Acta Med. Nagasaki 47 : 37−41

Preoperative Serum Levels of Sialyl Lewis^a, Sialyl Lewis^x, and Carcinoembryonic Antigens as Prognostic Factors after Resection for Primary Breast Cancer

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Sialyl Lewis^a (CA19-9) and sialyl Lewis^x antigens (CSLEX1) may play a role in tumor metastasis by serving as functional ligands in the cell adhesion system. To determine their prognostic value, we examined preoperative serum levels of CA19-9, CSLEX1, and carcinoembryonic antigen (CEA) in 64 female patients with primary breast cancer who underwent radical mastectomy. The patients were divided into two groups, termed the low- and high-antigen groups based on a value selected as a diagnostic cut-off. Correlation between the serum antigen levels, various established clinicopathologic factors, and prognosis were studied by univariate and multivariate analysis. The high-CEA group was at a more advanced stage (including T factor, N factor, M factor, and Stage) than the low-CEA group. Patients with high serum levels of CEA had shorter disease-specific intervals than those with low serum levels (P <0.0001), whereas disease-specific intervals did not differ between low- and high-CA19-9 or CSLEX1 groups. A Cox's regression multivariate analysis revealed a high serum CEA level as an independent factor for worse outcome, separate from Stage. In conclusion, an elevated preoperative serum CEA level was a predictor for poor outcome after radical mastectomy for breast cancer, while CA19-9 and CSLEX1 were not.

ACTA MEDICA NAGASAKIENSIA 47 : 37−41, 2002

Key Words: carcinoembryonic antigen, CEA, sialyl Lewis^a, CA19-9, sialyl Lewis^x, CSLEX1, breast cancer, prognostic value

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Introduction

The composition of cell surface carbohydrates usually changes with tumor development.[1)] Among the tumor-associated carbohydrate molecules that accumulate substantially in solid tumors, sialyl Lewis^a and sialyl Lewis^x antigens are representative examples of type 1 and type 2 terminal structures, respectively.[1,2)] Several studies indicate that tumor cells expressing sialyl Lewis^a and/or sialyl Lewis^x antigens adhere to cultured endothelial cells activated by certain interleukins.[3−5)] This observation supports the idea that sialyl Lewis^a and/or sialyl Lewis^x antigens may actually play a role in the adhesion of cancer cells to endothelial cells, resulting in hematogenous metastasis. Sialyl Lewis^a and/or sialyl Lewis^x antigens accumulate in breast cancer tissues.[6−8)] In addition, increased expression of sialyl Lewis^x antigen, as demonstrated by immunohistochemistry, has been shown to correlate with poor prognoses in breast cancer patients after surgery.[6)] Although circulating sialyl Lewis^a and/or sialyl Lewis^x antigens are also detected in the blood streams of breast cancer patients, their prognostic value in breast cancer patients has not been extensively discussed.[9,10)]

In this study, we examined the preoperative serum levels of sialyl Lewis^a and sialyl Lewis^x antigens in breast cancer patients in order to clarify the role of these two carbohydrate antigens as prognostic factors after radical mastectomy. We also evaluated CEA levels in the same patients because CEA has been considered as one of the most useful tumor markers for a wide variety of malignancies, including breast carcinoma.[11−15)] The prognostic values of sialyl Lewis^a, sialyl Lewis^x antigens, and CEA were compared.

Patients and Methods

Patients

Sixty-four female patients with primary breast cancers who underwent either Halsted or modified radical mastectomy at Nagasaki University Hospital and Sasebo Municipal Hospital between April 1985 and December 1986 were enrolled in this study. All patients had locoregional or systemic untreated breast cancer. Patients who had a synchronous or metachronous cancer of the breast, or evidence of other organ malignancies, were excluded from this study. We prospectively evaluated the prognostic values of sialyl Lewis^a, sialyl Lewis^x antigens, and CEA in the patients.

