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Predictive and Prognostic Value of CYFRA 21-1 for Advanced Non-small Cell Lung Cancer Treated with EGFR-TKIs

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Abstract. *Background: Tyrosine kinase inhibitors (TKIs) directed against epidermal growth factor receptor (EGFR) are important in the treatment of non-small cell lung cancer (NSCLC), especially those harboring EGFR mutations. But little is known regarding the clinical value of serum tumor marker levels measured prior to treatment. Patients and Methods: We retrospectively reviewed 95 patients with advanced NSCLC treated with EGFR-TKIs, and inspected the relationship between serum tumor marker levels and clinical outcome. Results: Forty-three patients with an elevated serum level of cytokeratin 19 fragment (CYFRA 21-1) had shorter progression-free (PFS) and overall (OS) survival than 52 patients with normal serum CYFRA 21-1 levels (99 vs. 123.5 days p=0.011; and 385 vs. 607 days, respectively, p=0.001). Regardless of EGFR mutation status, patients had shorter progression-free survival when serum CYFRA 21-1 was elevated. Conclusion: Serum CYFRA 21-1 level may be a predictive factor for patients with NSCLC treated with EGFR-TKIs, regardless of EGFR mutation status.*

Tyrosine kinase inhibitors (TKIs) directed against epidermal growth factor receptor (EGFR) are very effective against non-small cell lung cancer (NSCLC) harboring *EGFR* mutations (1-6). Erlotinib, one of the first-generation EGFR-TKIs, is a treatment for NSCLC without EGFR mutation (7). Beyond EGFR mutation however, there are no clear predictive clinical markers for the therapeutic effect of EGFR-TKIs.

Meta-analysis has demonstrated that an elevated serum level of cytokeratin 19 fragment (CYFRA 21-1) may be associated with poor prognosis in patients with NSCLC (8). CYFRA 21-1 is usually used as a tumor marker in lung cancer, especially in squamous cell tumors (9). It has also been reported that an increase in pretreatment serum level of CYFRA 21-1 and carcinoembryonic antigen (CEA) are associated with poor outcome in patients with NSCLC treated with erlotinib (10). There is little information, however, about the predictive value of serum tumor markers for patients with advanced NSCLC treated with EGFR-TKIs. The present study aimed to examine the predictive and prognostic value of pretreatment serum CYFRA 21-1 and CEA levels in patients with advanced NSCLC treated with first- and second-generation TKIs (gefitinib, erlotinib and afatinib).

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Key Words: Cytokeratin 19 fragment, tumor marker, EGFR-TKI, non-small-cell lung cancer.

Patients and Methods

We retrospectively screened cases of advanced NSCLC treated with EGFR-TKI therapy at Nagoya City University Hospital (Japan) between June 2006 and August 2016. All patients had stage IIIB or IV disease. Patients received gefitinib (250 mg/day), erlotinib (150 mg/day), or afatinib (40 mg/day) until detection of progressive disease (PD) or intolerable toxicity. Dose interruption or reduction was permitted in cases of treatment-related toxicity. Our Institutional Ethics Committee approved the protocol of this study (No. 1301), and all medical data were anonymized.

Serum CYFRA 21-1 and CEA levels were measured prior to initial chemotherapy. The serum CEA level was measured by using a commercial electrochemiluminescence immunoassay on the HISCL-5000 system (Sysmex, Hyogo, Japan). Serum CYFRA 21-1 level was measured using a commercial electrochemiluminescence

immunoassay on a LUMIPULSE Presto II system (Fujirebio Inc., Tokyo, Japan). Measurements were performed at our hospital, using cut-off values of 3.5 ng/ml for CYFRA 21-1, and 5.0 ng/ml for CEA, which were the upper limit of normal values at our hospital.

Statistical analysis. Progression-free survival (PFS) was defined as the time from the first day of TKI treatment until the date of disease progression, death, or last follow-up. Overall survival (OS) was defined as the time from the first day of TKI treatment until the date of death or last follow-up. PFS and OS were analyzed using the Kaplan–Meier method and compared using the log-rank test, with $p<0.05$ considered statistically significant. Multivariate analysis using a Cox proportional hazards model was performed to identify associations between clinical characteristics and survival, using a probability of $p=0.10$ as the threshold for adding a covariable to or removing it from the model, with $p<0.05$ again considered significant. All statistical analyses were performed with EZR (Saitama Medical center, Jichi Medical University, Saitama, Japan), which is a graphical use interface for R (the R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics (11).

