CYFRA 21-1 as a Predictive Marker for Non-small Cell Lung Cancer Treated with Pemetrexed-based Chemotherapy

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Abstract. *Background: Pretreatment serum tumor marker levels predict outcome in non-small cell lung cancer (NSCLC). However, little is known about the clinical value of such measurements for patients treated with pemetrexed plus a platinum-derivative. Patients and Methods: We retrospectively screened 100 chemotherapy-naïve patients with advanced non-squamous NSCLC treated with pemetrexed plus a platinum-derivative in relation to the pretreatment level of cytokeratin 19 fragment (CYFRA 21-1) and carcinoembryonic antigen (CEA). Results: Sixty one patients with a high CYFRA 21-1 level had statistically shorter progression-free and overall survival than 39 patients with a normal CYFRA 21-1 level (median progression-free survival=127 days vs. 191 days, respectively; p=0.046; median overall survival=360 days vs. 781 days, respectively, p<0.001). Serum CEA level was not related to survival. Conclusion: Serum CYFRA 21-1 level is a predictive and prognostic marker in patients with NSCLC treated with pemetrexed plus a platinum-derivative.*

Pemetrexed is one of the most widely used cytotoxic agents, and is especially effective for non-squamous non-small cell lung cancer (non-SqNSCLC) (1). In first-line studies, pemetrexed plus a platinum-derivative was better tolerated

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and offered superior survival than conventional treatment (2, 3). Moreover, it was demonstrated that pemetrexed maintenance therapy prolonged overall survival (OS) and was well tolerated (4). Pemetrexed plus a platinum-derivative is recommended as standard therapy for advanced non-SqNSCLC. However, clinical predictive markers for the therapeutic effect of pemetrexed-based chemotherapy have not been established.

It has been shown in meta-analysis that cytokeratin 19 fragment (CYFRA 21-1), which is usually used as a tumor marker of lung cancer, is a prognostic indicator of NSCLC (5, 6). Fiala *et al.* reported that the pretreatment serum level of CYFRA 21-1 and other tumor markers predict shorter OS in patients treated with pemetrexed-based chemotherapy (7). However, little is known about the predictive value of serum tumor markers for advanced patients with non-SqNSCLC treated with pemetrexed plus a platinum-derivative.

The aim of the present study was to analyze the predictive and prognostic value of the pretreatment serum level of CYFRA 21-1 and of carcinoembryonic antigen (CEA) in patients with advanced-stage non-SqNSCLC treated with pemetrexed plus a platinum-derivative in a first-line setting.

Patients and Methods

In this retrospective cohort study, we screened cases of chemotherapy-naïve patients with advanced non-SqNSCLC treated with pemetrexed plus a platinum derivative at Nagoya City University Hospital and the Kainan Hospital (Japan) between 2010 and 2015. Approval for the study was obtained from our Institutional Ethics Committee (no. 1301), and routine medical data were analyzed under complete anonymity. All patients had locally advanced (stage IIIB) or metastasized (stage IV) non-SqNSCLC. Patients received pemetrexed at a dose of 500 mg/m^2 on day 1 and either cisplatin at a dose of 75 mg/m^2 on day 1 or carboplatin at the

area under the concentration-time curve (AUC) 6 on day 1 every 3 weeks. Induction treatment with platinum derivative was scheduled for up to 4-6 cycles, followed by maintenance pemetrexed monotherapy in patients with non-progressive disease. Addition of pemetrexed monotherapy was at the treating physician's discretion.

The serum CYFRA 21-1 and CEA levels were measured before the initial chemotherapy. The serum CYFRA 21-1 level was measured using a commercial electrochemiluminescence immunoassay on LUMIPULSE Presto II system (Fujirebio Inc., Tokyo, Japan) or Cobas 8000 system (Roche Diagnostics, Tokyo, Japan). The serum CEA level was measured using a commercial electrochemiluminescence immunoassay on HISCL-5000 system (Sysmex, Hyogo, Japan) or Cobas 8000 system (Roche Diagnostics). Measurements were made at laboratories in the two hospitals, 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 the two hospitals.

