Optimal Cutoff Points of CYFRA21-1 for Survival Prediction in Non-small Cell Lung Cancer Patients Based on Running Statistical Analysis

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Abstract. *Purpose: To determine pretreatment serum CYFRA21-1 levels as indicators of poor prognosis in patients with non-small cell lung cancer (NSCLC). Methods: 1,202 consecutive patients, diagnosed pathologically with NSCLC from January 1999 to December 2009, were entered in this study. To obtain optimal cutoff points of CYFRA21-1 for these endpoints, a running log-rank statistical method was applied. Results: The cutoff level for the maximum log-rank statistical value of one-year survival in patients with NSCLC was 18.0 ng/ml. These results could be applied to patients with squamous cell carcinoma. In multivariate analysis, elevated (>18.0 ng/ml) levels of CYFRA21-1 was confirmed as being an unfavourable prognostic factor. Conclusion: CYFRA21-1 assay has a clinical significance for identifying patients with poor prognosis among those with early and advanced NSCLC. Elevated serum CYFRA21-1 levels at the time of diagnosis may be a useful noninvasive marker for identifying the risk of early death from NSCLC.*

Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases, presenting as locally advanced in approximately 25-30% of cases and as metastatic disease in approximately 40-50% of cases. After radical treatment for seemingly localised disease, 20% of these patients develop an early distant relapse, probably due to systemic micro-

metastases that were present at the time of initial staging. One- and two-year survival rates are still used as the indices of survival for advanced NSCLC patients (1, 2).

Cytokeratin is a cytoskeletal structure expressed in epithelial cells, including bronchial epithelia (3). More than 20 subunits of cytokeratin are known and are expressed differently in several types of epithelia (4). Of these cytokeratins, cytokeratin 19 fragment (CYFRA21-1) levels in serum have already been evaluated as a useful tumour marker for non-small cell lung cancer (NSCLC) (5-18). Several studies have shown that elevated serum level of CYFRA21-1 is an independent prognostic factor of the risk of cancer death (8-18). The elevated serum levels of CYFRA21-1 could serve as a separate measure of biological aggressiveness and may have an important role in guiding treatment recommendations and in patient selection for clinical trials. However, regarding the use of CYFRA21-1 as a prognostic tool, neither the way of defining its cutoff points nor the use of multiple cutoff points for various survival conditions have been discussed in the published literature to date.

In evaluating the prognostic significance of elevated serum levels of CYFRA21-1, all the previous studies used manufacturer recommended cutoff levels of CYFRA21-1 for diagnosis of lung cancer (8-10, 12-18). Only one previous study analysed novel cutoff levels for poor prognosis (11). To calculate an optimal cutoff point, that study applied the receiver operation characteristic (ROC) method. This method, however, proved to be inapplicable because follow-up cases were not treated adequately in the survival analysis. In order to circumvent this weakness, a running log-rank statistic was calculated for each possible cutoff point on the basis of serum CYFRA21-1 level (19). The most optimal cutoff level was then determined as that corresponding to the maximum log-rank statistical value. As mentioned above, one- and two-year

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survival rates are still used as the index of the survival for advanced NSCLC patients (1, 2). This study evaluated the CYFRA21-1 level which attained the maximum log-rank statistical value of one-, two-, and three-year survival time.

Patients and Methods

Study population. A retrospective study of serum CYFRA21-1 levels was conducted in patients with NSCLC. 1.202 consecutive patients, diagnosed pathologically with NSCLC at the Divisions of Respiratory Medicine and Thoracic surgery, Tsukuba University Hospital and Division of Respiratory Medicine, Tsukuba Medical Center Hospital from January 1999 to December 2009, were entered in this study. Staging procedure was performed for all patients according to TNM classification (20) using chest computed tomography (CT), brain magnetic resonance imaging (MRI), bone scan as well as ultrasonograpy and/or CT of the abdomen. Peripheral venous blood samples collected from patients with NSCLC were used for the CYFRA21-1 assay. The samples were stored at -30°C until use. This study was approved by the Institutional Review Boards of the participating hospitals.

Measurement of CYFRA21-1 levels. Blood sampling was performed within the 1-month period preceding therapy. The serum CYFRA21-1 level of all blood samples was determined using a chemiluminescent enzyme immunoassay method (Lumipulse I CYFRA, Fujirebio Inc, Tokyo, Japan). According to the manufacturer, the upper limit of the normal CYFRA21-1 level is 3.5 ng/ml. The assay was performed by technicians who had no clinical information regarding the samples.

