Circulating Cell Adhesion Molecules and Death in Patients With Coronary Artery Disease

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Background—Vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and E-selectin mediate adhesion and transmigration of leukocytes to the vascular endothelial wall and may promote plaque growth and instability. In a prospective study, we evaluated the effect of soluble adhesion molecules on the risk of future cardiovascular events among patients with angiographically documented coronary artery disease (CAD).

Methods and Results—We obtained baseline samples from a prospective cohort of 1246 patients with CAD. Besides various markers of inflammation, soluble VCAM-1 (sVCAM-1), sICAM-1, and sE-selectin were determined. Follow-up information on cardiovascular events was obtained (mean, 2.7; maximum, 4.1 years). Independently higher levels of sVCAM-1 (1932 versus 1128 ng/mL; \( P \leq 0.0001 \)), sICAM-1 (353 versus 287 ng/mL; \( P \leq 0.015 \)), and sE-selectin (81 versus 63 ng/mL; \( P \leq 0.003 \)) were observed in patients with future death from cardiovascular causes. In a multivariate model, fatal risk was 2.1-fold (1.1 to 4.0) higher in patients within the top quartile of baseline sVCAM-1 concentrations compared with lower quartiles. This association was present independent of general inflammatory response as reflected by low or high C-reactive protein (hs-CRP) levels. In a model that simultaneously controlled for all inflammatory and soluble adhesion markers determined, only sVCAM-1 remained independently significant for future fatal cardiovascular events, with a 2.8-fold increase in risk (\( P \leq 0.003 \)).

Conclusions—Soluble adhesion molecules sVCAM-1, sICAM-1, and sE-selectin were significantly related to future death from cardiovascular causes among patients with documented CAD. Especially sVCAM-1 added to the predictive value of classic risk factors and hs-CRP in determining the risk of future cardiovascular death. (Circulation. 2001;104:1336-1342.)

Key Words: cell adhesion molecules ■ survival ■ heart diseases ■ risk factors ■ thrombosis

A dhesion of circulating leukocytes to the endothelial cells and subsequent transendothelial migration is suggested as an important step in initiation and aggravation of atherosclerotic lesions.1,2 This process is predominantly mediated by cellular adhesion molecules, which are expressed on the endothelial membrane in response to several inflammatory stimuli.3 Focal expression of vascular cellular adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and E-selectin has been demonstrated in human atherosclerotic plaques.4 In addition, expression of VCAM-1 was identified on activated endothelium and vascular smooth muscle cells in the course of atherogenesis.5,6 Expression of VCAM-1 occurs particularly in the neovascular endothelium7 and is strongly associated with increased intimal leukocyte accumulation.8 Moreover, blockade of VCAM-1 has been reported to reduce neointimal formation.9

Although their pathological role remains further uncertain, elevated levels of soluble adhesion molecules have been detected in plasma of patients with stable angina and acute coronary syndrome.10 Clinical data about these soluble forms are still limited. Whereas elevated levels of sICAM-1 and sE-selectin were associated with future cardiovascular events in apparently healthy individuals11,12 this relation could not be demonstrated for sVCAM-1 in the same cohort.13 By contrast, all soluble adhesion molecules are raised in unstable angina and elevation sustained for up to 6 months.14

To further explore the relation of soluble adhesion molecules and future cardiovascular risk, we evaluated in a large prospective cohort of patients with documented coronary artery disease (CAD) whether elevated baseline levels of soluble adhesion molecules are associated with future risk of fatal cardiovascular events. We especially aimed to investigate whether measurement of one of these soluble adhesion markers added to the predictive value of classic risk factors or high sensitive C-reactive protein (hs-CRP) in determining risk of future cardiovascular death.
Methods