Statistical analysis. The response rate (RR) was defined as the best overall response according to Response Evaluation Criteria In Solid Tumours (RECIST) (8). The disease control rate (DCR) was defined as the percentage of patients who did not have progressive disease. Survival was estimated using the Kaplan–Meier method. Progression-free survival (PFS) was measured from the date of the first course of initial chemotherapy to the date of first disease progression, of death, or of the last follow-up. OS was measured from the date of the first course of initial chemotherapy to the date of death or of the last follow-up. Differences in RR and DCR between groups were evaluated using Fisher's exact test. A log-rank test was performed to evaluate the significance of differences in PFS and OS between the groups. As a level of statistical significance, a value of $p=0.05$ was used. Univariate analyses were carried out by means of log-rank tests. Multivariate analysis using a Cox proportional hazards model was used to establish the association between the clinical variables and survivals, and $p=0.10$ was the significance level used for adding or deleting a covariable from the model. As the level of statistical significance in the model, $p=0.05$ was used. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user 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 (9).

Results

The study included 100 patients whose characteristics are shown in Table I. The median age was 67 years (range= 40-81 years). Thirty-three patients (33%) were female, and 25 (25%) were never smokers. Most patients had adenocarcinoma, and most patients had stage IV disease. Ninety-eight patients (98%) had a good performance status (PS) (0 or 1). Twenty-one patients (21%) had epidermal growth factor receptor (*EGFR*) mutation, and four patients (4%) had anaplastic lymphoma kinase (*ALK*) translocation; no patient had both gene mutations.

Sixty-one (61%) patients had a serum CYFRA 21-1 level that was above the normal upper limit of 3.5 ng/ml before the initial chemotherapy. The serum CEA level was elevated (>5.0 ng/ml) in 89 patients before the initial chemotherapy.

Table I. *Patient characteristics (n=100).*

Characteristic	Value
Age (years)	
Median	67
Range	40-81
Gender, n	
Male	67
Female	33
Smoking status, n	
Current or former smoker	75
Never smoked	25
Histological type, n	
Adenocarcinoma	97
Large cell carcinoma	3
Disease stage, n	
IIIB	2
IV	98
ECOG PS, n	
0-1	97
2	3
EGFR mutation status, n	
Positive	21
Wild-type	79
ALK translocation status, n	
Positive	4
Negative	96
No. of prior systemic regimens, n	
0	88
1	12
Type of prior systemic therapy, n	
Platinum-based therapy	0
EGFR tyrosine kinase inhibitor	12
ALK inhibitor	0
Regimen, n	
CDDP+PEM	35
CBDCA+PEM	65
No. of induction chemotherapy cycles	
Median	4
Range	1-6
Pemetrexed maintenance therapy, n	
Yes	39
No	61
CEA (ng/ml)	
Median	14.1
Range	0.7-5235.5
CYFRA 21-1 (ng/ml)	
Median	5.0
Range	0.5-684.0

ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth facter receptor; *ALK*, anaplastic lymphoma kinase; CDDP, cisplatin; CBDCA, carboplatin; PEM, pemetrexed; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment.

At the time of analysis, the median follow-up was 426 days (range=24-1374 days). There was no difference in RR between patients with a high and those with a normal CYFRA 21-1 level (36.1% *vs*. 33.3%, *p*=0.8323). Patients
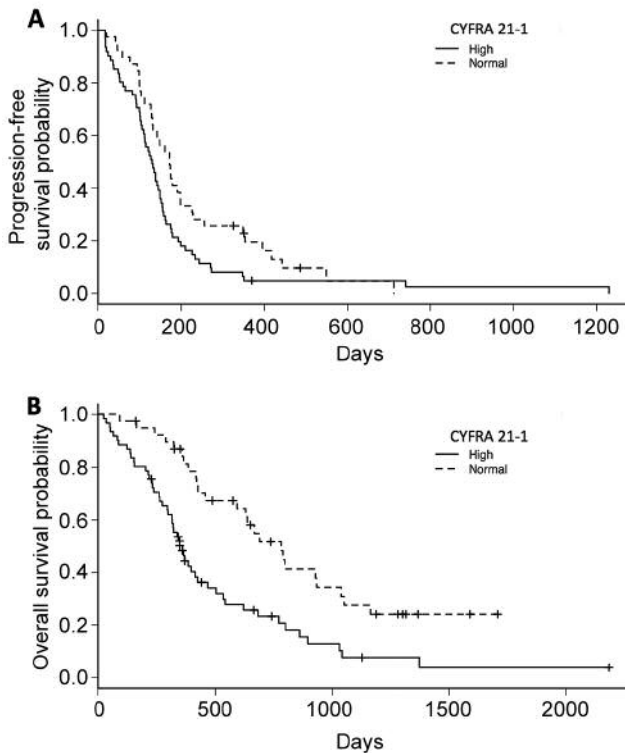
Figure 1. *Kaplan–Meier survival curves of progression-free (A) and overall (B) survival according to serum cytokeratin 19 fragment (CYFRA 21-1) level. The median PFS in patients with high and normal serum CYFRA 21-1 levels was 127 days [95% confidence interval (CI)=103-152 days] and 191 days (95% CI=103-349 days), respectively (p=0.046). The median OS in patients with high and normal levels of CYFRA 21-1 was 360 days (95% CI=299-425 days) and 781 days (95% CI=457-1038 days), respectively (p<0.001).*