Statistical methods. To obtain optimal cutoff levels of CYFRA21-1, running log-rank statistics was applied (19). Running log-rank statistics produced for each cutoff point of serum level of CYFRA21-1 were plotted against survival and tested for statistical significance *via* permutations of the data. In brief, the patients were divided into two groups: patients with serum CYFRA21-1 levels more than the cutoff point and those with serum CYFRA21-1 levels equal to or less than the cutoff point. Log-rank statistical values between the groups were calculated for each possible cutoff point on the basis of serum CYFRA21-1 up to level that covered 90% of the patients by 1.0 ng/ml increments. The CYFRA21-1 level which attained the maximum log-rank statistical value of one-, two- and three-year survival time between the two groups was evaluated as an optimal cutoff point. All statistical analyses were performed using the SAS 9.1.3 for Windows and R (R-2.7.0) computer packages (Cary, North Carolina, USA). *p*-values less than 0.05 were considered to be statistically significant.

Results

Table I shows the basic characteristics of NSCLC patients. Of the 1,202 NSCLC patients, 906 were men. Median age was 68 years (range: 21-94 years). Among them were 280 stage IA-IB and 728 stage IIIB and IV. Histological types were: 733 patients with adenocarcinoma (AD), 399 with squamous cell carcinoma (SQ), 60 with large cell carcinoma, and 10 with other types as defined by the WHO classification system.

Table I. *Characteristics of patients with non-small cell lung cancer.*

No. of patients	1.202
Age (year)	median: 68 range: 21-94
Gender	
Male	906 (75.4%)
Female	296 (24.6%)
Performance status	
0-1	963 (80.2%)
2-4	238 (19.8%)
Histology	
Adenocarcinoma	733 (61.0%)
Squamous cell carcinoma	399 (33.2%)
Large cell carcinoma	60 (5.0%)
Other	10 (0.8%)
Clinical stage	
IA-B	280 (23.3%)
IIA-B	58 (4.8%)
IIIA	136 (11.3%)
IIIB	285 (27.7%)
IV	443 (36.9%)

The serum CYFRA21-1 levels differed significantly according to clinical stage (Kruskal-Wallis test, *p*=0.0001). Maximum serum level of CYFRA21-1 was 562.1 ng/ml, and CYFRA21-1 level up to 20.0 ng/ml covered 94.2% of all 1.202 NSCLC patients.

As shown in Figure 1, the maximum log-rank statistical value of one-year survival in patients with NSCLC was 0.455, which gave an optimal cutoff point for CYFRA21-1 level of 18.0 ng/ml. The maximum log-rank statistical value of two- and three-year survival in patients with NSCLC was 0.485 and 0.489, which gave optimal cutoff points of 3.0 ng/ml and 1.0 ng/ml, respectively. In patients with AD, the maximum log-rank statistical value of one-year survival was 0.531 which gave an optimal cutoff point of 5.0 ng/ml. The maximum log-rank statistical value of two- and three-year survival in patients with AD was 0.524 and 0.504 which gave optimal cutoff points of 5.0 ng/ml and 5.0 ng/ml, respectively. In patients with SQ, the maximum log-rank statistical value of one-year survival was 0.476 which gave an optimal cutoff point of 18.0 ng/ml (Figure 2). The maximum log-rank statistical value of two- and three-year survival in patients with SQ was 0.665 and 0.723 which gave optimal cutoff points 1.0 ng/ml and 1.0 ng/ml, respectively.

Confirmation of the above results was performed using uni- and multivariate analysis. Elevated CYFRA21-1 levels (>18.0 ng/ml) in NSCLC patients showed statistical significance in survival (*p*=0.001, for uni- and multi-variate analysis). Elevated CYFRA21-1 levels (>18.0 ng/ml) in patients with SQ also conferred a poor prognosis (*p*=0.001 for uni- and multivariate analysis; data not shown).