Results

The clinicopathological characteristics of the 95 patients are shown in Table I. All patients had a performance status of 0 or 1. Fifty-four patients carried an *EGFR* mutation (29 had exon 19 deletion, 23 had exon 21 L858R, and two had exon 21 L861Q), 34 patients had wild-type *EGFR*, and in seven patients the *EGFR* mutation status was not evaluated. Forty-three patients had an elevated serum level of CYFRA 21-1 (>3.5 ng/ml) prior to the start of EGFR-TKI treatment, and 69 patients had an elevated serum level of CEA (>5.0 ng/ml). Both PFS and OS were significantly shorter in the group with an elevated level of CYFRA 21-1 than those with normal serum levels (99 *vs*. 123.5 days, $p=0.011$; and 385 *vs*. 607 days, $p=0.001$, respectively; Figure 1A and B). In contrast, there was no significant difference in PFS or OS between those who had elevated or normal CEA level (124 *vs*. 97 days, $p=0.757$; and 542 *vs*. 357 days, $p=0.059$, respectively; Figure 1C and D). In univariate analysis, CYFRA 21-1 level and *EGFR* mutation were significant factors for PFS, while CYFRA 21-1 level, *EGFR* mutation, and tumor pathology were significant factors for OS (Table II). In multivariate analysis, CYFRA 21-1 and *EGFR* mutation were significant factors for both PFS [hazard ratio (HR)=2.17, $p<0.001$; HR=2.92, $p=0.001$, respectively; Table III] and OS (HR=2.29, $p=0.001$; and HR=4.72, $p<0.001$, respectively; Table IV).

In patients with *EGFR* mutation, PFS and OS were both found to be significantly shorter in those with elevated CYFRA 21-1 than those with normal levels (168 *vs*. 244.5 days, $p=0.032$; and 471 *vs*. 1023 days, $p<0.001$, respectively; Figure 2A and B). On the other hand, in patients with wild-type/unknown *EGFR* status, there was again a significantly shorter PFS but only a trend for shorter OS in those with elevated CYFRA 21-1 compared to those with normal levels (30 *vs*. 53.5 days, $p=0.027$; and 106 *vs*. 252.5 days, $p=0.115$, respectively; Figure 2C and D). There was no association between CEA and length of PFS or OS in *EGFR* mutation-positive patients (193 *vs*. 362.5 days, $p=0.297$; and 767 *vs*. 1314 days, $p=0.951$, respectively), nor in those with wild-type/unknown *EGFR* status (48 *vs*. 45.5 days, $p=0.66$; and 194 *vs*. 285 days, $p=0.827$, respectively).

Table I. *Patient characteristics (n=95).*

Characteristic	Value
Median age (range), years	66 (43-88)
Gender, n	
Male	44
Female	51
Smoking history, n	
Current or former smoker	45
Never smoker	50
Disease stage, n	
IIB	6
IV	89
Pathology, n	
Squamous cell carcinoma	12
Non-squamous cell carcinoma	83
Adenocarcinoma	73
Large cell carcinoma	1
Non-small cell carcinoma	9
EGFR mutation status, n	
Mutated	54
Ex19 Del	29
Ex21 L858R	23
Ex21 L861Q	2
Wild-type/unknown	41
Wild-type	34
Unknown	7
No. of prior treatment s, n	
0	37
1	22
2	12
More	24
Type of TKI, n	
Gefitinib	38
Erlotinib	55
Afatinib	2
Median CEA (range), ng/ml	15.6 (1.1-4609)
Median CYFRA 21-1 (range), ng/ml	3.2 (0.6-83.6)

EGFR, Epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment.

