Serum Soluble Vascular Cell Adhesion Molecule-1: Role as a Surrogate Marker of Angiogenesis

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Background: Angiogenesis, the development of new blood vessels from pre-existing vasculature, is a prerequisite for tumor growth and metastasis. Surrogate markers for angiogenesis would be useful for studying the effectiveness of antiangiogenesis drugs. We examined the potential of three serum glycoproteins—vascular cell adhesion molecule-1 (VCAM-1), endothelial selectin (E-selectin), and von Willebrand factor (VWF)—to serve as markers for angiogenesis. *Methods:* Preoperative serum levels of VCAM-1, E-selectin, and VWF were measured by enzyme-linked immunosorbent assay in 93 women with early breast cancer and were compared with microvessel density in each tumor, histologic features, and recurrence after surgery. Serum samples were taken from 55 women with advanced breast cancer who were commencing hormonal therapy, both immediately before therapy and 3 months later. Changes in serum levels of VCAM-1, E-selectin, and VWF were compared with the response of the disease to hormonal therapy assessed 6 months after the start of hormone therapy or at disease progression. All *P* values are two-sided. *Results:* In women with early breast cancer, serum levels of VCAM-1 (but not of E-selectin or VWF) correlated closely with microvessel density in tumors ($r = .65$; $P<.001$), and women who developed early recurrence had higher preoperative levels of serum VCAM-1 than those who remained disease free ($P = .01$). Serum VCAM-1 levels rose in women with advanced breast cancer whose disease progressed ($P<.001$) but remained unchanged or fell in women with advanced breast cancer whose disease remained stable or showed a partial response to hormonal therapy. *Conclusion:* Serum VCAM-1 appears to be a surrogate marker of angiogenesis in breast cancer. Its measurement may, therefore, help in the assessment of antiangiogenesis drugs currently in phase II trials. [J Natl Cancer Inst 2000;92:1329–36]

Angiogenesis plays a central role in tumor growth and metastasis *(1–3)*. Consequently, pharmaceutical research into new cancer treatments has focused on the development of drugs that target the tumor vasculature and inhibit angiogenesis. Many antiangiogenesis drugs are undergoing phase I/II trials, but assessment of the response to pharmacologic intervention is difficult because the only currently available method of quantifying angiogenesis requires removal of the tumor and counting of microvessel density after staining with antibodies to endothelial cell antigens *(4,5)*. This technique is applicable only to primary tumors and is impractical as a method of monitoring response to antiangiogenesis therapy in metastatic disease.

Surrogate markers of angiogenesis would facilitate the assessment of the response to angiogenesis inhibitors *(1)*. Candidates for such biomarkers include glycoproteins that are produced and secreted by activated endothelial cells. These glycoproteins include vascular cell adhesion molecule-1 (VCAM-1) *(6)*, endothelial selectin (E-selectin) *(7)*, and von Willebrand factor (VWF) *(8)*.

VCAM-1 is a 90-kd transmembrane glycoprotein that is expressed transiently on vascular endothelial cells in response to vascular endothelial growth factor (VEGF) and other cytokines *(6)*. Endothelial expression of VCAM-1 plays a major role in adhesion of leukocytes to the endothelium in inflammation. In addition, endothelial cells expressing VCAM-1 bind melanoma cell lines, suggesting that VCAM-1 may function as an adhesion molecule to facilitate metastasis *(6)*. Although VCAM-1 is expressed predominantly on activated endothelial cells, it is also found on dendritic cells and proximal renal tubule cells *(6)*.

E-selectin (CD62E) (previously known as endothelial leukocyte adhesion molecule [ELAM-1]) is a transmembrane glycoprotein that, like VCAM-1, is expressed on endothelial cells in response to VEGF *(9)*. E-selectin mediates adhesion of neutrophils, monocytes, and memory T cells to the endothelium and has, like VCAM-1, been implicated in metastasis *(10)*.

VWF is produced by endothelial cells and platelets; however, it is not an adhesion molecule. Physiologic levels of VWF in-

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crease with platelet adhesion at the site of injury. VWF then forms a complex with factor VIII that facilitates normal blood clotting.

Soluble forms of several adhesion molecules (e.g., leukocyte-selectin [CD62L] and platelet-selectin [CD62P]) and growth factor receptors (e.g., c-erbB2) are known to be shed from the cell surface *(11)*; VCAM-1 and E-selectin both have soluble forms. Soluble forms of VCAM-1 have been detected both *in vitro (12)* and *in vivo (11,13–15)*. Serum levels of soluble VCAM-1 are raised in patients with various malignancies, including breast and gastric cancers *(16,17)*. The soluble form of E-selectin is present at higher levels in the serum of patients with certain cancers than in cancer-free control subjects *(16)*. In addition, the serum VWF concentration has been shown to be increased in patients with advanced breast cancer as compared with normal control subjects *(15)*. Koch et al. *(18)* have demonstrated that soluble forms of VCAM-1 and E-selectin released by leukocyte adhesion to endothelial cells are chemotactic attractants for endothelial cells.

