Comparison of the serum fibrin-fibrinogen degradation products with cytokeratin 19 fragment as biomarkers in patients with lung cancer

HEE JIN SO[1], SEOK-IL HONG[1,2], JIN KYUNG LEE[1,2], YOON HWAN CHANG[1,2], SUN JUNG KANG[1] and YOUNG JUN HONG[1,2]

[1]Department of Laboratory Medicine, Korea Cancer Center Hospital, Seoul 139-709; [2]Radiation Cancer Medicine Program, University of Science and Technology, Daejeon 305-350, Republic of Korea

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Abstract. Lung cancer is one of the main causes of cancer-related mortality. The identification of early diagnostic biomarkers improved outcomes for lung cancer patients. Serum fibrin-fibrinogen degradation products (FDP) levels are elevated in numerous malignancies due to hemostatic alterations. The serum FDP levels were compared to the levels of cytokeratin 19 fragment antigen (CYFRA 21-1), another well-established biomarker. The serum samples from 193 lung cancer patients, 84 healthy controls and 106 patients with benign respiratory diseases were obtained. The serum FDP level was measured using the DR-70 immunoassay and the CYFRA 21-1 level was measured by electrochemiluminescence using the Roche Analytics E170. Receiver operating characteristics curves were used to assess the predictive sensitivity and specificity. The mean serum FDP level in lung cancer patients (35.01±229.02 μg/ml) was significantly higher compared to the 190 non-cancerous subjects (0.60±0.75 μg/ml; P=0.039). The mean serum CYFRA 21-1 level in lung cancer patients (4.50±6.67 ng/ml) was also significantly higher compared to the non-cancerous subjects (1.40±0.83 ng/ml; P<0.05). FDP exhibited clinical sensitivity and specificity of 86 and 75%, respectively, at an optimal cut-off at 0.67 μg/ml. CYFRA 21-1 exhibited clinical sensitivity and specificity of 77 and 74%, respectively, at a cut-off of 1.65 ng/ml. The serum FDP area under the curve (0.87) was slightly higher compared to CYFRA 21-1 (0.83). Therefore, it is apparent that serum FDP is comparable to CYFRA 21-1 as a lung cancer biomarker and can be used for clinical practice.

Correspondence to: Dr Young Jun Hong, Department of Laboratory Medicine, Korea Cancer Center Hospital, 75 Nowon-ro, Nowon-gu, Seoul 139-709, Republic of Korea
E-mail: clinchem@kcch.re.kr

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Introduction

Lung cancer is a major cause of cancer-related mortality in humans worldwide (1). In Korea, 21.7% of cancer mortality in 2010 was due to lung cancer and the number of new cases is predicted to continue to rise (2,3). As the majority of lung cancer patients have advanced disease at diagnosis, they are not candidates for curative surgery. In addition, the methods for the early detection of lung cancer have been proven to be elusive, and as a result, prognostic improvement of this type of cancer has not been successfully achieved (4). Therefore, it is necessary to identify early diagnostic biomarkers to improve the clinical outcome of lung cancer patients.

In the normal state, hemostasis and angiogenesis are physiological processes that are strictly regulated to adjust to tissue remodeling and wound healing requirements. However, this ability is destroyed when cancer cells proliferate (5). For tumor growth, invasion and metastasis, the activation of exogenous coagulation and fibrinolysis is required. Therefore, the topical generation of thrombin and fibrinolysis are extremely important factors for the growth and spread of tumors (6,7). Tumor cells release either coagulation factors, which directly activate the coagulation pathway resulting in thrombin formation, or plasminogen activators (PA), which directly activate the fibrinolytic system. Thrombin acts as a growth factor for tumor cells and facilitates tumor angiogenesis leading to fibrin formation. The deposition of fibrin in cancer tissues acts as a barrier against inflammatory cells that may destroy the tumor. PA generates plasmin that promotes invasion and migration of tumor cells into the circulation. As plasmin is also an active serine protease in fibrinolysis (8), PA affects the production of fibrin-fibrinogen degradation products (FDP) in cancer cells. Numerous studies have demonstrated that serum FDP is elevated in patients with various types of cancer (9-11). Therefore, measuring serum FDP can be useful for tumor detection.

