Advances in Brief

Serum Sialyl Lewis X-i Antigen Levels in Non-Small Cell Lung Cancer: Correlation with Distant Metastasis and Survival

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Abstract

To evaluate the correlation between serum levels of sialyl Lewis X-i antigen and distant metastasis and survival in patients with non-small cell lung cancer (NSCLC), we measured the serum levels of the tumor marker in 371 patients with untreated NSCLC. The sialyl Lewis X-i antigen level was measured using a RIA kit. In patients with adenocarcinoma or other NSCLC subtypes, there was a correlation between serum sialyl Lewis X-i antigen and stage of the disease ($P = 0.0001$ and $P = 0.0015$, respectively). Levels of the marker varied significantly depending on the number of metastatic organs in adenocarcinoma ($P = 0.0089$) and in other NSCLC subtypes ($P = 0.002$). Univariate analysis showed that survival of NSCLC patients with high (more than 100 units/ml) sialyl Lewis X-i antigen levels was significantly poorer than that of patients with low antigen levels ($P = 0.0001$). Multivariate analysis using Cox's proportional hazard model showed that high sialyl Lewis X-i antigen levels correlated significantly with poor survival ($P = 0.004$). Our data suggest that a high serum level of sialyl Lewis X-i antigen seems to be an indicator of the presence of metastasis and might indicate the need for a careful investigation of all putative metastatic sites. The serum levels of sialyl Lewis X-i antigen may reflect the extension of metastasis and would be helpful in considering treatment options.

Introduction

There have been some interesting reports on the association between metastasis and carbohydrate antigens expressed on the tumor cell surface (1, 2). Presently, changes in the expression of glycoconjugates have been observed in a variety of cancers, and aberrant glycoproteins and glycolipids, which are absent or minimal in normal tissues, are expressed in various cancer tissues (3–5). Recently, some investigators demonstrated that the metastatic lesions contain more sialyl Lewis X-i antigen, one of the carbohydrate antigens, than the primary tumor, which suggested a role of sialyl Lewis X-i antigen in distant metastasis (6–10). It has been demonstrated that elevated levels of sialyl Lewis X-i antigen in sera from patients with NSCLC,[2] especially in patients with locally advanced or metastatic disease, were higher than those from patients with an early stage of the disease (11, 12). Some studies have indicated that the number of metastatic organs is one of the most important factors for both response to chemotherapy and survival in advanced NSCLC (13–15).

In this study, we focused on serum levels of sialyl Lewis X-i antigen in patients with NSCLC, with special reference to distant metastasis and survival. It is well known that sialyl Lewis X-i antigen is expressed more frequently on the cell surface of adenocarcinoma than other NSCLC subtypes (16, 17). Also, at initial diagnosis, adenocarcinoma has a tendency to start with metastatic spread more frequently than squamous cell carcinoma. Therefore, we analyzed the findings closely as to the histological subtype to demonstrate whether increased serum levels of sialyl Lewis X-i antigen might be observed in patients with NSCLC subtypes other than adenocarcinoma. We revealed that the serum levels of sialyl Lewis X-i antigen may reflect the extension of metastasis, and higher levels of the antigen seems to predict shorter survival.

Materials and Methods

Patients' Characteristics. Three hundred seventy-one consecutive untreated NSCLC patients referred to Tsukuba University Hospital between July 1987 and December 1995 were entered in this study (Table 1). All of the patients had been pathologically confirmed to be NSCLC. Clinical stage (18, 19) of the cancer was determined according to a complete physical examination, chest X-ray, computerized tomography of the head and thorax, abdominal computed tomography and/or ultrasound, as well as a radionuclide bone scan. Patients with metastatic disease were subclassified into three groups according to the number of metastatic organs as follows: 73 patients with one metastatic organ, 51 patients with two metastatic organs, and 32 patients with three or more metastatic organs (Table 1). Serum samples were obtained from patients prior to any clinical treatment.

Serum Assay. The levels of sialyl Lewis X-i antigen were measured by RIA on serum samples stored at −20°C, using a commercial kit (Otsuka Assay Laboratories, Tokushima, Japan). All assays were performed without clinical information.

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[2] The abbreviations used are: NSCLC, non-small cell lung cancer; ROC, receiver operating characteristic.