Discussion

In this retrospective study, EGFR-TKI-treated patients with an elevated serum level of CYFRA 21-1 had shorter PFS and OS times than those with normal levels. In contrast, an

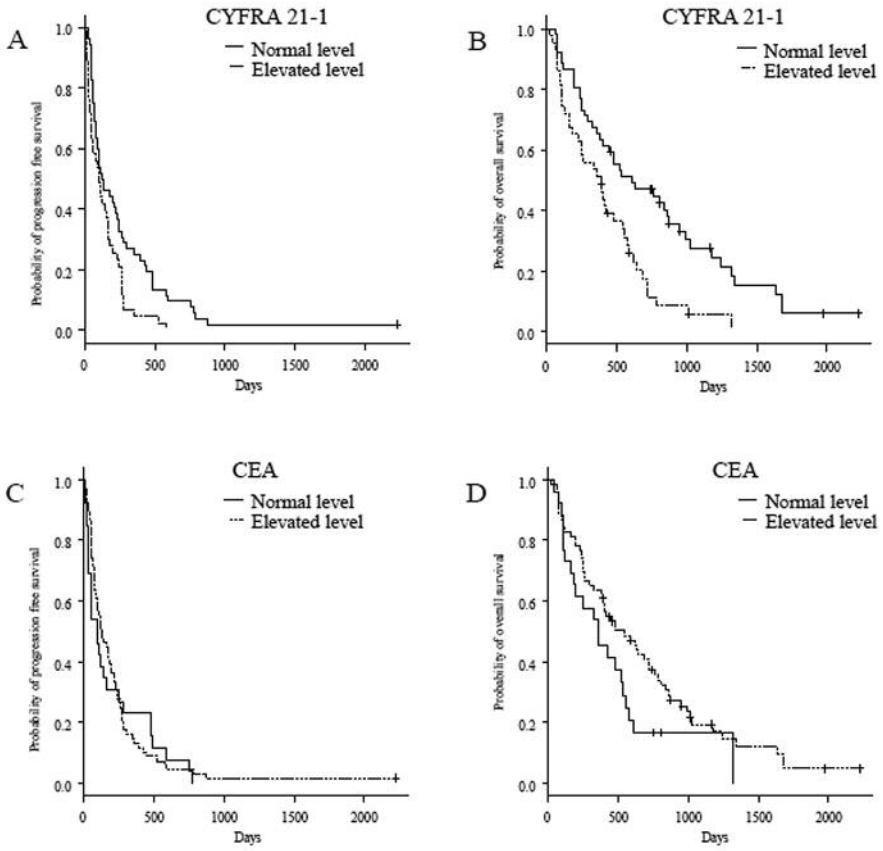

Figure 1. *Kaplan–Meier plots of progression-free (PFS) (A, C) and overall (OS) (B, D) survival according to serum cytokeratin 19 fragment (CYFRA 21-1) level (A, B) and carcinoembryonic antigen (CEA) level (C, D). The median PFS durations in the group with normal and that with elevated serum CYFRA 21-1 were 123.5 [95% confidence interval (CI)=83-226] days and 99 (95% CI=46-164) days, respectively (p=0.011) (A). The corresponding median OS durations were 607 (95% CI=374-873) days and 385 (95% CI=175-542) days, respectively (p=0.001) (B). The median PFS durations in the group with normal and that with elevated serum CEA were 97 (95% CI=33-164) days and 124 (95% CI=83-192) days, respectively (p=0.757) (C). The corresponding median OS durations were 357 (95% CI=158-526) days and 542 (95% CI=374-718) days, respectively (p=0.059) (D).*

elevated serum level of CEA was not found to influence the duration of PFS or OS. In multivariate analysis, CYFRA 21-1 and *EGFR* mutation were significant factors for both PFS and OS. It is known that EGFR-TKIs for patients with advanced NSCLC harboring *EGFR* mutation improve PFS compared with chemotherapy (1-6). In our subgroup analysis, an elevated level of serum CYFRA 21-1 was associated with shorter PFS and OS in *EGFR*-mutated patients, and shorter PFS in those with with wild-type/unknown *EGFR* status. Thus, CYFRA 21-1 was a predictive factor not only in EGFR mutation-positive patients treated with *EGFR*-TKIs, but also in those with wild-type/unknown *EGFR* status.

