Clinical Usefulness of CYFRA Assay in Diagnosing Lung Cancer: Measurement of Serum Cytokeratin Fragment

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We evaluated the diagnostic usefulness of measurement of the soluble cytokeratin 19 fragment, a new tumor marker, in 391 patients with lung cancer and in 424 patients with benign lung diseases. Serum concentrations of cytokeratin 19 fragment were measured by a sandwich ELISA (CYFRA). The cut-off value was defined as 3.5 ng/ml, which is associated with a specificity of 85% for benign lung diseases. CYFRA had a high sensitivity (57.5%) in all subjects with lung carcinoma, and had a higher sensitivity for squamous cell carcinoma (73.1%, n=141) than squamous cell carcinoma-related antigen (61.0%). CYFRA was associated with a relatively high sensitivity (42.1%) in early-stage squamous cell carcinoma (stage I, based on the classification of the Japan Lung Cancer Society), but the CYFRA titer was higher in advanced squamous cell carcinoma than in early-stage squamous cell carcinoma. Our findings suggest that CYFRA is potentially useful for diagnosis and monitoring of lung carcinoma, especially for squamous cell carcinoma.

Key words: Tumor marker — Cytokeratin fragment — Lung cancer

A number of tumor markers are used for the diagnosis and monitoring of pulmonary carcinoma, including carcinoembryonic antigen (CEA), squamous cell carcinoma-related antigen (SCC), neuronspecific enolase (NSE), and sialyl Le^x-i antigen (SLX). However, these markers have not been found to be adequate for definitely diagnosing malignancy, and are therefore used only as supplementary diagnostic methods. Because these markers have a low sensitivity in diagnosing early pulmonary carcinoma, combinations of several tumor markers have been investigated in an attempt to improve diagnosis.

We investigated the usefulness of CYFRA 21-1, a kit for measuring the serum concentration of the soluble cytokeratin 19 fragment (CYFRA), a new tumor marker. Cytokeratin, which is also called α-keratin, is a component of the cytoskeleton in epithelial cells.[1, 2]

MATERIALS AND METHODS

Subjects were out-patients or in-patients at some time during the period from January 1988 to February 1994 at any of the 7 participating facilities of the Japan CYFRA Research Group. We studied 391 subjects with histologically confirmed primary pulmonary carcinoma (Table I), and 424 subjects with benign pulmonary diseases (Table II). Patients with metastatic pulmonary carcinoma or a history of malignant tumors at sites other than the respiratory system, patients with two or more different tumors simultaneously and patients with pulmonary carcinoma who had undergone previous treatment were excluded. Thirty-six cases with pulmonary carcinoma were monitored before and after surgery, chemotherapy, or irradiation therapy.

Serum concentrations of CYFRA were determined with a 2-step sandwich ELISA kit (Enzymun-Test CYFRA 21-1, Boehringer-Mannheim GmbH, Mannheim, Germany).[1, 2] The average concentration (n=440) in healthy individuals was 1.29±0.04 ng/ml (mean± SEM), with no significant difference between males and females (1.29±0.06 vs. 1.28±0.06) or between smokers and non-smokers (1.10±0.06 vs. 1.22±0.06). CEA, SCC and NSE, which served as control tumor markers, were measured by enzyme immunoassay (EIA), radio-immunoassay (RIA) and EIA methods, respectively.

Data are represented as mean±SEM. The Bartlett test and the Kruskal-Wallis test (non-parametric method) were used to evaluate the significance of differences in this study.

Table I. Subjects with Pulmonary Carcinoma

Type	Stage						Total
	I	II	IIIA	IIIB	IV	Unknown	
Lung cancer	47	35	87	83	119	20	391
Non-small cell	45	32	82	78	100	0	337
Squamous cell	20	19	33	33	36	0	141
Adenocarcinoma	23	12	41	34	51	0	161
Large cell	2	1	8	11	13	0	35
Small cell	2	3	5	5	19	20	54

Table II. Subjects with Benign Lung Diseases

Disease	Number of patients
Pulmonary tuberculosis	85
COPD[a]	52
Sarcoidosis	42
Interstitial pneumonia	41
Pneumonia	40
Bronchial asthma	34
Bronchiectasis	26
Other	104
Total	424

a) COPD: Chronic obstructive pulmonary disease.

