Relations of a Marker of Endothelial Activation (s-VCAM) to Function and Mortality in Community-Dwelling Older Adults

Kim M. Huffman,1,2,3 Carl F. Pieper,3 Virginia B. Kraus,2,3 William E. Kraus,3,4 Gerda G. Fillenbaum,3,5 and Harvey J. Cohen3

1Department of Physical Medicine and Rehabilitation, Veterans Affairs Medical Center, Durham, North Carolina.
2Division of Rheumatology, Center for Aging and Human Development, and 3Division of Cardiovascular Medicine, Duke University Medical Center, Durham, North Carolina.
5Geriatric Research Education and Clinical Center, Veterans Affairs Medical Center, Durham, North Carolina.

Address correspondence to Kim M. Huffman, PhD, Box 3327, Duke University Medical Center, Durham, NC 27710. Email: huffm007@mc.duke.edu

Background. We wished to determine if a marker of endothelial dysfunction/activation soluble vascular cell adhesion molecule (s-VCAM)—was related to functional status and mortality in community-dwelling older adults independent of the known effects of markers of inflammation and coagulation.

Methods. Data came from the third and fourth in-person waves of the Duke Established Populations for Epidemiologic Studies of the Elderly. Participants (aged ≥ 71 years) had participated in a blood draw (N = 1,551) from which concentrations of s-VCAM, interleukin-6, and D-dimer were determined. Information was gathered in-person on demographics, health behaviors, chronic health conditions, and functional status (Katz, Rosow–Breslau, Nagi). Death was determined through the National Death Index. Multivariable regression analysis was used to examine the adjusted association of s-VCAM with functional status; Cox proportional hazards models ascertained hazard of mortality.

Results. Controlled analyses indicated that cross-sectionally, but not longitudinally (4 years later), greater s-VCAM concentrations were associated with poorer function as measured by the Katz and Rosow–Breslau scales (p < .05 for both), independent of interleukin-6 and D-dimer. In controlled analyses, s-VCAM (p = .002), D-dimer (p = .008), and interleukin-6 (p = .01) were independently related to 4-year mortality; 1 SD increase in log concentration conferred 1.2-, 1.1-, and 1.2-fold increases in mortality, respectively. The greatest hazard of mortality was observed within the first year after measurement. s-VCAM concentrations were not predictive of 15-year mortality.

Conclusions. Independent of inflammation and coagulation markers, endothelial dysfunction serves as a marker of, and potentially contributes causally to, poor function and death in community-dwelling older adults.

Key Words: S-VCAM—D-dimer—IL-6.

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THERE is an evolving literature about the impact of physiologic processes, and biomarkers of these processes, on function and mortality in aging populations. Previously, increased inflammation and coagulation have been related to functional decline and earlier mortality independent of associations with demographics and specific diseases (1–10). Because endothelial dysfunction has been linked to a number of diseases associated with aging, type 2 diabetes (11), sleep apnea (12), ischemic stroke (13), Alzheimer’s and vascular dementia (14), metastatic malignancies (15), and severe hip and knee osteoarthritis (16), we sought to determine if a marker of endothelial dysfunction, as indicated in this case by vascular cell adhesion molecule (VCAM), might impact functional decline and mortality independent of inflammation and coagulation.

VCAM is a transmembrane glycoprotein expressed on the surface of activated endothelial cells and vascular smooth muscle cells (17). VCAM recruits leukocytes to areas of inflammation by promoting adhesion of circulating inflammatory cells to endothelium (reviewed in 18). Expression of VCAM is typically promoted by inflammatory molecules, including tumor-necrosis factor alpha and interleukin-1 beta (reviewed in 17,18). When endothelial cells are activated by inflammatory cytokines, soluble VCAM (s-VCAM) is generated via proteolytic cleavage at or near the site where the protein inserts into the endothelial cell membrane (17,19). The proportion of the membrane-bound form released as s-VCAM from activated cells is estimated to be as much as 20% of total cellular VCAM (19). s-VCAM can induce T-cell chemotaxis, contributing to sy-
novial inflammation in rheumatoid arthritis (20) and recruit eosinophils to lungs in animal models of asthma (21). Given these pathologic roles of s-VCAM and the association of elevated s-VCAM with abundant endothelial activation, detection of elevated concentrations of s-VCAM is commonly equated with endothelial dysfunction (17,18).