Table 1 Patient characteristics

	No. of patients
Total no. of patients	371
Gender: male/female	270/101
Mean age, years (range)	65 (22–87)
Histology	
Adenocarcinoma	198
Squamous cell carcinoma	140
Large cell carcinoma	22
Others	11
Stage	
I	48
II	24
IIIA	43
IIIB	100
IV	156
One metastatic organ	73
Two metastatic organs	51
Three metastatic organs or more	32
Performance status	
0	56
1	195
2	55
3	45
4	20
Treatment	
Surgery	99
Radiotherapy	37
Chemotherapy	148
Best supportive care	87

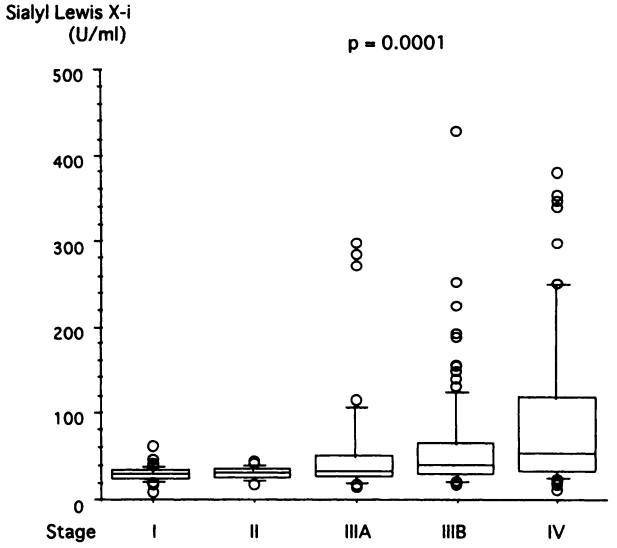

Fig. 1 Percentile distribution of serum levels of sialyl Lewis X-i antigen at the time of primary diagnosis in 371 patients with NSCLC. Each *box* contains the variable distribution between the 25th and 75th percentiles, with the median value indicated with a *line* in the *box*. The *bars* extending above and below the box indicate the 90th and 10th percentiles, respectively.

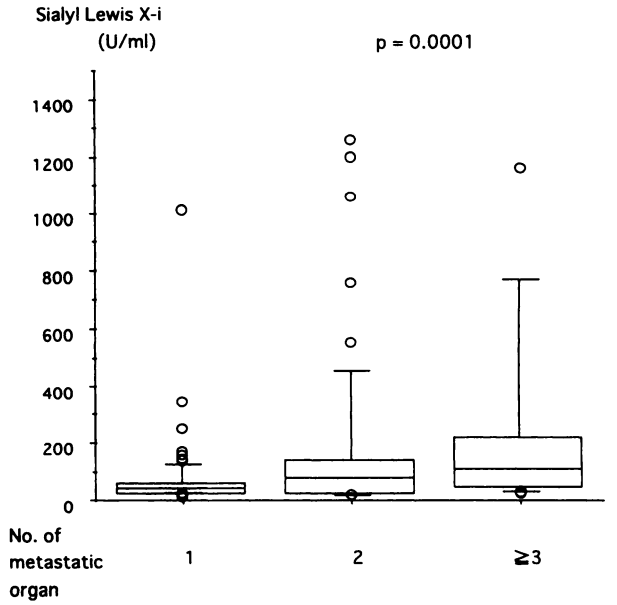

Fig. 2 Percentile distribution of serum levels of sialyl Lewis X-i antigen at the time of primary diagnosis in stage IV adenocarcinoma of the lung. Each *box* contains the variable distribution between the 25th and 75th percentiles, with the median value indicated with a *line* in the *box*. The *bars* extending above and below the box indicate the 90th and 10th percentiles, respectively.

Statistical Analysis. Mann-Whitney *U* test or Kruskal-Wallis test was applied to elucidate the difference between two independent groups or more; the proportion was compared by χ^2 test. Only results with $P < 0.05$ were regarded as significant. The cutoff levels for the serum sialyl Lewis X-i antigen were determined by constructing a ROC curve analysis (20). Survival was recorded from the date of the measurement of sialyl Lewis X-i antigen to the date of death or last follow-up, and survival curves were calculated according to the method of Kaplan and Meier (21). Single variable survival analysis was calculated using the logrank test and generalized Wilcoxon test. Cox's regression was used to simultaneously evaluate various prognostic factors (22).