RESULTS

CYFRA titers in pulmonary caricnoma patients Fig. 1 shows CYFRA titers (ng/ml) on a logarithmic scale for pulmonary carcinoma patients as well as benign pulmo-

nary disease patients. High concentrations were observed in squamous cell carcinoma patients (20.30 ± 4.32 ng/ml, mean $\pm$ SEM) and adenocarcinoma patients (9.88 ± 1.94 ng/ml), and the former showed especially high levels. On the other hand, the concentrations were 5.17 ± 0.86 ng/ml in small cell carcinoma patients, and low levels were observed in pulmonary benign disease patients (2.48 ± 0.12 ng/ml).

CYFRA titers in pulmonary benign disease patients CYFRA titers in the pulmonary benign disease patients are shown in Fig. 2 (logarithmic scale). Somewhat raised levels were observed in pulmonary tuberculosis, interstitial pneumonia, and pneumonia patients, but low levels were observed in most of the pulmonary benign disease patients.

Setting a cut-off value of CYFRA titer and its sensitivity in pulmonary carcinoma Fig. 3 shows receiver operating
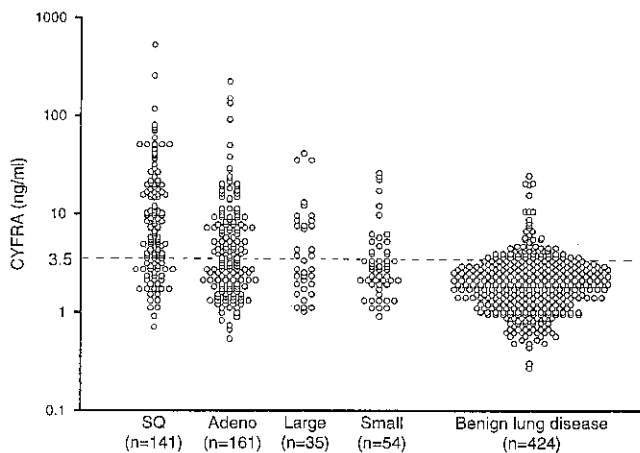

Fig. 1. CYFRA titers in patients with pulmonary carcinoma and benign lung diseases. SQ, squamous cell carcinoma; Adeno, adenocarcinoma; Large, large cell carcinoma; Small, small cell carcinoma.
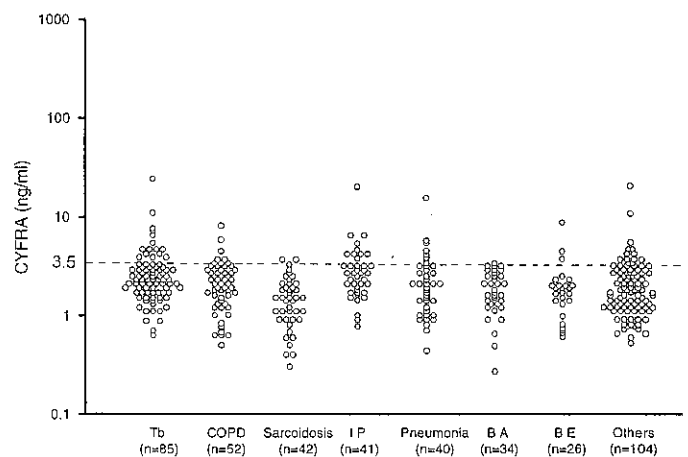