The recommendations of the American Joint Committee on Cancer Classification and Stage grouping were used to classify the tumors.[16] Each tumor was histopathologically classified according to its histology as a papillo-tubular, solid-tubular, scirrhous, or mucinous carcinoma, using the General Rules for Clinical and Pathological Recording of Breast Cancer presented by the Japanese Breast Cancer Society.[17]

Patients with stage II-IV tumors received adjuvant cytotoxic chemotherapy (oral administration of 5-fluorouracil (5-FU) or 5-FU analogues) after surgery, while patients with stage I tumors did not. Data from patients who died of causes other than breast cancer were censored in the survival analysis. No patient died within 30 postoperative days. Written informed consent was obtained from each patient.

Measurement of serum antigen levels

In order to detect the presence of circulating cancer markers, venous blood was obtained after an overnight fast. The blood samples were separated immediately by centrifugation, and the remaining blood sera were stored at -80℃ until use. The serum levels of sialyl Lewis^a antigen (CA19-9) and CEA were measured in the Otsuka Assay Laboratory (Tokushima, Japan) using commercially available radioimmunoassay kits: specifically, Centocor CA19-9 RIA kit (Centocor, Malvern, PA, USA)18) and CEA Roche 2 (Nippon Roche K.K., Tokyo, Japan)[19], respectively. The serum levels of sialyl Lewis^x antigen (CSLEX1) were measured using the fluorescent enzyme immunoassay we described previously[20]. The data obtained were based on the simultaneous assay for these three antigens using the same set of sera.

The cut-off values recommended by the manufacturers for diagnostic use are 37 U/ml for CA19-9[18] and 2.5 ng/ml for CEA[19], while 149 U/ml for CSLEX1 was reported previously[20]. For each antigen, we classified the patients into two groups: a high-antigen group, with serum antigen concentrations greater than the selected cut-off value, and a low-antigen group, with concentrations less than the cut-off value.

Statistical analysis

Statistical analyses were performed using the computer program STATISTICA™ (StatSoft, Tulsa, Oklahoma, USA). A variable, age, was classified into two groups based on its median (53.0 years). The χ^2 test or Fisher's exact test was performed to assess association levels between expected and detected frequency. In evaluating the prognostic value of CA19-9, CSLEX1, and CEA, the disease-specific intervals served as the study endpoint. The influence of each variable on the disease-specific interval was calculated according to the Kaplan-Meier method,[21] and differences between disease-specific intervals were tested for significance using the log rank test.[22] The prognostic relevance of a single factor was determined by the application of the univariate Cox's regression analysis, whereas antigen status and well-established factors (pathologic stage of disease after primary therapy and histologic type)[16] were analyzed by using multivariate Cox' regression analysis[23]. All tests were two-tailed and a P value of less than 0.05 was considered significant.

Results

Comparison of clinicopathologic features between the low- and high-antigen groups

Among the 64 patients, high levels of each antigen were observed as follows: CA19-9 in 7 patients (10.9%), CSLEX1 in 19 (29.7%), and CEA in 12 (18.8%).

There was a statistically significant difference in the proportion of histologic types when comparing low- and high-CA19-9 groups. There was a statistically significant difference in the proportion of age and menopausal status between low- and high-CEA groups. The high-CEA group was at a more advanced stage (including T factor, N factor, M factor, and pathological Stage) than the low-CEA group. However, there were no differences in the other variables compared between any of the low- and high-antigen groups (Table 1).

The number of patients with high antigen levels who underwent Halsted or modified radical mastectomy was as follows: 4 (22.2%) or 3 (6.5%) for CA19-9, 6 (33.3%) or 13 (28.3%) for CSLEX1, and 4 (22.2%) or 8

(17.4%) for CEA, respectively (P=0.090, P=0.56, and P =0.73, respectively). Thus, there was no difference between groups with regard to operative procedure performed.

Table 1. Comparison of clinicopathological features of tumors with frequency of patients in high-antigen group.

