

Relation between the Serum E-Selectin Level and the Survival Rate of Patients with Resected Non-small Cell Lung Cancers

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E-Selectin is an inducible adhesion molecule, which is expressed on cytokine-activated endothelial cells and is thought to interact with cancer cells to initiate metastases. The relationship between serum E-selectin levels and prognoses in 101 patients with resected non-small cell lung cancers (NSCLCs) was studied, and survival curves were compared in relation to E-selectin levels and expression of two carbohydrate antigens, Sialyl Lewis^x (SLX) and Sialyl Lewis^a (CA19-9), which were immunohistochemically detected in resected specimens in 65 of the 101 cases. The serum E-selectin level on admission was 48.9±25.7 ng/ml (mean±SD, n=101), and the E-selectin-positive rate was 22.7%, being correlated with the progression of T-factor. The high E-selectin group showed a significantly worse survival rate than the normal E-selectin group. Multivariate analysis confirmed the significant prognostic value of E-selectin. The mean postoperative E-selectin level in 52 cases (36.93 ng/ml) was significantly lower than the preoperative E-selectin level (43.57 ng/ml), indicating that certain NSCLCs might induce the expression of E-selectin. In cases expressing carbohydrate antigens (SLX, CA19-9), the high E-selectin group showed a significantly worse survival curve than the normal E-selectin group. On the other hand, there was no significant difference in the survival curve between the high and normal E-selectin groups when carbohydrate antigens were negative. These results suggest that patients who have high serum E-selectin levels, especially with carbohydrate antigen-positive NSCLC, might be expected to have poor prognoses.

Key words: Lung cancer — Adhesion molecule — Carbohydrate antigen — Prognosis — Surgical resection

E-Selectin is an adhesion molecule expressed on cytokine-activated endothelial cells, and is released into the bloodstream as soluble E-selectin. It was originally discovered as a homing receptor named Endothelial Leukocyte Adhesion Molecule 1 (ELAM-1), which mediates the migration of neutrophils and monocytes to inflammatory foci.¹⁾ The carbohydrate antigens Sialyl Lewis^x (SLX) and Sialyl Lewis^a (CA19-9), which had previously been regarded as tumor markers,^{2,3)} were found to interact with E-selectin as ligands.

It is well-known that various cancer cells have carbohydrate antigens on their surface membranes.⁴⁾ Since one of the crucial steps in the development of blood-borne metastasis is thought to be the attachment of malignant cells to endothelial cells, it was postulated that the interaction of the carbohydrate antigens with E-selectin might represent an initiating process of metastasis. Laboratory experiments have indicated that carbohydrate antigens

present on highly metastatic cancer cells do bind to E-selectin on endothelial cells. Clinically, however, the roles of the carbohydrate antigens SLX and CA19-9, and E-selectin, in the mechanism of metastasis of cancer have not been fully elucidated.

We investigated the relationship between the serum level of soluble E-selectin and the prognosis of patients with resected non-small cell lung cancers (NSCLCs), and we also examined the expression of carbohydrate antigens on individual cancers in the present study.

MATERIALS AND METHODS

Sera and patients Blood samples were obtained on admission from 101 patients with NSCLCs, who had consecutively undergone curative tumor resection and lymph node dissection after admission. Each blood sample was promptly centrifuged to isolate the serum, which was stored at -80°C until assay. Blood samples were also collected from 52 patients about 4 weeks after surgery. In order to avoid the influence of postsurgical inflammation, sera with positive C-reactive protein (CRP) were excluded from the postoperative samples.

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These 101 patients consisted of 67 males and 34 females, whose average age was 65.0 years (range: 40 to 85 years). Clinical and pathological ratings were made following the General Rule for Clinical Pathological Record of Lung Cancer (4th edition).⁵⁾ In pathological staging, 46 cases were at stage I, 11 at stage II, 32 at stage IIIA, and 12 at stage IIIB. There was no patient at stage IV. Histologically, 68 cases were adenocarcinoma, 24 were squamous cell carcinoma, 3 were large cell carcinoma, and 6 were adenosquamous carcinoma. All resected cases were followed up at the outpatient clinic after hospital discharge. Patients in whom a tumor stump was exposed microscopically at a surgical margin were omitted from this study, and patients who died of non-cancer causes were also excluded.

