

Activated leukocyte cell adhesion molecule: A novel biomarker for breast cancer

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Activated leukocyte cell adhesion molecule (ALCAM) has been implicated in tumorigenesis. Our goal was to examine the levels of ALCAM, in addition to the classical breast cancer tumor markers carbohydrate antigen 15-3 (CA15-3) and carcinoembryonic antigen (CEA), in serum by quantitative enzyme-linked immunosorbent assay for diagnosis in breast cancer patients. The 3 proteins were measured in serum of 100 healthy women, 50 healthy men and 150 breast carcinoma patients. The diagnostic sensitivity and specificity of the tests were calculated and the association of serum marker concentrations with various clinicopathologic variables was examined using nonparametric Kruskal-Wallis tests. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic performance of the biomarkers. ALCAM, with area under the curve (AUC) of 0.78 [95% CI: 0.73, 0.84] outperformed CA15-3 (AUC = 0.70 [95% CI: 0.64, 0.76]) and CEA (AUC = 0.63 [95% CI: 0.56, 0.70]). The incremental values of AUC for ALCAM over that for CA15-3 were statistically significant (Delong test, $p < 0.05$). Combining CA15-3 and ALCAM yielded a ROC curve with an AUC of 0.81 (95% CI [0.75, 0.87]). Serum ALCAM appears to be a new biomarker for breast cancer and may have value for disease diagnosis.

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Key words: ALCAM; breast cancer; immunoassay; immunoglobulin superfamily; tumor marker

More than 1 million new breast cancer cases are diagnosed each year.¹ It is a heterogeneous disease with a wide range of histological, clinical and molecular presentations. Unfortunately, other than definitive diagnosis by biopsy and histopathology, no diagnostic or screening test is presently suitable for early detection of breast cancer.² The ability to detect human malignancy *via* a simple blood test has long been a major objective in medical screening. Carbohydrate antigen 15-3 (CA15-3) and carcinoembryonic antigen (CEA), discovered more than 2 and 4 decades ago, respectively, are the most commonly used tumor markers for breast cancer.^{3–5} CA15-3 and CEA levels in serum are recommended for monitoring therapy of advanced breast cancer.² However, these cancer biomarkers have proven to be ineffective in detecting the early stages of the disease because of low diagnostic sensitivity and specificity.^{6–8} Clearly, new tumor markers are urgently needed for screening, diagnosis, prognosis or selection of therapy of breast cancer patients.

We have previously performed an extensive proteomic analysis of tissue culture supernatants from 3 different breast cancer cell lines.⁹ More than 1,000 proteins were identified in our proteomic analysis. Based on literature searches, from the top 100 differentially expressed proteins (as defined by spectral counting), 46 of the molecules had been previously examined as a serological breast cancer marker. The remaining proteins were scrutinized to select proteins for further investigation that was found only in the conditioned media of breast cancer cell lines and absent in MCF-10A (seminormal breast epithelial cell line). This filtering criterion resulted in 30 candidates for further analysis. Of these 30 candidates, 11 of them had reagents available to develop an immunoassay

to measure the levels of these proteins in biological fluids. Analyzing the serum of healthy individuals and patients with breast cancer resulted in one promising candidate molecule: activated leukocyte cell adhesion molecule (ALCAM) (Supporting Information Fig. 1).

Cell adhesion molecules are cell surface receptors that mediate cell-cell and cell-substrate interactions. These molecules can be grouped into 4 families: integrins, cadherins, selectins and the immunoglobulin superfamily (Ig-SF).¹⁰ Alterations in cellular adhesion and communication can contribute to uncontrolled cell growth.¹¹ In this respect, ALCAM is a type 1 transmembrane glycoprotein of the Ig-SF.¹² The molecular weight of ALCAM is 65 kDa but with *N*-glycosylation at 8 putative sites, the mature ALCAM molecule has a molecular weight of 110 kDa.¹³ Five extracellular Ig domains, a transmembrane region and a short cytoplasmic tail make up the ALCAM protein that resembles E-cadherin in motif-arrangement.¹² ALCAM mediates both heterophilic (ALCAM-CD6) and homophilic (ALCAM-ALCAM) cell-cell interactions.¹⁴ The extracellular structures of ALCAM provide 2 structurally and functionally distinguishable modules, 1 involved in ligand binding (to CD6)¹⁵ and the other in avidity.^{14,16} Physiologically, ALCAM is expressed in activated leukocytes and neural, epithelial and hematopoietic progenitor cells.¹⁷ Functionally, ALCAM may act as a cell surface sensor to register local growth saturation and to regulate cellular signaling and dynamic responses.¹⁸ ALCAM-CD6 interaction is required for optimal activation of T-cells suggesting a possible ALCAM involvement in the immunologic response to tumor cells.¹⁹ ALCAM may favor interactions between tumor and endothelial cells.¹⁸