Table II. *Univariate analysis of survival by log-rank test.*

Factor	n	Median PFS (days)	*p*-Value	Median OS (days)	*p*-Value
Age					
≥75 Years	87	134	0.201	420	0.380
<75 Years	13	172		503	
Gender					
Male	67	137	0.894	426	0.186
Female	33	144		468	
Smoking status					
Never smoked	25	137	0.79	683	0.017
Current or former smoker	75	141		420	
Driver mutation*					
Yes	25	148	0.204	799	0.018
No	75	137		414	
Pretreatment					
Yes	12	148	0.503	347	0.065
No	88	134		468	
Regimen					
CDDP+PEM	35	128	0.528	544	0.754
CBDCA+PEM	65	149		385	
CYFRA 21-1					
≤3.5 ng/ml	39	172	0.035	781	<0.001
>3.5 ng/ml	61	129		360	
CEA					
≤5.0 ng/ml	25	111	0.963	365	0.664
>5.0 ng/ml	75	149		468	

PFS, Progression-free survival; OS, overall survival; CDDP, cisplatin; PEM, pemetrexed; CBDCA, carboplatin; CYFRA 21-1, cytokeratin 19 fragment; CEA, carcinoembryonic antigen. *Epidermal growth factor receptor mutation or anaplastic lymphoma kinase translocation.

with a high CYFRA 21-1 level tended to have a lower DCR than patients with a normal CYFRA 21-1 level (77% *vs.* 92.3%, *p*=0.0587). Patients with a high level of CYFRA 21-1 also experienced statistically shorter PFS compared with those with a normal level (log-rank *p*=0.035), with a median PFS of 129 days [95% confidence interval (CI)=103-152 days] *vs.* 172 days (95% CI=103-349 days) respectively (*p*=0.046) (Figure 1A). The median duration of PFS of patients with high and normal serum CEA levels was 149 days (95% CI=129-172 days) and 111 days (95% CI=99-158 days), respectively (*p*=0.963).

To determine potential predictive factors among clinical characteristics including the serum CYFRA 21-1 and CEA level in the patients with non-SqNSCLC treated with pemetrexed and platinum derivatives, we performed a univariate analysis on the clinical variables listed in Table II. Stage, histological type and performance status were excluded from the analysis because they were too one-sided. A high level of CYFRA 21-1 was statistically the only factor

with predictive efficacy (*p*=0.035). As shown in Figure 1B, the median duration of OS for patients with high and normal levels of CYFRA 21-1 was 360 days (95% CI=299-425 days) and 781 days (95% CI=457-1038 days), respectively (*p*<0.001). The median duration of OS of patients with high and normal serum CEA levels was 468 days (95% CI=370-668 days) and 365 days (95% CI=290-771 days), respectively (*p*=0.664).

Potential prognostic factors were subjected to a univariate survival analysis (Table II). Never-smoked status (*p*=0.017), presence of driver mutations (*EGFR* mutation or *ALK* translocation), and a normal serum CYFRA 21-1 level were associated with better OS. To determine independent prognostic factors, we performed multivariate analyses (Table III). Lack of driver mutation (*p*<0.022), no pretreatment (*p*=0.008) and a normal serum CYFRA 21-1 level (*p*=0.007) were statistically independent prognostic factors for better OS. In the population without driver mutations, the median duration of OS in patients with a high

Table III. *Multivariate analysis of overall survival by a Cox proportional hazards model.*

Factor	HR (95% CI)	*p*-Value
Smoking status		
Never smoked	1.00	
Current or former smoker	1.30 (0.69-2.48)	0.418
Driver mutation*		
Yes	1.00	
No	2.95 (1.17-7.43)	0.022
Pretreatment		
Yes	1.00	
No	0.23 (0.08-0.68)	0.008
CYFRA 21-1		
≤3.5 ng/ml	1.00	
>3.5 ng/ml	2.01 (1.21-3.33)	0.007

HR, Hazard ratio; CI, confidence interval; CYFRA 21-1, cytokeratin 19 fragment. *Epidermal growth factor receptor mutation or anaplastic lymphoma kinase translocation.

