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Original Research

Analysis of serum cfDNA concentration and integrity before and after surgery in patients with lung cancer

Yihui Fan[1, 2], Minxin Shi[2], Saihua Chen[2], Guanjun Ju[2], Lingxiang Chen[3], Haimin Lu[2], Jian Chen[2], Shiying Zheng[1*]

[1] Department of Thoracic and Cardiovascular Surgery, The First Affiliated Hospital of Soochow University, Medical College of Soochow University, 215006 Suzhou, Jiangsu Province, China
[2] Department of Thoracic Surgery, The Affiliated Tumor Hospital of Nantong University, 226019 Nantong, Jiangsu Province, China
[3] Department of Medical Oncology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, 210009 Nanjing, Jiangsu Province, China

*Correspondence to: qrju40@163.com

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Abstract: The content and integrity of cell-free DNA (cfDNA) before and after surgery in patients with lung cancer were determined to investigate its clinical significance. Peripheral blood was collected from 120 patients with lung cancer who were treated in our hospital from March 2016 to November 2018, including 50 cases before operation and 70 cases after operation. 60 healthy subjects served as controls. Quantitative PCR was used to determine the cfDNA level of each group. The relationship between cfDNA levels and the clinical features of lung cancer patients was determined. Receiver Operating Curves were used to determine the sensitivity and specificity of cfDNA, CEA, NSE and CYFRA21-1 in lung cancer. The concentration and integrity of cfDNA before surgery in patients with lung cancer were significantly higher than those after surgery and those in healthy control group. The cfDNA concentration in patients with lung cancer after surgery was significantly higher than that in the control group, but there was no statistical difference in cfDNA integrity between the two groups. There was no significant correlation between cfDNA concentration/integrity and gender, age, tumor type, tumor stage, and expressions of CA199, CA125, and CA153 in patients with lung cancer before or after surgery. However, there were significant correlations between the expression levels of CEA, NSE, and CYFRA21-1 and cfDNA concentration. The expression levels of CEA and CYFRA21-1 were significantly correlated with cfDNA integrity before surgery, while the correlations were not significant after surgery. The concentration and integrity of cfDNA increased significantly in serum of lung cancer patients. The concentration and integrity of cfDNA in patients with lung cancer after surgery were significantly lower than those before surgery. Thus, cfDNA has high application value in the diagnosis and evaluation of lung cancer.

Key words: Lung cancer; cell-free DNA; CEA; NSE; CYFRA21-1; ROC curve.

Introduction

Lung cancer is one of the most malignant tumors with the fastest growth in morbidity and mortality. About one-third of patients worldwide who die of cancer every year are lung cancer patients. About 80% of lung cancer patients have non-small cell lung cancer (NSCLC), including squamous cell carcinoma, adenocarcinoma and large cell carcinoma, while 20% of the lung cancer patients have small cell lung cancer (SCLC) (1, 2). At present, chemotherapy is still the main treatment method for non-small cell lung cancer. However, chemotherapy can only prolong the survival time of patients and improve their quality of life, but generally it cannot cure NSCLC. Traditional laboratory medicine uses pathology, cytology, and spiral CT to diagnose lung cancer in patients. However, due to the lack of specific clinical symptoms at the early and middle stages of most lung cancer, nearly two-thirds of patients have metastasized cancer at the time of diagnosis, thereby missing the best treatment time. Therefore, the 5-year survival of lung cancer patients is only 8 to 16% (3, 4). Consequently, there is need for development of early diagnosis and screening methods, which constitute an important step towards improving the treatment outcome of lung cancer.

