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Soluble adhesion molecules (E-selectin, ICAM-1 and VCAM-1) in breast carcinoma

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Abstract

Adhesion molecules are important in cell–cell and cell–basement membrane interactions. They are intimately involved in inflammatory reactions and a role in tumour progression has been postulated. E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) play a role in cell adhesion to the vascular endothelium, and may have a role in tumour cell dissemination. Soluble forms of these molecules have been described and this study was established to examine these adhesion molecules in patients with breast carcinoma. Serum was obtained from 92 patients with breast carcinoma and 31 age-matched patients with benign breast disease. All samples were obtained prior to surgery. Soluble levels of E-selectin, ICAM-1, and VCAM-1 were significantly elevated in patients with Stage 4 disease compared with controls. (E-selectin 88.6 (47.9) versus 51.4 (18.4) ng/ml; $P<0.001$: ICAM-1 447 (249) versus 244 (79) ng/ml; $P<0.001$: VCAM-1 779 (159) versus 552 (135) ng/ml; $P<0.001$ results expressed on mean (SEM) SD placed above this.). The prognostic value of the adhesion molecules was examined. In patients with Stage 2 disease, elevated VCAM-1 was predictive of decreased survival, even when corrected for T and N status. Adhesion molecules are elevated in patients with advanced disease and elevation in VCAM-1 has prognostic significance in patients with breast carcinoma.

Keywords: Breast carcinoma; Adhesion molecules; E-selectin; ICAM-1; VCAM-1; Prognosis

1. Introduction

Tumour progression and the development of metastases involves several steps which include uncontrolled cell growth, loss of homophilic intercellular interactions, migration of tumour cells into vessels or lymphatics, interaction with surface constituents of platelets and leucocytes, adhesion to lymphatic or vascular endothelium at distant sites, migration into the parenchyma, induction of neoangiogenesis and cell growth [1,2].

Intercellular adhesion molecules play a role in the process of invasion and the development of metastases. Four major classes of adhesion molecules have been described. Selectins are transmural proteins involved in the early phase of the adhesion cascade and constitute one class. They allow the establishment of weak, low-affinity bonds between endothelial cells and leucocytes resulting in the phenomenon of leucocyte rolling. They are subdivided into three subgroups E, P and L denoting their association with endothelial cells, platelets and leucocytes, respectively. E-selectin is expressed in low concentrations on endothelial cells and is synthesised *de novo* and rapidly upregulated following stimulation by inflammatory mediators [3–6].

The second class of adhesion molecules is the immunoglogulin-like superfamily, and these are membrane proteins, which are organised into pleated sheets. They are known as the intercellular adhesion molecules and include intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). The activity and expression of these molecules is upregulated following inflammation. Interactions between integrins and intercellular adhesion molecules result in strong bonds, which facilitate adhesion and subsequent diapedesis of leucocytes. ICAM-1 is involved in leucocyte adhesion and VCAM-1 is involved in adhesion and diapedesis [3–6].

The third class of adhesion molecules are the integrins, which are transmembrane proteins composed of two subunits α and β. The fourth class is the cadherins,

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which are mediators of cell–cell interactions. They are calcium-dependent transmembrane glycoproteins that form homophilic interactions [1,3–6]. Other adhesion molecules have also been identified including the sialomucin family and CD44 and may serve multiple adhesion functions.

The adhesion molecules are intimately involved in inflammatory reactions, but more recently a role in tumour progression has been postulated. E-selectin, ICAM-1 and VCAM-1 may play a role in tumour cell adhesion to the vascular endothelium, which precedes extravasation of cells and the development of metastases. Recently, soluble forms of these molecules have been described [7–10].

This study was established to examine soluble adhesion molecules in a group of patients presenting with breast carcinoma, to correlate the levels of these molecules with stage and other tumour parameters at presentation and to examine the prognostic significance of soluble adhesion molecules.

