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Plasma Concentration of Soluble Vascular Cell Adhesion Molecule-1 and Subsequent Cardiovascular Risk

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OBJECTIVES The purpose of this study was to evaluate whether soluble vascular cell adhesion molecule-1 (sVCAM-1) is a marker for increased cardiovascular risk.

BACKGROUND Soluble forms of cellular adhesion molecules (CAMs) may be useful markers of endothelial activation and local or systemic inflammation. Recent studies indicate that plasma concentration of soluble intercellular adhesion molecule-1 (sICAM-1) is elevated many years before a first myocardial infarction (MI) occurs. However, only a few prospective studies have evaluated whether sVCAM-1 is also a marker for increased cardiovascular risk.

METHODS Baseline plasma samples were obtained prospectively from 14,916 healthy participants in the Physicians' Health Study. In a nested, case-control study design, the plasma concentration of sVCAM-1 was measured in 474 men with confirmed MI during the nine-year follow-up period, and in an equal number of control subjects who remained free of reported cardiovascular disease and who were matched for age, smoking status and length of follow-up.

RESULTS No significant difference in the median baseline sVCAM-1 concentration was found between case and control subjects (638 vs. 634 ng/ml; p = NS). Cardiovascular risk was similar between patients with sVCAM-1 levels in the highest quartile and those in the lowest quartile, in both crude (relative risk [RR] 1.28, 95% confidence interval [CI] 0.85 to 1.92) and adjusted (RR 1.17, 95% CI 0.71 to 1.91) matched-pairs analyses.

CONCLUSIONS In contrast to previous data on sICAM-1, we found no evidence of an association between sVCAM-1 levels and the risk of future MI in a large cohort of apparently healthy men. These data suggest important pathophysiologic differences between sVCAM-1 and sICAM-1 in the genesis of atherothrombosis. (J Am Coll Cardiol 2000;36:423–6) © 2000 by the American College of Cardiology

Leukocyte binding to cellular adhesion molecules (CAMs) on the surface of vascular endothelial cells appears to be one of the earliest events in the atherosclerotic process (1–4). Increased endothelial cell expression of CAMs has been demonstrated in response to a number of inflammatory cytokines, including interleukin-1, interleukin-4, tumor necrosis factor-alpha, interferon-gamma, lipopolysaccharide and oxidized low density lipoprotein (LDL) (1,5–7). Pathologic studies have demonstrated CAMs within and adjacent to atherosclerotic plaque (8–12), and clinical studies have suggested a role for CAMs in plaque disruption and subsequent acute coronary events (1,5,13,14).

After cytokine activation, CAMs are released from the surface of endothelial cells and leukocytes, probably by proteolytic cleavage (15,16). Plasma levels of these "shed" CAMs can now be measured using commercially available immunoassays (15). Although the pathogenic role of these circulating CAMs in disease states remains unclear, these molecules may serve as markers of endothelial activation and local or systemic inflammation (17,18). For example, in cross-sectional studies, the plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) correlate with the extent of underlying atherosclerosis (19–22). Moreover, two prospective cohort studies indicate that baseline levels of sICAM-1 are increased many years before a first myocardial infarction (MI) occurs (13,14). In each of these studies, an apparent threshold effect was found, such that increased risk was present among those with the highest levels of sICAM-1. In one of the studies, increased risk was observed only after two years of follow-up, suggesting that an increased level of sICAM-1 is a very early marker of risk (13).

In contrast to sICAM-1, there is a lack of prospective data on the relation between sVCAM-1 and subsequent coronary risk (14). We therefore sought to test this hypothesis directly, using baseline plasma samples obtained from a large cohort of apparently healthy men who were prospectively observed for the occurrence of a first MI.

METHODS

We used a nested, case-control study design within the Physicians' Health Study (PHS)—a randomized, double-blind, placebo-controlled trial evaluating low dose aspirin

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Abbreviations and Acronyms

CAM	=	cellular adhesion molecule
CRP	=	C-reactive protein
HDL	=	high density lipoprotein
LDL	=	low density lipoprotein
MI	=	myocardial infarction
PHS	=	Physicians' Health Study
sICAM-1	=	soluble intercellular adhesion molecule-1
sVCAM-1	=	soluble vascular cell adhesion molecule-1

and beta-carotene in the primary prevention of cardiovascular disease and cancer (23). Overall, the PHS consisted of 22,071 apparently healthy male physicians who were free of a previous MI stroke, transient ischemic attack and cancer at study entry. Before randomization, 14,916 (67.6%) men provided a baseline plasma specimen that was collected in EDTA and stored at −80°C until the time of assay.

Hospital records were reviewed by a blinded end points committee for all events of incident MI reported after study enrollment. When fatal infarction occurred, autopsy reports and death certificates were reviewed when appropriate. Myocardial infarction was confirmed when symptoms met the World Health Organization criteria, in association with either elevated cardiac enzymes or characteristic electrocardiographic changes.