A previous meta-analysis reported that an elevated serum level of CYFRA 21-1 is associated with shorter OS in patients with advanced NSCLC (8), an observation that our findings are consistent with. It has been shown that CYFRA 21-1 is a predictive marker of PFS in patients with NSCLC with *EGFR* mutation who were treated with EGFR-TKIs (12). An elevated serum level of CYFRA 21-1 has also been associated with poor PFS in patients with NSCLC treated with EGFR-TKIs (10). However in this study, because *EGFR* status was not considered, it was difficult to determine how much effect CYFRA 21-1 had on PFS and OS in patients with NSCLC with wild-type/unknown *EGFR* status. Our study showed that elevation of serum CYFRA 21-1 was also a negative predictive factor for PFS in patients with NSCLC with wild-type *EGFR* or unknown *EGFR* status. Since elevated CYFRA 21-1 has been associated with the presence of mediastinal lymph node metastases and poor performance status (9), this marker may reflect tumor volume. In contrast, serum CEA had no association with PFS or OS in our study. Not only tumor volume but also tumor characteristics may have an important role in determining patient survival.

Table II. *Univariate analysis of progression-free (PFS) and overall (OS) survival by log-rank test.*

Factor	Category	n	PFS		OS	
			Median (days)	*p*-Value	Median (days)	*p*-Value
Age	<75	69	105	0.626	471	0.668
	≥75	26	136.5		397	
Gender	Female	51	116	0.475	519	0.227
	Male	44	100.5		329.5	
Smoking history	Never	50	123.5	0.22	630	0.054
	Current or former	45	99		330	
Disease Stage	IIIB	6	184	0.283	366	0.291
	IV	89	105		471	
Pathology	Non -squamous	83	124	0.098	476	<0.001
	Squamous	12	74		145.5	
EGFR mutation status	Mutated	54	214.5	<0.001	777	<0.001
	Wild-type/unknown	41	46		228	
No. of prior treatments	0/1	59	174	<0.001	636	<0.001
	2/more	36	46		233.5	
CEA	≤5.0 ng/ml	26	97	0.757	357	0.059
	>5.0 ng/ml	69	124		542	
CYFRA 21-1	≤3.5 ng/ml	52	123.5	0.011	607	0.001
	>3.5 ng/ml	43	99		385	

EGFR, Epidermal growth factor receptor; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment.

Table III. *Multivariate analysis of progression-free survival by Cox proportional hazards regression.*

Factor	Category	HR (95% CI)	*p*-Value
Pathology	Non-squamous	1.00	0.169
	Squamous	0.61 (0.31-1.23)	
EGFR mutation status	Mutated	1.00	0.001
	Wild-type/unknown	2.92 (1.54-5.52)	
No. of prior treatment	0/1	1.00	0.12
	2/more	1.60 (0.88-2.91)	
CYFRA 21-1	≤3.5 ng/ml	1.00	<0.001
	>3.5 ng/ml	2.17 (1.38-3.40)	

HR, Hazard ratio; CI, confidence interval; *EGFR*, epidermal growth factor receptor; CYFRA 21-1, cytokeratin 19 fragment.

Table IV. *Multivariate analysis of overall survival by Cox proportional hazards regression.*

Factor	Category	HR (95% CI)	*p*-Value
Smoking history	Never	1.00	0.621
	Current or former	0.87 (0.51-1.49)	
Pathology	Non-squamous	1.00	0.679
	Squamous	1.18 (0.53-2.61)	
EGFR mutation status	Mutated	1.00	<0.001
	Wild-type/unknown	4.72 (2.09-10.66)	
No. of prior treatments	0/1	1.00	0.858
	2/more	1.07 (0.51-2.23)	
CYFRA 21-1	≤3.5 ng/ml	1.00	0.838
	>3.5 ng/ml	1.07 (0.57-1.99)	
Pathology	Non-squamous	1.00	0.001
	Squamous	2.29 (1.40-3.74)	

HR, Hazard ratio; CI, confidence interval; *EGFR*, epidermal growth factor; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment.