Fig. 2. CYFRA titers in patients with benign lung diseases. Tb, tuberculosis; IP, interstitial pneumonia; BA, bronchial asthma; BE, bronchiectasis. Others comprise 5 cases of hypersensitivity pneumonitis, 5 cases of pulmonary aspergillosis, 5 cases of diffuse panbronchiolitis, 3 cases of pulmonary cryptococcosis, etc. High levels were observed in one case of hypersensitivity pneumonitis and in one case of pulmonary alveolar proteinosis.
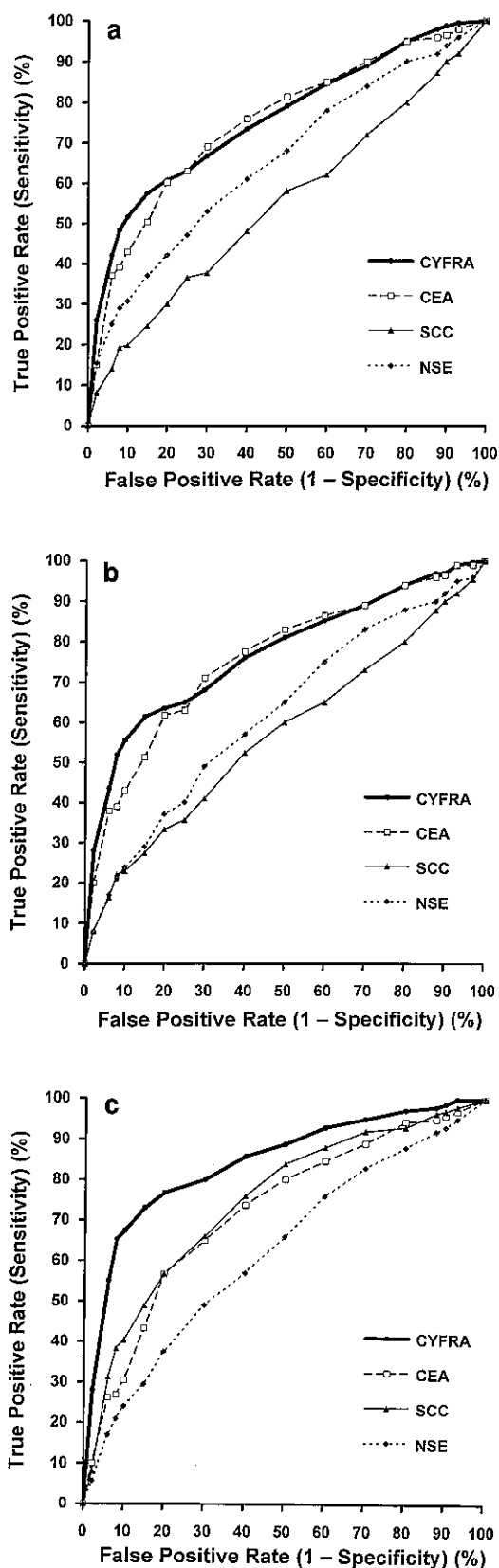
characteristic (ROC) curves for CYFRA, SCC, and CEA obtained from the data in all subjects with pulmonary carcinoma (Fig. 3a), non-small cell pulmonary carcinoma (Fig. 3b), and squamous cell carcinoma (Fig. 3c), against pulmonary benign diseases. The ROC curve for CYFRA is similar to that for CEA in all subjects with pulmonary carcinoma and non-small cell lung carcinoma, while the sensitivity of CYFRA at the false positive rate of 5–15% which is clinically used is better than that of CEA (Fig. 3a and 3b). Fig. 3c clearly shows the superiority of CYFRA over CEA and SCC in squamous cell carcinoma.

Based on these ROC curves, the most efficient rate of diagnosis was obtained at 3.5 ng/ml, which was set as the cut-off value. CYFRA was associated with a specificity of 85% at this concentration. The cut-off values for control tumor markers were set at 2.0 ng/ml for SCC, 4.6 ng/ml for CEA and 7.0 ng/ml for NSE based on the same specificity value of 85%.