Cell-free DNA, also known as circulating DNA, was discovered in 1948 by Mandel and Metais. It is a small, endogenous, heterologous and double-stranded DNA that is free of cells (1, 5). Usually, cfDNA is produced by apoptosis and necrosis, in the form of DNA-protein complexes or free fragments in body fluids such as blood, urine, pleural effusion, ascites, and bronchoalveolar lavage fluid (6-8). The length of cfDNA is generally 130-180 bp, and the blood content is below 100 ug/L, with an average of 30 ug/L. The cfDNA content of blood increases correspondingly in pathological conditions such as infection, inflammation and malignant tumor (9). The free DNA released by the tumor has the same characteristics as the DNA of the tumor cell itself, and reflects the characteristic changes in the tumor. Thus, blood cfDNA provides a comprehensive and accurate genetic map to compensate for the heterogeneous defects associated with pathological biopsy, and it macroscopically reflects the disease state. The detection of cfDNA is non-invasive, and cfDNA can be

easily obtained from peripheral blood. These characteristics make it a biomarker with significant advantages in lung cancer diagnosis, monitoring of tumor treatment efficacy, and evaluation of prognosis (10, 11).

This study determined changes in cfDNA concentration and integrity in peripheral blood of patients with lung cancer before and after surgery, and discussed their clinical application values in the diagnosis of lung cancer.

Materials and Methods

Sample selection

Fifty lung cancer patients who had not undergone surgery between March 2016 and November 2018 were selected as study subjects. Thirty-four (34) of the 50 patients (68%) were males, while 16 (32%) were females, and their ages ranged from 40 to 83 years, with a mean of 63.08±8.37 years. In addition, 70 lung cancer patients undergoing surgery at the same period were enrolled. They comprised 44 (63%) males and 26 (37%) females, aged 44 to 85 years, with a mean age of 65.03±9.58 years. There was no significant difference in gender and age between the two groups ($p > 0.05$).

Inclusion criteria: The following categories of patients were included in the study: (1) all patients diagnosed with lung cancer by histopathological examination, cytology examination and CT; (2) patients with intact medical records, and (3) patients with KPS score > 60 points.

Exclusion criteria: The excluded patients were: (1) those with other malignant tumors apart from lung cancer; (2) patients with serious diseases such as heart, liver and kidney diseases; (3) patients with acute or chronic infectious diseases, and (4) those with mental illness who were unable to participate in normal medical activities. A set of 60 healthy people during the same period served as a control group. These comprised 36 (60%) males and 24 (40%) females, with ages ranging from 43 to 84 years (mean age = 64.23±8.59 years). All participants in the trial signed informed consent prior to enrollment in the study. This clinical trial was performed following approval by the Clinical Research Ethics Committee of our hospital.

Serum separation and cfDNA extraction

Venous blood samples were collected in non-EDTA-coated tubes and sera were separated by centrifuging at 1600g for 10 minutes at room temperature. The supernatants were transferred to new tubes and centrifuged at 16000g for 10 minutes at 4°C, and the serum samples were carefully removed without disturbing lower residual layer. A minimum aliquot of 200 µl serum was used for DNA extraction immediately or stored at -80°C freezer. Serum samples were thawed on ice and spun at 10,000g for 3 minutes at room temperature before DNA purification. The DNA was purified from 200 µl of serum and eluted with 50 µl elution buffer using QIAamp DNA Blood Mini Kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. The DNA samples were quantified and kept at -20°C in a freezer prior to use.

Quantitative Polymerase Chain Reaction (Q-PCR)

The Q-PCR was performed on a LightCycler LC480 PCR machine (Roche Molecular Systems, Inc. Pleasanton, CA, USA). To measure the concentration of serum cfDNA, the repetitive LINE 1(Long interspersed nuclear element 1) 97 bp (both for short and long), and LINE1 259bp (only for long) DNA fragments were amplified as described previously (12). The LINE1 97bp primer amplified apoptotic and non-apoptotic DNA fragments, while the LINE1 259bp primer amplified non-apoptotic DNA fragments only. The total amount of serum DNA was represented by the QPCR result with LINE1 97bp primer. The DNA integrity index was calculated as the ratio of the result of LINE1 259 and LINE1 97 QPCR result. Serial concentrations of diluted standardized solution of human genomic DNA (Thermo Fisher Scientific, Waltham, MA, USA) were used as a standard curve reference. The concentration of cfDNA in each sample was calculated from the standard curve. The Q-PCR reaction was performed in triplicate, and the mean values were used for further analysis. The Q-PCR reaction mixture (20 µl) contained 1µl DNA template, 0.5 µl of each forward and reverse primer (LINE1 97 or LINE1 259), 10 µl UltraSYBR Mixture (Cwbiotech, Beijing, China), and 8 µl double-distilled water. Cycling conditions were 1 minute at 95°C, 35 cycles of 95°C for 8 seconds, and 60°C for 15 seconds. Each plate consisted of a serum DNA sample, a negative control (water template) and 7 serially diluted standard DNA solutions.