Results

Sialyl Lewis X-i Antigen in Patients with NSCLC. The serum sialyl Lewis X-i antigen levels significantly differed according to clinical stage (Kruskal-Wallis test, $P = 0.0001$; Fig. 1). To confirm a possible correlation between serum levels of sialyl Lewis X-i antigen and more extensive disease, we divided the 156 patients with stage IV into three groups according to the number of metastatic organs. As shown in Fig. 2, the distribution of serum sialyl Lewis X-i antigen levels varied significantly among three groups (Kruskal-Wallis test, $P = 0.0001$).

The serum levels of sialyl Lewis X-i antigen in patients with two metastatic organs or more (median, 82.0 units/ml; interquartile range, 35.0–174.8 units/ml) were significantly higher than those in patients with one metastatic organ (median, 43.0 units/ml; interquartile range, 28.8–64.8 units/ml; $P = 0.0001$). In ROC curve analysis of discrimination between patients with one metastatic organ and those with two metastatic organs or more, the optimal cutoff level was set to 100 units/ml, which gave a sensitivity of 86.3% and specificity of 54.2%.

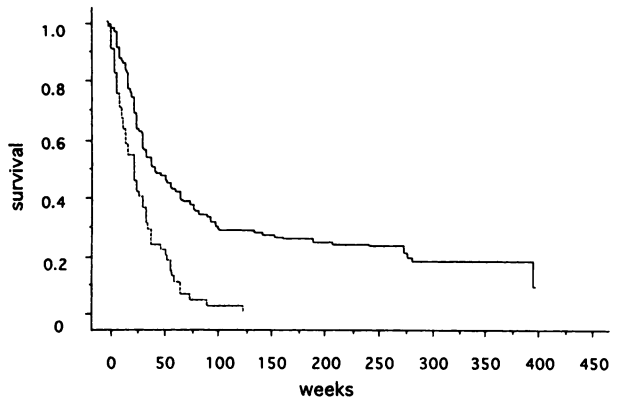

Fig. 3 Survival curves of 371 patients with NSCLC based on their sialyl Lewis X-i antigen levels (*upper line*, patients with sialyl Lewis X-i antigen levels less than 100 units/ml; *lower line*, patients with sialyl Lewis X-i antigen levels more than or equal to 100 units/ml).

Sialyl Lewis X-i Antigen in Patients with Adenocarcinoma. We analyzed the distribution of sialyl Lewis X-i antigen levels in patients with adenocarcinoma of the lung. The serum sialyl Lewis X-i antigen levels significantly differed according to clinical stages ($P = 0.0001$). The distribution of the serum sialyl Lewis X-i antigen levels varied significantly according to the number of metastatic organs ($P = 0.0089$).

The serum levels of sialyl Lewis X-i antigen in patients with two metastatic organs or more (median, 109.0 units/ml; interquartile range, 47.9–213.0 units/ml) were significantly higher than those in patients with one metastatic organ (median, 52.5 units/ml; interquartile range, 28.0–95.5 units/ml; $P = 0.0027$). In ROC curve analysis of discrimination between patients with one metastatic organ and those with two or more metastatic organs, the optimal cutoff level was set to 100 units/ml, which gave a sensitivity of 73.7% and specificity of 46.7%.

Sialyl Lewis X-i Antigen in Patients with Other NSCLC Subtypes. We also analyzed the distribution of sialyl Lewis X-i antigen levels in patients with 140 squamous cell carcinoma, 22 large cell carcinoma, and 11 others. The serum sialyl Lewis X-i antigen levels significantly differed according to clinical stages ($P = 0.0015$). The distribution of serum sialyl Lewis X-i antigen levels varied significantly according to the number of metastatic organs ($P = 0.002$).

Relationship between Serum Sialyl Lewis X-i Antigen and Survival. To evaluate the relationship between serum levels of sialyl Lewis X-i antigen and survival, we performed univariate analysis of 371 patients with NSCLC using the logrank test and generalized Wilcoxon test. Patients with a serum sialyl Lewis X-i antigen level more than or equal to 100 units/ml proved to have a shorter survival than patients with a sialyl Lewis X-i antigen less than this value (logrank test, $P = 0.0001$; generalized Wilcoxon test, $P = 0.0001$; Fig. 3). To further clarify the independent prognostic importance of the categories, Cox's proportional hazard model was used for analysis. For each variable, the proportional hazard assumption was tested graphically. With Cox's model analysis, an advanced stage ($P = 0.0001$), male ($P = 0.0037$), and a higher level of sialyl Lewis X-i antigen ($P = 0.0040$) were confirmed as significant determinants of survival (Table 2).