Detection of soluble E-selectin in serum The level of circulating E-selectin was measured in duplicate using a commercial ELISA kit (R&D Systems, Minneapolis, MN).

Expression of carbohydrate antigens on tumors Formaldehyde-fixed, paraffin-embedded blocks of tumor tissues from 65 patients were sectioned and treated with SLX (FH6, Otsuka Pharmaceutical, Tokushima) and CA19-9 (NS19-9, CIS Bio International, Cedex, France) as primary antibodies for immunohistochemical observation. Diluted antibody solution (SLX 1:10 and CA19-9 1:10) was overlaid on each section and incubated at room temperature for 2 h. After the application of the labeled streptavidin biotinyl peroxidase (LSAB) complex (DAKO LSAB kit, Carpinteria, CA), the sections were stained with 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB), then counter-stained with Mayer's hematoxylin. Normal serum obtained from mice was used instead of each primary antibody as the negative control. Grading of the expression of carbohydrate antigens in tumor tissues was defined as follows: (-), positive cells were less than 10%; (1+), 10–50%, and (2+), 50% or more. The grade (-) was regarded as negative, and the grades (1+) and (2+) were regarded as positive. If either or both of SLX and CA19-9 were positive, the tissue was classified as carbohydrate antigen-positive. If neither SLX nor CA19-9 was positive, the tissue was classified as carbohydrate antigen-negative. Immunohistochemical findings were evaluated independently by two pathologists who were blinded as to the clinical outcomes.

Statistical analysis Statistical analyses were made using statistical software (StatView, Abacus, Tokyo) on a personal computer (Macintosh, Apple, Tokyo). The one sample sign test was used for testing the significance of the difference between observed mean value and the mean value from healthy volunteers, the paired *t* test or Wilcoxon signed rank test for paired comparison, and the unpaired *t* test or Mann-Whitney's *U* test for unpaired comparison. The χ^2 test was used for the contingency

table. Cumulative survival curves were calculated by Kaplan-Meier method and statistically evaluated by use of the logrank test and generalized Wilcoxon test. In the simple variate and multivariate survival analyses, the Cox proportional hazards model was used. The criterion of significance was $P < 0.05$.

RESULTS

Level of soluble E-selectin and positive rate The mean serum level of soluble E-selectin on admission was 48.9 ± 25.7 ng/ml (mean \pm SD). According to the one sample *t* test, this value was not significantly different from the mean value from healthy volunteers, 46.25 ± 17.1 ng/ml, which was given in the instruction manual for users of the ELISA kit. No significant differences were observed in the serum level of E-selectin among the patient groups stratified by gender, histological type, T-factor, N-factor, the presence or absence of pulmonary metastasis and pathological stage.

All 101 cases were then divided into 2 groups using the mean + SD as the cut off level for E-selectin: one was the normal E-selectin group (E-selectin under 63.35 ng/ml) and the other was the high E-selectin group (63.35 ng/ml and over). When values higher than this cut-off level were defined as positive, 22.7% of all cases belonged to the positive E-selectin group.

As before, all cases were categorized by gender, histological type, T-factor, N-factor, the presence or absence of pulmonary metastasis and pathological stage, and we examined whether the positive rate of E-selectin differs in each category. However, there was no statistically significant difference in the positive rate of E-selectin in individual categories except for the category expressing T-factor (Table I). The incidence of E-selectin-positive cases increased as the expression of T-factor progressed.

Cumulative survival rate in all cases The mean survival rate of the high E-selectin group was significantly worse than that of the normal E-selectin group (Fig. 1). As mentioned above, we could not find any statistically significant background factor except for T-factor.

Survival analysis Survival analysis was carried out for a simple variate prognostic factor using the proportional hazards model. The results showed that T-factor, N-factor, the presence or absence of pulmonary metastasis, pathological stage and the serum level of preoperative E-selectin were significant prognostic factors (Table II). Multivariate analysis was then applied to these significant prognostic factors, and it was found that pathological stage and serum level of preoperative E-selectin were independent and significant prognostic factors (Table III).