Detection of tumor biomarkers

Electrochemiluminescence was used for the determination of tumor biomarkers. Serum samples were obtained by centrifugation of fasting venous blood, using fully automated Electrochemiluminometer E170 and assorted kits (Roche, Switzerland). The reference ranges for each item were: cancer antigen (CA)199 < 39 U/ml, CA125 < 35 U/ml, carcino-embryonic antigen (CEA) < 3.5 ng/mL, neuron-specific enolase (NSE) < 16.3 ng/mL, cytokeratin fragment antigen 21-1 (CYFRA21-1) < 3.3 ng/mL, and cancer antigen CA153 < 25 U/ml.

Statistical analysis

The cfDNA quantification results are expressed as mean ± standard deviation ($\bar{x}$ ± SD). Kruskal-Wallis rank sum test was used for comparison between groups. Count data were compared using *r*-test, while measurement data were compared using *t*-test. The ROC curve was used to assess cfDNA quantification as a screening tool for patients with colorectal cancer, and the area under the ROC curve was used to calculate the accuracy in the difference between two different diseases for different critical values. All statistical analyses were done with SPSS 21.0 software. Values of $p < 0.05$ were considered statistically significant.

Results

Patients' clinical characteristics

The clinicopathological features of the patients before and after surgery are shown in Table 1. Twenty-six of the 50 patients with lung cancer who did not undergo surgery (52%) had adenocarcinoma, 10 patients (20%) were SCLC cases, while remaining 14 patients (28%)

Table 1. Clinical features of lung cancer patients.

Variable	Before surgery	After surgery	*p*
Gender			
Male	34	44	0.7865
Female	16	26	
Age			
≥65	26	44	0.4365
<65	24	26	
Tumor type			
Adenocarcinoma	26	42	
SCLC	10	12	0.8253
squamous carcinoma	14	16	
Disease stage			
I/II	18	30	0.7897
III/IV	32	40	
CA199			
≥39 U/ml	30	38	0.7928
<39 U/ml	20	32	
CA125			
≥35 U/ml	24	30	0.7944
<35 U/ml	26	40	
CEA			
≥5 ng/mL	26	16	0.0285
<3.5 ng/mL	24	54	
NSE			
≥16.3 ng/mL	32	22	0.0181
<16.3ng/mL	18	48	
CYFRA21-1			
≥3.3 ng/mL	24	14	0.0273
<3.3 ng/mL	26	56	

had squamous carcinoma. Disease grading was performed for all enrolled patients according to the criteria of the World Health Organization grading standard for lung cancer. The results revealed that 18 (36%) cases categorized as stage I/II, and 32 (64%) cases in stage III/IV. Regarding the 70 lung cancer patients receiving surgery, 42 of them (60%) had adenocarcinoma, 12 (17%) had SCLC, and 16 (23%) had squamous carcinoma. Results from disease grading showed that 30 of the patients (42.86%) were categorized in stage I/II, while 40 patients (57.14%) were in stage III/IV. These clinicopathological features did not differ significantly in patients with lung cancer, when compared before and after surgery ($p > 0.05$).