Fig. 4 shows the sensitivities of tumor markers for each type of tumor. In all subjects with pulmonary carcinoma, the sensitivity of CYFRA was 57.5%, which was higher
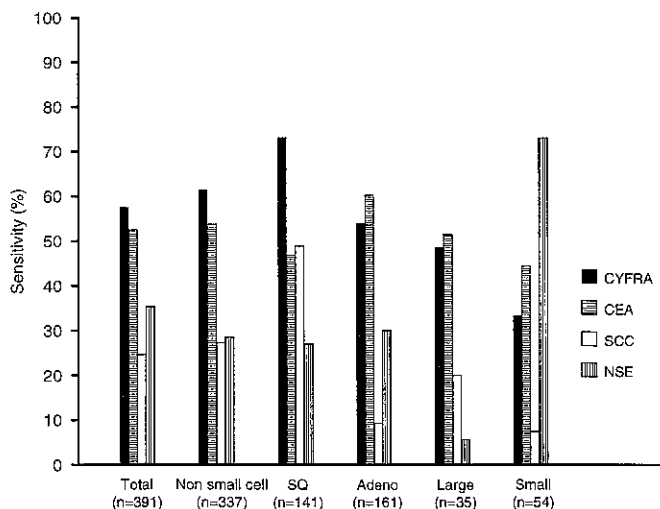

Fig. 4. Sensitivity of tumor markers in pulmonary carcinomas. Cut-off values: CYFRA; 3.5 ng/ml, CEA; 4.6 ng/ml, SCC; 2.0 ng/ml and NSE; 7.0 ng/ml (85% specificity).

Fig. 3. a: ROC curves for CYFRA and other tumor markers in subjects with lung cancer (n=391) and subjects with benign lung diseases (n=334). b: ROC curves in subjects with non-small cell lung carcinoma (n=337) and subjects with benign lung diseases (n=334). c: ROC curves in subjects with squamous cell carcinoma (n=141) and subjects with benign lung disease (n=334).
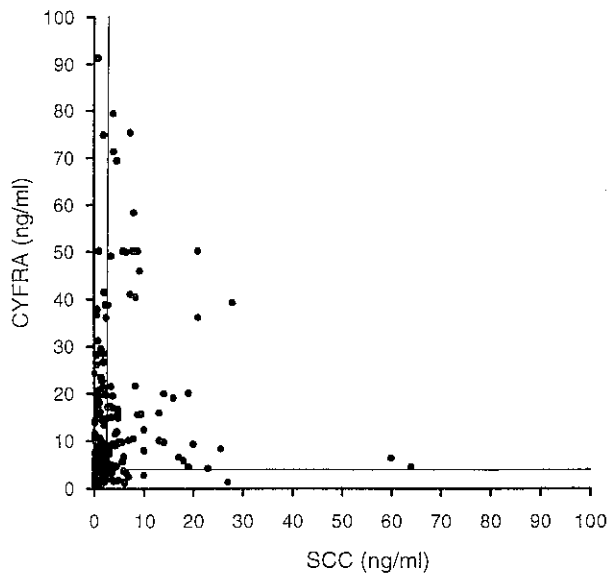
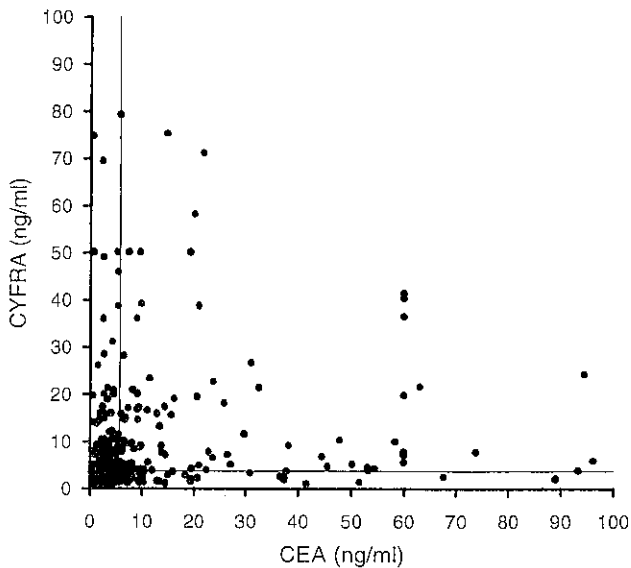Fig. 5. Correlation between CYFRA and SCC (n=337; r= 0.21).