Although immunohistochemical studies demonstrate that VCAM-1 and E-selectin are found on the membranes of malignant breast endothelial cells, they are not found on breast epithelial cells *(19)*. Soluble VCAM-1 and E-selectin have been implicated in the mediation of angiogenesis *(18)*. We, therefore, hypothesized that levels of VCAM-1 and E-selectin in the serum may provide surrogate markers of angiogenesis occurring in breast cancer. By contrast, we anticipated that VWF, which is released by all endothelial cells, would be a pan-endothelial marker that would not accurately report angiogenesis. To determine the potential of these serum glycoproteins to serve as biomarkers for angiogenesis, we examined whether serum levels of VCAM-1, E-selectin, or VWF are associated with standard prognostic factors in women with early and advanced breast cancers. We also tested whether microvessel density in tumors from women with early breast cancer correlates with serum levels of any of these endothelial cell-derived glycoproteins.

PATIENTS AND METHODS

Patients with early breast cancer were recruited from a breast clinic at the University Hospital of South Manchester, U.K. Patients with advanced breast cancer were recruited from a breast oncology clinic at the same hospital. These studies were approved by the South Manchester Ethics Committee, and all patients gave written informed consent.

Patients With Early Breast Cancer

Women with newly diagnosed breast cancer (n = 93) provided venous blood immediately before surgery (axillary lymph node clearance combined with either mastectomy or wide local excision). The mean age of the patients at diagnosis was 57.8 years (range = 32–95 years). Of the women with early breast cancer, 22 were premenopausal and 71 were postmenopausal. Blood was taken from a control group of 29 women with benign breast disorders (cyclical mastalgia [n = 17] or fibrocystic change [n = 12]) who had attended the same clinic. The commercial enzyme-linked immunosorbent assays (ELISAs) used to measure VCAM-1 and E-selectin had already reported a normal range of levels in healthy control women (VCAM-1: median value = 553 ng/mL [range = 395–714 ng/mL]; E-selectin: median value = 46.3 ng/mL [range = 29.1–63.4 ng/mL]). We assayed serum levels in the 29 women with benign breast disorders and found that levels were in the normal range in these women as well.

An experienced breast pathologist assessed hematoxylin–eosin-stained specimens for tumor type, size, and grade *(20)* without knowledge of serum levels of VCAM-1, E-selectin, or VWF. The total number of lymph nodes in the axillary clearance specimens and the total number of involved lymph nodes were recorded for each patient. The development and site of a recurrent carcinoma were recorded at the date of presentation. Estrogen receptor was assessed in all tumors

with the use of a standard immunohistochemical staining technique utilizing a primary mouse monoclonal antibody to human estrogen receptor (M7042; DAKO, Copenhagen, Denmark) as described previously *(21)*.

Patients With Advanced Breast Cancer

Sequential serum samples were taken from 55 women with estrogen receptor-positive cancer who were commencing or switching hormonal therapy for progressing or newly diagnosed advanced breast cancer. All of the women had evidence of metastatic disease in the skeleton, as diagnosed by isotope bone scan and plain radiographs. In addition, 28 of the women had disease in one or more other sites (lung [n = 12], liver [n = 10], soft tissue [n = 8], adrenal [n = 1], or brain [n = 1]). Blood was taken immediately before the patient started or changed hormonal therapy and 3 months later. The women had a mean age of 56.4 years (range = 37–81 years). At the time of breast cancer diagnosis, 13 of the women were premenopausal, and 42 were postmenopausal. Response to treatment was assessed by standard radiologic criteria at 3 and 6 months after initiation of or change in hormonal therapy or at disease progression *(22)*. Each patient was assessed by a medical oncologist (A. Howell) without knowledge of serum concentrations of VCAM-1, E-selectin, and VWF.

VCAM-1, E-Selectin, and VWF Assays

Blood from all patients was centrifuged for 20 minutes at 3500g at 4 °C immediately after phlebotomy. The separated serum was then stored at −20 °C in 1-mL aliquots. Before analysis, samples were thawed slowly and mixed gently.

VCAM-1 and E-selectin were assayed with the use of commercial ELISA kits (R&D Systems Ltd., Oxford, U.K.) *(11,12)*. Intra-assay precision for VCAM-1 and E-selectin assays was 6.7% and 5.1%, respectively. Interassay precision for VCAM-1 and E-selectin assays was 7.0% and 6.2%, respectively.