Participants who provided a baseline plasma sample and who had a confirmed MI during the nine-year follow-up period were included in this study. Control subjects were selected randomly from those study participants who provided baseline blood specimens and who remained free of reported cardiovascular disease. Each case was matched with one control on the basis of length of follow-up (six-month intervals), age (within two years) and smoking status (past, current or never). Overall, 474 pairs of MI cases and control subjects were included in this analysis.

After thawing at room temperature, baseline plasma from each study participant was assayed for sVCAM-1, using a commercially available ELISA (R&D systems, Minneapolis, Minnesota) (coefficient of variation 9% to 11%). Total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and C-reactive protein (CRP) were also measured on baseline plasma specimens. At the time of study entry, patients reported on age, smoking status, weight, systolic and diastolic blood pressure, history of diabetes, hypercholesterolemia and family history of MI.

Differences in baseline characteristics were compared using the chi-square test for categoric variables and the Student *t* test for continuous variables. Logistic regression analyses, conditioned on the matching variables, were used to control for potential confounding variables and randomized treatment assignment. Tests for trend were used to evaluate for evidence of increased coronary risk across increasing quartiles of sVCAM-1, with the distribution of sVCAM-1 defined by the control group. Analyses were performed for all patients and for the subgroup of nonsmokers. Age-adjusted correlations were determined between sVCAM-1 and total cholesterol, HDL cholesterol, triglycerides and CRP. Finally, the results were stratified according to year of follow-up to determine if the effect of sVCAM-1 on the incidence of MI varied over time.

Table 1. Baseline Characteristics

	MI Cases (n = 474)	Control Subjects (n = 474)	p Value
Age (yrs)*	58.1	57.9	—
Smoking status*			
Never	44.4%	44.4%	—
Past	40.8%	40.8%	—
Current	14.8%	14.8%	—
Body mass index (kg/m^2)	25.4	25.0	0.002
Diabetes	5.1%	2.3%	0.03
Hypercholesterolemia	14.2%	10.0%	0.06
Hypertension	28.6%	17.6%	0.001
Family history of CAD	18.0%	11.7%	0.007

*Matching factor. Data are presented as the mean value or percentage of cases/controls.
CAD = coronary artery disease; MI = myocardial infarction.

RESULTS

The baseline characteristics of the MI cases and control subjects are shown in Table 1. As expected, the men with MI had a higher prevalence of known cardiac risk factors at baseline.

Overall, sVCAM-1 levels ranged from 55 to 2,192 ng/ml, in a moderately rightward skewed distribution. There were no significant differences between MI cases and control subjects in terms of mean (669 vs. 656 ng/ml; p = NS) or median (638 vs. 634 ng/ml; p = NS) baseline levels of sVCAM-1.

In a crude, matched-pairs analysis, no difference in the incidence of MI was observed between subjects with sVCAM-1 levels in the lowest quartile and those in any of the three higher quartiles (Table 2). In addition, we found no evidence of an association between increasing levels of sVCAM-1 and future coronary risk, both among all study subjects and nonsmokers (Table 2). When adjustment was made for body mass index, history of hypercholesterolemia, hypertension and a family history of coronary artery disease, there remained no association between sVCAM-1 and MI (Table 2).

To determine whether a relation between sVCAM-1 and MI developed with longer follow-up time, the analysis was stratified by years of follow-up. The relative risk for MI associated with the highest quartile of sVCAM-1 was not statistically significant at any time during the follow-up period (Fig. 1).

Total cholesterol was significantly correlated with sVCAM-1, although the magnitude of the association was small (r = 0.09, p = 0.02). In contrast, no significant correlation was observed between sVCAM-1 and either HDL cholesterol or triglyceride levels. No significant association was found between sVCAM-1 and CRP.

Table 2. Crude and Adjusted Relative Risks of First Myocardial Infarction, According to Baseline Concentration of Soluble Vascular Cell Adhesion Molecule-1

	Quartile 1 (<525 ng/ml)	Quartile 2 (525–634 ng/ml)	Quartile 3 (634–748 ng/ml)	Quartile 4 (>748 ng/ml)	p Trend
Crude analysis (all patients)					
RR (95% CI)	1.0	1.16 (0.79–1.69)	1.07 (0.72–1.60)	1.28 (0.85–1.92)	0.30
p Value		0.44	0.75	0.23	
Crude analysis (nonsmokers)					
RR (95% CI)	1.0	1.02 (0.68–1.55)	0.96 (0.62–1.50)	1.18 (0.76–1.83)	0.48
p Value		0.91	0.87	0.45	
Adjusted analysis (all patients)					
RR (95% CI)	1.0	1.00 (0.63–1.59)	1.06 (0.66–1.73)	1.17 (0.71–1.91)	0.48
p Value		1.0	0.80	0.54	
Adjusted analysis (nonsmokers)					
RR (95% CI)	1.0	0.78 (0.46–1.31)	0.82 (0.47–1.43)	0.96 (0.55–1.66)	0.87
p Value		0.35	0.49	0.88	

CI = confidence interval; RR = relative risk.

