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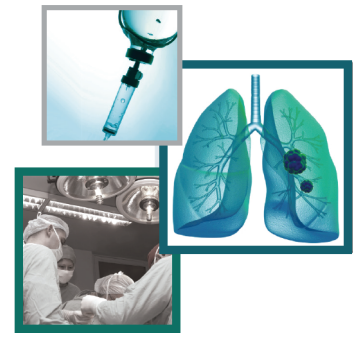
Lung Cancer Management



Diagnostic value of combined detection of plasma cfDNA concentration and integrity in NSCLC

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Aim: To evaluate the value of combined detection of plasma cfDNA concentration and integrity in the early diagnosis of NSCLC. **Methods:** Real-time fluorescence quantitative PCR was used to determine the concentration and integrity of plasma cfDNA in 71 NSCLC patients and 53 healthy people. **Results:** Combined detection of plasma cfDNA concentration and integrity had higher diagnostic power in differentiating NSCLC patients with stage I/II from healthy people than detection of plasma cfDNA concentration alone or integrity alone. The AUC, sensitivity and specificity of the combined detection of plasma cfDNA concentration and integrity were 0.781, 0.62 and 0.85. **Conclusion:** Combined detection of plasma cfDNA concentration and integrity could improve the diagnostic value in NSCLC detection.

Plain language summary: The discovery of cfDNA has opened up a wide range of new possibilities for the diagnosis of cancer. CfDNA provides a noninvasive diagnostic approach for early screening, early detection and monitoring of patients with cancer. Currently, the application of cfDNA in clinical practice for NSCLC patients has been widely reported, which mainly focused on DNA methylation detection, oncogenic driver gene mutation detection. However, few studies have evaluated the diagnostic value of combined detection of plasma cfDNA concentration and integrity for NSCLC patients. Our study suggests that the combination of plasma cfDNA concentration and integrity has higher AUC value in differentiating NSCLC patients from healthy individuals than plasma cfDNA concentration alone or integrity alone.

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Keywords: LINE1 element • non-small-cell lung cancer • plasma cfDNA • quantitative real-time PCR

Lung cancer continues to be the primary cause of cancer-related deaths globally. In the 2020 year, it was estimated that there were approximately 1.8 million deaths, accounting for 18.00% of all cancer deaths [1]. Lung cancer is mainly made up of two histologic subtypes: SCLC and NSCLC, with the latter accounting for about 80% of all lung cancer cases [2]. The early symptoms of lung cancer are often not easily recognizable, leading to most people at an advanced stage when diagnosed. Therefore, the crucial factors for improving the survival rate of lung cancer are early detection, early diagnosis and early treatment.

Currently, the primary methods used to screen for early lung cancer include chest x-ray, chest computed tomography (CT) and blood tumor markers [3–8]. However, chest x-ray has limited effectiveness in detecting small nodules, chest CT can be costly, and blood tumor markers have lower specificity in the early detection of lung cancer, therefore, it is crucial to develop new screening strategies for lung cancer.

Recently, blood-derived circulating cell-free DNA (cfDNA) has gained recognition as a promising biomarker for detecting various malignancies [9–12]. It is widely recognized that apoptotic cells are the primary source of cfDNA in healthy individuals. The size of cfDNA fragments released by apoptotic cells usually ranges from 185 to 200 bp [13]. In contrast, the main source of cfDNA in cancer patients were necrotic cells, apoptotic cells and autophagy, thus, the size of cfDNA fragments in cancer patients varies [14,15]. Therefore, detecting the concentration levels of different lengths of cfDNA could be a promising biomarker for malignant tumor. Numerous studies have

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found that the concentration of cfDNA was higher in various malignancies such as prostate cancer [16], colorectal cancer [17], breast cancer [18], lung cancer [19] and hepatocellular carcinoma [20]. Furthermore, the cfDNA integrity index, which was calculated as the ratio of longer DNA fragments to shorter DNA fragments, was also significantly higher in various cancer patients than in healthy individuals [21–24]. These findings suggested that plasma/serum cfDNA concentration or integrity had important clinical value in the detection and prognosis of malignant tumor.