The majority of FDP tests use latex agglutination, turbidimetry or reflectometry, which mainly quantify the D and E fragments of fibrin in plasma samples. The DR-70 immunoassay, a commercially available polyclonal anti-FDP antibody-based immunoassay, quantifies all the products of

cancer-induced FDP, including the D and E fragments and D-dimers in serum. Thus, the DR-70 immunoassay is more sensitive than conventional FDP tests (12). The cytokeratin 19 fragmentation antigen, CYFRA 21-1, is a polypeptide that recognizes soluble cytokeratin 19 fragments and is a well-established biomarker for lung cancer. Cytokeratin 19 is an acidic type I cytokeratin that is expressed in all simple epithelia and in carcinomas, including lung cancer, and it is a sensitive tumor marker, particularly for non-small cell lung cancer (13-16).

Although the DR-70 immunoassay has been reported to be clinically sensitive for the detection of several malignancies (9,12,17-23), extremely few studies have investigated the clinical efficiency of DR-70 for the detection of lung cancer. A number of studies have demonstrated that hemostatic alterations are frequently observed in lung cancer patients and the degree of coagulation and fibrinolysis activation has been correlated with the clinical progression of the disease (5,24,25). The aim of the present study was to investigate the diagnostic value of the DR-70 immunoassay in lung cancer and to compare the sensitivity and specificity of FDP with CYFRA 21-1 as biomarkers of lung cancer.

Materials and methods

Patients. Serum samples were obtained from 193 patients diagnosed with lung cancer between July 2007 and December 2009 at Korea Cancer Center Hospital (Seoul, Korea) and from 84 healthy controls from the blood specimen biobank of the hospital. An additional 106 serum samples from patients with benign respiratory diseases (86 asthma patients, 10 chronic obstructive pulmonary disease, eight tuberculosis, one pneumonia and one aspergillosis patient) were provided by Soonchunhyang University Bucheon Hospital Biobank (Bucheon, Korea). All the samples of benign lung diseases were obtained at the time of diagnosis, whereas the serum from patients with cancer were collected prior to the surgery. Lung cancer diagnosis and staging was determined using whole-body computed tomography, percutaneous needle aspiration and bronchoscopy with biopsy. Histopathological evaluation was performed according to the revised World Health Organization classification of lung tumors (26). The characteristics of the study population are shown in Table I. The median age of the patients with cancer was 62 years (range, 8-81 years) with a male-to-female ratio of 7.8:1. The male-to-female ratio and the median age of the patients in the benign lung disease group were 3.1:1 and 47 years (range, 17-80 years), respectively, whereas these values for the healthy control group were 7.4:1 and 45 years (range, 28-75 years), respectively. Smoking history was not available for 101 patients (12 lung cancer patients, five benign lung disease group and all 84 healthy control group patients). Among the remaining 282 patients, the smoker vs. non-smoker ratios were 2.1:1 in the lung cancer patients and 0.5:1 in patients with benign lung diseases. The approval for the use of human sera was obtained by the Institutional Review Board of Korea Institute of Radiological and Medical Sciences (K-1111-002-026).

Detection of FDP and CYFRA 21-1. The concentrations of all the forms of FDP in the serum were measured with the DR-70 immunoassay (AMDL Inc., Tustin, CA, USA) in accordance with the manufacturer's instructions. DR-70 is an enzyme-linked immunosorbent assay-based sandwich method. Briefly, 100 μl of serum was diluted 1:200 and incubated in the wells coated with affinity-purified rabbit anti-DR-70 antibodies (AMDL Inc.,) for 30 min at room temperature. The wells were washed and subsequently incubated with 100 μl horseradish peroxidase-conjugated anti-DR-70 antibodies. Following additional washes, 3,3',5,5'-tetramethylbenzidine was added and the wells were incubated in the dark at 25˚C for 15 min. The reaction was terminated by adding 100 μl stop solution and the intensity of the color formed was read at 450 nm.

Serum CYFRA 21-1 was determined by an electrochemiluminescence immunoassay system Roche Modular Analytics E170 module (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis. The differences in serum concentration between the groups were analyzed by the Student's t-test and one-way analysis of variance (ANOVA). Adjustment of age and gender was also performed by two-way ANOVA. The optimal cut-off values for FDP and CYFRA 21-1 were obtained by receiver operating characteristics curve analysis. Diagnostic performance was described in terms of sensitivity, specificity and area under the curve (AUC) by receiver operating characteristics analysis. The association between FDP and CYFRA 21-1 in lung cancer patients was assessed by Pearson's correlation test. $P<0.05$ was considered to indicate a statistically significant difference. All the statistical data analyses were performed using SALT Version 2.0 (Istech Inc., Korea) software.