The aim of our study was to investigate if ALCAM, either alone, or in combination with the classical breast cancer biomarkers (CA15-3 and CEA) represent a new strategy for breast cancer diagnosis with high sensitivity and specificity in serum, using quantitative methodologies. The association between serum marker concentrations with various clinicopathologic parameters was also examined.

Additional Supporting Information may be found in the online version of this article.

Abbreviations: ALCAM, activated leukocyte cell adhesion molecule; AUC, area under the curve; CI, confidence interval; CV, coefficient of variation; ELISA, Enzyme-linked immunosorbent assay; ER, estrogen receptor; Ig-SF, immunoglobulin superfamily; OR, odds ratio; PgR, progesterone receptor; PSA, prostate-specific antigen; ROC, receiver operating characteristic.

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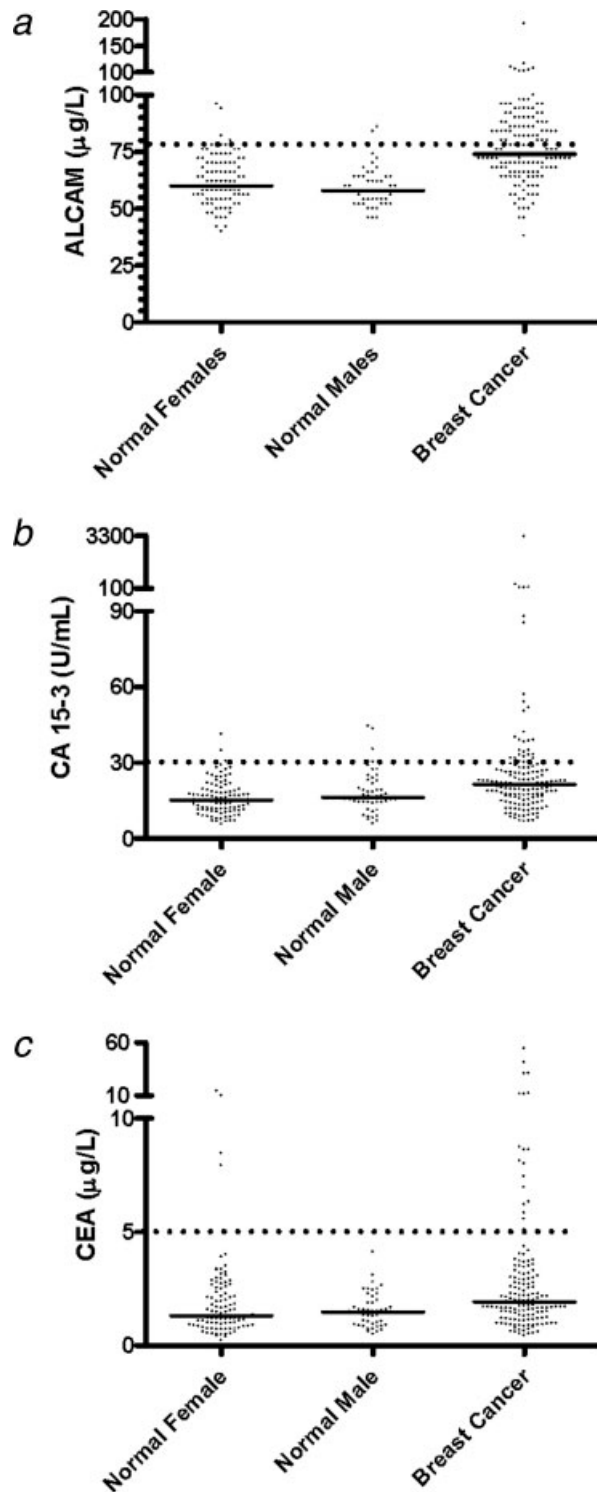


FIGURE 1 – Distribution of markers (a) ALCAM, (b) CA15-3 and (c) CEA in the 3 groups (normal female, normal male and breast carcinoma) examined by an immunoassay specific to the molecule. The solid horizontal line indicates the median value for each of the groups. The dotted horizontal line indicates the cutoff values to discriminate cancer from control subjects (a) ALCAM: 78 $\mu\text{g/L}$, 95% specificity cutoff; (b) CA15-3: 30 U/mL and (c) CEA: 5 ng/mL.