Study Population
Between November 1996 and June 2000, 1453 patients who underwent coronary angiography at the II. Medical Department of the University Clinic Mainz or the Bundeswehrzentralkrankenhaus Koblenz were enrolled in a cardiovascular registry (AtheroGene Study). Finally, 1303 patients with at least 1 stenosis >30% diagnosed in a major coronary artery were included; 150 patients had no evidence of angiographically visible stenosis. For the present study, we further excluded 57 patients with acute myocardial infarction within the preceding 48 hours; therefore, the final study cohort consisted of 1246 patients. Exclusion criteria of the AtheroGene study were evidence of hemodynamically significant valvular heart disease, surgery, or trauma within the prior month, known cardiomypathy, known malignant diseases, febrile conditions, or oral anticoagulant therapy within the prior 4 weeks. Diabetes mellitus was diagnosed in patients with dietary treatment or antidiabetic medication or current fasting blood sugar >125 mg/dL, hypertension in patients who had received antihypertensive treatment or had been diagnosed as hypertensive (RR >160/90 mm Hg), and hyperlipoproteinemia in patients who had been given lipid-lowering medication or had a history of cholesterol levels >240 mg/dL. Smoking was classified as current smoking, past smoking (stopped ≥ 12 months ago), or never smoking. Unstable angina was classified by Braunwald classification (class B or C).

A total of 1240 of 1246 patients (99.5%) were followed up in a mean of 2.7 (max: 4.1) years. Follow-up information was obtained about death from cardiovascular causes (n = 88), death of causes not related to heart disease (n = 21), and nonfatal myocardial infarction (n = 65). Information about the cause of death or clinical events was obtained from hospital or general practitioner charts.

Study participants were of German nationality. The study was approved by the ethics committee of the University of Mainz. Participation was voluntary, and each study subject gave written informed consent.

Laboratory Methods
Blood was drawn from all study subjects under standardized conditions before coronary angiography was performed. Samples were stored at −80°C until analysis. Serum sICAM-1, plasma sVCAM-1 (EASIA, Biosource Europe), and plasma sE-selectin (Bender Medical Systems) were measured with the ELISA technique according to the manufacturer’s instructions. C-reactive protein was determined by a highly sensitive, latex particle–enhanced immunoassay (detection range, 0 to 20 mg/L, Roche Diagnostics) and fibrinogen by derived method. Lipid serum levels were measured immediately by routine methods.

Statistical Considerations
Because of skewed distribution of each inflammatory variable, the significance of any differences for sVCAM-1, sICAM-1, sE-selectin, fibrinogen, or hs-CRP was assessed in independent groups by Mann-Whitney U test for univariate analysis or log-normalized variables if appropriate. Differences in proportions were evaluated by the χ² test. In all survival analyses, the end point was death from cardiovascular causes, and data on patients who died of other causes were censored at the time of death. To identify variables as independent predictors of death from cardiovascular causes, we calculated various multivariate Cox regression models with stepwise adjustment for univariate predictors or variables possibly confounding the examined variable. Hazard risk ratio (HRR) and 95% CI are reported with 2-tailed probability values; P < 0.05 was considered to be significant. All computations were carried out with SPSS, version 10.07.

Results

Baseline Characteristics According to Clinical Outcome
Table 1 demonstrates patient characteristics according to clinical outcome. A history of hyperlipidemia paradoxically predicted lower risk after CAD diagnosis, which is caused by a higher percentage of statin prescription in patients defined as being hyperlipidemic (63%). The high percentage of ACE inhibitor use in the group with cardiovascular death might be explained by the higher percentage of patients with low left ventricular ejection fraction in this group. Furthermore, lower use of percutaneous transluminal coronary angioplasty in this group may reflect a high-risk population, which precludes interventional procedures.

Cardiovascular Risk Factors and Soluble Adhesion Molecules
Table 2 presents the associations between soluble adhesion molecules, inflammatory markers, and coronary risk factors. Levels of sVCAM-1 showed a strong correlation with hs-CRP and a modest correlation with fibrinogen levels, and the latter variables also had a strong interdependence. In unstable angina, levels of the myocardial necrosis marker troponin I correlated highly significant with hs-CRP but showed no interdependence with sVCAM-1. Other risk factors such as diabetes and history of hypertension were significantly associated with levels of sVCAM-1 (1200 versus 1121 ng/mL, P = 0.014, respectively, 1162 versus 1074 ng/mL, P = 0.024) but not with levels of sICAM-1 or sE-selectin. Patients receiving statin therapy at least 4 weeks before study enrollment had significantly lower levels of sVCAM-1 (1050 versus 1198 ng/mL, P < 0.0001) or hs-CRP (0.36 versus 0.54 mg/dL, P < 0.0001).