Changes in E-selectin level before and after surgery In order to find out whether surgical operation affects the postoperative serum level of E-selectin, serum E-selectin

Table I. Comparison of Clinicopathological Factors According to E-Selectin

	Normal E-selectin <i>n</i> =78	High E-selectin <i>n</i> =23	Positive rate (%)	<i>P</i> value
Gender				
Male	50	17	25.4	NS ^{a)}
Female	28	6	17.6	
Histological type				NS ^{a)}
AD	52	16	23.5	
SQ	18	6	25.0	
LA	2	1	33.3	
ADSQ	6	0	0	
T-factor				<i>P</i> <0.05 ^{b)}
T1	30	4	11.8	
T2	30	7	18.9	
T3	11	9	45.0	
T4	7	3	30.0	
N-factor				NS ^{b)}
N0	47	12	20.3	
N1	10	5	33.3	
N2	21	6	22.2	
Pulmonary metastasis				NS ^{b)}
Negative	69	20	22.5	
Positive	9	3	25.0	
Stage				NS ^{b)}
I	38	8	17.4	
II	10	1	9.1	
IIIA	21	11	34.4	
IIIB	9	3	25.0	

NS, not significant; AD, adenocarcinoma; SQ, squamous cell carcinoma; LA, large cell carcinoma; ADSQ, adeno-squamous carcinoma.

a) χ^2 test.

b) Mann-Whitney's *U* test.

levels were measured on postoperative days 1, 3, 7 and 14 in 5 cases. The E-selectin level in the samples collected on admission was substituted for the preoperative E-selectin level. The scattered values of preoperative E-selectin tended to show a slight decrease after the operation and became stable in 2 weeks (Fig. 2). The mean serum level of E-selectin obtained from 52 cases 4 weeks after resection (before discharge from hospital) was 36.93 ng/ml, which was significantly lower than the mean preoperative value of 43.57 ng/ml (paired *t* test and Wilcoxon signed rank test).

Carbohydrate antigens Background factors were compared between the carbohydrate antigen-positive and negative groups in 65 cases (Table IV). There was no significant difference between the two groups except for T-factor. The T-factor was slanted to the T1 side in the carbohydrate antigen-positive group.

Survival curves stratified by preoperative E-selectin levels and carbohydrate antigens There was no significant difference in the cumulative survival rate between the

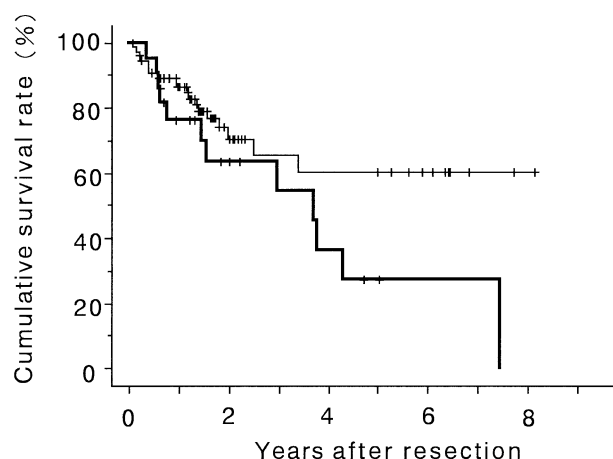


Fig. 1. Survival curves for 101 patients stratified by preoperative serum E-selectin levels. The survival rate of the group with high E-selectin was significantly worse than that of the normal E-selectin group (*P*<0.01, Wilcoxon test). — normal E-selectin group, *n*=78, - - - high E-selectin group, *n*=23.