TABLE 1 – SPEARMAN'S RANK CORRELATION COEFFICIENTS AMONG 3 MARKERS FOR FEMALE CONTROLS AND CASES

	Female controls			Cases		
	CA15-3	CEA	ALCAM	CA15-3	CEA	ALCAM
CA15-3	1			1		
CEA	-0.091	1		0.161 ¹	1	
ALCAM	0.082	0.348 ¹	1	0.2 ¹	0.371 ¹	1

¹ $p < 0.05$.

Material and methods

Patients and specimens

The clinical material used consisted of 150 serum samples from breast cancer patients (age, 34–82 years; median, 62 years), 100 serum samples from normal, apparently healthy women (age, 24–56 years; median, 40 years), and as an additional control, 50 serum samples from normal healthy men (age, 23–61 years; median, 48 years). Serum samples from healthy subjects were obtained through the Preventive Medical Unit at Venice General Hospital. The subjects were asked to donate blood samples for studies on tumor biomarkers and then asked to fill out a complete patient form. Any subjects that had a history of any serious or chronic illnesses or neoplasms were eliminated from the sample collection. The breast carcinoma serum samples were obtained on the morning prior to surgery and prior to any treatment. Peripheral blood samples were drawn by venopuncture after overnight fasting between 8 and 8:30 AM. Serum was collected in a Becton Dickinson vacutainer, code n°367896, containing a coagulation coactivator and kept at ambient temperature (20–25°C) before centrifugation. They were centrifuged at 1,500g at ambient temperature (20–25°C) after 45 min and within 2 hr were aliquoted and snap frozen in liquid nitrogen and immediately stored at -80°C . Of the 150 breast carcinoma patients, 32 were Stage 1, 57 were Stage 2A or 2B, 27 were Stage 3A or 3B and stage information was not available for the remaining 34. Supporting Information Table I contains detailed information on the 150 breast cancer patient samples including their histology, grading (Elston-Ellis), diameter (cm), pT, pTNM, stage and age. Clinical grades 1, 2 and 3, corresponding to 26, 62 and 56 patients, respectively, were included in this study. The clinical characteristics of the breast cancer patients are described later. Serum samples, obtained from Venice, Italy, from all patients were stored at -80°C until further analysis. Our protocols have been approved by the review boards of the participating institutions.

Measurement of ALCAM, CA15-3 and CEA in serum

The concentration of ALCAM in serum was measured by using a highly sensitive and specific noncompetitive “sandwich-type” enzyme-linked immunosorbent assay (ELISA), developed in our laboratory. The assay is based on mouse monoclonal antibody capture and biotinylated mouse monoclonal detection antibody (both obtained from R&D Systems, Minneapolis, MN). The assay has a detection limit of 0.05 $\mu\text{g/L}$ and a dynamic range of up to 10 $\mu\text{g/L}$. Precision was less than 10% within the measurement range. Serum samples were analyzed in triplicate with inclusion of 2 quality control samples in every run. In addition, CA15-3 and CEA were measured using a commercially available automated ELISA kit (Elecsys CA15-3 and CEA Immunoassay, respectively; Roche Diagnostics, Indianapolis, IN). The upper limit of normal for CA15-3 for this method is 30 U/mL and for CEA is 5 ng/mL.

Data analysis and statistics

The relationships between biomarkers and patient and tumor characteristics were examined with the Kruskal-Wallis test, a non-parametric method for examining differences among multiple groups. Spearman's rank correlation coefficient was used to assess the correlations among biomarkers. Logistic regression was performed to calculate the odds ratio (OR) that defines the relation between biomarkers and case or control status. OR were calcu-