Serum concentrations of VWF were determined by ELISA, with the use of a technique described previously *(23)*. The rabbit antihuman VWF antibody for this assay was obtained from DAKO (High Wycombe, U.K.).

Microvessel Count: Assessment of Angiogenesis

Microvessels in tumors from women with early breast cancer were visualized by immunostaining sections with antibodies to CD31, an endothelial cell antigen. Sections (5 μm) were cut from formalin-fixed, paraffin-embedded specimens of primary breast cancers. The specimens were dewaxed in xylene followed by four changes of ethanol, after which they were washed in tap water prior to staining. Endogenous peroxidase activity was blocked by treating the sections with 3% hydrogen peroxide in deionized water for 10 minutes. Tissue sections were put in 0.1 M citrate buffer (pH 6.0) and placed on a rotating table in a microwave oven. Heat pretreatment was carried out with two 15-minute cycles each at medium-high output (600 W). The sections were allowed to cool at room temperature and washed in Tris-buffered saline (TBS) prior to immunostaining. Nonspecific binding was blocked with 1% normal goat serum in TBS for 10 minutes. Serial sections were incubated with a 1 : 20 dilution of primary antibody (JC/70A monoclonal antibody to CD31; DAKO, Copenhagen) *(24–26)*. The slides were washed with TBS for 5 minutes, incubated with a 1 : 100 dilution of biotinylated secondary goat anti-mouse antibody (KO492; DAKO, Goldstrup, Denmark) in 1% normal goat serum in TBS for 30 minutes, and washed in TBS for 2–3 minutes. The streptavidin–biotin complex (1 : 100 in TBS; DAKO, Copenhagen) was applied for 30 minutes. The slides were then washed with TBS, treated with 0.08% diaminobenzidine (Sigma Chemical Co., St. Louis, MO) and hydrogen peroxide (0.3%) in deionized water, counterstained with hematoxylin, dehydrated, and mounted with DPX (a mixture of disterene, plasticizer, and xylene; BDH, Leicester, U.K.). Sections for which the primary or secondary antibody had been omitted were used as negative controls.

Microvessel density was quantified by light microscopy of labeled slides without knowledge of patient details. The most vascular areas in a tumor (i.e., the hot spots) were located at low magnification, and the vessels in these regions were counted with the use of a Chalkley point eyepiece graticule at 400× magnification *(21)*. Any brown-staining endothelial cells or group of cells in contact with a spot in a graticule was counted as an individual vessel. The mean of four Chalkley counts for each tumor was calculated and used in statistical analysis. Microvessel density was assessed without knowledge of serum levels of VCAM-1, E-selectin, or VWF. Microvessel scores with the use of Chalkley counting ranged from 1 to 4 vessel counts, with 4 being the highest score and 1 being the lowest. We have previously validated this technique *(21)*.

Statistical Methods

Statistical analysis was performed with the use of Pearson correlations for all paired continuous variables and Student's *t* tests for categorical analysis. Serum measurements of all three glycoproteins demonstrated skewed distributions; therefore, logarithms of the geometric means of variables were used in the analysis. Data for tumor grade and lymph node status for women with early breast cancer were assessed with the use of a one-way analysis of variance. For women with advanced breast cancer, logarithms of the geometric mean difference were analyzed with the use of a paired Student's *t* test. VCAM-1, E-selectin, and VWF serum levels were compared with vessel counts with the use of chi-square tests. All *P* values are two-sided.

RESULTS

Serum VCAM-1, E-Selectin, and VWF Levels in Women With Early Breast Cancer

To investigate whether serum concentrations of activated endothelial cell molecules are associated with cancer, we compared serum levels of VCAM-1, E-selectin, and VWF in women with early breast cancer with those in control women with benign breast disease. In women with early breast cancer, serum concentrations of VCAM-1 (mean = 769.5 ng/mL; 95% confidence interval [CI] = 651–887 ng/mL), E-selectin (mean = 54.3 ng/mL; 95% CI = 48.1–60.6 ng/mL), and VWF (mean = 141.6 ng/mL; 95% CI = 126.8–156.5 ng/mL) were statistically significantly higher than those in control women (VCAM-1: mean = 483 ng/mL [95% CI = 448–518 ng/mL]; E-selectin: mean = 43.5 ng/mL [95% CI = 40.3–46.8 ng/mL]; VWF: mean = 112.2 ng/mL [95% CI = 93.8–128.6 ng/mL]) (*P* = .001, *P* = .003, and *P* = .031, respectively). In addition, serum levels of soluble VCAM-1 and E-selectin, but not of VWF, were significantly higher in women with histologic axillary lymph node metastases (40 [43%] of the 93 women with early breast cancer) than in lymph node-negative women or control women (*P* = .003 and *P* = .047, respectively; Fig. 1). VCAM-1 levels also were associated with the extent of tumor differentiation. Serum from women with well-differentiated tumors (grade 1 [n = 12]) had lower VCAM-1 levels (mean = 592 ng/mL; 95% CI = 520–664 ng/mL) than serum from women with higher tumor grades (grade 2 [n = 34] or grade 3 [n = 47]) (mean = 793 ng/mL; 95% CI = 675–911 ng/mL) (*P* = .003, two-sided Student's *t* test following logarithmic transformation). By contrast, serum E-selectin and VWF levels did not relate to tumor grade.