Variables	No. in high-antigen group (%)					
	CA19-9 antigen		CSLEX1		CEA	
	(n=7)	*P* value*	(n=19)	*P* value*	(n=12)	*P* value*
Age (years)		0.43		0.27		0.022
<53 (n=32)	2 (6.3)		12 (37.5)		2 (6.3)	
≥53 (n=32)	5 (15.6)		7 (21.9)		10 (31.3)	
Menopausal status		0.24		0.76		0.0057
Premenopausal (n=34)	2 (5.9)		12 (35.3)		2 (5.9)	
Postmenopausal (n=30)	5 (16.7)		7 (23.3)		10 (33.3)	
Histology		0.019		0.14		0.17
Papillo-tubular (n=8)	0 (0)		0 (0)		0 (0)	
Solid-tubular (n=44)	3 (6.8)		15 (34.1)		8 (18.2)	
Scirrhous/Mucinous (n=12)	4 (33.3)		4 (33.3)		4 (33.3)	
T		0.046		0.11		0.0068
T1 (n=26)	3 (11.5)		4 (15.4)		2 (7.7)	
T2 (n=26)	1 (3.8)		10 (38.5)		4 (15.4)	
T3/T4 (n=12)	3 (25.0)		5 (41.7)		6 (50.0)	
N		0.67		0.41		0.0059
N0 (n=44)	4 (9.1)		12 (27.3)		4 (9.1)	
N1/N2 (n=20)	3 (15.0)		7 (35.0)		8 (40.0)	
M		0.21		0.51		0.033
M0 (n=62)	6 (9.7)		18 (29.0)		10 (16.1)	
M1 (n=2)	1 (50.0)		1 (50.0)		2 (100)	
Stage		0.062		0.096		0.0012
I (n=20)	3 (15.0)		2 (10.0)		2 (10.0)	
II (n=34)	1 (2.9)		14 (41.2)		4 (11.8)	
III (n=8)	2 (25.0))		2 (25.0)		4 (50.0)	
IV (n=2)	1 (50.0)		1 (50.0)		2 (100)	

* *P* value based on the χ^2 test or Fisher's exact test.

Comparison of disease-specific interval after surgery between low- and high-antigen groups

At the time these data were analyzed, the median follow-up was 58.3 (range: 10.4-138.7) months. Of the 64 patients, 48 were alive and 2 patients had died of other causes, while 14 had died of breast cancer (some patients had more than one cause of death): 6 with local recurrence, 6 with bone metastasis, 2 with lung metastasis, and 1 with liver metastasis.

Patients with high serum levels of CEA had shorter disease-specific intervals than those with low serum levels (P<0.0001), whereas disease-specific intervals did not differ between low and high CA19-9 or CSLEX1 groups (Fig. 1).

Patients with stage II tumors who had high serum CEA levels had shorter disease-specific intervals than those with low serum levels. In contrast, the disease-specific interval of patients with stage III/IV tumors did not differ between low and high CEA antigen groups. In addition, no patients with stage I tumors had died of breast cancer during the 130 months follow-up (Fig. 2).