The levels of the six tumor biomarkers in the 50 patients with preoperative lung cancer were as follows: 30 cases (60%) were positive for CA199, while 20 cases (40%) were negative; 24 cases (48%) had positive expression of CA125, while 26 cases (52%) were negative; 26 cases (52%) had positive expression of CEA, while the expression of CEA was negative in 24 patients (48%). In 32 patients (64%) there was positive NSE expression, while NSE expression was negative in 18 patients (36%); there were 24 cases (48%) with positive expression of CYFRA21-1, and 26 cases (52%) with negative expression of CYFR21-1; 22 patients (44%) were positive for CA153, while 28 patients (56%) were CA153 negative. In the 70 patients with lung cancer after surgery, 38 (54.29%) had positive expression of CA199, while 32 (45.71%) were CAI negative; 30 patients (42.86%) had positive expression of CA125, while 40 patients (57.14%) were CA125 negative. There were 16 cases (22.86%) with positive expression of CEA, while 54 cases (77.14%) were with negative expression of CEA. Regarding NSE expression, 22 patients (31.43%) had positive expression of NSE, while 48 patients (68.57%) were NSE negative. Positive expression of CYFRA21-1 was seen in 14 patients (20%), while 58 patients (80%) were CYFRA21-1 negative; 38 patients (54.29%) had positive expression of CA153, while 32 patients (45.71%) were CA153 negative. The expressions of CEA, NSE and CYFRA21-1 in serum of patients with lung cancer differed significantly, when compared before and after surgery, while there were no significant differences in the expressions of CA199, CA125 and CA153, when pre- and post-surgery values were compared.

cfDNA concentration and integrity in serum of healthy individuals and lung cancer patients

The results of Q-PCR for determination of cfDNA concentration and integrity are shown in Figure 1. The cfDNA concentration in healthy people was 5.78 ± 2.19 ng/mL, while the cfDNA integrity was 1.28 ± 0.54. The cfDNA concentration of lung cancer patients before surgery was 36.59±26.49 ng/mL, while the cfDNA integrity was 5.85±4.48. After surgery, the cfDNA concentration of patients with lung cancer was 8.14±2.98 ng/mL, while the cfDNA integrity was 1.59±0.75. The cfDNA concentration and cfDNA integrity of patients with lung cancer before surgery were significantly higher than those in patients with lung cancer after surgery, and was also significantly higher in healthy control ($p < 0.05$). Moreover, the cfDNA concentration of lung cancer pa-

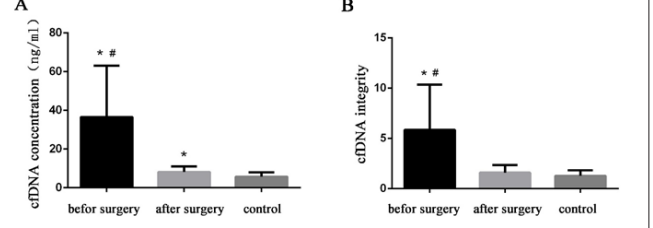

Figure 1. Concentration and integrity of cfDNA in serum of patients with lung cancer. (*$p < 0.05$), compared with the control group; #$p < 0.05$, compared with post-surgery group.)

Table 2. Correlation between serum cfDNA concentration and clinical characteristics.

Variable	Before surgery	After surgery
Gender		
Male	33.22±21.02	8.24±3.19
Female	43.74±36.19	7.98±2.71
P	0.3654	0.8074
Age		
≥65	37.76±21.62	8.40±3.30
<65	35.32±31.90	7.70±2.41
P	0.8231	0.5090
Pathological type		
Adenocarcinoma	37.34±29.89	7.96±2.82
SCLC	32.57±17.05	7.89±4.64
Squamous carcinoma	38.06±28.42	8.82±2.10
P	0.9346	0.7760
Disease stage		
I/II	31.19±21.05	7.87±2.78
III/IV	39.62±29.30	8.35±3.18
P	0.4567	0.6479
CA199		
≥39 U/ml	42.40±28.69	8.76±3.10
<39 U/ml	27.87±21.20	7.40±2.75
P	0.185	0.1823
CA125		
≥35 U/ml	39.12±29.84	8.78±3.32
<35 U/ml	34.25±23.96	7.66±2.69
P	0.655	0.2768
CEA		
≥3.5 ng/mL	48.61±30.74	10.41±3.17
<3.5 ng/mL	23.56±11.98	7.47±2.62
P	**< 0.05**	**< 0.05**
NSE		
≥16.3 ng/mL	45.76±28.98	10.22±3.27
<16.3 ng/mL	20.28±7.91	7.19±2.34
P	**< 0.05**	**< 0.01**
CYFRA21-1		
≥3.3 ng/mL	48.68±32.05	10.84±3.17
<3.3 ng/mL	25.43±13.43	7.48±2.57
P	**< 0.05**	**< 0.01**
CA153		
≥25 U/ml	38.41±30.12	8.87±2.28
<25 U/ml	35.15±24.33	7.28±3.05
P	0.7671	0.1184