CYFRA 21-1 is a fragment of the cytokeratin 19 protein that forms part of the cytoskeleton of epithelial cells (9). Activated proteases in malignant epithelial cells increase cytokeratin degradation, with the consequent release of cytokeratin fragments, particularly fragment 19, into the blood (14). Cytokeratin 19 is thought to be expressed more widely in cases of poorly differentiated squamous cell cancer (15), where its serum levels have been shown to provide significantly greater sensitivity and specificity for the purpose of diagnosis than other histological markers (9). Due to the histological heterogeneity of NSCLC, it is often difficult to distinguish squamous from non-squamous cell cancer using small biopsies or cytological specimens (16). Pemetrexed, which has more efficacy in patients with non-squamous NSCLC, in combination with a platinum-derivative therapy, has been associated with shorter PFS and

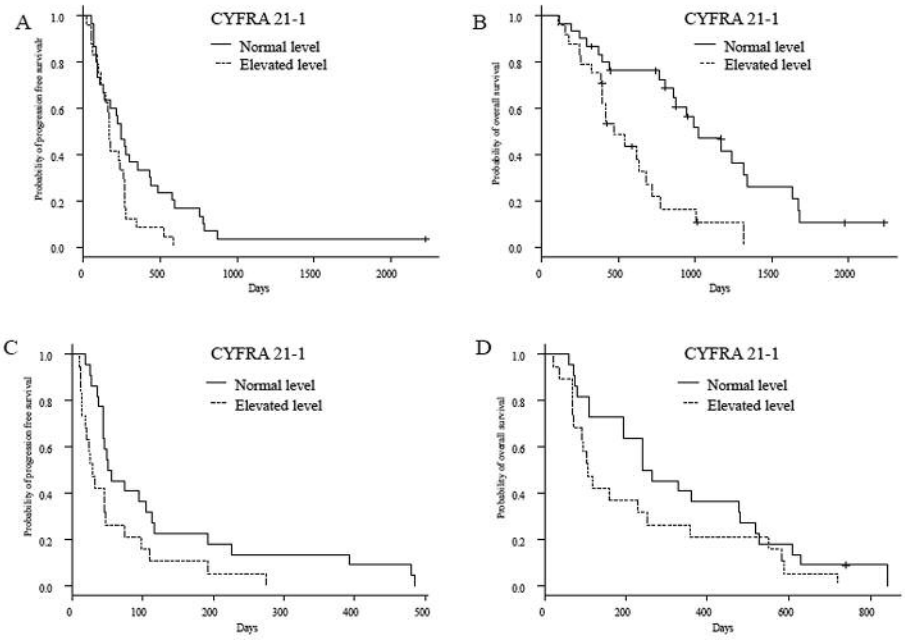

Figure 2. *Kaplan–Meier plots of progression-free (PFS) (A, C) and overall (OS) (B, D) survival according to serum level of cytokeratin 19 fragment (CYFRA 21-1) in the group with mutation of epidermal growth factor receptor (EGFR) (A, B) and that with wild-type/unknown EGFR status (C, D). For those with EGFR mutation, the median PFS durations in those with normal and those with elevated serum CYFRA 21-1 were 244.5 [95% confidence interval (CI)=131-428] days and 168 (95% CI=113-265) days, respectively (p=0.032) (A). The corresponding median OS durations were 1023 (95% CI=805-1340) days and 471 (95% CI=385-685) days, respectively (p<0.001) (B). The median PFS durations in the group with normal and that with elevated serum CYFRA 21-1 were 53.5 (95% CI=45-113) days and 30 (95% CI=15-48) days, respectively (p=0.027) (C). The median OS in the normal and elevated serum CYFRA 21-1 group were 252.5 (95% CI=109-481) days and 106 (95% CI=67-251) days, respectively (p=0.115) (D).*

OS, and worse disease control when the serum level of CYFRA 21-1 is elevated (17). Given this observation, higher levels of CYFRA 21-1 may be indicative of a squamous cell cancer component, explaining such differences in survival with this treatment approach.

It is important to highlight that this study has several limitations. Firstly, this was a single-institute retrospective study, so it is possible that selection bias may have affected our findings. Secondly, the molecular basis for how cytokeratin 19 plays a role in the growth and invasion of squamous cells in lung cancer is not yet understood.

In conclusion, elevated serum CYFRA 21-1 was associated with shorter PFS and OS of patients with NSCLC treated with EGFR-TKI. Importantly however, CYFRA 21-1 was associated with shorter PFS even in patients with wild-type or unknown *EGFR* status, who were poor responders to this therapy. Thus, the serum level of CYFRA 21-1 may be useful to help determine the appropriate use of EGFR-TKI therapy in all patients with NSCLC, regardless of *EGFR* mutation status. Further research into the molecular basis for this observation, along with prospective clinical studies, are now warranted to verify these findings.

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