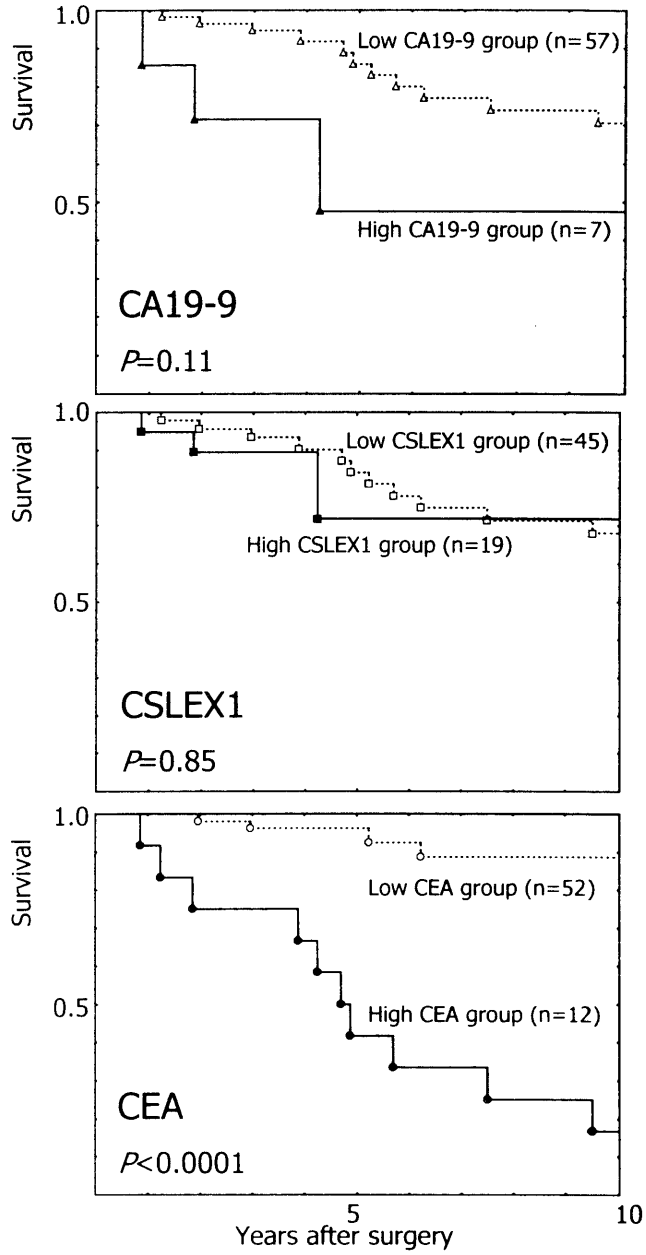

Figure 1. Comparison of disease-specific intervals between low- and high-antigen groups.
after resection for breast cancer

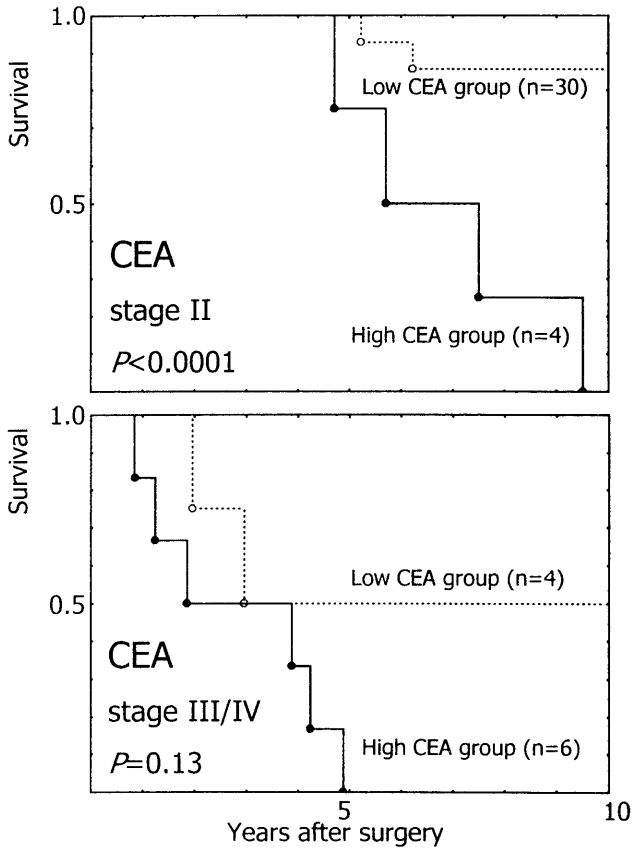

Figure 2. Disease-specific intervals of patients with stage I, stage II, or stage III/IV breast cancer, and with high or low antigen levels after radical mastectomy.

Table 2. Prognostic variables for survival in Cox's regression analysis.

	Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)*	*P* value	HR (95% CI)*	*P* value
Age at diagnosis (years)				
<53 (n=32)	1		1	
≥53 (n=32)	2.31 (0.72-7.38)	0.16	0.72 (0.09-5.59)	0.75
Histology				
Papillo-tubular/Solid-tubular	1		1	
Scirrhous/Mucinous	2.38 (0.74-7.63)	0.14	1.90 (0.30-12.24)	0.50
Stage				
I/II	1		1	
III/IV	12.17 (4.17-35.56)	<0.0001	14.11 (2.90-68.53)	0.0010
Serum CA19-9 status				
Low group	1		1	
High group	3.15 (0.87-11.33)	0.079	1.86 (0.11-30.77)	0.67
Serum CSLEX1 status				
Low group	1		1	
High group	1.15 (0.31-4.24)	0.83	0.71 (0.05-10.80)	0.80
Serum CEA status				
Low group	1		1	
High group	12.16 (3.79-39.02)	<0.0001	8.43 (2.11-33.71)	0.0026

*HR, hazard ratio; CI, confidence interval.