Table II. Survival Analysis Using Simple Variate Analysis

Variable	Hazard ratio	95% confidence interval	P value
Gender (female/male)	0.825	0.428–1.589	NS
Age	1.008	0.977–1.040	NS
Histological type (AD/SQ)	0.914	0.409–2.042	NS
Carbohydrate antigen (positive/negative)	0.987	0.409–2.386	NS
Expression of SLX (positive/negative)	0.542	0.210–1.403	NS
Expression of CA19-9 (positive/negative)	1.410	0.586–3.394	NS
T-factor	1.611	1.170–2.217	<i>P</i> <0.01
N-factor	1.505	1.051–2.156	<i>P</i> <0.05
Pulmonary metastasis (positive/negative)	2.497	1.099–5.674	<i>P</i> <0.05
Stage	1.756	1.320–2.336	<i>P</i> <0.01
E-Selectin (high/normal)	2.226	1.186–4.178	<i>P</i> <0.01

NS, not significant; AD, adenocarcinoma; SQ, squamous cell carcinoma; SLX, Sialyl Lewis^x; CA19-9, Sialyl Lewis^a.

Table III. Multivariate Analysis

Variable	Hazard ratio	Regression coefficient	SE	γ^2	P value
Stage	1.693	0.527	3.568	12.731	<i>P</i> <0.01
E-Selectin (high/normal)	1.962	0.674	2.054	4.220	<i>P</i> <0.05

SE, standard error.

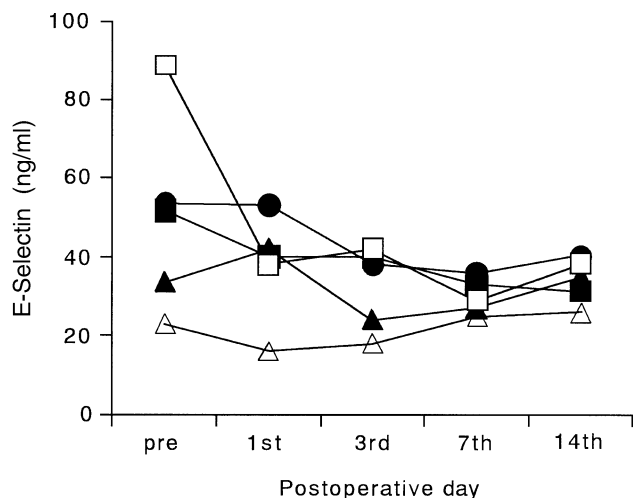


Fig. 2. Time course of changes in serum E-selectin in 5 cases. The E-selectin level upon hospital admission was substituted for the preoperative level of E-selectin. Scattered values of preoperative E-selectin settled with time. Different symbols are used for individual cases.

carbohydrate antigen-positive and negative groups (data not shown). In carbohydrate antigen-positive cases, the survival curve of the high preoperative E-selectin group was significantly worse than that of the normal preoperative E-selectin group (Fig. 3). In carbohydrate antigen-

negative cases, on the other hand, there was no significant difference in the cumulative survival curve between the high and normal preoperative E-selectin groups (Fig. 4).

DISCUSSION

Some inflammatory cytokines such as interleukin 1, tumor necrosis factor and endotoxin interact with cultured human vascular endothelial cells to increase leukocyte adhesion, and E-selectin was shown to be involved as a leukocyte-binding inducible structure on the endothelial cell surface.¹⁾ There are two species (115 and 97 kDa) of E-selectin, and both are glycoproteins. These E-selectin species are thought to play an important role in the adhesion between activated endothelial cells and neutrophils, monocytes, natural killer cells or some kinds of T cells. Structurally, E-selectin has a lectin-like domain, an epidermal growth factor-like domain and a complement-binding domain,⁶⁾ similarly to L-selectin (CD62L) or P-selectin (CD62P). Thus, E-selectin has been classified as a member of the selectin family, and termed E-selectin or CD62E. E-Selectin has attracted a great deal of attention, because it is thought to be involved in the pathology and clinical features of leukocyte dysfunction diseases, such as adult respiratory distress syndrome (ARDS), post reperfusion myocardial injury, etc.