Our group has previously demonstrated relations for both inflammation, as indicated by interleukin-6 (IL-6), and coagulation, as indicated by d-dimer, with functional decline and mortality (3,4,9,22). Given these relations, we wished to determine the associations of s-VCAM with function and mortality, independent of the impacts of IL-6 and d-dimer. We hypothesized that concentrations of s-VCAM, a marker of endothelial dysfunction and activation, would be related to functional status and mortality independent of disease state, demographics, and markers of inflammation and coagulation in a sample of community-dwelling older adults.

**METHODS**

**Participant Population**

The National Institute on Aging–funded Duke Established Populations for Epidemiologic Studies of the Elderly has been described previously (3,23).

**Measures**

Demographic information (self-reported age, sex, race, and education) was collected at enrollment (1986). At the third in-person interview (1992–1993), the baseline for the current study, height and weight were measured, and body mass index (kg/m²) was calculated. Other information gathered at the same time included the following.

**Blood collection subsample.**—Blood was collected in ethylenediaminetetraacetic acid–containing tubes, centrifuged, and plasma was stored at −70°C in 0.5-mL aliquots. As previously reported, 67% (1,727) of those interviewed had a successful blood sample obtained (3). Of the 1,727, s-VCAM could be measured for 1,551. This sample of 1,551 constitutes the analysis sample for the present study.

**Functional status.**—Assessments of functional status included Katz (24), Rosow–Breslau (25), and Nagi (26) scales. Katz reports on five activities of daily living: bathing, dressing, transferring from bed to chair, toileting, and eating. The abbreviated Rosow–Breslau assesses participants’ abilities to do heavy house work, walk up a flight of stairs, and walk a half mile. The Nagi scale assesses fine and gross motor activity, including abilities to extend the arms above the shoulders, manipulate small objects, stoop or kneel, carry a 10-pound item, and push a large object. Items were scored dichotomously, with higher scores indicating poorer function. Function was reassessed during an in-person interview 4 years later (1996–1997).

**Cognitive status and depression.**—Cognitive status was assessed in terms of errors on the Short Portable Mental Status Questionnaire (27). Depression was assessed with a modified version of the Center for Epidemiologic Studies—Depression scale (28), in which the original 4-point scale for each item was dichotomized (condition present versus absent). A score of 9 on this abbreviated measure has been found to be equivalent to a score of 16 on the original (29). Higher scores indicated more cognitive impairment or depression.

**Self-rated health.**—Self-rated health was assessed with the question “Overall, how would you rate your health.” Four responses were possible: “excellent,” “good,” “fair,” and “poor.”

**Chronic conditions.**—The presence of five chronic medical problems (cancer, heart attack, stroke, diabetes, and hypertension) was assessed with the question “Did a doctor ever tell you that you had . . .”

**Sleep.**—Sleep problems were assessed by asking participants how often they had difficulty falling asleep. Three responses were possible: “rarely or never,” “sometimes,” and “most of the time or always.”

**Smoking and alcohol.**—Smoking status was determined by asking whether the participant currently smoked cigarettes. Alcohol use was assessed with a question on whether individuals consumed two or more drinks of beer, wine, or liquor each week.

**Biomarker Analyses**

Concentrations of IL-6, d-dimer, and s-VCAM were measured by enzyme-linked immunoassays (Quantikine, R&D Systems, Minneapolis, MN; Dimertest Tripwell ELIA kit, American Diagnostical, Greenwich, CT; and R&D Systems, Minneapolis, MN, respectively). Analyses of IL-6 and d-dimer concentrations and characteristics were reported previously (3,4), whereas subsequent analyses of s-VCAM were possible for 1,551 individuals with sufficient plasma remaining after the initial biomarker analyses were completed (30). Computed from raw s-VCAM data, the intraassay coefficient of variation, calculated as the mean coefficient of variation for 57 samples run in duplicate, was 3.91%. The interassay coefficient of variations for low, medium, and high concentrations were 7.8%, 3.1%, and 0.14%, respectively; these were determined from the low, medium, and high concentration standards across 20 plates.