2. Patients and methods

Serum was drawn in the morning from 92 patients with breast cancer and 31 age-matched patients with benign breast disease. All the samples were taken prior to any operative intervention. None of the control patients had evidence of autoimmune diseases, vasculitides, liver or renal problems. Demographic data were collected prospectively on each patient and entered onto a database. Tumour histopathology and oestrogen receptor (ER) levels were collected and entered prospectively. The blood was spun at 4 °C and serum was collected, aliquoted, and stored at −70 °C until analysis was performed. Levels of circulating E-selectin, ICAM-1 and VCAM-1 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, Oxon, UK) and plates were read using an ELISA plate reader and concentrations were calculated automatically from standard curves. CA15-3, and tissue polypepticle specific antigen (TPS) measurements were performed routinely in the laboratory and this cohort of patients had C-reactive protein (CRP) measured as part of another study.

Patients were categorised and analysis performed by stage, TNM criteria, grade (Elston and Ellis's modifications of Bloom and Richardson criteria [11]), ER status (measured using a cytosol-based ligand-binding assay), and the presence of lymphovascular invasion (assessed on haematoxylin and eosin (H&E) sections). All tumours were ductal carcinomas. Statistical analysis was performed using ANOVA with Bonferroni correction, the Levene test, and Kruskal–Wallis one-way ANOVA, Chi Square, and Spearmans correlation with significance assumed at the 5% level. Soluble adhesion molecule levels were measured in 31 patients with benign breast disease. The 90th percentile was calculated and levels above this were regarded as elevated. Survival was examined using the 90th percentile as a cut-off. Survival analysis was performed using Kaplan–Meier and Cox regression analysis.

3. Results

Measured levels of soluble adhesion molecules were significantly higher in patients with more advanced disease (Table 1). When subdivided according to individual TNM criteria (Table 2), significant elevations were seen in E-selectin, but not in ICAM-1 or VCAM-1 in patients with more advance tumour stages. No significant differences were seen when nodal status was examined. All the adhesion molecules were significantly elevated in patients with metastatic disease. Only VCAM-1 was significantly elevated in patients with high-grade disease and there was no significant elevation in the soluble adhesion molecules associated with histological evidence of lymphovascular invasion.

There was a significant correlation between E-selectin and tumour size, preoperative CA15-3, ICAM-1, VCAM-1, platelet count and CRP (Table 3). There was a significant correlation between ICAM-1 and age, preoperative CA15-3, preoperative TPS, VCAM-1 and CRP. There was a significant correlation between VCAM-1 and age, preoperative CA15-3, ICAM-1 and CRP.

Table 1
Soluble cell adhesion molecule levels E-selectin, ICAM-1 and VCAM-1 subdivided and compared by Union International Against Cancer (UICC) stage

	Stage	*N*	Median (IQR)	*P* value
Selectin	Benign	31	54.2 (35.4–62.2)	
	1	20	41.4 (33.9–51.1)	
	2	56	53.6 (39.0–74.4)	
	3	6	38.8 (37.4–43.0)	
	4	10	79.1 (69.0–93.2)	0.001
ICAM-1	Benign	31	207 (180–323)	
	1	20	284 (204–355)	
	2	56	256 (232–326)	
	3	6	272 (222–320)	
	4	10	370 (314–448)	0.002
VCAM-1	Benign	31	532 (445–631)	
	1	20	563 (465–642)	
	2	56	604 (532–707)	
	3	6	560 (503–645)	
	4	10	790 (584–898)	0.009

ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1. E-selectin, ICAM-1 and VCAM-1 in ng/ml.
P: Statistical analysis was performed using Kruskal–Wallis and comparisons were made between variables in the groups indicated.

Table 2
E-selectin, ICAM-1, and VCAM-1 levels subdivided according to tumour, nodes, metastases (TNM) criteria, grade and the presence or absence of lymphovascular invasion