Soluble Adhesion Molecules and Death From Cardiovascular Causes
In the overall study population median levels of sVCAM-1, sICAM-1, and sE-selectin were significantly higher among patients who died of cardiovascular causes compared with those who did not (Table 1). Although there was a graded dose-response relation observed for all soluble adhesion molecules determined, mainly the upper two quartiles (sICAM-1, sE-selectin), respectively, the top quartile (sVCAM-1) at enrollment had the highest probability of death from cardiac causes during the entire follow-up period (Figure 1). For this reason, further analysis was performed with levels exceeding the 50th and 75th percentiles as prespecified cut points. As shown in Table 3, the risk factor–adjusted HRR associated with baseline sVCAM-1 concentrations exceeding the 75th percentile was 2.6 (95% CI 1.5 to 4.4, P = 0.001). With the 50th percentile used as the cut point, HRRs associated with baseline sICAM-1 or sE-selectin concentrations exceeding this threshold were 2.1 (95% CI, 1.3 to 3.5, P = 0.003) and 2.2 (95% CI, 1.3 to 4.0, P = 0.005), respectively. After further adjustment for relevant clinical and therapeutic features, the predictive power of soluble adhesion molecules was attenuated but remained independently significant. Furthermore, the elevation of sVCAM-1 levels in those who had fatal cardiovascular events was independently present in all subgroups evaluated, including patients with stable (1470 versus 1102 ng/mL, P adjusted = 0.002) and unstable angina (1919 versus 1187 ng/mL, P adjusted = 0.003) as well as those with single-vessel disease (2211 versus 1083 ng/mL, P adjusted = 0.002) and multivessel disease (1698 versus 1047 ng/mL, P adjusted = 0.0001).
1141 ng/mL, \( P \) adjusted = 0.01). By contrast, the other soluble adhesion molecules did not achieve independent significance in all subgroups evaluated, and hs-CRP was strongly associated with future fatal events, mainly in patients with multivessel disease but not in those with single-vessel disease.

Figure 2 shows the HRRs of future fatal cardiovascular events according to log-normalized levels of each inflammatory marker and soluble adhesion molecules. In the first model adjusting for the strongest confounders, each of the 5 inflammatory measures except fibrinogen remained indepen-
dently significant. In a Cox regression model that further simultaneously controlled for log-normalized values of each inflammatory variable, only sVCAM-1 (HRR, 2.8; 95% CI, 1.4 to 5.4; \( P < 0.003 \)) was found to be independent predictive.

**Soluble Adhesion Molecules and Combined End Points**

When the combined end point of patients who died of cardiovascular causes or nonfatal myocardial infarction was analyzed, after adjustment for classic risk factors, clinical, and therapeutic features, log-normalized levels of sVCAM-1 (HRR, 2.3; 95% CI, 1.5 to 3.4; \( P < 0.0001 \)) and hs-CRP (HRR, 1.34; 95% CI, 1.09 to 1.9; \( P = 0.03 \)) remained independent predictors.

**High Sensitive C-Reactive Protein, Soluble Adhesion Molecules, and Clinical Outcome**

Figure 3 illustrates that the predictive value of sVCAM-1 on fatal cardiovascular events is independent of hs-CRP levels. After adjustment for most potential confounders, the top quartile of sVCAM-1 was associated with a 4.0-fold increase in risk (95% CI, 1.4 to 11.5; \( P < 0.01 \)) in patients less than hs-CRP median levels and with a 2.2-fold increase in risk (1.05 to 4.8; \( P = 0.038 \)) in patients greater than or equal to hs-CRP median levels. Top quartiles of sICAM-1 and sE-selectin also added to the predictive value of hs-CRP but did not achieve independent significance. Figure 4 presents the HRRs of future fatal cardiovascular events in study subjects categorized as being above or below the 75th percentile cut-point for hs-CRP and sVCAM-1. Patients with elevation of sVCAM-1 without elevated hs-CRP levels had an independent significant 3.1-fold increase in risk (95% CI, 1.4 to 7.0; \( P = 0.004 \)). Highest risk of future cardiovascular death was associated with elevation of both hs-CRP and sVCAM-1 (HRR, 3.9; 95% CI, 1.6 to 9.5; \( P = 0.003 \)). A likelihood-ratio test was used to compare models including mortality predictors and hs-CRP alone to the fit of models based on additional measurement of sVCAM-1. Models including both hs-CRP and sVCAM-1 were significantly better in the prediction of future fatal risk than were models without sVCAM-1 (\( P = 0.003 \)).