Since the selectin family has a lectin-like domain, it had been speculated that selectin families may bind with carbohydrate chains, and it was established subsequently

Table IV. Comparison of Background Factors According to Immunohistochemical Carbohydrate Antigen Expression

	CA negative <i>n</i> =25	CA positive <i>n</i> =40	<i>P</i> value
E-Selectin			
Mean±SD (ng/ml)	49.0±22.5	43.5±21.0	NS ^{a)}
Positive rate	28%	15%	NS ^{b)}
Gender			
Male	19	29	NS ^{b)}
Female	6	11	
Histological type			
AD	12	22	NS ^{b)}
SQ	10	12	
LA	2	1	
ADSQ	1	5	
T-factor			
T1	3	15	<i>P</i> <0.05 ^{c)}
T2	13	17	
T3	9	3	
T4	0	5	
N-factor			
N0	12	24	NS ^{c)}
N1	5	6	
N2	8	10	
Pulmonary metastasis			
Negative	22	36	NS ^{c)}
Positive	3	4	
Stage			
I	7	20	NS ^{c)}
II	5	5	
IIIA	12	9	
IIIB	1	6	

CA, carbohydrate antigen; NS, not significant; AD, adenocarcinoma; SQ, squamous cell carcinoma; LA, large cell carcinoma; ADSQ, adenosquamous carcinoma.

a) Unpaired *t* test.

b) χ^2 test.

c) Mann-Whitney's *U* test.

that SLX⁷⁾ and CA19-9⁸⁾ are the carbohydrate chains binding with E-selectin. In this connection, it is noteworthy that some kinds of cancers such as lung cancer⁹⁾ and colon cancer¹⁰⁾ often have these carbohydrate antigens. Thus, it seemed likely that the interaction between E-selectin and these carbohydrate antigens might play an important role in blood-borne metastasis of malignant cells.¹¹⁾

In a series of basic studies using cultured cells, it has been proved that E-selectin plays a significant role in the binding of human cancer cells to activated endothelial cells.¹²⁾ In addition, in a study in which serum concentrations of circulating E-selectin in 110 cancer patients were measured, the level of soluble E-selectin was found to be

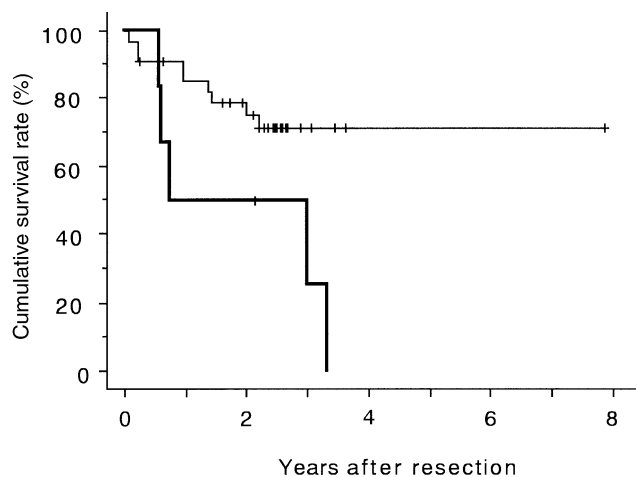


Fig. 3. Survival curves for carbohydrate antigen-positive cases stratified by serum E-selectin levels. The survival rate of the high E-selectin group was significantly worse than that of the normal E-selectin group (*P*<0.01, Wilcoxon test). — normal E-selectin group, *n*=34, — high E-selectin group, *n*=6.

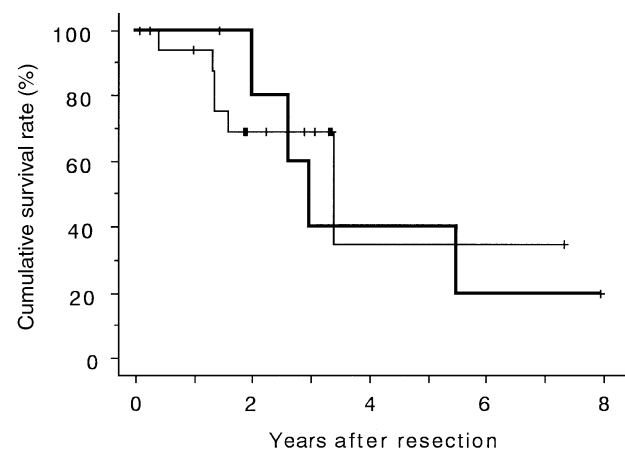


Fig. 4. Survival curves for carbohydrate antigen-negative cases stratified by serum E-selectin levels. There was no significant difference in the survival curve between the high and normal E-selectin groups (*P*>0.05, Wilcoxon test). — normal E-selectin group, *n*=18, — high E-selectin group, *n*=7.