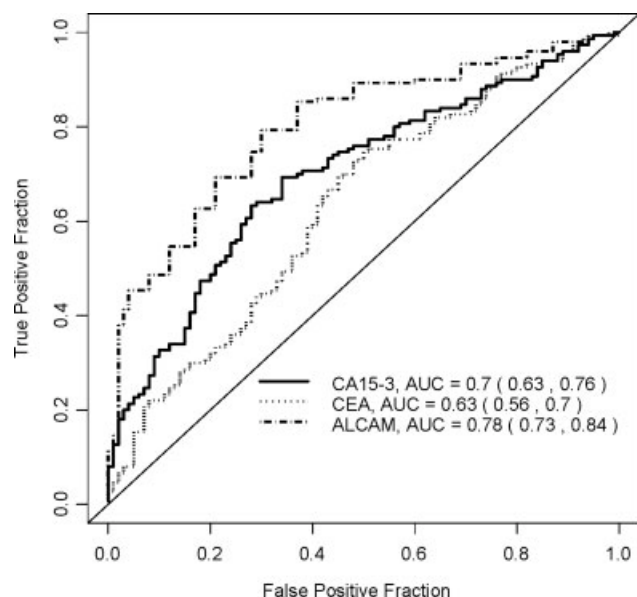


FIGURE 2 – ROC curves for the 3 markers (CA15-3, CEA and ALCAM).

lated on log-transformed biomarkers and were represented with their 95% confidence interval (95% CI) and 2-sided p -values.

To further evaluate the diagnostic usefulness of the markers for dichotomous classification, we considered receiver operating characteristic (ROC) curve analysis. For each ROC curve, we calculated the area under the curve (AUC). Bootstrap method was used to calculate the CIs for AUC. The ROC analysis was first conducted on individual markers and then in combination, to explore the potential that a marker panel can lead to improved performance. We considered an algorithm that renders a single composite score using the linear predictor fitted from a binary regression model. This algorithm has been justified to be optimal under the linearity assumption²⁰ in the sense that ROC curve is maximized (*i.e.*, best sensitivity) at every threshold value. Since an independent validation series was not available for this study, the predictive accuracy of the composite scores was evaluated based on resampling of the original data. All analyses were performed using Splus 8.0 software (Insightful Corp., Seattle, WA).

Results

ALCAM ELISA assay development

To ensure that the immunoassay was suitable for measuring clinical serum samples, the recovery, reproducibility, linearity, crossreactivity and serum sample stability were examined. Recombinant human ALCAM protein was added into the general diluent (control), normal serum (male and female) and into serum of breast cancer patients at different concentrations and measured with the ALCAM immunoassay. A recovery of 90–100% was observed in these samples. The assay also showed negligible crossreactivity to another adhesion molecule of the Ig-SF, B-cell adhesion molecule,¹⁴ displayed excellent linearity with serial dilutions and showed <10% coefficient of variation (CV) for intra- and inter-assay variability studies (Supporting Information Fig. 2). Finally, the design of the stability study consisted of collecting serum at different time points (2 weeks, 4 weeks and fresh samples) and storing them at 4°C, –20°C and –80°C. ALCAM levels were measured in these samples using the immunoassay. No difference was observed among the samples stored at the different temperature conditions and among the different time point collections, compared to the freshly obtained samples.

Association of biomarkers with age

As cases and controls were not matched for age, we first explored if marker values differed by age. The comparisons between cases and controls were based on data from females only. Although no change with age was observed for CA15-3 concentrations, the level of CEA appeared to increase with age for both cases and controls. With respect to ALCAM, there was a trend for marker level to increase with age for cases but not for controls (see Supporting Information Fig. 3).

Correlations among biomarkers

Spearman's rank correlation coefficients were used to assess the correlations among markers for female controls and cases, respectively, and the results are listed in Table I. CEA appeared to be weakly correlated with ALCAM in both cases (Spearman $r = 0.371$, $p < 0.001$) and controls (Spearman $r = 0.348$, $p = 0.001$), whereas CA15-3 was weakly correlated with ALCAM among cases only (Spearman $r = 0.2$, $p = 0.015$).

Association of biomarkers with tumor characteristics for cases

The association of ALCAM, CA15-3 and CEA with patient and tumor characteristics such as age, tumor diameter, estrogen receptor (ER) and progesterone receptor (PgR) status, grade, histology, ratio of lymph node positive (lpos) and total lymph nodes (ltot), menopausal status, and stage were examined. A significant association was obtained for the following clinicopathologic variables: age (≤ 50 , 51–60, 61–70 and > 70), menopausal status (pre and postmenopausal), and stage (I, II, III). The distributions of each marker in cases for these variables are given in Table II. Postmenopausal women displayed higher values of CEA and ALCAM (all $p < 0.001$). Also, levels of ALCAM were not significantly associated with stage whereas CEA and CA15-3 were significant. Finally, although a statistically significant p -value was not obtained for an association between ALCAM values and tumor grade, a general trend was observed with elevated ALCAM levels corresponding to increased tumor grade (Supporting Information Fig. 4).