Results

Serum levels of tumor markers. The mean serum FDP level in lung cancer patients was 35.01±229.02 μg/ml (range, 0.30-2200.00 μg/ml), which was significantly higher compared to the non-cancerous subjects, including patients with benign lung diseases and healthy controls (0.60±0.75 μg/ml; range, 0.20-8.90 μg/ml; P=0.039). The mean serum CYFRA 21-1 level was significantly higher in lung cancer patients (4.50±6.70 ng/ml; range, 0.50-51.30 ng/ml) compared to the non-cancerous subjects (1.40±0.83 ng/ml; range, 0.20-5.70 ng/ml; $P<0.05$) (Fig. 1). No significant differences for the tumor markers were observed between the patients with benign lung diseases and the healthy control group (Fig. 2).

Association between the tumor markers and pretreatment clinicopathological characteristics in lung cancer. The mean levels of each tumor marker stratified by clinicopathological characteristics of lung cancer patients are listed in Table II. The levels of FDP and CYFRA 21-1 were significantly higher in current smokers compared to non-smokers (P=0.044 and $P<0.001$, respectively). There were no significant differences in the serum levels of FDP or CYFRA 21-1 based on age, gender and pathological cancer type or tumor stage, with the exception of a significant increase in the serum levels of CYFRA 21-1 in squamous cell carcinoma compared to the other types of cancer.

Table I. Characteristics of the study population.

Characteristics	Lung cancer patients (n=193)	Benign lung disease patients (n=106)	Healthy controls (n=84)
Gender. n			
Male	171	80	74
Female	22	26	10
Age, years (range)	62 (8-81)	47 (17-80)	45 (28-75)
Current smoking, n			NE
Yes	123	33	
No	58	68	
Unknown	12	5	
Types, n (%)			
SCLC	7 (3.6)		
ADC	63 (32.6)		
SCC	72 (37.3)		
Others[a]	51 (26.4)		
Stage, n (%)			
I	58 (30.1)		
II	32 (16.6)		
III	38 (19.7)		
IV	65 (33.7)		

[a]Large cell carcinoma, carcinoid tumor or malignant hemangioendothelioma. NE, not evaluated; SCLC, small cell lung cancer; ADC, adenocarcinoma; SCC, squamous cell carcinoma.