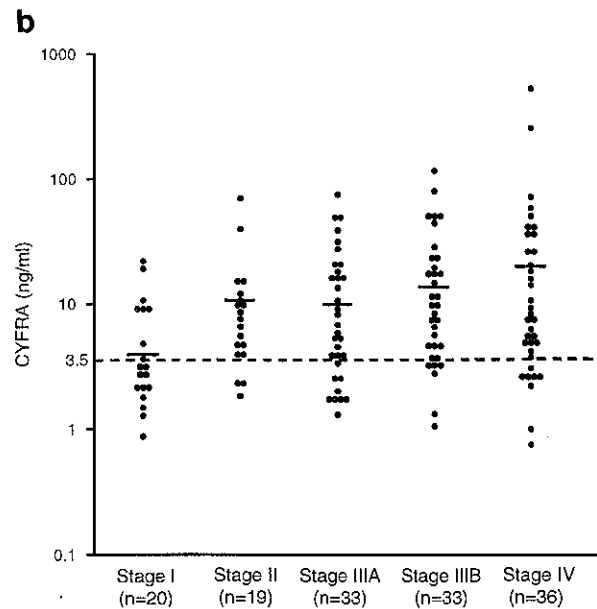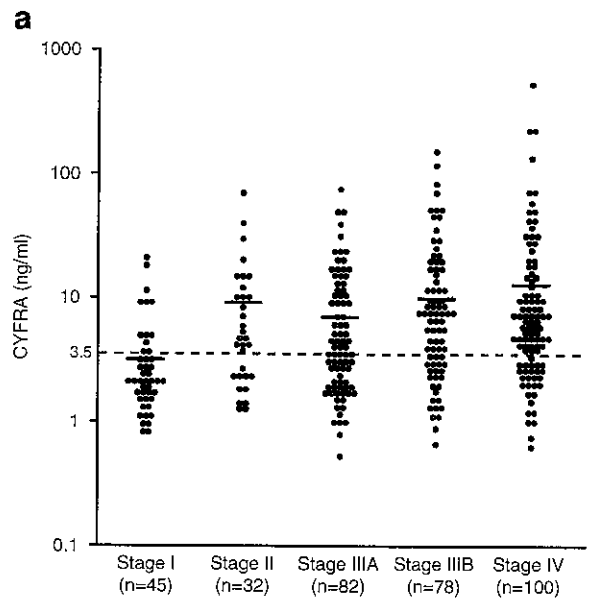Fig. 6. Correlation between CYFRA and CEA (n=337; r= 0.24).

Fig. 7. a: CYFRA titers in non-small cell lung carcinoma according to clinical stage. $P<0.001$ among each stage (Kruskal-Wallis test). b: CYFRA titers in subjects with squamous cell carcinoma according to clinical stage. $P<0.05$ among each stage (Kruskal-Wallis test).

than that of CEA (52.4%), SCC (34.3%), or NSE (16.9%) at the same value of specificity (85%). The sensitivity of CYFRA in subjects with non-small cell carcinoma (61.4%) was also higher than those of the other three markers (CEA 53.7%, SCC 37.1%, NSE 9.8%, respectively). The ROC curves clearly demon-

strated that CYFRA is superior to the others in squamous cell carcinoma (Fig. 3c), in which the sensitivity of CYFRA (73.1%) was much higher than that of SCC (61.0%).