**Discussion**

In this prospective data deriving from a large cohort of patients with angiographically documented CAD, baseline sVCAM-1, sICAM-1, and sE-selectin concentrations are elevated among patients with future fatal cardiovascular events. Of all inflammatory markers and lipid values evalu-
ICAM-1 is expressed additionally by fibroblasts and hematopoietic cells. Despite these caveats, elevated levels of ICAM-1 are produced exclusively by the vascular wall components, and statin intake. Model 2 further includes all inflammatory markers simultaneously.

The origins of circulating adhesion molecules are not entirely clear, but they may derive from shedding or proteolytic cleavage from endothelial cells and thus reflect increased expression of membrane-bound adhesion molecules. Our findings that soluble adhesion molecules and especially sVCAM-1 are strongly and independently predictive for future cardiovascular events in patients with documented CAD extends previous observations describing the predictive value of unspecific inflammatory markers on future cardiac events. However, whereas the causative role of hs-CRP in promoting the inflammatory component in development of plaque rupture remains controversial, our data implicate a direct mediator of an inflammatory vessel wall process. In contrast to E-selectin and VCAM-1, which are produced exclusively by the vascular wall components, ICAM-1 is expressed additionally by fibroblasts and hematopoietic cells. Despite these caveats, elevated levels of soluble cell adhesion molecules may mirror a low-grade vascular inflammatory response in the setting of stable and unstable angina more directly than other markers of inflammation and may be even more useful in predicting clinical outcome. Several arguments may support this hypothesis.

First, all circulating adhesion molecules measured in our study are elevated in patients at risk for future fatal cardiovascular events. This association was independent of classic risk factors and clinical features. Second, despite the moderately strong association of sVCAM-1 and hs-CRP, the measurement of sVCAM-1 added to the predictive value of hs-CRP in determining the risk of future fatal cardiovascular events. Even patients with low hs-CRP levels were found to be at significantly higher risk, with elevated sVCAM-1 levels, and, by contrast, elevation of hs-CRP levels alone without raised levels of sVCAM-1 were not predictive for future fatal cardiovascular events. Moreover, models including both hs-CRP and sVCAM-1 provide a significantly better method to predict risk than did models that used hs-CRP and lipid values alone. Third, if incorporating all serum variables determined in this study into one Cox predictive multivariate model in a log-transformed fashion, only sVCAM-1 remained strongly associated with future risk. Last, in the subgroup of patients with unstable angina, levels of hs-CRP correlated with troponin I values. This indicated that at least in part elevation of hs-CRP levels are caused by the effect of ischemia. By contrast, levels of sVCAM-1 and other adhesion molecules did not correlate with troponin I values. This result suggests that elevation of these markers may derive from endothelial activation or damage and not as a consequence of perfusion or reperfusion injury.