We next compared serum levels of VCAM-1, E-selectin, and VWF with microvessel density scores of tumors from women with early breast cancer. These scores, as assessed on paraffin sections by CD31 staining, ranged from 1 to 4, with 29% of the tumors scoring as highly vascular (counts of 3–4). Serum levels of VCAM-1, but not of E-selectin or VWF, correlated with microvessel density in early breast cancer (*r* = .65; *P*<.001 [Fig. 2]).

Other properties of tumors did not show an association with any of the three endothelial cell molecules. Of the 93 women with early breast cancer, 62 (67%) had tumors that stained strongly for estrogen receptors. However, receptor status did not relate to serum levels of VCAM-1, E-selectin, or VWF. In addition, tumor size (mean = 22 mm; 95% CI = 19–25 mm) did not correlate with serum levels of VCAM-1 (*r* = .213), E-selectin (*r* = .314), or VWF (*r* = .173). Tumor type, age, and menopausal status also did not show an association with serum levels of VCAM-1, E-selectin, or VWF (data not shown).

Recurrence did show an association with serum VCAM-1 levels (Table 1). Of the 93 women with early breast cancer, 16 have subsequently developed recurrent breast carcinoma (locoregional [n = 4], bone alone [n = 10], or mixed bone and visceral [n = 2]) after a median follow-up from the date of surgery of 38 months (range = 22–72 months). The median time to recurrence was 21 months (range = 8–53 months). Preoperative serum VCAM-1 levels were higher in those women who developed early recurrence than in those who have remained cancer free to the end of the study (*P* = .01) (Table 1).

Serum VCAM-1, E-Selectin, and VWF Levels in Women With Advanced Breast Cancer

Levels of angiogenesis in women with advanced breast cancer cannot be assessed by microvessel density because the technique does not allow sequential assessment of angiogenesis in

Fig. 1. Relationship of serum vascular cell adhesion molecule-1 (VCAM-1) and endothelial selectin (E-selectin [ESEL]) levels to lymph node status. In women undergoing surgery for early breast cancer, both VCAM-1 and E-selectin levels are significantly raised in women with involved axillary lymph nodes (n = 40) when compared with lymph node-negative women (n = 53) undergoing surgery for early breast cancer. The **line marked N** refers to the mean serum values for women with benign breast disease. **Error bars** show 95% confidence intervals.
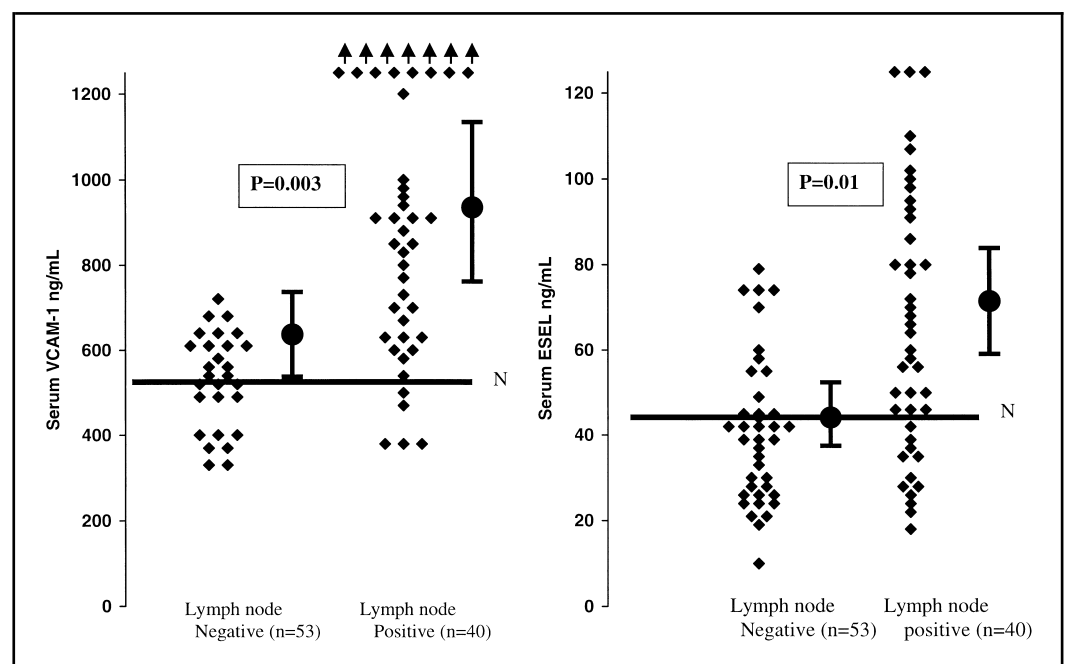
Fig. 2. Relationship of serum vascular cell adhesion molecule-1 (VCAM-1) to microvessel density in early breast cancer. Serum VCAM-1 levels correlate closely ($r = .65$; $P<.001$) with microvessel density counted with the use of a Chalkley eyepiece following immunohistochemical staining for the endothelial CD31 antigen. The r value refers to the Pearson correlation coefficient.
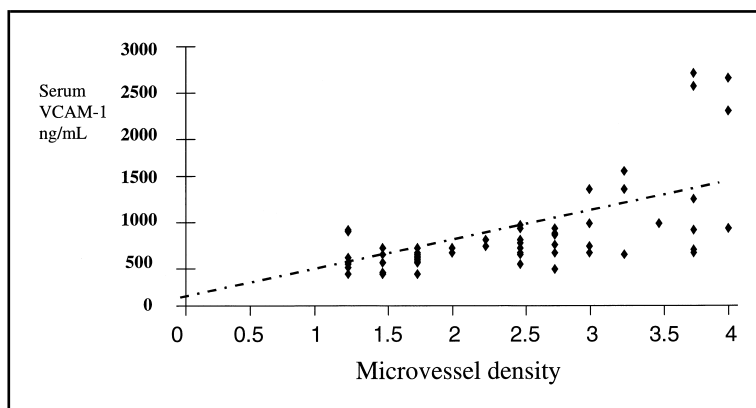
Table 1. Factors associated with breast cancer recurrence in women with early breast cancer