Variable	Groups	*N*	E-selectin		ICAM-1		VCAM-1	
			Median (IQR)	*P* value	Median (IQR)	*P* value	Median (IQR)	*P* value
T	1	33	40.9 (36.8–51.0)		278 (208–353)		592 (470–680)	
	2	49	59.1 (40.0–77.0)		264 (234–340)		599 (537–710)	
	3	4	50.2 (37.9–73.9)		272 (229–409)		687 (512–882)	
	4	6	85.6 (49.5–103.8)	0.008	309 (240–486)	NS	684 (569–945)	NS
N	0	51	50.7 (37.0–72.0)		584 (234–343)		595 (530–710)	
	1	38	47.8 (38.1–78.6)		253 (222–334)		584 (501–707)	
	2	3	107.3 (43.0–211.0)	NS	320 (314–1107)	NS	999 (645–1231)	NS
M	0	85	47.8 (37.2–71.5)		258 (222–329)		589 (525–704)	
	1	7	79.1 (71.2–91.4)	0.002	370 (321–438)	0.001	790 (575–884)	0.04
Grade	1	8	36.8 (26.5–51.7)		236 (202–259)		556 (405–615)	
	2	18	52.1 (42.4–72.3)		284 (214–316)		574 (475–632)	
	3	39	63.0 (39.0–78.6)	NS	288 (234–340)	NS	652 (530–785)	0.04
Invasion	0	68	49.1 (37.7–72.2)		272 (223–344)		594 (530–719)	
	1	24	53.9 (37.2–77.7)	NS	279 (235–337)	NS	625 (501–708)	NS

ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; NS, non-significant. E-selectin, ICAM-1 and VCAM-1 in ng/ml. Invasion refers to lymphovascular invasion. Statistical analysis was performed using Kruskal–Wallis and comparisons were made between variables in the groups indicated.

Table 3
Correlations between the different parameters examined

	E-selectin	ICAM-1	VCAM-1
Age	NS	$r=0.359$; $P=0.001$	$r=0.336$; $P=0.001$
Tumour size (cm)	$r=0.319$; $P=0.002$	NS	NS
Platelet count	$r=0.294$; $P=0.007$	NS	NS
C reactive protein	$r=0.392$; $P<0.001$	$r=0.366$; $P<0.001$	$r=0.228$; $P=0.01$
ER	NS	NS	NS
CA15-3	$r=0.322$; $P=0.004$	$r=0.325$; $P=0.003$	$r=0.256$; $P=0.02$
TPS	NS	$r=0.336$; $P=0.005$	NS
ICAM-1	$r=0.439$; $P<0.001$	NS	$r=0.416$; $P<0.001$
VCAM-1	$r=0.212$; $P=0.02$	$r=0.416$; $P<0.001$	NS

ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1. *r*, correlation coefficient; *P*, significance value; NS, non-significant. ER, oestrogen receptor.

The number of patients in each stage with elevated soluble adhesion molecule levels is given in Table 4. All of the tumour markers, E-selectin ($P<0.04$), ICAM-1 ($P<0.002$) and VCAM-1 ($P<0.0001$) had prognostic significance when the whole group was examined. When Stage 2 patients alone were examined, only VCAM-1 retained prognostic significance ($P=0.001$) (Figs. 1 and 2). This remained on multivariate analysis when controlled for T and N status.

4. Discussion

In the present study, soluble levels of E-selectin, ICAM-1 and VCAM-1 were elevated in patients with advanced breast cancer. Only VCAM-1 appeared to have independent prognostic significance in patients with early breast cancer.

The attachment of circulating tumour cells to the endothelium precedes the development of metastases and it has been postulated that these cells utilise the same endothelial surface receptors used by leucocytes, i.e. selectins and intercellular adhesion molecules. Tumour cells may induce expression of adhesion mol-

Table 4
Numbers of patients divided by stage with soluble adhesion molecule levels above the 90th percentile for those with benign disease

	Number	Elevated E-selectin	Elevated ICAM-1	Elevated VCAM-1
Stage 1	20	2 (10%)	2 (10%)	3 (15%)
Stage 2	56	13 (23%)	3 (5%)	9 (16%)
Stage 3	6	0 (0%)	0 (0%)	1 (17%)
Stage 4	10	7 (70%)	4 (40%)	6 (60%)

ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

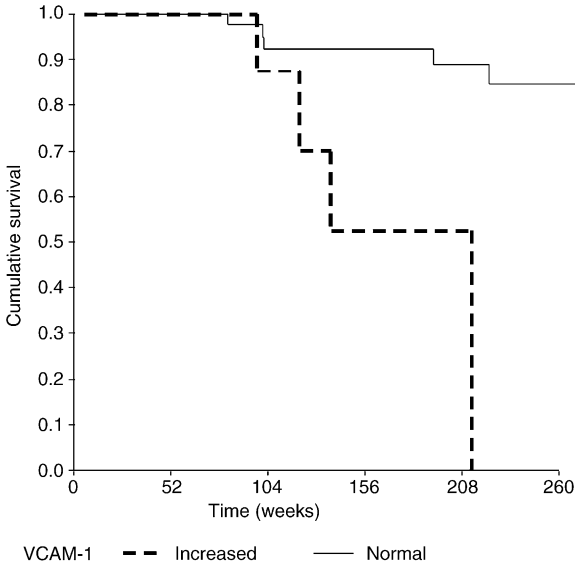
ecules to facilitate the adhesion of tumour aggregates [11].

Increased expression of E-selectin has been described on endothelial cells adjacent to many tumours including lymphomas and colorectal tumours. E-selectin is involved in mediating the adhesion of breast, colonic and renal carcinoma cells to the endothelium and inhibition reduces adherence [12,13]. Breast cancer cell lines induce the expression of E-selectin on vascular endothelium [14]. E-selectin is detectable in serum and previous studies have demonstrated higher levels in patients with breast, ovarian and gastrointestinal cancers [7]. Similar results were seen in the present study. The highest levels were observed in patients with hepatic metastases and previous studies have suggested that E-selectin expression is a risk factor for the development of metastases [15,16]. E-selectin expression was a predictor of recurrence in melanoma. Muraki and colleagues [17] found high serum E-selectin levels were associated with a low incidence of metastases in renal cell carcinoma. In the present study, elevated levels of E-selectin were associated with a reduced survival in the group as a whole, but this merely reflected the association between elevated E-selectin levels and advanced stage. Elevated levels of E-selectin were not of prognostic significance when the stage of tumour was controlled for.

ICAM-1 is expressed at low levels on resting leucocytes and at virtually undetectable levels on vascular endothelium and other cell types. Its expression is rapidly upregulated by cytokines on a wide variety of cell types. Banner and colleagues [18] demonstrated increased ICAM-1 expression on stroma in colon carcinoma and this has also been demonstrated in other tumours [19]. ICAM-1 is postulated to play a role in the progression to metastasis. Santarosa and colleagues [20] found a correlation between the expression of ICAM-1 in renal carcinoma and recurrence. ICAM-1 was a marker whose expression increased as melanocytes transformed to melanomas and increased further as they progressed to metastases. ICAM-1 is also expressed on benign naevi and levels of soluble ICAM-1 were not a reliable indicator of tumour burden in melanomas [8,21].

ICAMs are involved in leucocyte recognition and destruction of tumour cells. The presence of high concentrations of ICAM-1 renders cells more liable to lysis by leucocyte-activated killer cells [22]. Antibodies to ICAM-1 inhibit T-cell-dependent cytotoxicity. Paradoxically, increased tumour cell expression of ICAMs and shedding of ICAMs may aid immunological escape. It has been proposed that increased ICAM-1 expression allows tumour cells to attach to circulating leucocytes and later adhere to the vascular endothelium at a distant site facilitating dissemination [23].

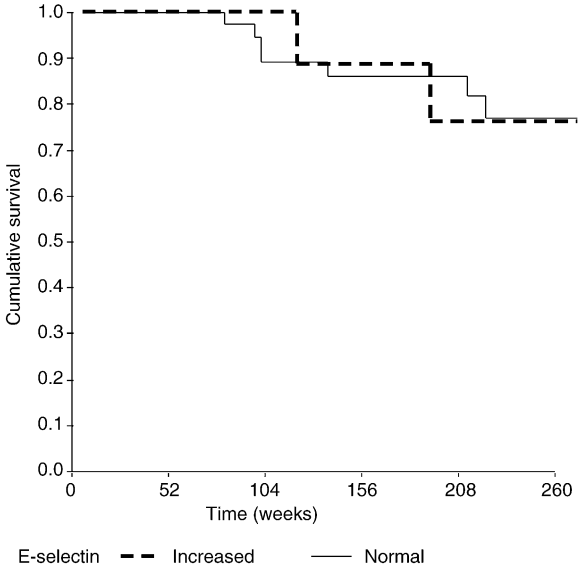
Recently, a soluble form of ICAM-1 has been described and elevated levels have been associated with advanced disease in gastric, colonic, gall bladder, pancreatic and renal carcinomas [20,24–27]. Increased levels have been correlated with relapse in Hodgkins disease and survival in hepatocellular carcinoma, malignant melanoma and metastatic breast carcinoma [16,25,26].



