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Serum CEA and CYFRA Levels in ALK-rearranged NSCLC Patients: Correlation With Distant Metastasis

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Abstract. *Aim: To clarify the correlation between serum levels of carcinoembryonic antigen (CEA) and cytokeratin 19 fragment (CYFRA) and metastasis and survival in anaplastic lymphoma kinase (ALK)-rearranged non-small cell lung cancer (NSCLC) patients. Patients and Methods: CEA and CYFRA levels in 131 ALK-rearranged NSCLC patients were determined using fluorescence in situ hybridization (FISH), real time-reverse transcription polymerase chain reaction, and immunohistochemistry, using biopsy specimens, cytology specimens, and plasma specimens. Cut-off value of each marker was determined as 10 ng/ml. Results: In logistic regression analysis, higher levels of both markers had a positive relationship with bone metastases, and higher levels of CYFRA was relevant to liver metastases, and multiple-organ metastases. However, these markers were not proven to be poor prognostic factors in Cox's proportional model analysis. Conclusion: Elevated serum CEA and CYFRA levels seem to provide useful clinical information about presence of bone and liver metastasis and multiple-organ metastases, although they were not a powerful indicator of prognosis. These two markers may suggest the extension of metastasis and would be helpful in considering treatment options.*

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Key Words: Serum tumor markers, carcinoembryonic antigen, CEA, cytokeratin 19 fragment, CYFRA, ALK rearranged NSCLC, distant metastasis, non-small cell lung cancer.

Carcinoembryonic antigen (CEA) has been widely used in clinical practice as a serum tumor marker for non-small cell lung cancer (NSCLC) patients (1). Cytokeratin 19 fragment (CYFRA) is also one of the representative serum tumor markers used for detection of NSCLC patients in clinical practice (1). CEA is a glycoprotein and CYFRA is a soluble

component of cytoskeleton cytokeratin (1). Although there is no tumor marker useful for the early diagnosis of NSCLC, there have been many reports that CEA and CYFRA reflect the extent of disease progression (1). Some of them have suggested that these tumor markers are unfavorable prognostic factors for NSCLC patients (1, 2-4). The clinical problem has been that for both markers there exist several assay kits and cut-off values. Considering the accuracy of clinical information provided by current tumor markers for lung cancer, comparing values to the decimal point measured with different kits is of little clinical necessity. If the measured value is markedly high, the sample must be diluted and re-measured. At that time, the measurement error increases due to dilution. These backgrounds must be taken into account when evaluating serum levels of tumor markers for lung cancer. However, elevated serum levels may provide important information that can be used in clinical practice, even if they are at rough levels. It is worthwhile to fully understand the characteristics and measurement methods of each tumor marker and use them in clinical practice of NSCLC.

In the last two decades, advances in molecular biology have led to discovery of several specific driver genes in many cancers and development of specific therapeutic agents (5, 6). This new trend is also noticeable in clinical practice for NSCLC patients. Long-term survival can be expected in NSCLC patients with driver genes for which specific therapeutic agents exist (5, 6). Anaplastic lymphoma kinase (ALK) fusion gene mutation is one of such driver genes, but its frequency is very rare (5, 6). Because of the rarity of ALK-rearranged NSCLC, it is important to collect and evaluate data in clinical practice in addition to results from clinical trials. Recently, an interesting study pointed out that liver and bone invasion were unfavorable prognostic factors in patients with ALK-rearranged gene mutations (7).

Until now, we accumulated and published actual clinical data on lung cancer treatment collected by multiple medical Institutions covering the residents of the Ibaraki prefecture with a population of 3 million (8-11). In this study, we investigated the significance of serum levels of CEA and CYFRA in ALK-rearranged NSCLC patients in actual clinical practice, with special interest in distant metastasis and prognosis. Since it was possible to investigate information of ALK-rearranged NSCLC patients in over one hundred patients, we herein share our medical information in real clinical practice.