tients after surgery was significantly higher than that of the healthy control group ($p < 0.05$).

Relationship between cfDNA concentration/integrity and clinical features of lung cancer patients

Analysis of the correlation between cfDNA and clinical features of lung cancer patients was performed after measuring cfDNA concentration and integrity. The results are shown in Table 2 (cfDNA concentration) and Table 3 (cfDNA integrity) . The cfDNA concentration/integrity was not associated with gender, age, tumor type, TNM stage, and expressions of CA199, CA125 and CA153 in patients with lung cancer before or after surgery ($p < 0.05$). Interestingly, the expression levels of CEA, NSE and CYFRA21-1 were associated with cfDNA concentration in lung cancer patients before and after surgery ($p < 0.05$). Furthermore, there was a significant correlation between the cfDNA integrity and CEA/CYFRA21-1 expressions in lung cancer patients before surgery ($p < 0.05$), while they were no significant difference in lung cancer patients after surgery ($p > 0.05$). Moreover, NSE expression was not correlated with cfDNA integrity in lung cancer patients before or after surgery ($p > 0.05$).

Receiver operator characteristic curve analysis of cfDNA levels in lung cancer patients

This study analyzed the ROC of different tumor markers and cfDNA in lung cancer before and after surgery. As shown in Figure 2, the ROC curves for the analyses of CEA, NSE, CYFRA21-1, cfDNA concentration and integrity were plotted. For lung cancer patients before surgery, the AUCs of the three tumor markers were as follows: 0.6982 (95% CI: 0.5862 - 0.8103) for CEA; 0.6821 (95% CI: 0.5771 - 0.7870) for NSE; and 0.7151 (95% CI: 0.6295 - 0.8006) for CYFRA21-1. The ROC curves for cfDNA concentration and integrity were 0.8209 (95% CI: 0.7375 - 0.9043), and 0.8345 (95% CI: 0.7413 - 0.9278). For lung cancer patients after surgery, the AUCs for CEA, NSE and CYFRA21-1 were 0.6990 (95% CI: 0.5695 - 0.8286), 0.6919 (95% CI: 0.5917 - 0.7921), and 0.7088 (95% CI: 0.6186 - 0.7990). The ROC curves for cfDNA concentration and integrity were 0.7958 (95% CI: 0.6994 - 0.8923) and 0.7286 (95% CI: 0.6063 to 0.8509). Before or after surgery, the AUC values of cfDNA concentration and integrity were significantly greater than CEA, NSE and CYFRA21-1 ($P<0.05$). In essence, serum cfDNA concentration and integrity had higher diagnostic values than CEA, NSE and CYFRA21-1, although those three routine tumor

Table 3. Correlation between integrity of cfDNA and clinical characteristics.