There was no significant correlation between CYFRA titers and serum C-reactive protein or creatinine levels in
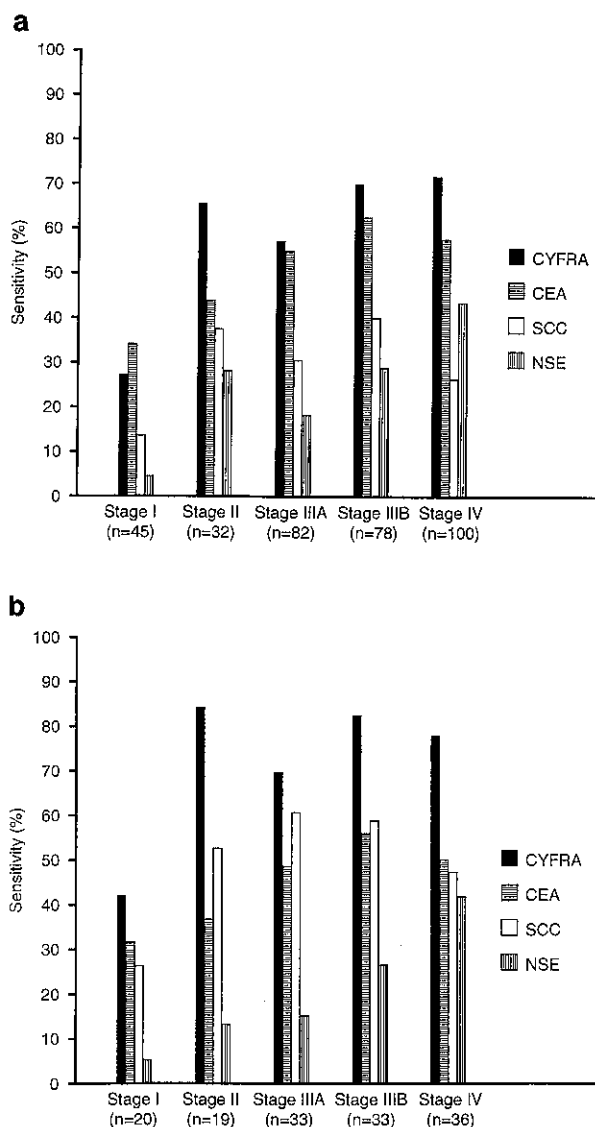
Fig. 8. a: Sensitivities of CYFRA, SCC, CEA and NSE in non-small cell lung carcinoma according to clinical stage. b: Sensitivities of CYFRA, SCC, CEA and NSE in squamous cell carcinoma according to clinical stage.

subjects with pulmonary benign diseases with CYFRA titers above the cut-off level (data not shown).

No correlation of CYFRA to CEA and SCC CYFRA was not significantly correlated with SCC (Fig. 5) or CEA (Fig. 6) in subjects with non-small cell carcinoma. **CYFRA titers and sensitivities according to clinical stage** Fig. 7a shows CYFRA titers for each stage in subjects with non-small cell carcinoma classified according to the Japan Lung Cancer Society. CYFRA titers increased progressively with clinical stage. The Bartlett test showed

a non-homogeneous distribution in each clinical stage. The Kruskal-Wallis test (non-parametric method) showed a significant difference in CYFRA titers ($P<$ 0.001) among these clinical stages.

Fig. 7b shows CYFRA titers for each stage in subjects with squamous cell carcinoma. CYFRA titers were high in stage I, and moreover, they increased progressively with clinical stage. The Kruskal-Wallis test showed a significant difference in CYFRA titers ($P<0.05$) among these clinical stages.

Fig. 8 shows sensitivities with CYFRA, SCC, and CEA in each stage in subjects with non-small cell carcinoma (Fig. 8a) and squamous cell carcinoma (Fig. 8b). CYFRA had a higher sensitivity than SCC and CEA in each clinical stage in subjects with non-small cell carcinoma and in subjects with squamous cell carcinoma.

CYFRA titers before and after therapy in pulmonary carcinoma Of the 36 cases with pulmonary carcinoma in whom CYFRA titers were measured, 20 cases showed high levels from the beginning. Before therapy, in 4 cases in whom measurement was performed more than twice before a definite diagnosis was made. CYFRA titers increased as the tumor increased in size and the disease advanced. Among the 30 cases who received chemotherapy and irradiation therapy, CYFRA titers decreased in all of 18 responders, but increased in 6 cases with progressive diseases, and CYFRA titers and tumor size were unchanged in the remaining 6 non-responders. CYFRA titers fluctuated according to tumor size in 13 of 16 patients who had CYFRA titers below the cut-off level before treatment. CYFRA titers returned to under the cut-off value in two patients who had curative operation, and also decreased in three patients in whom the tumor was resected. The titers increased in one patient who had open biopsy, but whose tumor could not be resected. CYFRA titers increased significantly when bone metastasis occurred in three patients. No patient showed an increase in CYFRA titers immediately after radiation therapy, chemotherapy or surgery.