Factor	Subjects with recurrent cancer (n = 16)	Disease-free subjects (n = 77)	P*
Median follow-up, mo (interquartile range)	40 (18–73)	37 (24–70)	NS
Median tumor size, mm (interquartile range)	17 (14–24)	20 (12–29)	NS
Tumor type, No.			
Lobular	2	13	NS
Ductal	14	64	NS
Lymph node-positive tumors, No.	13	27	$P = .0058$ $\chi^2 = 36.8$†
Estrogen receptor-negative tumors, No.	8	23	NS
Grade 3 tumors, No.	5	42	NS
Median microvessel density score (interquartile range)	3 (2.69–3.81)	2.38 (1.5–2.81)	$P = .19$ (Mann-Whitney)
Median serum VCAM-1,‡ ng/mL (interquartile range)	875 (714–1311)	599.5 (495–646.5)	$P = .01$ (Mann-Whitney)

*NS = not significant.

†Lymph node-positive tumors had a greater chance of recurrence than lymph node-negative tumors.

‡VCAM-1 = vascular cell adhesion molecule-1.

human metastases. Often biopsies cannot be performed on metastases and, if the lesion is excised, it cannot be assessed again. In animal models, angiogenesis is inhibited by hormonal therapy *(27,28);* therefore, we studied women with advanced breast cancer who were undergoing hormonal therapy to assess any changes in the levels of VCAM-1, E-selectin, and VWF. Of the 55 women with advanced breast cancer, 31 had a partial response (n = 10) or stable disease for more than 6 months (n = 21) after treatment with tamoxifen (n = 15), megestrol acetate

(n = 10), goserelin (n = 3), or anastrozole (n = 3). Among women who showed a partial response to therapy, their serum VCAM-1 levels fell by an average value of 276 ng/mL ($P = .043$), whereas serum E-selectin and VWF levels did not change (Table 2). Among women whose disease was stable, there were no changes in serum levels of any of the three endothelial cell molecules.

For the remaining 24 women with advanced breast cancer (who were taking tamoxifen [n = 10], megestrol acetate [n =

Table 2. Disease response and serum concentrations of vascular cell adhesion molecule-1 (VCAM-1), endothelial selectin (E-selectin), and von Willebrand factor (VWF) at 0 and 3 months in patients with advanced breast cancer undergoing hormonal therapy*

UICC response	Time, mo	Mean serum VCAM-1, ng/mL (95% CI)	Mean E-selectin, ng/mL (95% CI)	Mean VWF, ng/mL (95% CI)
Partial response (n = 10)	0	776.1 (592–960)	55.95 (44.5–67.4)	147.3 (112–182)
	3	500.4 (378–622)†	55.4 (40.2–70.8)	135.2 (93–177)
Stable disease (n = 21)	0	542.4 (451–633)	72.8 (65–80)	149.5 (123–176)
	3	604.8 (499–850)†	60.7 (52.4–69)	149.4 (118–180)
Progressive disease (n = 24)	0	568 (490–659)	46 (33–58)	121 (103–142)
	3	989 (758–1290)†	62 (33–107)‡	133 (121–146)‡

*UICC = Union Internationale Contre le Cancer; CI = confidence interval.