**Mortality Assessments**

Mortality was assessed via a combination of family statements and National Death Index searches (31).
Statistical Analyses

Handling missing data.—When data were missing, we imputed the mode for categorical variables, the median for ordinal variables, and the mean for continuous variables. The maximum number of missing values for a single variable was 52 for baseline function assessed with the Nagi scale.

Analysis.—Biomarker variables (s-VCAM, IL-6, and D-dimer) were log-transformed to approximate a normal distribution prior to modeling. Standardized z scores (raw data – population mean)/standard deviation) were created for log-transformed biomarker concentrations to allow comparison among the three biomarker parameter estimates. The impact on physical function outcomes (Katz, Rosow–Breslau, and Nagi scores) was assessed with Pearson correlations and, to account for covariates as described subsequently, with general linear modeling by ordinary least squares (regression) procedures. Cox-proportional hazards modeling was used for determining predictors of 4- (1996) and 15-year (2008) mortality. As a final test of the assumptions of this model, both the proportionality and the linearity assumptions were tested for the variables of interest. Analyses were controlled for age, sex, race, education, body mass index, cognitive status, depression, self-rated health, cancer, heart attack, stroke, diabetes, hypertension, sleeping difficulties, smoking, and alcohol use. To evaluate for potential s-VCAM by demographic or health factor interactions and to control for type I error, chunks of interactions were tested using multivariable tests including VCAM by x, where x was the vector of all covariates entered into the model for each outcome. If the overall test was positive, then, each two-way interaction was tested individually (32).

RESULTS

Cross-Sectional and Longitudinal (4-Year) Association of Biomarkers With Functional Status

Table 1 shows characteristics of the 1,551 participants for whom s-VCAM data were available. Cross-sectionally, greater s-VCAM concentrations (log s-VCAM) were related to poorer function regardless of measure (Table 2: $r_{Katz} = .14$, $p < .0001$; $r_{Rosow-Breslau} = .17$, $p < .0001$; $r_{Nagi} = .13$, $p < .0001$). After controlling for previously identified demographic and health predictors of current function (age, sex, race, education, body mass index, cognitive status, depression, self-rated health, cancer, heart attack, stroke, diabetes, hypertension, sleeping difficulties, smoking, and alcohol use), greater s-VCAM concentrations (log s-VCAM) were independently related to poorer function as measured by the Katz ($p = .003$) and Rosow–Breslau ($p = .0003$) scales, but not the Nagi ($p = .07$). Further, when controlling for IL-6 and D-dimer, s-VCAM remained an independent predictor of cross-sectional function as measured by the Katz and Rosow–Breslau scales ($p < .02$ for both; Table 2). Although s-VCAM was significantly correlated with IL-6 ($r = .13$, $p < .0001$) and D-dimer ($r = .12$, $p < .0001$), these were not levels that generally raise concerns about collinearity-induced problems with model estimation. For Nagi- and Rosow-Breslau–assessed function, there were no significant s-VCAM interactions for the individual demographic (age, race, and gender) or health factors; for Katz-assessed function, the only significant s-VCAM interaction was for cognitive status ($\beta [SE] = 0.046 [0.17], p = .006$).

Longitudinally, in controlled analyses with or without D-dimer and IL-6, s-VCAM was not related to functional status 4 years later, as assessed by Katz, Rosow–Breslau, or Nagi scales ($p > .05$ for all). However, 4 years after the current study baseline assessment, 338 (22%) of 1,551 participants were deceased, implying a potential survivor bias confounding the relation of s-VCAM with function.