Variables	Before surgery	After surgery
Gender		
Male	4.84±2.79	1.61±0.87
Female	8.00±6.51	1.57±0.51
P	0.1015	0.8827
Age		
≥65	5.89±2.99	1.54±0.65
<65	5.82±5.84	1.68±0.92
P	0.9688	0.6115
Pathological type		
Adenocarcinoma	6.03±5.63	1.52±0.53
SCLC	5.88±4.09	1.69±1.38
squamous carcinoma	5.51±2.40	1.72±0.71
P	0.9722	0.7766
Disease stage		
I/II	5.26±2.99	1.48±0.54
III/IV	6.19±5.20	1.68±0.88
P	0.6292	0.4332
CA199		
≥39 U/ml	7.21±5.32	1.70±0.87
<39 U/ml	3.82±1.43	1.46±0.56
P	0.063	0.3557
CA125		
≥35 U/ml	6.69±5.51	1.63±0.59
<35 U/ml	5.08±3.32	1.56±0.86
P	0.380	0.8009
CEA		
≥3.5 ng/mL	8.04±5.36	1.74±0.65
<3.5 ng/mL	3.48±0.87	1.55±0.78
P	**< 0.01**	0.5345
NSE		
≥16.3 ng/mL	6.70±4.95	1.55±0.60
<16.3 ng/mL	4.35±3.24	1.61±0.82
P	0.2170	0.8307
CYFRA21-1		
≥3.3 ng/mL	8.02±5.54	1.68±0.68
<3.3 ng/mL	3.85±1.74	1.57±0.77
P	**< 0.05**	0.7324
CA153		
≥25 U/ml	6.65±5.23	1.74±0.89
<25 U/ml	5.23±3.89	1.41±0.50
p	0.4437	0.1990

biomarkers also had certain auxiliary diagnostic values for lung cancer. Thus, cfDNA concentration and integrity can be used as ideal tumor markers with high clinical application values for lung cancer.

Discussion

In 1948, Mandel and Metais first reported the presence of cell-free nucleic acid (cfDNA) substances in human blood (5). Since then cfDNA has been used in many medical fields as a non-invasive prenatal genetic testing method for evaluating fetal DNA in the maternal blood circulation, analysis of circulating cell-free tumor DNA, detection of changes in genes, provision of potential targets for cancer-targeted therapy, and design of liquid biopsy in the field of targeted cancer therapy (13, 14). In recent years, studies have shown that cfDNA concentration has potential application value in the diagnosis and prognosis of patients with NSCLC (15-18). Some of these studies indicated that high cfDNA concentrations are associated with low survival in patients with NSCLC (15, 16). Other studies have shown that cfDNA levels rose rapidly as the disease progressed, and cfDNA levels declined after resection or successful drug treatment (19 - 21), suggesting that cfDNA has broad application prospects in the treatment monitoring. In addition, some researchers have studied changes in certain genotypes of cfDNA, and their association with NSCLC treatment response and survival, leading to the discovery of tumor-specific gene mutations such as KRAS or EGFR mutations which aid in NSCLC diagnosis and prognosis (22, 23). A number of studies have found that the concentration of cfDNA changed during tumorigenesis and treatment, with commensurate changes in the structure and sequence of cfDNA. Therefore, the determination of cfDNA integrity can further improve the accuracy of cfDNA detection (24 - 27).

Blood levels of cfDNA can be used for close monitoring of disease progression, thereby providing a personalized treatment plan. Liquid biopsy allows for frequent sampling of patients, and also provides an alternative method for tissue biopsy in cases where critically ill patients and tissue specimens are limited or when tissue specimens are not available for diagnosis (28-30).

This study investigated the association between the expressions of CA199, CA125, CEA, NSE, and CYFRA21-1, and clinical features of lung cancer patients. The results showed that the expressions of CEA, NSE, and CYFRA21-1 were significantly associated with the clinical features of lung cancer patients, but their specificities were not high enough. The simplest and most effective method for screening peripheral lung cancer is CT, but central lung cancer is not easily detec-