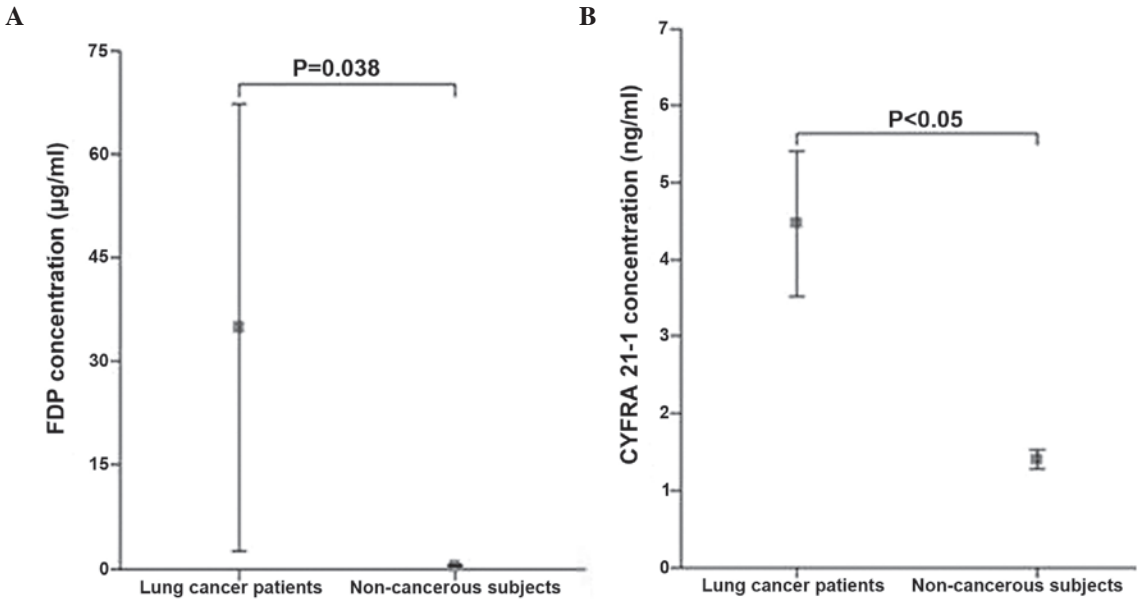

Figure 1. Comparison of the tumor marker levels between the lung cancer and non-cancerous patients. The line indicates the 95% confidence interval (CI) of the tumor marker levels in each group. The box represents the mean level. (A) The mean fibrin-fibrinogen degradation products (FDP) level was significantly higher in lung cancer patients (35.01±229.02 μg/ml; range, 0.30-2200.00 μg/ml) compared to non-cancerous subjects (0.60±0.75 μg/ml; range, 0.20-8.90; P=0.038). (B) The mean cytokeratin 19 fragment (CYFRA 21-1) level was significantly higher in lung cancer patients (4.50±6.67 ng/ml; range, 0.50-51.30 ng/ml) compared to non-cancerous subjects (1.40±0.83 ng/ml; range, 0.20-5.70 ng/ml; P<0.05).

Diagnostic performance of tumor markers for lung cancer. The accuracy, sensitivity and specificity of serum FDP for the diagnosis of lung cancer at a cut-off value of 0.67 μg/ml were 80, 86 and 75%, respectively (Fig. 3A). The diagnostic accuracy, sensitivity and specificity of CYFRA 21-1 in the same serum samples at a cut-off value of 1.65 ng/ml were 75, 77 and 74%, respectively (Fig. 3B). The AUC for FDP was 0.87 [95% confidence interval (CI), 0.83-0.90], which was higher than that of

Table II. FDP and CYFRA 21-1 concentrations in patients with lung cancer.

Variables (n)	FDP, μg/ml mean (range)	P-value	CYFRA 21-1, ng/ml mean (range)	P-value
Age, years				
≤62 (105)	10.3 (0.3-912.6)	0.131	3.9 (0.5-51.3)	0.263
>62 (88)	64.5 (0.3-2200.0)		5.1 (0.9-23.3)	
Gender				
Male (171)	39.4 (0.3-2200.0)	0.464	5.4 (0.5-123.0)	0.285
Female (22)	1.3 (0.3-2.9)		2.8 (1.0-14.6)	
Current smoking[a]				
Yes (123)	54.0 (0.3-2200.0)	0.044	5.6 (0.6-51.3)	<0.001
No (58)	1.7 (0.3-10.2)		2.5 (0.6-10.9)	
Type				
SCLC (7)	2.4 (0.7-9.4)	0.629	4.3 (1.8-9.0)	0.048
ADC (63)	46.2 (0.3-2200.0)		4.4 (0.9-51.3)	
SCC (72)	51.8 (0.3-1892.8)		7.7 (0.6-123)	
Others[b] (51)	1.9 (0.3-10.2)		2.3 (0.6-20.1)	
Stage				
I (58)	96.8 (0.3-2200.0)	0.095	4.9 (0.6-47.9)	0.460
II (32)	1.4 (0.3-2.7)		6.1 (1.2-23.3)	
III (38)	25.7 (0.3-912.6)		6.9 (1.3-123.0)	
IV (65)	1.9 (0.3-9.4)		3.6 (0.5-51.3)	

[a]Smoking history was unknown in 12 subjects; [b]large cell carcinoma, carcinoid tumor or malignant hemangioendothelioma. FDP, serum fibrin-fibrinogen degradation products; CYFRA 21-1, cytokeratin 19 fragment; SCLC, small cell lung cancer; ADC, adenocarcinoma; SCC, squamous cell carcinoma.

the AUC for CYFRA 21-1 at 0.83 (95% CI, 0.79-0.87). The sensitivity and specificity of serum FDP combined with serum CYFRA 21-1 were 95 and 57%, respectively.