Patients and Methods

Patients. Fifteen institutions located in the Ibaraki prefecture (area, 6,095 km^2; population, ~3 million) participated in the present retrospective study. We included patients who were diagnosed with ALK-rearranged NSCLC between April, 2008 and March, 2019. Treatment-naïve ALK-rearranged NSCLC patients whose serum CEA and CYFRA levels were measured at diagnosis were included in this study. NSCLC patients, who had any treatment before the approval of ALK fusion gene in 2012 and diagnosed as having the gene present, were included on this study. All the patients demonstrated histological or cytological evidence of NSCLC. Histopathological diagnoses were defined according to the World Health Organization (WHO) classification system and patients were staged according to the Union for International Cancer Control (UICC) tumor-node-metastasis (TNM) staging system. Metastatic sites were evaluated as bone, lung, brain, liver, extrathoracic lymph nodes, adrenal glands, and other uncommon sites. The clinical information of patient characteristics were evaluated using patient data extracted from the database of each Institution. Patient survival time was calculated from the date of initiation of first-line therapeutic drug to the date of death or last follow-up of the patient. The present observational study conformed to the Ethical Guidelines for Clinical Studies issued by the Ministry of Health, Labor and Welfare of Japan. This study was approved by the Institutional Review Board of the Mito Kyodo General Hospital (NO. 18-15) or independent Ethics Committees associated with each study Institute.

Table I. *Characteristics of 113 ALK-rearranged NSCLC patients for whom measurement of serum levels of CEA and CYFRA were available.*

Age (median, range), year	63, 32-84
Gender, M/F	73/40
Histopathological type	
AD/SQ/NSCLC	110/1/2
Performance status	
0-1/2 or more	103/10
Smoking habit	
Never/past or current	66/47
Pleural fluid	
Absent/present	84/29
Bone metastasis	
Absent/present	87/26
Liver metastasis	
Absent/present	107/6
Number of metastatic sites	
0-1/2 or more	80/33
Stage	
IA-B/IIA-B/IIIA-C/IVA-B	20/9/24/25/50
Surgical resection	
Received/not received	45/68
ALK-TKI therapy	
Received/not received	76/37
Serum CEA level at the time of diagnosis	
≤10 ng/ml/≥10 n/ml	72/41
Serum CYFRA level at the time of diagnosis	
≤10 ng/ml/≥10 n/ml	100/3

ALK: Anaplastic lymphoma kinase; NSCLC: non-small cell lung cancer; CEA: carcinoembryonic antigen; CYFRA: cytokeratin 19 fragment; AD: adenocarcinoma; SQ: squamous cell carcinoma; NSCLC: non-small cell lung cancer; TKI: tyrosine kinase inhibitor.

Measurement of ALK fusion gene, CEA, and CYFRA. ALK fusion gene mutation analysis was performed by the assay method normally used by each institution, such as fluorescence *in situ* hybridization (FISH), real time-reverse transcription polymerase chain reaction, and immunohistochemistry, using biopsy specimens, cytology specimens,

Table II. *Factors associated with bone metastasis in 113 ALK-rearranged NSCLC patients.*

Factors	Exp.	95% Confidence interval	*p*-Value
Age, less than 65, 65 years or more	0.479	0.158-1.453	0.1934
Gender, M/F	1.527	0.452-5.158	0.4952
Performance status			
0-1/2 or more	1.892	0.332-10.789	0.4728
Smoking habit			
Never/past or current	0.715	0.204-2.514	0.6016
Pleural fluid			
Absent/present	1.212	0.351-4.182	0.7607
CEA			
≤10 ng/ml/≥10 n/ml	4.002	1.280-12.511	0.0171
CYFRA			
≤10 ng/ml/≥10 n/ml	14.953	3.173-70.476	0.0006

ALK: Anaplastic lymphoma kinase; NSCLC: non-small cell lung cancer; Exp: exponential function; CEA: carcinoembryonic antigen; CYFRA: cytokeratin 19 fragment.

Table III. *Factors associated with liver metastasis in 113 ALK-rearranged NSCLC patients.*

Factors	Exp.	95% Confidence interval	*p*-Value
Age, less than 65, 65 years or more	2.742	0.350-21.482	0.3369
Gender, M/F	0.813	0.107-6.160	0.8415
Performance status			
0-1/2 or more	2.121	0.150-29.922	0.5777
Smoking habit			
Never/past or current	1.476	0.217-10.049	0.6906
Pleural fluid			
Absent/present	1.135	0.114-11.290	0.9141
CEA			
≤10 ng/ml/≥10 n/ml	0.510	0.057-4.587	0.5480
CYFRA			
≤10 ng/ml/≥10 n/ml	15.501	2.025-118.681	0.0083

ALK: Anaplastic lymphoma kinase; NSCLC: non-small cell lung cancer; Exp: exponential function; CEA: carcinoembryonic antigen; CYFRA: cytokeratin 19 fragment.

and plasma specimens. Blood samples were collected within 2 weeks before the initial treatment, and serum CEA and CYFRA levels were measured by chemiluminescence immunoassays. Serum levels of CEA and CYFRA measured with commercial kits used in Japan's insurance medical practice were evaluated. In this study, we considered the cutoff values of CEA and CYFRA as 10 ng/ml, as an easy-to-use measurement value in clinical practice.