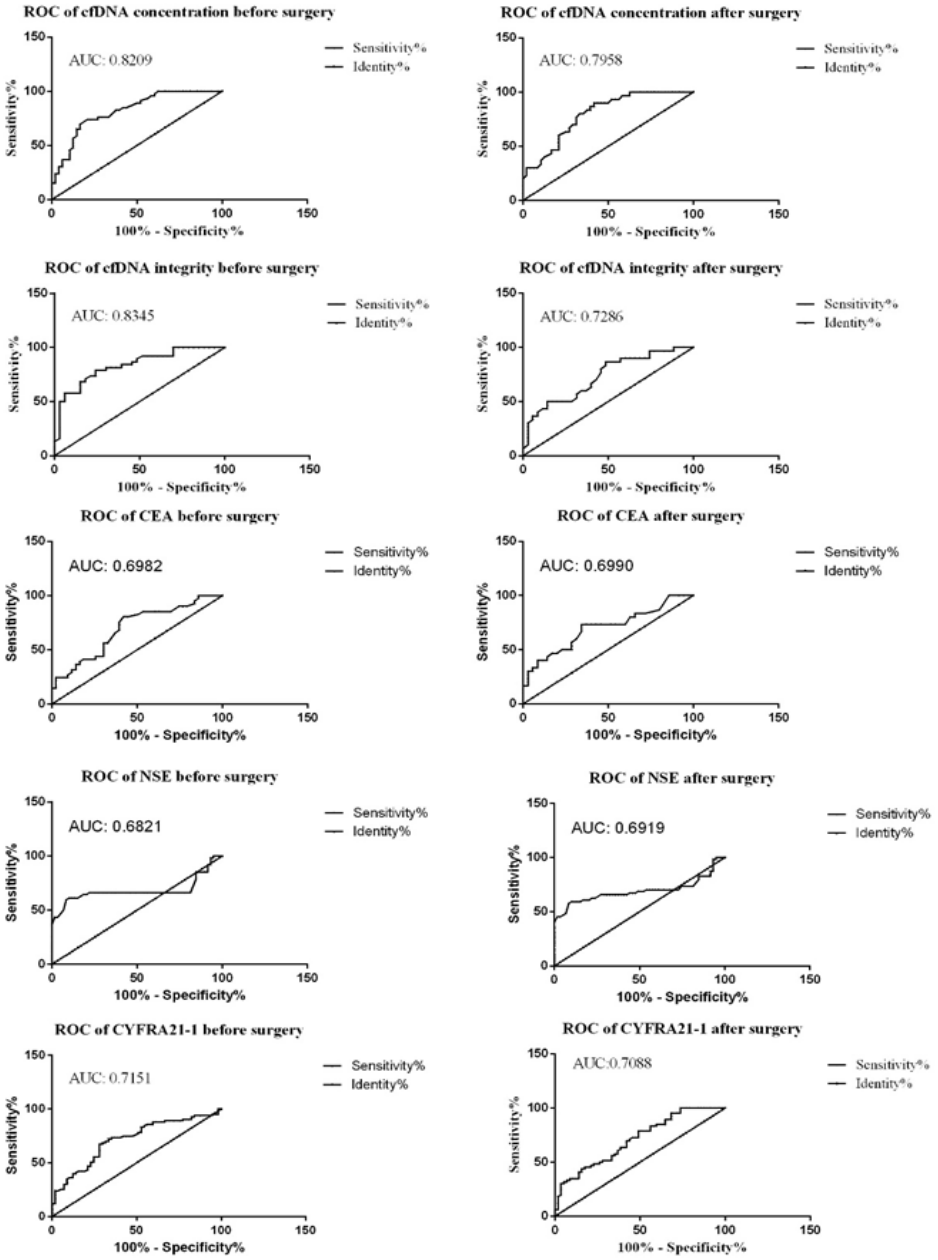

Figure 2. ROC curve analysis of tumor markers and cfDNA before and after surgery in lung cancer patients.

ted by this method, while bronchoscopy is not suitable for screening since it is a traumatic examination. Currently, tumor markers that have been used in clinical practice are associated with low sensitivity and specificity, indicating the necessity for continuous development of simpler, more sensitive and specific methods for quick screening and early diagnosis of tumors (31-33).