Correlation of serum FDP with CYFRA 21-1. There was no correlation observed between the serum FDP and CYFRA 21-1 levels in lung cancer patients (r=0.038; P=0.599).

Discussion

The present study demonstrated that the mean serum FDP level was higher in lung cancer patients compared to the non-cancerous subjects. Consistent differences in the level of components, such as FDP, are the pathophysiological basis for the detection of lung cancer using tumor markers. Additionally, FDP had a high lung cancer detection rate with a sensitivity of 85% and specificity of 75% at a cut-off value of 0.67 μg/ml. These results are comparable to those of CYFRA 21-1, which had a sensitivity and specificity of 77 and 74%, respectively, at a cut-off value of 1.65 ng/ml. In comparison to CYFRA 21-1, FDP appeared to be improved with regards to clinical performance expressed as AUC (0.87 vs. 0.83, respectively) for the detection of lung cancer.

The results of the present study are consistent with previous studies. In 1995, Fields *et al* (27) first reported the results of a clinical trial using the DR-70 immunoassay for the detection of lung cancer. The overall sensitivity of the assay was 66% at a specificity of 92%. The mean level of FDP in lung cancer patients was ~4 times higher compared to the normal controls. Wu *et al* (28) found that the FDP levels increased in lung cancer patients, with an 86% diagnostic sensitivity and a specificity of 96%. In contrast to the small sample sizes used in earlier studies assessing the DR-70 immunoassay for lung cancer detection (9,27-29), the sample size in the present study was considerably larger (n=193), allowing the application of more accurate parametric statistics.

The serum FDP levels were compared to CYFRA 21-1, which is a relatively well established lung cancer marker. To the best of our knowledge, this is the first study regarding the comparison between these two biomarkers. When considering FDP as a routine laboratory test for lung cancer, the present results could provide certain practical information. No correlation was found between FDP and CYFRA 21-1. The mechanisms by which these two biomarkers are generated during carcinogenesis are different and this may be the reason for the poor correlation. Although the combination of FDP with CYFRA 21-1 increased the diagnostic sensitivity <95%, this occurred at the expense of specificity. Accordingly, it would not be recommended to assess the two markers simultaneously for clinical purposes.

Kerber *et al* (18) demonstrated that the FDP levels increased as the stage of gastrointestinal cancer advanced. In addition, the level of FDP was positively correlated with the tumor load and the number of metastatic sites. In the