Statistical analysis. The survival rate of patients was analyzed by the Kaplan-Meier method and comparisons were performed using the log-rank test. The effects of clinicopathological factors on survival were analyzed using the Cox proportional hazards model. $p<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. During the study period, 129 patients were diagnosed as having ALK-rearranged NSCLC. Of the 129 patients, 113 NSCLC patients, who had both serum CEA and CYFRA measured at the time of diagnosis, were included in the study. Table I presents the characteristics of these patients. There were 40 (35.4%) males and 73 (64.6%) females. The median age was 63 (range=32-84) years, and 51 (45.1%) patients were ≥65 years old. FISH was the most common procedure to confirm ALK rearrangement. There were 103 (91.1%) patients with a good performance status (PS) (Eastern Cooperative Oncology Group 0-1). Sixty-six (58.4%) of them were no smokers. One hundred and ten (97.3%) had adenocarcinoma.

Serum levels of CEA and CYFRA and metastasis. In this study, cut-off values for CEA and CYFRA were set at 10 ng/ml, taking into account the versatility of measurement levels in several assay kits commonly available in clinical practice. There were 41 patients who had serum CEA levels ≥10 ng/ml. Thirteen patients had serum CYFRA levels ≥10 ng/ml. Tables II, III, and IV show the results of logistic regression analysis in serum levels of CEA and CYFRA and metastasis. In this analysis, higher levels of both CEA and CYFRA had a relationship with bone metastases, and higher levels of CYFRA were relevant to liver metastases, and multiple-organ metastases.

Prognostic factors. In univariate analysis, 'poorer PS (2 or more)', 'positive smoking habit', 'no surgical resection', and 'elevated CEA levels ≥10 ng/ml' were unfavorable prognostic factors. In Cox proportional hazards model analysis, however, none of these factors except for 'no surgical resection' were statistically significant unfavorable prognostic factors (Table V).

Discussion

ALK-rearranged NSCLC is a rare disease (5, 6). Unlike other clinical trials in NSCLC patients, phase III randomized controlled trials in ALK-rearranged NSCLC patients included up to 350 patients (12, 13). In most recent retrospective clinical practice studies, the number of patients evaluated was around 100 (14-20). Several studies on tumor markers in ALK-rearranged NSCLC patients have been reported (21-24). Many of these reports examined whether tumor markers could be useful for diagnosis (21-24). Also, the number of ALK-rearranged NSCLC patients studied was

Table IV. *Factors associated with multiple metastatic sites in 113 ALK-rearranged NSCLC patients.*

Factors	Exp.	95% Confidence interval	*p*-Value
Age, less than 65, 65 years or more	0.397	0.146-1.080	0.0704
Gender, M/F	0.975	0.330-2.881	0.9637
Performance status			
0-1/2 or more	1.607	0.332-8.034	0.5632
Smoking habit			
Never/past or current	2.065	0.696-6.130	0.1914
Pleural fluid			
Absent/present	3.244	1.110-9.479	0.0314
CEA			
≤10 ng/ml/≥10 n/ml	1.959	0.715-5.366	0.1907
CYFRA			
≤10 ng/ml/≥10 n/ml	8.050	1.746-37.127	0.0075

ALK: Anaplastic lymphoma kinase; NSCLC: non-small cell lung cancer; Exp: exponential function; CEA: carcinoembryonic antigen; CYFRA: cytokeratin 19 fragment.