(n=50) and those with normal (n=25) levels of CYFRA 21-1 was 349 days (95% CI=263-425 days) and 613 days (95% CI=418-934 days), respectively (*p*=0.009).

Discussion

This study showed that a high level of serum CYFRA 21-1 before initial chemotherapy was related to shorter PFS and OS, and a worse DCR with pemetrexed plus a platinum-derivative therapy in patients with advanced non-SqNSCLC. The duration of OS correlated with serum CYFRA 21-1 levels in multivariate analysis, whether patients had driver mutations or not, and whether patients took pretreatment of EGFR-TKI or not. Considering the survival benefit of EGFR-TKI for patients with *EGFR* mutation, the serum CYFRA 21-1 level was the only factor by which the effectiveness of chemotherapy could be predicted in this study.

In previous meta-analysis, high pretreatment serum CYFRA 21-1 levels were related to shorter OS in patients with advanced-stage NSCLC, and the findings of the present study are consistent with (5, 6). However, regarding PFS, only one study has reported that the serum CYFRA 21-1 level predicted the duration of PFS in patients treated with carboplatin plus gemcitabine-treated for advanced NSCLC (10). Thus, it is not clear whether CYFRA 21-1 is only a factor predictive of antimetabolite-based chemotherapies, or whether it is also a predictive of various other regimens (*e.g.* taxanes or topoisomerase inhibitors). On the other hand, in patients operated on for early-stage NSCLC, a high preoperative serum CYFRA 21-1 level was

associated with poor survival (5, 6). Considering that outcome, a high CYFRA level might lead to a worse outcome through tumor burden. Similarly, it is considered that a high tumor load leads to a worse outcome in patients with advanced-stage disease. However, the serum CEA level was not found to be related to survival in this study. These data suggest that not only tumor burden, but also tumor properties influence outcome.

Histological heterogeneity in lung cancer might also have an effect on outcome (11, 12). Pemetrexed treatment provides a better survival advantage for patients with tumors with a non-squamous histology than for those with a squamous histology (1). The fact that CYFRA 21-1 is a specific marker for squamous cell carcinoma (13, 14) suggests that elevated levels of serum CYFRA 21-1 indicate the presence of squamous cell components. For most patients with advanced NSCLC, histological classification of the tumor is based on a small biopsy or on cytological specimens. Pathogenic diagnosis of NSCLC based on a small biopsy has been shown to involve many false-positives/-negatives (15). In similar studies, it was reported that CYFRA 21-1 predicted the efficacy of EGFR-TKI treatment in patients with NSCLC harboring an *EGFR* mutation (16), and that patients whose tumors harbored an *EGFR* mutation with squamous histology had a shorter PFS (17). These data emphasize the importance of considering tumor heterogeneity in advanced lung cancer.

Our study has certain limitations. Firstly, not all patients with non-progressive disease in induction therapy underwent pemetrexed maintenance monotherapy. This may have influenced PFS and OS. Secondly, we adopted the upper limit of normal values at the two hospitals as the cut-off point for the serum tumor markers. However, these levels were decided from the viewpoint of diagnostic value. Therefore there may be better cut-off points for marker levels when considering predictive or prognostic value. Furthermore, it is expected that more effective therapeutic drugs for patients with high CYFRA 21-1 levels will be identified in the future. Other strategies, such as the addition of an anti-angiogenic inhibitor to pemetrexed-based chemotherapy or the use of an immunocheckpoint inhibitor, are considered worthy of consideration.

In conclusion, our study suggests that a high serum level of CYFRA 21-1 before treatment is a predictive and prognostic factor of therapy with pemetrexed combined with a platinum derivative therapy in patients with non-SqNSCLC.

Acknowledgements

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