Prospective data of soluble adhesion molecules are sparse. Whereas a prolonged elevation of soluble adhesion molecules in unstable angina and myocardial infarction up to 6 months is described and levels of sVCAM-1 are associated with death in diabetic patients, those were not related to future cardiac events in initially healthy people. However, an association between levels of sICAM-1 or E-selectin and future cardiac events in apparently healthy people could be demonstrated. A possible explanation for the heterogeneous results between the present study and previous studies concerning the predictive value of sVCAM-1 might be that our study population consists of patients with already documented disease.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cut Point, %</th>
<th>Model 1, HRR (95% CI)</th>
<th>P*</th>
<th>Model 2, HRR (95% CI)</th>
<th>P†</th>
<th>Model 3, HRR (95% CI)</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVCAM-1 (n = 1087)</td>
<td>50th</td>
<td>1140</td>
<td>2.2 (1.4–3.0)</td>
<td>0.018</td>
<td>2.2 (1.2–4.2)</td>
<td>0.014</td>
<td>1.7 (0.9–3.4)</td>
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<tr>
<td>75th</td>
<td>1588</td>
<td>2.6 (1.5–4.4)</td>
<td>0.001</td>
<td>2.6 (1.6–4.9)</td>
<td>&lt;0.001</td>
<td>2.1 (1.4–4.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>sICAM-1 (n = 1071)</td>
<td>50th</td>
<td>288</td>
<td>2.1 (1.3–3.5)</td>
<td>0.003</td>
<td>2.1 (1.3–3.5)</td>
<td>0.004</td>
<td>2.2 (1.2–3.9)</td>
</tr>
<tr>
<td>75th</td>
<td>389</td>
<td>1.8 (1.1–2.9)</td>
<td>0.03</td>
<td>1.7 (1.1–2.9)</td>
<td>0.03</td>
<td>1.6 (0.9–2.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>sE-selectin (n = 1087)</td>
<td>50th</td>
<td>63</td>
<td>2.2 (1.3–4.0)</td>
<td>0.005</td>
<td>2.3 (1.3–4.0)</td>
<td>0.005</td>
<td>1.9 (1.02–3.6)</td>
</tr>
<tr>
<td>75th</td>
<td>92</td>
<td>2.0 (1.2–3.5)</td>
<td>0.01</td>
<td>2.1 (1.2–3.5)</td>
<td>0.01</td>
<td>1.7 (0.9–3.3)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

HRR indicates hazard risk ratio.
*Model 1 controlled for age, sex, body mass index, history of hypertension, diabetes, current smoking, and HDL cholesterol levels.
†Model 2 additionally included clinical and therapeutic features such as extent of vessel disease, history of myocardial infarction, statin therapy, and interventional procedure.
‡Model 3 further adjusted for ejection fraction.

Figure 2. HRRs for death from cardiovascular causes according to log-normalized levels of various inflammatory markers. Model 1 tests effect of each serum marker individually, adjusted for age, sex, body mass index, classic risk factors, unstable angina, and statin intake. Model 2 further includes all inflammatory markers simultaneously.
CAD. In those subjects, the molecule marker sVCAM-1 may serve as an indicator of plaque burden or activity. However, expression of VCAM-1 mostly occurs on atherosclerotic plaques and is limited to activated vascular endothelial and smooth muscle cells. It therefore remains to be proven whether sVCAM-1 can be a predictive marker in initially healthy people without cardiovascular risk factors, which may lead to endothelial activation and VCAM expression. By contrast, ICAM-1 is additionally expressed in many cells of the hematopoietic lineage and fibroblasts and therefore appears to be a marker of a more general inflammatory condition. In light of this knowledge, it might be explainable that sICAM-1 is predictive—like hs-CRP or fibrinogen—also in initially healthy people, whereas sVCAM-1 has a strong predictive value mainly in those patients with atherosclerotic lesions or at least risk factor–induced activated endothelium.

Our study shares the limitations of nonrandomized observational studies such as unsuspected selection biases and confounding. In detail, levels of soluble adhesion molecules are subject to confounders such as age, smoking, and diabetes, and associations between soluble adhesion molecules with future fatal events might be a consequence of confounding. However, because statistically significant differences in baseline sVCAM-1 levels were present between case and control subjects in all subgroup analyses and significance was obtained after adjustment for all possible confounders, we believe that this is unlikely. Furthermore, measurement was performed at one time only. Therefore, we do not have the possibility to clarify the variability of soluble adhesion levels during a special time course. Furthermore, assessment of soluble adhesion molecules at the present time is an expensive and time-consuming procedure, and before entering a clinical setting, standardized and reproducible assays as well as a consistent series of prospective studies should be available. By contrast, these criteria are fulfilled by hs-CRP, which can be easily determined with low costs.

In conclusion, we found that levels of the soluble adhesion molecules sVCAM-1, sICAM-1, and sE-selectin were significantly related to future death from cardiovascular causes in patients with documented CAD. Especially sVCAM-1 added to the predictive value of classic risk factors and hs-CRP in determining the risk of future cardiovascular death. Inhibition of adhesion molecules and measurement of their soluble forms might provide a future direction of diagnostic and therapeutic strategies.

Appendix

The AtheroGene Group
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AtheroGene Recruitment Centers
Department of Medicine II, Johannes Gutenberg University, Mainz; Bundeswehrzentralkrankenhaus, Koblenz.
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References
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