†Changes in serum VCAM-1 levels over a 3-month period reflect the clinical response to hormonal therapy, as assessed by UICC criteria. Serum VCAM-1 levels remained stable in patients with stable disease, fell in patients who exhibited a partial response to therapy ($P = .043$), and rose in patients with progressive disease ($P<.001$).

‡Changes in serum E-selectin and VWF levels do not reflect the clinical response to hormonal therapy. However, serum E-selectin levels rose in patients with progressive disease ($P = .048$), as did serum VWF levels ($P = .051$).

10], or goserelin [n = 4]), the disease progressed over the test period. After 3 months, serum VCAM-1 and E-selectin levels rose statistically significantly from baseline initial values ($P<.001$ and $P = .048$, respectively). The average rise in VCAM-1 levels was 421 ng/mL, from an initial mean value of 568 ng/mL. The average rise in E-selectin levels was 16 ng/mL, from an initial mean of 46 ng/mL. Serum VWF levels rose as well, but the increase was not statistically significant ($P = .051$).

It has been shown previously that there is no difference in survival between patients who exhibit a partial response to hormonal therapy and those whose disease remains stable for 6 months *(29)*. Thus, the two groups were also combined for analysis. Statistical comparison between this combined group with women whose disease progressed indicated that only VCAM-1 demonstrated a difference between responding and nonresponding tumors at 3 months ($P<.001$). The positive predictive value of no increase in serum VCAM-1 levels, indicating a response to therapy, was 90% (26 of 29) (Table 3). The sensitivity and specificity of a decrease in serum VCAM-1 levels were 98% and 80%, respectively (Table 3).

DISCUSSION

In many disease processes, the activation of endothelial adhesion molecules facilitates the local entry of inflammatory cells, which in turn promotes tissue neovascularization, resulting in tumor growth and wound repair *(18,30,31)*. Soluble forms of endothelial adhesion molecules are also released from the endothelium on stimulation by cytokines and increase endothelial cell migration *(18)*. We, therefore, sought to define a possible relationship between the soluble forms of endothelial adhesion molecules and angiogenesis in breast cancer. In a study of the expression of adhesion molecules by breast cancer endothelial cells *(19)*, VCAM-1 was expressed on breast cancer endothelium but not on normal breast endothelium, and E-selectin was expressed at higher levels on breast cancer endothelium than on normal breast endothelium *(19)*. Other pan-endothelial molecules not associated with adhesion (e.g., VWF) showed no such differences in expression.

Our results demonstrate that serum levels of VCAM-1 are more closely associated than E-selectin and VWF serum levels with standard prognostic factors in early breast cancer. In addition, serum VCAM-1 levels correlate closely with tumor microvessel density, which is currently the standard method for measuring angiogenesis *(4,5,8)*. Moreover, changes in levels of VCAM-1 in the serum of women undergoing hormonal therapy for metastatic breast cancer also paralleled responses of the epithelial cells to the endocrine therapy, suggesting that a "switch off" of angiogenesis is an early step in inhibition of tumor growth.

A study *(17)* showed serum VCAM-1 to be of prognostic value in patients with gastric cancer. However, given the large acute-phase reaction seen in gastric neoplasia, it is difficult to separate the acute-phase response from angiogenesis in this context. In the clearer context of breast cancer, we found that high serum levels of VCAM-1 identified tumors at risk of early relapse as well as lymph node status and microvessel density.

Angiogenesis proceeds at the same time as tumor growth and metastasis *(1–3)*. The continuous formation of new capillaries, which is induced by tumor epithelial angiogenic factors *(1,2)*, can be quantified by measuring microvessels *(24–26)*. While endothelial cell activation *in vitro* can be recognized by a sprouting phenotype *(1,2,11)*, an angiogenic response in the cornea *(18)*, or endothelial chemotaxis *(18)*, recognition of endothelial cell activation *in vivo* is problematic *(1–3)*. Microvessel counts have been shown to be an independent prognostic marker in breast cancer *(8)*, but assessment of microvessel density relies on immunohistochemical staining of the primary tumor. Microvessel density thus provides a snapshot of angiogenesis that cannot be repeated once the primary tumor has been removed. The measurement of serum VCAM-1 levels, therefore, is potentially a simple surrogate method of determining levels of angiogenesis at any given time in women with early breast cancer. Serum VCAM-1 levels in women with advanced breast cancer potentially represent the state of continuing angiogenesis in metastases. The rise in VCAM-1 levels by 3 months correlated with hormone-nonresponsive disease, whereas a fall was seen in hormone-responsive disease, suggesting that a reduction in endothelial secretion of adhesion antigens occurs early in hormone-responsive tumors.