Table 2 Prognostic factors for 371 patients with NSCLC determined by the Cox proportional hazard model

Variables	Coefficient	SE	*P*
Gender	0.35933	0.12376	0.0037
Age	0.18222	0.11330	0.1078
Stage of disease	1.33654	0.13508	0.0001
Sialyl Lewis X-i (<100, ≥100)	0.50997	0.17726	0.0040

Because none of the patients with stages I and II disease had serum sialyl Lewis X-i antigen levels higher than 100 units/ml, we studied whether the antigen level is a statistically significant prognostic factor in patients with stage III or IV disease. Patients with stage IV disease (mean survival time, 34.8 weeks) had a shorter survival than those with stage III A and B (mean survival time, 53.4 weeks; $P = 0.0001$). The levels of sialyl Lewis X-i antigen in stage IV disease (median, 51.2 units/ml; interquartile range, 31.1–115.0 units/ml) were significantly higher than those in patients with stage III A and B (median, 36.2 units/ml; interquartile range, 26.4–56.0 units/ml; $P = 0.0001$). In univariate analysis, patients in stage III A and B as well as in stage IV with the antigen level higher than 100 units/ml had a statistically worse prognosis compared to patients with the lower antigen levels ($P = 0.0086$ and $P = 0.0246$, respectively). In Cox's model analysis, higher levels of the antigen (>100 units/ml) were confirmed as a significant determinant of survival among those with stage III A and B and stage IV patients ($P = 0.0013$).

We also performed univariate and multivariate analysis of 198 patients with adenocarcinoma of the lung. In both analyses, patients with a serum sialyl Lewis X-i antigen level over 100 units/ml proved to have a shorter survival than patients with a sialyl Lewis X-i antigen less than or equal to this value (logrank test, $P = 0.0001$; generalized Wilcoxon test, $P = 0.0001$; Cox's model analysis, $P = 0.017$).

Discussion

The estimation of distant metastasis could contribute to the making of treatment plans for patients, because the presence of distant metastasis was strongly associated with poor prognosis (13, 14). Some investigators have demonstrated that the number of metastatic organs was one of the most important prognostic factors for both response to chemotherapy and survival in advanced NSCLC, and in clinical trials, the number of metastatic organs should be considered as a factor in stratification (14, 15). Very recently, new agents that are conjugates of antibodies directed against Lewis antigens have been developed by several institutions (23, 24). Immunotherapy using a monoclonal Lewis Y antibody are under early clinical trials for human solid tumors (25, 26).

Our study demonstrated that, both in patients with adenocarcinoma or other NSCLC subtypes, there is a statistically significant correlation between serum sialyl Lewis X-i antigen and stage of the disease. Moreover, the marker varied significantly, depending on the number of metastatic organs. A high

serum level of serum sialyl Lewis X-i antigen seems to be an indicator of the presence of metastases and might indicate the need for a careful investigation of all putative metastatic sites. A high serum sialyl Lewis X-i antigen may indicate a high metastatic fraction, which is a feature usually linked to very aggressive clinical behavior. Serum levels of the antigen might also correlate with tumor burden and generally reflect disease spread, which were associated with an increasing number of metastatic organs. Alternatively, it must be stressed that a high pretreatment serum sialyl Lewis X-i antigen was associated with poor prognosis.

The biological function of Lewis X antigen had not been distinct; however, one of the functions was recently demonstrated to be a ligand of endothelial-leukocyte adhesion molecule-1 expressed on the cell surface of endothelium activated by cytokines such as tumor necrosis factor or interleukin 1 (27–31). The adherence of specific cancer cells to capillary endothelial cells of the target organs is considered as a mandatory step in the formation of metastasis. With respect to sialic acid, which is a constituent of sialyl Lewis X-i antigen, there also have been many studies suggesting a relation between sialic acid at the nonreduced end of carbohydrate chains and metastatic potential (13, 32–35). Needless to say, the levels of the antigen in the serum depend on several factors, such as production by cancer cells, amount of cancer cells producing the antigen, access to blood circulation, and clearance efficiency. We speculated that one of the possible explanations for the occurrence of the considerable overlap between the subgroups might be due to interactions of these factors. From the results of our own, aberrant expression of sialyl Lewis X-i antigen on NSCLC seems to have a functional role in the formation of metastasis and that increased serum levels of the antigen might be concerned with developing distant metastasis. A larger study design to compare this marker with other tumor markers such as carcinoembryonic antigen is needed to clearly define the role of sialyl Lewis X-i antigen in the management of NSCLC.

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