Cox's regression analysis for the prognostic value of serum antigen levels

To avoid the problem of co-linearity, variables such as menopausal status, T factor, N factor, and M factor, were excluded. The 6 remaining variables (age, histology, stage, serum CA19-9 status, serum CSLEX1 status, serum CEA status) were included in this analysis. According to an univariate Cox's regression analysis, stage and serum CEA status were associated with poor disease-specific survival (Table 2). To determine the independent prognostic value of the serum antigen, multivariate analysis was performed. Consequently, two independent variables, stage and serum CEA status, were found to be significant for predicting disease-specific survival (Table 2).

Discussion

Current therapeutic strategies for individual patients with primary breast cancer frequently are determined by the following variables: (1) the size (T factor) of the primary tumor; (2) the presence and extent of axillary lymph node metastases (N factor); (3) pathologic stage of the disease after primary therapy; and (4) the presence or absence of estrogen receptor and progesterone receptor activity.[16,24] It would be of great value to have an easily performed blood test that reliably predicts prognosis, independent of TMN stage.[25] For this reason, many circulating tumor markers, such as carcinoembryonic antigen (CEA)[11-15], CA15-3[11,13,15,26], E-selectin[9], c-erbB-2[15], and CYFRA 21-1[27], have been investigated for use in breast cancer patients. However, there are few known circulating tumor markers with established clinical utility.[25]

In the current study, multivariate Cox's regression analysis revealed that a high preoperative serum CEA level was an independent prognostic factor for disease-specific survival after radical mastectomy for breast cancer, whereas circulating CA19-9 or CSLEX1 did not correlate with disease-specific survival.

The prognostic value of the preoperative serum CA19-9 level in patients with gastric and colorectal cancer has been reported.[28-30] For example, Filella et al.[29] reported the prognostic value of circulating CA19-9 based on the disease-free interval after curative surgery in 162 colorectal cancer patients, and noted that CA19-9 provides more prognostic information than conventional staging methods (Dukes' classification). In contrast, the current study did not reveal the prognostic value of circulating CA19-9 in breast cancer patients. Narita et al.[6] also reported that sialyl

Lewis^a antigen expression in tumor tissue, as demonstrated by immunohistochemistry, did not correlate with overall survival or relapse-free survival in 300 breast cancer patients. Based on these findings, we believe that circulating sialyl Lewis^a antigen may have no prognostic value in breast cancer patients.

Narita et al.[6] also reported that increased expression of sialyl Lewis^x antigen in tumor tissue, as demonstrated by immunohistochemistry, correlated with a poor prognosis in breast cancer patients who underwent mastectomy. Matuura et al.[9] reported that increased serum levels of sialyl Lewis^x antigen are observed in patients with advanced and recurrent breast cancer, especially in those with distant metastasis. However, the current study showed no correlation between serum levels of sialyl Lewis^x antigen and tumor stage or survival after surgery. To date, a prognostic value of circulating sialyl Lewis^x antigen for survival in breast cancer patients has not been reported. Further investigation in a large number of patients is necessary to clarify this issue.

Several studies have reported that preoperative high serum levels of CEA correlate with a poor prognosis in breast cancer patients who underwent mastectomy.[13-15] In addition, preoperative serum CEA levels were shown to be an independent prognostic factor, separate from stage, by multivariate Cox's regression analysis.[13,15] The current study also confirmed the prognostic value for survival after surgery. Although Nakata et al.[27] reported no correlation between serum CEA levels and prognosis in breast cancer patients, this study is different from ours because it included patients with both primary and recurrent breast cancers. Therefore, we believe that a preoperative high serum CEA level is a useful predictor for a poor prognosis in patients with primary breast cancer who undergo radical mastectomy as a first-line therapy.

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