Angiogenesis takes place continuously at the advancing edge of tumors or metastatic deposits *(1,2)*. It may occur not just by endothelial cell proliferation but also by co-opting of host blood vessels, endothelial cell migration, and capillary cell morphogenesis *(1–3)*. Serum VCAM-1 levels in patients with advanced breast cancer represent an accurate measure of continuing en-

Table 3. Sensitivities, specificities, and positive predictive values of serum vascular cell adhesion molecule-1 (VCAM-1) measurements in patients with advanced breast cancer showing varying responses to hormonal therapy, according to Union Internationale Contre le Cancer (UICC) criteria

Change in serum VCAM-1 levels over 3-mo period	UICC response at 6 mo*			Sensitivity†	Specificity‡	Positive predictive value§
	Progressive disease (n = 24)	Stable disease (n = 21)	Partial response (n = 10)			
Increase	21	5	0	88% (21/24)	84% (26/31)	81% (21/26)
Stable‖	3	15	2	85% (29/34)	71% (15/21)	75% (15/20)
Decrease	0	1	8	98% (44/45)	80% (8/10)	89% (8/9)

*Response was assessed by an independent medical oncologist without knowledge of serum VCAM-1 levels.

†Sensitivity is defined as the proportion of patients with advanced breast cancer whose disease is progressing (or remaining stable or showing a partial response to endocrine therapy) at 6 months and whose serum VCAM-1 levels are increasing (or remaining stable or decreasing, respectively).

‡Specificity is defined as the proportion of patients whose disease is not progressing (or is not stable or is not responding partially to endocrine therapy) at 6 months and whose serum VCAM-1 levels are not increasing (or not stable or not decreasing, respectively).

§Positive predictive value is defined as the proportion of those patients whose serum VCAM-1 levels are increasing (or remaining stable or decreasing) and whose disease is progressing (or remaining stable or showing a partial response to endocrine therapy).

‖Serum VCAM-1 levels were considered stable if levels did not change by more than twice the interassay variability (i.e., 14%).

dothelial activation and angiogenesis, as indicated by the rise in VCAM-1 levels within 3 months in women whose breast cancer was progressing and by the fall in these levels in women whose breast cancer showed a partial response to endocrine therapy. These observations suggest that, in hormone-responsive tumors, a reduction in the surface area of activated endothelium leads to a reduction in shed VCAM-1 into the serum. Once blood vessels have become established in tumors, the endothelium expresses different antigens *(19,21,24)*. The factors controlling expression of all of these endothelial antigens—in particular, soluble VCAM-1—are not clear.

In tumors, endothelial VCAM-1 plays a major role in the adhesion of leukocytes to the endothelium, suggesting that cellular adhesion and angiogenesis are linked *(18,32)*. The adhesion molecule αvβ3 integrin is a marker of angiogenic vascular tissue in wound granulation tissue *(33)*, and both soluble E-selectin and soluble VCAM-1 are angiogenic in a corneal model *(18)*. The mechanism underlying the shedding of VCAM-1 and E-selectin is not yet known, but shedding is believed to occur after adhesion of inflammatory or tumor cells to activated vascular endothelium *(32)*. VCAM-1 expression by endothelial cells, although tissue and organ dependent *(34)*, is induced by VEGF, tumor necrosis factor-α, interleukin 1β, and interferon gamma—all of which have been implicated in the angiogenic response *(19,35,36)*. For example, Jallal et al. *(37)* have described the release of a 90-kd protein by tumor cells that increases the expression of VCAM-1 in tumor vessels. However, the same protein leads to tumor regression and infiltration by natural killer cells and macrophages when given locally or systemically in nude mice models *(37)*.

Increased expression of VCAM-1 on activated endothelium may facilitate transmigration of monocytes and T lymphocytes across the endothelium because these cell types express VLA4, the ligand for VCAM-1 *(38)*. The binding of monocytes to endothelial cells and the concomitant release of cytokines by both endothelial cells and monocytes have been reported to lead to the release of soluble VCAM-1 from the endothelial cells *(18)*; the soluble VCAM-1, in turn, binds to adjacent endothelial cells (via its ligand, VLA4), potentiating the angiogenic effects of cytokines released by the tumor itself on endothelial cells and enhancing tumor angiogenesis *(18)*. Increases in endothelial VCAM-1 expression may, therefore, be one mechanism to increase the immune response to tumors, but this mechanism is ultimately harmful to the host by increasing angiogenesis and metastasis *(37)*. Thus, the tumor may effectively hijack the normal tissue process of angiogenesis to provide itself with a blood supply.