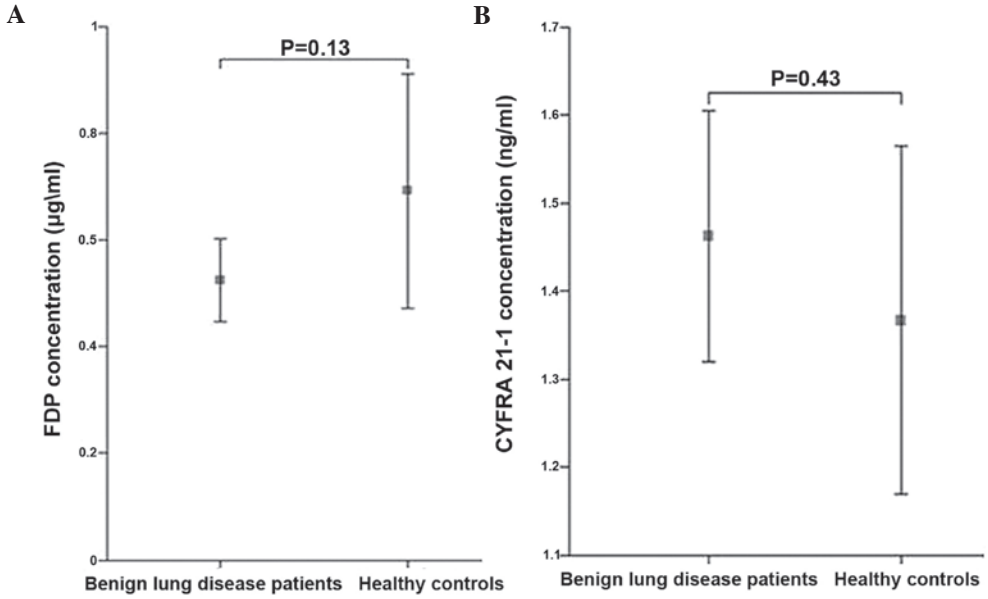

Figure 2. Comparison of the tumor marker levels in the benign lung disease patients and healthy controls. The line indicates 95% confidence interval (CI) of tumor marker levels in each group. The box represents the mean level. (A) The mean fibrin-fibrinogen degradation products (FDP) levels in the benign lung disease patients and healthy controls were 0.53±0.41 and 0.69±1.03 μg/ml, respectively. (B) The mean cytokeratin 19 fragment (CYFRA 21-1) levels in the benign lung disease patients and healthy controls were 1.46±0.75 and 1.37±0.92 ng/ml, respectively. No significant difference was observed between the levels of the two tumor markers in the benign lung disease patients and healthy controls.

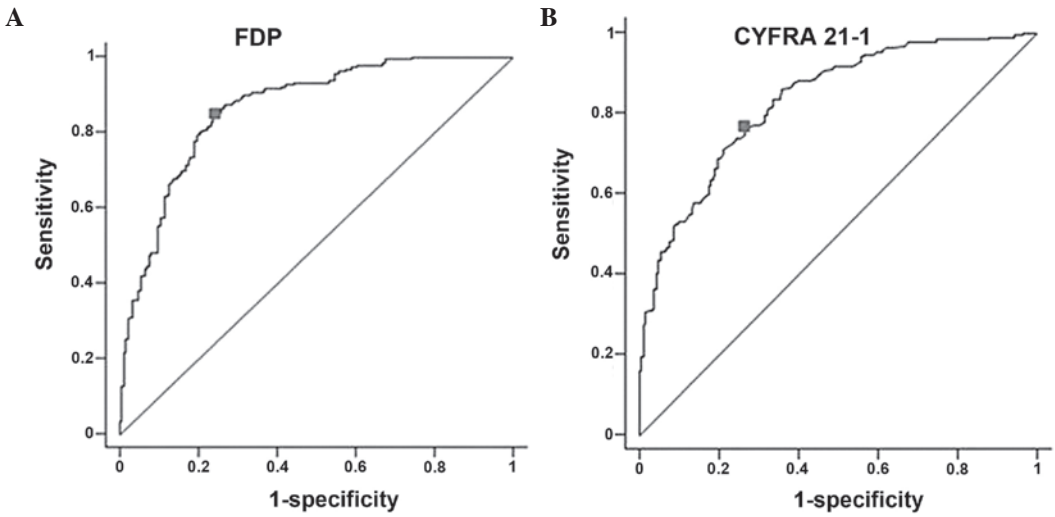

Figure 3. Receiver operating characteristic curves of the lung cancer tumor markers. (A) The area under the curve (AUC) of fibrin-fibrinogen degradation products (FDP) for the diagnosis of lung cancer was 0.87 (cut-off, 0.67 μg/ml). (B) AUC of cytokeratin 19 fragment (CYFRA 21-1) was 0.83 (cut-off, 1.65 ng/ml).

present study, the serum FDP levels increased in lung cancer patients, but the expression level was not correlated with the histological subtype of the tumor or the tumor stage. The lack of correlation in the present study may be due to the uneven number of patients among the histological subtypes or stages of lung cancer.

The present study has certain limitations. The significance of the serum FDP levels was not assessed as a marker for monitoring lung cancer progression. Furthermore, the smoking history of 101 subjects was unknown. The serum levels of FDP and CYFRA 21-1 have been previously reported to be unaffected by smoking (30). In the present study, a difference in the mean serum FDP levels based on smoking status was observed in lung cancer patients. However, this difference was not observed in patients with benign lung disease. The incomplete evaluation of the smoking history among the study subjects makes it difficult to establish a link between the serum FDP levels and smoking.

The present study focused on lung cancer patients in Korea, and to the best of our knowledge, it is the first study to compare lung cancer patients with healthy controls and patients with benign lung diseases. The comparison of serum FDP with an accepted lung cancer marker, CYFRA 21-1, also enhances the value of the study. Further studies, including survival rate analysis and long-term follow-up, are required to evaluate FDP as a monitoring marker for lung cancer. Taken together, the results of the present study indicate that the serum FDP levels measured by the DR-70 immunoassay can be used as a lung cancer marker in clinical laboratories.

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