Studies have shown that cancer patients have three- to five-times more free DNA in the plasma than healthy people, and these free DNA contain molecular properties and changes associated with tumors (34). The free DNA fragment produced by normal apoptosis is only about 100 bp. However, in disease state such as malignant tumor, the DNA fragment produced by cell shedding and necrosis is relatively large, with lengths of about 130-180 bp, and the free DNA content of the long fragment is relatively high. Increased cfDNA concentration in some malignant tumors is closely related to tumor progression. It has been revealed that in the diagnosis of cancer, long fragment cfDNAs are more sensitive and specific than short fragment cfDNAs (35). Similarly, results from multi-segment studies for colorectal cancer support this view (36). The present study analyzed the concentration and integrity of cfDNA in lung cancer patients and healthy people, as well as in lung cancer patients before and after surgery, and initially explored the correlation between lung cancer and serum cfDNA concentration and integrity. This study is the first to quantify the concentration and integrity of cfDNA before and after surgery in healthy and lung cancer patients. The results showed that the concentration and integrity of cfDNA in the serum of patients with lung cancer were significantly higher than those in healthy people. The concentration and integrity of serum cfDNA before surgery were significantly higher than their corresponding values after surgery, suggesting that cfDNA concentration is closely related to the occurrence and treatment of lung cancer. Thus, cfDNA assay is expected to be an effective molecular method for lung cancer screening.

The present study determined the correlation between

serum cfDNA and clinicopathological features before and after surgery in lung cancer patients, and found that serum cfDNA concentration was not significantly correlated with patient gender, age, tumor type, tumor stage, and expressions of CA199, CA125, CA153. However, it was significantly correlated with the expressions of CEA, NSE and CYFRA21-1; and the cfDNA integrity of patients with lung cancer was significantly closely related to the expressions of CEA and CYFRA21-1, suggesting that cfDNA is a reflection of active cancer proliferation.

To assess the specificity and sensitivity of cfDNA, ROC curves with three other tumor markers (CEA, NSE and CYFRA21-1) were used. The results confirmed that the AUCs of cfDNA before and after surgery were greater than those of CEA, NSE and CYFRA21-1, suggesting that cfDNA is more suitable as a diagnostic marker for lung cancer than CEA, NSE and CYFRA21-1.

Changes in peripheral blood cfDNA concentration, sequence integrity, and specific changes including oncogenes/tumor suppressor gene mutations, hypermethylation, microsatellite changes and chromosomal rearrangements are closely related to tumors. Compared with traditional histopathology, cytology, spiral CT and other examination methods, serum cfDNA has the advantages of simplicity, speed, minimal invasiveness and economy. Moreover, it has the potential value of assisting in early screening, prognosis evaluation and efficient monitoring of lung cancer indicators. Due to the high level of false positive results induced by high sensitivity of the cfDNA detection method, influence of different cfDNA extraction procedures on the detection results, and non-specific increase in cfDNA caused by non-neoplastic diseases, there are still many controversies about how to make cfDNA ideal for lung cancer diagnosis. Therefore, before cfDNA is widely used in clinical practice, researchers need to carry out more studies. However, it is believed that with support from a large number of basic and clinical studies, and improvements in technology and equipment, cfDNA-based liquid biopsy technique will play an increasingly important role in lung cancer diagnosis and screening.

This study is the first to analyze the serum cfDNA concentration and integrity before and after surgery in lung cancer patients, providing new ideas for the diagnosis and screening of lung cancer. It is expected to become a new diagnostic indicator for lung cancer. However, this study collected samples from pre-operative and post-operative lung cancer patients from the same hospital at the same time, with a small sample size. Although the results indicated that lung cancer has a certain correlation with cfDNA concentration and integrity, it is still necessary to collect a larger number of patient samples from different regions with larger time spans for verification, so as to obtain more comprehensive and reliable experimental results.

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Conflict of Interest
There are no conflict of interest in this study.

Author's contribution
All work was done by the authors named in this article and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Shiying Zheng. Yihui Fan, Minxin Shi, Guanjun Ju, Saihua Chen, Lingxiang Chen, Haimin Lu, Jian Chen, Shiying Zheng collected and analyzed the data. Yihui Fan wrote the manuscript. All authors read and approved the manuscript prior to publication.

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