Table V. *Unfavorable prognostic factors 113 ALK-rearranged NSCLC patients.*

Factors	Logrank test *p*-Value	Cox's proportional hazard model		
		Exp	95% Confidence interval	*p*-Value
Performance status				
0-1/2 or more	0.0234	1.706	0.637-4.570	0.2880
Smoking habit				
Never/past or current	0.0134	1.674	0.829-3.380	0.1507
Surgical resection				
Absent/present	0.0003	3.445	1.362-8.719	0.0090
CEA				
≤10 ng/ml/≥10 n/ml	0.0301	1.431	0.704-2.908	0.3221

ALK: Anaplastic lymphoma kinase; NSCLC: non-small cell lung cancer; Exp: exponential function; CEA: carcinoembryonic antigen; CYFRA: cytokeratin 19 fragment.

low in many reports, up to 54 patients (21-24). In all NSCLC patients without considering gene mutations, there have been reports examining the significance of CEA and CYFRA in distant metastasis and prognosis. To our knowledge, however, there have been no reports investigating the association of serum levels of CEA and CYFRA with metastasis and prognosis in ALK-rearranged NSCLC patients. This was the first study to include more than 100 ALK-rearranged NSCLC patients. Regarding prognostic factors, several researchers are conducting studies in ALK-rearranged NSCLC patients (7, 25-27). Among them, there was an interesting report from the research group of Jin *et al*. (7). According to their report, liver and bone invasions were significant unfavorable prognostic factors in ALK-rearranged NSCLC patients (7). The results of this study were so interesting that we examined the relationship between liver and bone invasion and elevated serum levels of tumor markers in our patients. In this study, we considered the cutoff values of CEA and CYFRA cutoff values of CEA and CYFRA as 10 ng/ml, as an easy-to-use measurement value in clinical practice. In the logistic regression analysis, the following three points became clear. First, patients with higher levels of both CEA and CYFRA were more likely to have bone metastases. Second, the higher-CYFRA group was more likely to have liver metastases. Third, the higher-CYFRA group was more likely to have two or more metastatic sites. With regard to prognosis as shown by uni- and multivariate analysis, the following two points were revealed: First, 'poorer PS (2 or more)', 'positive smoking habit', 'no surgical resection', and 'elevated CEA levels ≥10 ng/ml' were unfavorable prognostic factors. Second, in Cox proportional hazards model analysis, however, none of these factors except for 'no surgical resection' were statistically significant unfavorable prognostic factor. These results were considered clinically understandable. In other words, elevated serum CEA levels were interpreted as a result that suggested some clinical significance, but not a powerful indicator of prognosis.

There were several previous studies on the association between elevated serum levels of these markers and distant organ metastases (28-30). According to these reports, elevated levels of these markers might be considered an early warning sign in patients needing accurate imaging, as they are at higher risk of bone metastasis (28), liver metastasis (29), and several-organ metastases (30). Molecular biology studies show that CEA has a mechanism for distant organ metastasis *via* adhesive molecules (31). Sertić Milić and colleagues reported that the presence of soluble cytokeratin fragments in blood shed suggests a deviation from the primary site of the tumor (32). The results of these clinical and molecular biological studies and our results suggested that elevated serum levels of these markers could contribute to early detection of several-organ metastases including bone and liver metastases with careful imaging studies. This population-based, multi-institutional study covering a single prefecture has several limitations. Firstly, it was a retrospective study with patients from miscellaneous backgrounds. Secondly, the methods for examining ALK-fusion gene mutations were not unified. Thirdly, the limited number of patients and the short period of investigation were also limitations. Fourthly, there have been several measurement kits in CEA and CYFRA, and these kits were used at different facilities. Therefore, the cutoff values of these markers had to be determined with priority on the

usefulness in clinical practice. Fifth, the prognosis of ALK-rearranged NSCLC patients studied was good, and there were few patients who died during the study period, therefore a detailed prognosis could not be examined. ALK-rearranged NSCLC is a rare entity of NSCLC. Therefore, there may be "facts" regarding the treatment that cannot be grasped by clinical trials alone.

We do believe it is worthwhile to accumulate, collect and disclose clinical practice data to make these facts clear and we conducted this research based on this concept. ALK-rearranged NSCLC patients who had elevated serum CEA and CYFRA at diagnosis (in this study serum levels ≥10 ng/m) may have had bone, liver metastases, or distant metastases to several organs. This information may be useful for clinical practice, but verification in more patients is required.

Conflicts of Interest

The Authors do not have any conflicts of interest to disclose regarding this study.

Authors' Contributions

TN, TE, HS and NH designed the study. TN, TE, HY, KO, YY, KS, HY, KH, SO, HS, YY, TT, KS, RK, KK, HI, HW, TS, YF, SH, HN, and TY collected the data. TN, TE, SO, and HS analyzed the data and prepared the manuscript. All Authors approved the final version of the article.

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