Association of biomarkers with breast cancer

The distributions of the 3 markers, as measured by immunoassays, in cases and controls, are shown in Figure 1. Distributions of the patients with breast cancer differed from controls (female or male) for ALCAM, but to a lesser degree for the other 2 markers. The median values of males and females were similar for all 3 markers. When comparing the ALCAM values between normal women ($n = 100$) and patients with breast cancer ($n = 150$) by the nonparametric Mann Whitney test (2-tailed), the medians were significantly different (median normals = 60 $\mu\text{g/L}$; median cancer = 74 $\mu\text{g/L}$; $p < 0.0001$). For CA15-3, the medians were significantly different (median normals = 15 units/mL; median cancer = 21 units/mL; $p < 0.0001$). Finally for CEA, the medians were different (median normals = 1.3 $\mu\text{g/L}$; median cancer = 1.9 $\mu\text{g/L}$; $p = 0.0003$). The association of the markers with cancer was further considered with linear regression models of logarithm-transformed marker values as a function of clinical status (cancer vs. noncancer; females only) and age. Adjusting for age, the mean levels of log(CA15-3) and log(ALCAM) were significantly higher in cancer; levels of log(CEA) did not differ between cancer and controls.

We also considered logistic regression models to further characterize the associations between markers and breast cancer, adjusting for age. Similar to the results from linear regression, we found that 2 individual markers, CA15-3 (OR = 1.12, 95% CI [1.04, 1.19]) and ALCAM (OR = 1.42, 95% CI [1.14, 1.77]) univariately predicted breast cancer, but this was not the case for CEA (OR = 0.99, 95% CI [0.95, 1.05]). In a logistic regression model, which included age and all 3 markers, we found that CA15-3 and ALCAM independently predicted breast cancer. Results from the logistic regression models are given in Table III.

TABLE II – MARKER DISTRIBUTIONS BY TUMOR CHARACTERISTICS FOR CASES

	No. of patients	CA15-3 median	Q31 ¹	CEA median	Q31 ¹	ALCAM median	Q31 ¹
Age							
≤50	36	20.36	5.11	1.59	0.59	66.00	7.00
51–60	34	21.78	6.06	1.82	0.99	77.00	11.50
61–70	40	18.90	4.34	2.02	0.75	75.00	9.00
70+	40	22.92	6.12	2.62	0.96	82.00	8.25
<i>p</i> value ²		0.31		0.01		<0.001	
Menopausal status							
Pre	30	21.30	5.35	1.03	0.56	66.00	6.00
Post	103	20.61	6.06	2.14	0.98	78.00	9.50
<i>p</i> value ²		0.92		<0.001		<0.001	
Stage							
I	32	17.20	5.93	1.48	0.61	72.00	11.25
II	57	19.46	4.66	1.75	0.86	74.00	8.00
III	27	23.40	10.32	2.47	1.10	72.00	11.50
<i>p</i> value ²		0.003		0.004		0.88	
ER							
Negative		21.34	5.36	1.70	1.07	72.00	8.75
Positive		21.48	6.20	2.00	0.84	76.00	9.50
<i>p</i> value		0.55		0.4		0.64	
PgR							
Negative	19	21.45	8.12	1.67	1.08	84.00	8.50
Positive	131	21.46	5.43	1.97	0.86	74.00	9.50
<i>p</i> value		0.27		0.72		0.09	

¹Q31, semi-interquartile range: computed as one half the difference between the 75th percentile (Q3) and the 25th percentile (Q1). – ²*p* value: computed from global nonparametric Kruskal-Wallis test for testing the association between a marker and a clinical variable.