Numbers at risk at each time period

Time period (weeks)	0	52	104	156	208
VCAM-1 normal	47	43	35	30	24
VCAM-1 increased	9	8	7	5	4

Fig. 1. Survival in patients with Stage 2 disease, with normal or increased VCAM-1 levels ($P = 0.001$).



Numbers at risk at each time period

Time period (weeks)	0	52	104	156	208
E-selectin normal	43	39	32	27	22
E-selectin increased	13	12	10	8	6

Fig. 2. Survival in patients with Stage 2 disease, with normal or increased E-selectin levels ($P = $ NS).

In the present study, elevated levels of ICAM-1 were found in patients with advanced disease, similar to the reported results in many different types of tumours. However, elevated levels of ICAM-1 had no prognostic significance when the stage of disease was controlled for.

VCAM-1 is expressed on activated human endothelial cells and is upregulated in response to tumour necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) and lipopolysaccharide (LPS). It is involved in the adhesion of lymphocytes, monocytes and eosinophils to the vascular endothelium. It is upregulated on metastatic, but not on non-metastatic tumour cells, and it mediates the adhesion of melanoma cells to the endothelium [28,29]. A soluble form of VCAM-1 has also been described. Banks and coleagues [9] demonstrated that soluble VCAM-1 levels were significantly higher in tumours compared with controls. This is similar to the results from the present study. Byrne and colleagues [30] demonstrated that serum VCAM-1 correlated with tumour microvessel density in early breast cancer. They also demonstrated that VCAM-1 had prognostic significance, both in early and advanced disease. Similar results were found in the present study, elevated VCAM-1 had prognostic significance, when the disease stage was controlled for.

The origin of soluble adhesion molecules is unclear. Possible sources include tumour cells or the endothelium at the site of primary tumour or secondary deposit. Human melanoma cell lines release ICAM-1 into the serum of nude mice [31]. ICAM-1 expression and release was examined in 11 renal carcinoma cell lines. All 11 expressed ICAM-1, but only five cell lines released it and there was an inverse correlation with cellular expression. IL-1, TNF and gamma interferon (IFN) cause the release of soluble adhesion molecules from endothelial cells or tumours [31–33].

Adhesion molecules may be involved in mediating cytotoxic immune responses. VCAM-1 is a potential endothelial ligand for cytotoxic effector lymphocytes. Griffioen and colleagues [34] demonstrated downregulation of ICAM-1 on tumour infiltrating endothelial cells in renal carcinoma and hypothesised a tumour-derived escape mechanism from cytolytic effector leucocytes by downregulation of vascular adhesion molecules and decreased responsiveness of the endothelial cells to pro-inflammatory cytokines.

The significance and function of soluble cell adhesion molecules is unclear. Shedding of ICAM-1 by tumour cells may facilitate immunological escape from cytotoxic T cells and natural killer cells and promote the development of metastases [35]. Soluble E-selectin and soluble VCAM-1 also play a role in angiogenesis. Activated leucocytes bind to endothelial cells and result in the shedding of adhesion molecules. These molecules bind to adjacent endothelial cells and exert an angiogenic effect [36,37].

Adhesion molecules and receptors from different tissues and organs vary and heterogeneity has been recognised in the mechanisms of tumour cell interaction with the endothelium. The nature of these interactions may be relevant in determining the site at which tumour emboli lodge. Interference with adhesion and signalling represent a future direction for the development of anticancer and antimetastatic treatment protocols.

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