Table II. *Uni- and multivariate analyses of prognostic factors in patients with non-small cell lung cancer.*

Factor	Uni-variate analysis (log-rank test)	Multivariate analysis (Cox's proportional hazard model)		
	p-Value	Hazard ratio	95%CI	*p*-Value
Stage (IA-IIIA, IIIB-IV)	0.001	2.72	1.48-5.01	0.001
Smoking (smoker, non-smoker)	0.004	0.20	0.07-0.60	0.004
Performance status (0-1, 2-4)	0.001	2.43	1.43-4.14	0.001
CYFRA21-1 (≤18.0, >18.0 ng/ml)	0.001	2.02	1.14-3.58	0.001
Treatment (surgery, other)	0.001	1.59	0.81-3.12	0.175

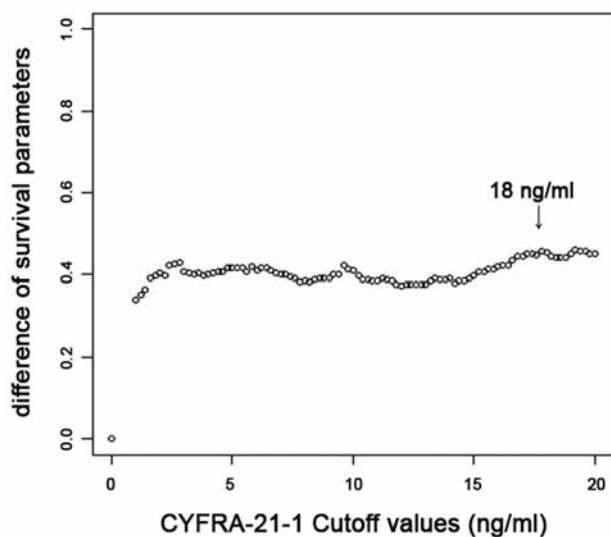95% CI: 95% Confidence interval.

Figure 1. *Maximum log-rank statistical value of 1-year survival in patients with non-small cell lung cancer.*
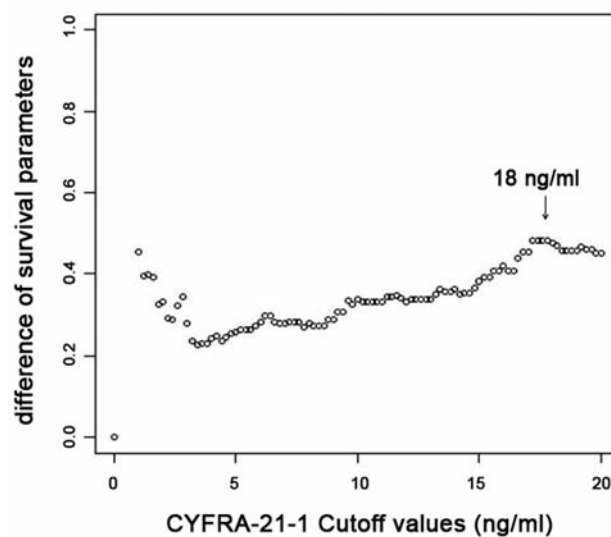

Figure 2. *Maximum log-rank statistical value of 1-year survival in patients with squamous cell carcinoma.*

Discussion

CYFRA21-1 is an immunometric assay measuring fragments of cytokeratin 19 and has been suggested to correlate with tumour burden and disease progression in NSCLC 12, 16). Prognostic significance of CYFRA21-1 has been discussed with conflicting results (5, 7, 11, 13, 14, 16-18). Most previous studies showed a prognostic significance for CYFRA21-1 (11, 13, 14, 16-18), while some studies did not (5, 7). In most of them, the prognostic significance of CYFRA21-1 was evaluated only for patients with resectable or resected NSCLC (9, 11, 18), or only for those with advanced disease (14, 17). In four large previous studies including more than one hundred NSCLC patients and which evaluated the prognostic significance of CYFRA21-1, the proportion of patients with early (stage IA-B) and advanced disease (stage IIIB-IV) was 8.7%-15.6%, and 62.7%-76.0%, respectively (6, 8, 10). However it would be preferable if the prognostic significance were evaluated in a large NSCLC patient population including both early and advanced clinical stages. Therefore, the present study evaluated serum CYFRA21-1 levels in NSCLC patients with operable stage as well as those with advanced or metastatic diseases. Above all, the major question dealt with in this study was whether patients who have elevated pretreatment serum CYFRA21-1 levels will have an unfavourable prognosis. If so, the next issue of this study was to determine pretreatment serum CYFRA21-1 levels that can be used as indicators of poor prognosis. Although currently the best predictor of outcome for patients with NSCLC is the TNM classification, the prognosis of patients within each stage of disease may vary considerably (1, 2). Early metastasis is a well-known feature of poor prognosis in potentially resectable NSCLC (21). The dissemination of malignant cells to distant organs *via* lymph nodes or blood vessels in NSCLCs can occur at an early stage of primary tumour growth and a significant number of early

