inflammation and upregulation in nonendothelial cells rather than an anatomically localized event in atheromatous vascular beds. Indeed, CRP, a sensitive marker of systemic inflammation, is positively correlated with sICAM-1, although the present report and our previous study of incident myocardial infarction, the OR associated with elevated sICAM-1 was undiminished by adjustment for CRP, and elevations in both biomarkers appeared to identify individuals at the greatest risk. These findings, coupled with the existence of experimentally documented biologic mechanisms, the magnitude of the observed effect, and the probable endothelial origin of soluble CAMs, suggest an independent role for ICAM-1 in the clinical development of peripheral atherosclerosis and raise the possibility that antiadhesive therapies may prevent or ameliorate progression of this disease.

Second, our observation that sICAM-1 levels are primarily elevated in the 2 years immediately preceding the development of symptomatic PAD, although somewhat limited by the number of case-control pairs assessed, is a finding that requires further investigation. Previously reported time-dependent associations between sICAM-1 and the development of myocardial infarction in healthy individuals have suggested that sICAM-1 elevation appears to be an early phenomenon in coronary atherothrombosis. Mechanisms involved in acute coronary syndromes that commonly result from sudden luminal occlusion by thrombus formation may differ from those involved in the pathogenesis of peripheral arteriosclerosis, which is generally associated with gradual luminal narrowing. ICAM-1 amplification in comparatively large areas of involvement in peripheral atherosclerosis as opposed to the coronary circulation may occur late in disease onset, during which biomechanical regulation of ICAM-1 may predominate.

Third, our null findings for sVCAM-1 should not be construed to imply the absence of a physiological role in atherogenesis. Animal models of nascent atherosclerosis indicate that VCAM-1 is a mediator of plaque initiation, and histopathological studies demonstrate preferential expression of VCAM-1 in association with inflammatory cell infiltration in intimal neovascularature of complex lesions. Therefore, atherosclerotic lesions that do not exhibit substantial intimal neovascularization may not sustain appreciably elevated levels of this marker. In addition, cross-sectional studies of associations between soluble CAMs and the extent of PAD indicate that sVCAM-1 as opposed to sICAM-1 may be more predictive of angiographically or echographically determined atherosclerotic burden while being less well correlated with clinical staging. In this regard, epidemiological data have shown that among patients with overt coronary artery disease, in contrast to studies of healthy individuals, elevated sVCAM-1 levels may discriminate those at high risk for subsequent cardiovascular events.

Potential limitations of the present study merit consideration. First, the use of self-reported symptomatic PAD as our
primary a priori end point may have resulted in misclassification bias. However, our study participants were physicians, a group in whom validation rates for several other self-reported vascular and nonvascular end points have consistently been excellent.6 Furthermore, any potential misclassification introduced on this basis would, if anything, tend to bias these data toward the null. Second, although plasma concentrations of soluble isoforms of cell-bound adhesion molecules are thought to derive from proteolytic cleavage and “shedding” from endothelial cells,24 it is currently unknown whether systemic release of soluble CAMs varies with vascular origin, and factors influencing clearance of these immunomarkers remain uncertain. Furthermore, the shedding process may be different for different CAMs, such that levels of sVCAM-1 may be disproportionately attenuated compared with sICAM-1 purely on this basis. In addition, because our blood samples were stored at −80°C until analysis, we cannot exclude the possibility of protein degradation. However, observed levels of sICAM-1 and sVCAM-1 in these data are similar to values obtained in studies using fresh plasma, and if unaccounted sources of protein instability were present, their effects would minimally impact the validity of the present results because all samples were handled identically, and relative differences between cases and controls should not be materially altered. Third, because the present study cohort comprised otherwise healthy men, our results may not be generalizable to women, who experience equivalent overall rates of PAD. However, at least with regard to myocardial infarction and stroke, sICAM-1 levels have been predictive in women as well as men.11

In conclusion, elevated levels of sICAM-1 are associated with subsequent risk of symptomatic PAD in otherwise healthy men. These data, while confirming the association between endothelial activation, inflammation, and systemic atherosclerosis, raise the possibility that cellular immune mechanisms and the associated temporal sequence of events in clinical PAD progression may differ from those related to acute coronary occlusion.

Acknowledgments

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References

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