Association of Biomarkers With 4- and 15-Year Mortality

In fully controlled analyses, s-VCAM was independently related to 4-year mortality; this relationship was independent of previously identified demographic and health predictors of mortality, including D-dimer and IL-6 ($p < .05$;
Table 2. Regression Estimates for Self-Reported Function at Baseline*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Unadjusted Pearson correlations</th>
<th>Adjusted for covariates</th>
<th>Multivariable model adjusted for covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-VCAM, pg/mL (log)</td>
<td>Nagi parameter estimate (SE)</td>
<td>Nagi parameter estimate (SE)</td>
<td>Nagi parameter estimate (SE)</td>
</tr>
<tr>
<td>d-dimer, mg/L (log)</td>
<td>Katz parameter estimate (SE)</td>
<td>Katz parameter estimate (SE)</td>
<td>Katz parameter estimate (SE)</td>
</tr>
<tr>
<td>IL-6, pg/mL (log)</td>
<td>Rosow-Breslau parameter estimate (SE)</td>
<td>Rosow-Breslau parameter estimate (SE)</td>
<td>Rosow-Breslau parameter estimate (SE)</td>
</tr>
<tr>
<td>p</td>
<td>p value</td>
<td>p value</td>
<td>p value</td>
</tr>
</tbody>
</table>

Notes: IL-6 = interleukin-6; s-VCAM = soluble vascular cell adhesion molecule.

*Standardized z-scores for biomarkers are modeled to allow comparison among the three biomarker parameter estimates.

§Analyses were controlled for age, sex, race, education, body mass index, cognitive status, depression, self-rated health, cancer, heart attack, stroke, diabetes, hypertension, sleeping difficulties, smoking, and alcohol use.

§§Estimates are presented for a single multivariable model for each measure of function such that the regression estimates, including the covariates and the three biologic variables simultaneously included.

DISCUSSION

Here, function, as measured with the Katz and Rosow–Breslau measures, was related to s-VCAM independent of the relations for d-dimer and IL-6. Also, as previously demonstrated, d-dimer and IL-6 concentrations were related to function, independent of one another (9). Most remarkably, in a sample of community-dwelling adults 71 years of age and older, s-VCAM, d-dimer, and IL-6 concentrations were independently related to mortality within 4 years, even after functional status and demographic and health status predictors of mortality had been taken into account.

Previous work has implicated s-VCAM as a marker of endothelial dysfunction associated with reduced survival. s-VCAM concentrations have been associated with disease-associated mortality in metastatic gastric cancer (15) and sickle-cell disease (18) as well as cardiovascular mortality in coronary artery disease (34) and type 2 diabetes (35). Although markers of inflammation and coagulation have been associated previously with declining function and mortality (3–10,22), to our knowledge, this is the first investigation linking s-VCAM concentrations to all-cause mortality in a general (not disease-specific) older population while controlling for the presence of multiple comorbidities that might account for such an association.

Endothelial dysfunction and decreases in endothelium-dependent dilation have been associated with aging, in the absence of overt disease (36,37). Mechanisms have been attributed to decreases in nitric oxide availability, endothelial nitric oxide synthase expression, and tetrahydrobiopterin...
activity as well as increases in oxidative stress, endothelin-1–mediated vasoconstriction, and inflammation (36,37). Thus, in our cohort of 71+ year olds, elevated s-VCAM concentrations, independent of disease and inflammation, might have contributed to decreased function and earlier mortality by reflecting subclinical vascular dysfunction. Future investigations are necessary to confirm this hypothesis, as well as to test the effects of attempts to improve endothelial function (exercise, growth hormone, diet, caloric restriction, etc. [reviewed in 36,37]) on physical function and mortality.

Although s-VCAM concentrations were predictive of 4-year mortality, this effect was due largely to association of s-VCAM concentrations with mortality observed during the first year. This observation highlights several noteworthy conclusions related to mortality predictions. First, it is important to verify the assumption of proportionality to ensure that predictive capability of a marker at a later time is not due to effects of the marker at initial or early times. Second, the change in the hazards over time suggests that either s-VCAM concentrations change dynamically or the relation between s-VCAM concentrations and mortality is mutable. The concentrations of IL-6 and d-dimer are stable within an individual over at least 36 days (38), but these concentrations, as well as those of s-VCAM, would be expected to change with the changing health status of an individual and therefore predict more proximal events. Third, it is important, but unknown whether additional predictive capability might be derived from serial longitudinal measurements of s-VCAM, d-dimer, or IL-6 over time.