DISCUSSION

Several tumor markers, including CEA, SLX, SCC, and NSE, are used as aids to the diagnosis of pulmonary carcinoma. NSE has a relatively high sensitivity (60 to 70%) in small cell lung cancer.[3, 4] Neuroblastomas are also NSE-positive, and NSE concentrations are sometimes increased in patients with carcinoid, germinoma, and kidney tumors, suggesting that NSE has a high tissue specificity. NSE is associated with a positive rate below 5% in benign pulmonary diseases.

SCC is used for diagnosis of squamous cell carcinoma. However, its sensitivity is low with a high false-positive rate.[5] CEA is widely used for diagnosis of pulmonary

carcinoma, although it has a low tissue specificity and a high false-positive rate.[6]

Cytokeratin, also called α-keratin, forms an 8 nm keratin filament with β-keratin, which is, together with actin microfilaments and microtubules, a component of the cytoskeleton in epithelial cells.[1, 2] In malignant epithelial cells, activated protease increases the degradation of cytokeratin, resulting in the release of large amounts of cytokeratin fragments in the blood, especially fragment 19.[7]

CYFRA, cytokeratin fragment 19, has been measured by two methods, RIA,[8] and ELISA.[9, 10] In this study, we selected ELISA and set a cut-off value of 3.5 ng/ml for lung cancer against benign lung diseases (85% specificity). CYFRA appeared to be a useful tumor marker for total pulmonary carcinoma: its sensitivity (57.5%) was similar to that of CEA in the present study and in a previous report (55.0%).[10] In the present study, CYFRA also showed a good sensitivity (61.4%) in non-small cell lung carcinoma.

In the previous study in France, the CYFRA cut-off value was set at 3.6 ng/ml, at which the sensitivity was 52% and the specificity was 87% for total lung cancer.[8] In Germany, the cut-off value was set at 3.3 ng/ml and sensitivity and specificity were 61% and 78%, respectively, for total lung cancer.[9] Our data are somewhat different from these reports, probably because the populations of subtypes of lung cancer are different in the three countries. More subjects with malignant or benign lung diseases were included in this study than in the previous three studies.[8–10]

In this study, relatively high levels of CYFRA were observed in pulmonary tuberculosis and interstitial pneumonia. The mechanism of this effect is still unclear. It is noteworthy that the concentrations of CEA and SCC are also elevated when inflammations or interstitial lung diseases exist. In this study, SCC levels were more sensitive to the amount of sputa and CEA levels were more

influenced by the presence of interstitial pneumonia, as compared with CYFRA levels (data not shown).

As this study showed that CYFRA is not influenced by smoking, it is suggested that CYFRA would be more effective than CEA and SCC in screening for pulmonary carcinoma. In the present study, CYFRA had a sensitivity of 73.1% in squamous cell carcinoma, and CYFRA and SCC were not significantly correlated. Our results suggest that CYFRA is more useful than SCC in diagnosing squamous cell carcinoma.

Our results showed that CYFRA is a tissue-specific tumor marker with a sensitivity equal to or higher than that of CEA for total pulmonary carcinomas. It is possible that the sensitivity of diagnostic assays for total pulmonary carcinoma could be improved by combining CYFRA (for non-small cell lung carcinoma) with NSE (for small cell lung carcinoma). Because CYFRA had a high sensitivity in stage I, it may be useful for the early detection of pulmonary carcinoma and for differential diagnosis of pulmonary coin lesions.

We found that the CYFRA titer and sensitivity of CYFRA increased progressively with clinical stages, suggesting that CYFRA may be useful for clinical staging and as a prognostic indicator. In a previous study, SCC in each clinical stage had a lower sensitivity than CYFRA in this study, and the sensitivity of SCC has not been found to increase with clinical stage.[5]

CYFRA is not affected by cell damage, because the increase in cytokeratin fragments does not result from cytokeratin release from degenerated cells, but from the increased degradation of cytokeratin filaments by activated protease in tumor cells. We observed no increase in CYFRA titers after surgery, chemotherapy, or radiation therapy.

In conclusion, it is suggested that CYFRA is potentially useful for diagnosis and monitoring of lung carcinoma, especially for squamous cell carcinoma.

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