The diagnostic values of the 3 markers

ROC curve analysis (Fig. 2) was used to quantify the diagnostic value of the 3 markers. All 3 markers have AUC significantly better than 0.5, with ALCAM having the best performance (AUC = 0.78, 95% CI [0.73,0.84]). The superiority of ALCAM over the other 2 markers was also evident when we considered sensitivities at fixed values of 90% and 80% specificities, respectively (Table IV). For example, at specificity of 80%, ALCAM yielded a sensitivity of 60%, compared with 48% for CA15-3. Likewise, at 90% specificity, ALCAM displayed higher sensitivity than CA15-3 and CEA. Combining CA15-3 and ALCAM, based on the linear predictors from a logistic regression model, yielded a ROC curve with an AUC of 0.81 (bootstrap 95% CI [0.75, 0.87]). Combining CA15-3, ALCAM and CEA did not result in any improvement in ROC curves compared with CA15-3 and ALCAM. Resampling methods which aimed to adjust for over-fitting²¹ did not yield substantially different results.

Discussion

Most primary cancers show loss of expression of adhesion molecules to allow for a critical step in metastasis to occur: detachment of the invading cell from its neighbors.^{22,23} However, a number of potential reasons exist for observing elevated levels of adhesion molecules such as ALCAM in cancer patients *vs.* normal individuals. First, increased homotypic intercellular adhesion (due to elevated levels of these molecules) may favor the metastatic process since cell aggregates, rather than single cells breaking away from the primary tumor, have a greater chance of survival in the circulation and of lodging in other organs.²⁴ Second, it is known that cell adhesion is necessary for the metastatic spread of cancer cells to new organs (secondary tumor establishment).²³ As well, overproduction of adhesion molecules may disrupt the normally operative intercellular adhesion forces, allowing more cell movement and the adoption of a less ordered tissue architecture.²⁵ An illustration of this is CEA, a member of the Ig-SF that is expressed in a variety of secretory tissues.^{26,27} Interestingly, expression of CEA is increased in colon carcinomas and it may be important to processes of intercellular recognition.^{28,29} It has been suggested that this might either result in disturbance of normal

TABLE III – RESULTS FROM LOGISTIC REGRESSION MODELS

Marker	Univariate ¹		Multivariate ²	
	OR	95% CI	OR	95% CI
CA15-3	1.12	(1.04,1.19)	1.09	(1.02,1.18)
CEA	0.99	(0.95,1.05)	0.94	(0.89,1.00)
ALCAM	1.42	(1.14,1.77)	1.39	(1.09,1.78)

OR, odds ratio; CI, confidence interval.

¹Logistic model with logarithm of the marker and age as predictors. – ²Logistic model with logarithm of all 3 markers and age as predictors.

intercellular adhesion or provide advantages in further steps of metastasis²⁵ such as conceivably facilitating establishment of a secondary tumor.^{23,30}

ALCAM expression has been explored in a number of different tumor types displaying a clear upregulation in some tumors and downregulation in others. In addition, variable levels of ALCAM expression have been found at different stages of tumor development in the same type of malignancies. In melanoma, ALCAM has been suggested to exhibit a role in cell invasion and neoplastic progression.³¹ In prostate carcinoma, ALCAM gene was found upregulated in high Gleason grade prostate cancers compared with benign prostatic hyperplasia cases.³² However, 1 study observed an upregulation of ALCAM in low-grade tumors.³³ Yet, another study on prostate cancer found ALCAM to predict prostate-specific antigen (PSA) relapse.¹⁷ In colon cancer, using IHC, no significant correlation with patient age, tumor grade, stage or nodal status and ALCAM expression was observed, but membranous ALCAM expression correlated significantly with shortened patient survival.³⁴

There have been a few studies investigating ALCAM expression in breast cancer. Low levels of ALCAM mRNA correlated with nodal involvement, high grade and worse prognosis.^{35,36} At the protein level, laser scanning cytometry and confocal microscopy showed that high levels of ALCAM correlated with small tumor diameter, low grade and the presence of hormone receptors, which supported the view that this adhesion molecule is a tumor suppressor with prognostic significance.¹² However, an IHC

TABLE IV – ROC ANALYSIS FOR BIOMARKERS

Marker	AUC	95% CI	Sensitivity			
			90% Specificity	95% CI	80% Specificity	95% CI
CA15-3	0.70	(0.64,0.76)	0.32	(0.19,0.44)	0.48	(0.32,0.63)
CEA	0.63	(0.56,0.70)	0.22	(0.12,0.31)	0.32	(0.22,0.41)
ALCAM	0.78	(0.73,0.84)	0.47	(0.38,0.57)	0.60	(0.48,0.73)
Combined ¹	0.81	(0.75,0.87)	0.52	(0.39,0.64)	0.67	(0.54,0.80)