Endocrine agents (e.g., tamoxifen and medroxyprogesterone acetate) are known to induce tumor regression by acting through steroid receptors in tumor epithelial cells *(27,28)*. In addition, medroxyprogesterone acetate inhibits tumor growth and neovascularization in the rabbit cornea *(39)*. Tamoxifen has also been shown to inhibit angiogenesis and endothelial cell growth in human tumors *(27)* and in animal models *(28)*, although whether the inhibition occurs directly or indirectly, via inhibition of epithelial cell secretion of cytokines, is unclear. Whatever the mechanisms, our data suggest that inhibition of endothelial cell activation and a fall in serum VCAM-1 levels are key early events in the response of tumors to hormonal therapy. The fall in serum VCAM-1 levels occurred early and was not associated with a flare or rise in responding tumors, which is often seen with clinical epithelial cell markers, such as MUC1 and CA15-3 *(40)*.

In patients undergoing hormonal therapy, changes in serum VCAM-1 levels provide a surrogate measure of angiogenesis, allowing rapid assessment of response to therapy rather than the static measurement obtained by microvessel density. Chemotherapy should theoretically reduce angiogenesis even more expeditiously than hormonal therapy, in which reduction in serum VCAM-1 levels occurs within 3 months. We are currently examining whether measurement of serum VCAM-1 levels in women undergoing primary medical chemotherapy for locally advanced breast carcinoma provides an earlier marker of response to therapy than does clinical examination or radiologic assessment.

Angiogenesis in women with advanced breast cancer is inhibited by endocrine therapy in hormone-responsive tumors *(27,28)*. In this study, serum VCAM-1 levels but not E-selectin levels rose rapidly in women whose disease progressed on endocrine therapy but remained static in women whose disease remained stable or who had a partial response to therapy. The level of VWF, a pan-endothelial cell marker, although raised in breast cancer, did not correlate with VCAM-1 levels, microvessel density, or tumor response. Like VCAM-1, E-selectin is also expressed at higher levels in activated endothelium within breast tumors as compared with normal breast endothelium *(19)*, but serum VCAM-1 (not E-selectin) more closely reflected vessel counts, prognostic factors, and clinical progression.

The finding that VCAM-1 levels were a good marker of angiogenesis but E-selectin levels were a poor marker was not entirely surprising because antiangiogenesis drugs have been shown to increase the expression of E-selectin but not VCAM-1 on breast tumor endothelial cells *(41)*. The reason for this effect is unknown, but it may be due to increased endothelial turnover or increased intravascular shedding of VCAM-1 from activated endothelial cells relative to E-selectin. No increases in serum VCAM-1 levels were seen in patients with hormone-responsive tumors, but increases in serum VCAM-1 levels observed in five patients who were subsequently determined to have stable disease at 6 months (Table 3) may reflect a late response of these tumors to hormonal therapy, a well-recognized phenomenon *(22)*. Levels of several epithelial tumor markers (e.g., CA15-3 and carcinoembryonic antigen [CEA]) may rise initially in responding tumors in the so-called "flare response" *(40)*. Because the major source of VCAM-1 in tumors is endothelial cells, not epithelial cells *(9)*, serum VCAM-1 may prove to be an early and sensitive marker of disease progression in hormone-nonresponsive tumors. By contrast, we have not seen any increases in VCAM-1 levels in the serum of patients with hormone-responsive tumors.

Ideally, we would have compared serum VCAM-1 levels with immunohistochemical expression of VCAM-1 to confirm that increases in serum VCAM-1 levels were derived directly from the endothelium. Unfortunately, there is, as yet, no reliable antibody for VCAM-1 staining in paraffin sections. Frozen-section immunohistochemistry studies show that 10%–40% of breast cancers have endothelial cells that express VCAM-1, depending on the antibody and techniques used *(19,34,42)*, which implies that serum VCAM-1 is rapidly turned over and secreted from activated endothelium. The currently used breast tumor markers (e.g., CA15-3 and CEA) are secreted by epithelial cells *(40)*. The combination of an endothelial marker (i.e., serum

VCAM-1) and epithelial markers may be better than multiple epithelial tumor marker measures in predicting breast cancer response to hormonal therapy because measurement of two different tumor cell compartments will likely improve early assessment of response.

Our data, from what is, to our knowledge, the first systematic survey of angiogenic markers in breast cancer patients, indicate that serum soluble VCAM-1 is an accurate marker of tumor angiogenesis in breast cancer. For a biomarker to be useful as a monitor of a pathological process, it must be easily measurable, must reflect accurately the pathological process that it is designed to measure, and must provide the clinician with an answer that can be easily interpreted. Serum VCAM-1 fulfills these criteria as a clinical measure of tumor angiogenesis in breast cancer.

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