Table 3. Adjusted Hazard Ratios for 4- and 15-Y Mortality*

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>4–Y Mortality</th>
<th>15–Y† Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td></td>
<td>(95% Confidence Interval)</td>
<td>(95% Confidence Interval)</td>
</tr>
<tr>
<td></td>
<td>p Value</td>
<td>p Value</td>
</tr>
<tr>
<td>s-VCAM, pg/mL (log)</td>
<td>1.193 (1.065, 1.336) .0023</td>
<td>1.066 (0.979, 1.161) .14</td>
</tr>
<tr>
<td>d-dimer, µg/L (log)</td>
<td>1.138 (1.035, 1.252) .0079</td>
<td>0.947 (0.874, 1.027) .19</td>
</tr>
<tr>
<td>IL-6, pg/mL (log)</td>
<td>1.209 (1.047, 1.397) .0098</td>
<td>1.046 (0.956, 1.143) .33</td>
</tr>
</tbody>
</table>

Notes: IL-6 = interleukin-6 and s-VCAM = soluble vascular cell adhesion molecule.

* Standardized z scores for biomarkers are modeled to allow comparison among the three biomarker parameter estimates. Estimates are presented for a single multivariable model for each of 4- and 15-y mortality such that the hazard ratios for biologic variables are independent of one another. All analyses were controlled for age, sex, race, education, body mass index, Rosow-Breslau–measured function, cognitive status, depression, self-rated health, cancer, heart attack, stroke, diabetes, hypertension, sleeping difficulties, smoking, alcohol use, d-dimer, and IL-6. s-VCAM = soluble vascular cell adhesion molecule; BMI = body mass index; and IL-6 = interleukin-6.

† Fifteen-year mortality conditional on having survived 4 y.

**Figure 1.** High s-VCAM was associated with increased mortality. Adjusted survival over time is shown for low and high baseline s-VCAM plasma concentrations (divided at the sample mean). Cox proportional hazards modeling evaluated hazard ratios for a 1 SD increase in s-VCAM (log s-VCAM) concentrations for 4-y mortality (HR = 1.2, p = .002) controlling for age, sex, race, education, BMI, Rosow-Breslau–measured function, cognitive status, depression, self-rated health, cancer, heart attack, stroke, diabetes, hypertension, sleeping difficulties, smoking, alcohol use, d-dimer, and IL-6. s-VCAM = soluble vascular cell adhesion molecule; BMI = body mass index; and IL-6 = interleukin-6.

Limitations

Because the participants are a subsample of individuals who survived 6 years after initial enrollment and who had blood collected, there is a question whether they are representatives of the population of community-dwelling adults 71 years and older. As previously reported, those without a blood draw were typically older and sicker suggesting a possible ceiling effect on the generalizability of the findings (4). Also, whereas the long-term follow-up is a strength of
the Established Populations for Epidemiologic Studies of the Elderly cohort, the lack of shorter follow-up intervals for a more granular assessment of the temporal course of function and the relatively small number of deaths within the first year make it difficult to determine with precision the time-dependent effect of s-VCAM concentrations on function and mortality. Further, causal inferences regarding the impact of greater s-VCAM concentrations on function and mortality should be interpreted with caution. However, the consistency of these findings with prior findings of greater concentrations of s-VCAM in disease populations (11–16,39,40) supports the face validity of our findings. Further, the strength of the associations between s-VCAM, function, and mortality, despite adjustment for multiple potential confounders, supports our hypothesis that endothelial dysfunction is related to functional decline and contributes to death in older community-dwelling adults. Nonetheless, additional investigations designed to prospectively follow s-VCAM concentrations, function, and mortality with greater granularity over time are necessary to refine an understanding of the time interval over which s-VCAM can provide informative predictions of mortality and to determine if s-VCAM is causally related to (a biomarker) or merely a risk indicator of declining function and mortality.

In summary, in this cohort of community-dwelling older adults, greater blood concentrations of s-VCAM were related to poorer function and to short-term (≤1 year) mortality independent of comorbidities, inflammation, and coagulation markers. These findings imply that endothelial dysfunction is potentially a causal contributor to functional decline and death in elders.

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