DISCUSSION

In this prospective study, we observed no association between baseline sVCAM-1 levels and risk of future MI among apparently healthy men. Specifically, mean and median levels of sVCAM-1 at baseline were virtually identical among those who subsequently had an MI and those who remained free of cardiovascular disease. Moreover, no evidence of an association was observed even among those with the very highest baseline levels.

Differences between sVCAM-1 and sICAM-1. The current results for sVCAM-1 are clearly distinct from those results previously reported in this cohort for sICAM-1 (13). In our previous study, we found that elevated baseline concentrations of sICAM-1 were independently associated with an increased risk of MI, and that this risk increased over time. Furthermore, concentrations of sICAM-1 were strongly associated with concentrations of CRP, a marker of systemic inflammation that has been shown to be an independent risk factor for adverse cardiovascular events in this and other cohorts (24–27). In contrast, our current null data for sVCAM-1 are consistent with prospective data derived from the Atherosclerosis Risk In Communities (ARIC) study (14). In that analysis, as in the PHS, sICAM-1 but not sVCAM-1 was significantly associated with future MI. The consistency of these two large, prospective studies suggests that important pathophysiologic differences exist between VCAM-1 and ICAM-1 in the genesis of atherothrombosis.

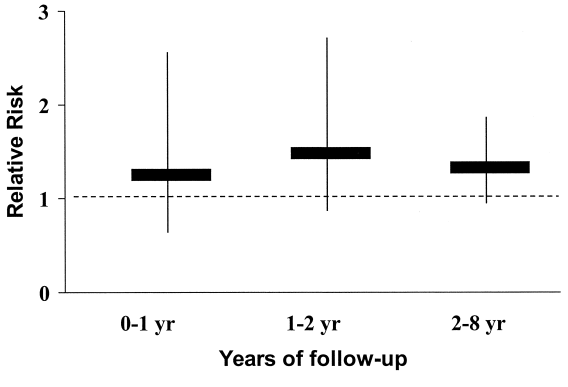

Figure 1. Relative risk (95% confidence interval) for first MI associated with a baseline sVCAM-1 concentration ≥ the 75th percentile, according to year of follow-up.

Vascular cell adhesion molecule-1 is a member of the immunoglobulin superfamily and the receptor for the ligand very late antigen-4, a $beta_1$ integrin found only on the surface of mononuclear cells (5). In a rabbit model of atherogenesis, VCAM-1 was highly expressed on endothelial cells at very early stages of atherogenesis (2–4). Histopathologic studies in humans, however, have generally not found high level VCAM-1 expression (8), except in areas of intimal neovascularization (9). These studies have shown clear differences between VCAM-1 and ICAM-1 expression in normal and atherosclerotic arteries. Although VCAM-1 is not expressed on normal endothelium, ICAM-1 is constitutively expressed at low levels (8,11,28). In human atheroma, ICAM-1 is highly expressed by both endothelial cells and subendothelial macrophages. In contrast, VCAM-1 is found in fewer than one-third of lesions, with expression predominantly restricted to endothelial cells and occasional spindle-shaped cells (8). The number of cells within the atheroma expressing VCAM-1 is therefore markedly less than the number expressing ICAM-1.

Other indirect lines of evidence point toward an association between atherosclerosis and sICAM-1, but not sVCAM-1. Although smoking is clearly associated with increased sICAM-1 levels (13,29), we did not find an association between smoking and sVCAM-1 levels. Similarly, although increased sICAM-1 is also associated with elevated levels of CRP (25), we found no association between VCAM-1 and this sensitive inflammatory marker.

Potential study limitations. The design of our study prevents direct assessment of the role of membrane-bound VCAM-1 in coronary atherosclerosis. It is possible that in contrast to ICAM-1, levels of soluble VCAM-1 do not reflect expression of membrane-bound VCAM-1. Indeed, some evidence exists that the "shedding" process may be different for different CAMs. For example, Pigott et al. (16)

found a greater proportion of soluble to membrane-bound VCAM-1 than ICAM-1 (16), suggesting that VCAM-1 may be cleaved from the cell surface more readily than ICAM-1.