staged NSCLC patients die of aggressive progression of the disease (21). In evaluating the prognostic significance of CYFRA21-1, most previous studies used the cutoff level that was recommended by the manufacturer of the assay kit, based on the specificity of the marker as the diagnostic tool to distinguish between lung cancer and normal individuals (8, 13-15). For example, Barlési et al. used a cutoff level of 3.5 ng/ml (14), and both Muley et al. and Merle et al. used a cutoff of 3.3 ng/ml (13, 15). In a large prospective study in 621 patients by Pujol et al., the cutoff level was 3.6 ng/ml (10). Only Reinmuth et al. determined a cutoff CYFRA21-1 level using classification and regression tree survival analysis, resulting in a best predictive cutoff value of 3.57 ng/ml (11). The difference in the optimal CYFRA21-1 level for prognostic value between Reinmuth et al. (11) and the present study may be due to differences in the statistical analysis as well as the number of NSCLC patients and clinical stages of them evaluated. The analysis by Reinmuth et al. (11) included only 67 patients with completely resected NSCLC patients, and the proportion of stage IA-B and IIIB-IV cases were 85% and 0%, respectively. The present study included 1,202 patients, and the proportion of cases with early and advanced disease was 22.8% and 58.4%, respectively.

Recently, an optimal cutoff point for the diagnosis of lung cancer was determined using a ROC curve by choosing the value which attains the shortest length of (0, 1) point in the x-y plane, with the x axis representing the false positive proportion and the y axis representing the true positive proportion (22, 23). This method, however, does not cover the situation when censored data arise. To overcome this difficulty, running log-rank statistics (19) was applied in the present study. The value which attained the maximum statistic value between groups was naturally considered to be the best cutoff point for CYFRA2-1 levels. In this study, two notable results were found. Firstly, the maximum log-rank statistical value of one-year survival in NSCLC patients gave an optimal cutoff point of 18.0 ng/ml. The same cutoff level was also given by the maximum log-rank statistical value of one-year survival in SQ patients. Rather than the upper level for diagnosis, these findings could provide more important clinical information on identifying patients with poor prognosis. It seems reasonable that the optimal CYFRA21-1 point for poor prognosis, which was evaluated in this study, was higher than the manufacturer recommended cutoff value for diagnosis of NSCLC. Secondly, the cutoff levels for the maximum log-rank statistical value of two- and three-year survival in NSCLC, AD, and SQ patients were almost the same as the manufacturer recommended cutoff value for diagnosis. This result seemed to be somewhat unexpected; however, it may mean that if a patient had lung cancer with a higher CYFRA21-1 level than the manu-facturer recommended cutoff value, they would have a considerably unfavorable prognosis, irrespective of the histological type of NSCLC.

In spite of these significant findings, this study has limitations that need to be addressed before serum CYFRA21-1 can be used clinically at the time of diagnosis to predict subsequent mortality. Firstly, changes in serum CYFRA21-1 levels in serial measurement may be of great importance; however, this conclusion is only speculative, as there is no definite information on the serial measurements of CYFRA21-1 and changes in its level. Secondly, this study included NSCLC patients treated with various kinds of therapies. Thirdly, the development of these therapies may have influenced the prognosis of the patients. Therefore, these limitations may have affected the results of the present study.

In conclusion, elevated levels of CYFRA21-1 (>18.0 ng/ml) in NSCLC patients suggest a poor prognosis. In patients with SQ, CYFRA21-1 levels (>18.0 ng/ml) also predict poor prognosis. These findings suggest that CYFRA21-1 assay have a clinical significance for identifying patients with a poor prognosis among those with early and advanced NSCLC. In other words, elevated serum CYFRA21-1 levels at the time of diagnosis may be a useful noninvasive marker for identifying the risk of early death from NSCLC. A careful and large-sized clinical study may be necessary to corroborate these results.

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