significantly higher in groups with ovarian, breast and gastrointestinal cancers and lower in a myeloma group, as compared with healthy subjects.¹³⁾ Another report concluded that the concentration of soluble E-selectin was significantly elevated in the sera of breast cancer patients with distant metastases.¹⁴⁾

Whether the soluble E-selectin level in lung cancer patients is significantly higher than that in normal volunteers could not be assessed in the present study, because

no healthy Japanese control was included in the study. According to the instruction for users attached to the ELISA kit used, however, there was no significant difference in the mean soluble E-selectin level between our Japanese lung cancer group and Western healthy volunteers.

Soluble E-selectin in serum is thought to be derived from the E-selectin expressed on endothelial cells, not directly from cancer cells. Therefore, E-selectin would be less useful as a tumor marker than other cancer-related antigens. In fact, no significant difference was found in soluble E-selectin level at different pathological stages and in different histological groups. Moreover, the positive rate of soluble E-selectin was as low as 22.7%.

The mean value of postoperative E-selectin was significantly lower than the mean preoperative value. This decrement is presumably due to the surgical excision of the tumor, suggesting that some kinds of NSCLCs might induce the expression of vascular E-selectin either directly or indirectly. This is not contradictory to the finding that the positive rate of E-selectin was higher in the group with a greater expression of T-factor. The high E-selectin group showed a significantly poorer survival rate than the normal E-selectin group. Though there was no significant relation between clinicopathological factors and the positive rate of E-selectin except for T-factor, this poor survival in the high E-selectin group cannot be accounted for solely by T-factor. The multivariate analysis showed that the preoperative serum E-selectin level was an independent prognostic factor ranking with the disease stage.

In immunohistological studies of the expression of carbohydrate antigens on isolated lung cancer tissue, pulmonary adenocarcinoma was found to express SLX frequently.^{9,15)} Since the type 1 carbohydrate chain (SLX) and the type 2 chain (CA19-9) were lumped together in the present study, no significant difference was found among different histological types.

Tumors not expressing binding sites for Le^x antigen have been reported to show fewer metastases and to have significantly better prognoses than tumors with other carbohydrate profiles.¹⁶⁾ It has also been reported that the SLX expression at stage I of NSCLCs might be an important determinant of recurrence.¹⁷⁾ However, no correlation between the expression of carbohydrate antigens and the postoperative survival of patients with pulmonary adenocarcinoma was found in another study.¹⁸⁾ In our study, there was no significant difference in the postoperative cumulative survival rate between the carbohydrate antigen-positive and negative groups.

In the combination study of serum E-selectin and immunohistological expression of carbohydrate antigens, E-selectin was significantly related to the prognoses of patients in the carbohydrate antigen-positive group, whereas there was no relationship between E-selectin and the prognoses of patients in the carbohydrate antigen-negative group. The group in which carbohydrate antigens were positive and E-selectin was high showed T1 in 1 case, T2 in 3 cases, and T3 and T4 in 1 case each.

In this study, although the number of cases studied was not large, we found that patients who had high serum E-selectin levels, especially with carbohydrate antigen-positive NSCLC, tended to have poor prognoses, even though carbohydrate antigen alone was found to have no prognostic value. Our findings are consistent with the hypothesis that carbohydrate antigen-positive cancers are liable to metastasize remotely under the influence of E-selectin.

Measurement of the expression of E-selectin and its ligands is expected to provide clinically useful information for the planning of appropriate and effective adjuvant chemotherapies¹⁹⁾ and follow-up examinations to control the postoperative recurrence of NSCLCs.

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