¹Linear combination of CA15-3 and ALCAM.

analysis showed that high cytoplasmic ALCAM expression was associated with shortened patient disease-free survival.³⁷ Yet, a further study found that ALCAM-ALCAM interactions between breast cancer cells were important for survival in the primary tumor and that a loss of ALCAM was associated with programmed cell death.³⁸ Finally, Ihnen *et al.*³⁹ discovered that patients with high ALCAM mRNA expression who did not receive chemotherapy tended to have a worse prognosis, suggesting that high ALCAM expression levels may be a marker for prediction of the response to adjuvant chemotherapy in breast cancer. Indeed, the discordant data between RNA and protein levels of ALCAM in breast cancer and even discordance among different protein expression studies suggest the need for additional research to evaluate the role of ALCAM in breast cancer.

The finding of decreased levels of ALCAM in breast cancer tissue compared with normal breast tissue is not contradictory to our results of elevated levels of ALCAM in serum of breast cancer patients. It is possible that ALCAM levels decrease in tissue but is elevated in serum. For example, although *PSA* gene transcription is downregulated in prostate cancer, *PSA* protein levels in the circulation of prostate cancer patients increase because of the disruption of the anatomic barriers between the glandular lumen and capillaries. In addition, our data of increased ALCAM levels in cancer *vs.* healthy controls is in line with data about ALCAM expression in prostate and colon cancer and melanoma. It should also be noted that we are the first to report presence of ALCAM in serum of breast cancer patients. Until now, all studies regarding ALCAM expression have been performed at the transcript level or using IHC or confocal microscopy. In this study, we developed a robust and highly sensitivity immunoassay to measure ALCAM in biological fluids.

It is generally agreed upon that no single cancer biomarker will provide all necessary information for optimal cancer diagnosis. The current trend is to focus on the identification of multiple biomarkers that can be used in combination. The present data provides evidence that serum ALCAM represents a novel biomarker for breast cancer. This biomarker displays higher diagnostic sensitivity for breast cancer than the currently used tumor markers CA15-3 and CEA (Table IV). Moreover, among the 120/150 cancer patients examined who displayed normal levels of CA15-3

(< 30 U/mL), 48 of them (40%) had elevated levels of ALCAM (values of 78 µg/L or greater; the cutoff for 95% specificity). For this reason, CA15-3 measurements will benefit from combining ALCAM measurements, to increase the diagnostic sensitivity of each of the markers alone. As well, assuming a 95% specificity, the statistical power of our study ($n = >100$ for both control and cases) will allow the detection of a 20% difference between mean values of ALCAM levels in breast cancer patients and controls. The difference between the ALCAM means in this study was >20%, within the power of our study. In addition, a correlation of elevated ALCAM levels with increasing age was observed in breast carcinoma patients (Table II). However, there was no correlation between ALCAM levels and age of normal women (data not shown). This suggests that the difference in age between cases and controls is not a confounding factor in this study.

In conclusion, we show evidence that serum ALCAM concentration represents a novel biomarker for breast carcinoma, which has potential utility as a diagnostic tool. The combination of ALCAM with CA15-3 improved the diagnostic sensitivity. It is important to note that both CA 15-3 and CEA levels in serum are related to tumor size and nodal involvement and are recommended by international bodies such as ASCO (American Society of Clinical Oncology) for monitoring therapy of advanced breast cancer; it is not recommended as a diagnostic marker for breast cancer. However, when evaluating the potential of a new marker such as the one presented in this study for breast cancer, a benchmark molecule is needed for comparison. Since the only markers available for breast cancer were CA 15-3 and CEA, both markers were used to compare the diagnostic discriminatory ability. Moreover, the availability of a reliable immunoassay, such as the one developed in this study, for measuring serum ALCAM may facilitate further studies to establish the clinical usefulness of this marker in breast cancer. For example, examining the levels of ALCAM in other serum samples such as those obtained from patients pre and postsurgery as well as serial serum samples collected from patients undergoing therapy may be beneficial in evaluating the biomarker potential of ALCAM as a prognostic or predictive marker of therapy. Further validation studies that integrate ALCAM with imaging modalities, namely mammography, may reveal potential clinical utility of ALCAM for breast cancer.

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