A second potential limitation is our use of frozen plasma samples for analysis. The distribution of baseline sVCAM-1 values in the current study is within the expected range for this assay (± 1 SD range 395 to 714 ng/ml), however, suggesting that the frozen specimens were suitable samples for analysis. As with any null study, consideration must be given to the possibility that the study was not sufficiently powered to detect small differences in risk. The current study, however, had >80% power to detect a relative risk as low as 2.0. Finally, although we found no association between sVCAM-1 and cardiovascular events through eight years of follow-up, it is possible that even longer follow-up would be necessary to detect such an association. This is at least theoretically plausible in light of the proposed role for sVCAM-1 very early in the atherosclerotic process (2–4).

Conclusions. The plasma concentration of sVCAM-1 does not appear to be a marker for future MI among apparently healthy men. When interpreted with our ICAM-1 results, these data strongly suggest that there are important distinctions between different sCAMs in the genesis of atherothrombosis. Future studies, both at the basic and clinical level, should focus on the mechanisms and implications of these differences.

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REFERENCES

1. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
2. Cybulsky MI, Gimborne MA. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 1991; 251:788–90.
3. Li H, Cybulsky MI, Gimbrone MA Jr., Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. Arterioscler Thromb Vasc Biol 1993;13:197–204.
4. Sakai A, Kume N, Nishi E, Tanoue K, Miyasaka M, Kita T. P-selectin and vascular cell adhesion molecule-1 are focally expressed in aortas of hypercholesterolemic rabbits before intimal accumulation of macrophages and T lymphocytes. Arterioscler Thromb Vasc Biol 1997;17:310–6.
5. Jang Y, Lincoff AM, Plow EF, Topol EJ. Cell adhesion molecules in coronary artery disease. J Am Coll Cardiol 1994;24:1591–601.
6. Bevilacqua MP, Nelson RM, Mannori G, Cecconi O. Endothelial-leukocyte adhesion molecules in human disease. Annu Rev Med 1994;45:361–78.
7. Springer TA. Adhesion receptors of the immune system. Nature 1990;346:425–33.
8. Davies MJ, Gordon AJH, Gearing AJH, et al. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. J Pathol 1993;171:223–9.
9. O'Brien KD, Allen MD, McDonald TO, et al. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques: implications for the mode of progression of advanced coronary atherosclerosis. J Clin Invest 1993;92:945–51.
10. O'Brien KD, McDonald TO, Chait A, Allen MD, Alpers CE. Neovascular expression of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in human atherosclerosis and their relation to intimal leukocyte count. Circulation 1996;93:672–82.
11. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the apoE-deficient mouse. Arterioscler Thromb Vasc Biol 1998;18:842–51.
12. van der Wal AC, Das PK, Tigges AJ, Becker AE. Adhesion molecules on the endothelium and mononuclear cells in human atherosclerotic lesions. Am J Pathol 1992;141:1427–33.
13. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule-1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351:88–92.
14. Hwang S, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997;86:4219–25.
15. Gearing AHJ, Hemingway JK, Pigott R, Hughes J, Rees AJ, Cashman SJ. Soluble forms of vascular adhesion molecules E-selectin, ICAM-1 and VCAM-1: pathological significance. Ann NY Acad Sci 1992;667: 324–31.
16. Pigott R, Dillon LP, Hemingway IH, Gearing AJH. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochem Biophys Res Commun 1992;187:584–9.
17. Blann AD, McCollum CN. Circulating endothelial cell/leukocyte adhesion molecules in atherosclerosis. Thromb Haemost 1994;72: 151–4.
18. Blann AD, Lip GYH. Endothelial integrity, soluble adhesion molecules and platelet markers in type 1 diabetes mellitus. Diabet Med 1998;15:634–42.
19. Rohde LE, Lee RT, Rivero J, et al. Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. Arterioscler Thromb Vasc Biol 1998;18:1765–70.
20. Morisaki N, Saito I, Tamura K, et al. New indices of ischemic heart disease and aging: studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hypercholesterolemia and ischemic heart disease. Atherosclerosis 1997;131:43–8.
21. De Caterina R, Basta G, Lazzerini G, et al. Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:2646–54.
22. Peter K, Nawroth P, Conradt C, et al. Circulating vascular adhesion molecule-1 correlates with the extent of human atherosclerosis in contrast to circulating intercellular adhesion molecule-1, E-selectin, P-selectin, and thrombomodulin. Arterioscler Thromb Vasc Biol 1997;17:505–12.
23. Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. N Engl J Med 1989;321:129–35.
24. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. N Engl J Med 1994;331:417–24.
25. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973–9.
26. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 1998;98:731–3.
27. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. Circulation 1998;97:425–8.
28. Chester AH, Morrison KJ, Yacoub MH. Expression of vascular adhesion molecules in saphenous vein coronary bypass grafts. Ann Thorac Surg 1998;65:1685–9.
29. Blann AD, Steele C, McCollum CN. The influence of smoking on soluble adhesion molecules and endothelial cell markers. Thromb Res 1997;85:433–8.