Currently, the application of liquid biopsy in clinical practice for NSCLC patients has been widely reported, which mainly focused on DNA methylation detection [25,26], oncogenic driver gene mutation detection [27,28]. Although there have been some studies of the cfDNA concentration or integrity in NSCLC, little is known about the diagnostic value of combined detection of plasma cfDNA concentration and integrity in NSCLC patients. The objective of this study was to assess the diagnostic value of combined plasma cfDNA concentration and integrity for the early detection of NSCLC. The *LINE1* sequence was chosen as the target due to its high abundance in the human genome, accounting for 17% of the genome [29]. Therefore, quantitative real-time PCR (qPCR) of *LINE1* elements can significantly improve the cfDNA detection accuracy. This study measured plasma cfDNA concentration and integrity in 71 NSCLC patients and 53 healthy controls. Our results showed that the plasma cfDNA concentration (*LINE1*-97 bp and *LINE1*-266 bp) in NSCLC patients was significantly higher than that of healthy controls, while the plasma cfDNA integrity (*LINE1*-266/97) in NSCLC patients was significantly lower than that of healthy controls. In addition, the combined detection of plasma cfDNA concentration and integrity can significantly improve the accuracy of early NSCLC diagnosis.

Materials & methods

Plasma samples & clinical pathological information

Plasma samples from 53 healthy People and 71 patients with NSCLC, including stage I (n = 23), II (n = 14), III (n = 14), and IV (n = 20) were assessed. Blood was drawn before therapeutic intervention. Staging was based on postoperative histopathology findings for stage 0 to III, and imaging diagnoses were used for stage IV. All NSCLC patients are staged using the Union for International Cancer Control (UICC) criteria. Patients with NSCLC were selected by the database coordinator based on those patients treated between 2020 and 2023 at Daping Hospital of Army Medical University. All participants in this study provided written informed consent before enrollment. This study was approved by the Medical Ethics Committee of Daping Hospital of Army Medical University and was carried out according to the relevant guidelines and regulations.

Plasma samples processing & cfDNA extraction

Two milliliter (2 mL) of antecubital venous blood was collected in EDTA-containing tubes before therapeutic intervention. To eliminate cellular residues as much as possible, all blood samples were centrifuged at 1000 × g for 10 min and then 10000 × g for 10 min. The supernatant plasma was immediately stored at -80°C until extraction. Then, cfDNA was extracted from 1 mL of plasma and eluted in 60 µL of H_2O by using QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany) according to the manufacturer's instructions. The extracted plasma cfDNA was stored at -80°C until use.

Quantification of *LINE1* elements by qPCR

The target of amplification in this study was the human *LINE1* repeat sequence (Gene ID: 54596). The qPCR of *LINE1* elements (*LINE1*-qPCR) was performed using two different primer sets, amplifying short (*LINE1*-97 bp) and long (*LINE1*-266 bp) products, respectively. The primer sets for *LINE1*-97 bp and *LINE1*-266 bp amplicons were obtained from the literature [20]. The *LINE1*-97 bp primer sets amplified both long and short DNA fragments, and the *LINE1*-266 bp primer sets only amplified long DNA fragments. The reaction mixture for each *LINE1*-qPCR consisted of 2 µL of cfDNA template, 2 µL of the forward and reverse primers (0.5 µM), 10 µL of FastStart Essential DNA Green Master (Roche, Switzerland) and 6 µL of RNase – free water in a total of 20 µL volumes. The qPCR amplification was performed with precycling heat activation of DNA polymerase at 95°C for 10 min, followed 40 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 30 sec and extension at 60°C for 30 sec in a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). The absolute equivalent amount of *LINE1* fragments in each sample was determined by using a standard curve with serial dilutions (i.e., 10 ng to 1 pg) of prepared genomic DNA from healthy volunteers' peripheral blood leukocytes. A negative control (no cfDNA template) was also performed in each reaction plate. All qPCR assays were performed without knowing the identity of the specimen, and mean values were calculated from duplicate reactions.

Table 1. The clinical characteristics of patients with NSCLC.

Clinical characteristics	NSCLC patients (n = 71)	
	No.	%
Age (years)		
≤60	34	47.89
>60	37	52.11
Sex		
Male	41	57.75
Female	30	42.25
UICC primary tumor		
T1	26	36.62
T2	22	30.99
T3	10	14.08
T4	13	18.31
UICC regional lymph nodes		
N0	39	54.93
N1	10	14.08
N2	11	15.49
N3	11	15.49
UICC distant metastasis		
M0	51	71.83
M1	20	28.17
UICC stage		
I	23	32.39
II	14	19.72
III	14	19.72
IV	20	28.17

UICC: Union for International Cancer Control.

Measurement of plasma cfDNA integrity

Because the LINE1-97-qPCR results represent the total amount of cfDNA; LINE1-266-qPCR results represent amounts of cfDNA released from tumor cells. Therefore, plasma cfDNA integrity index was calculated as the ratio of *LINE1*-qPCR results (*LINE1*-266-qPCR/LINE1-97-qPCR). Because the annealing sites of LINE1-97 bp amplicons were within the LINE1-266 bp amplicons annealing sites, thus the qPCR ratio (DNA integrity) is 1.0 when plasma cfDNA template was not truncated and 0.0 when all plasma cfDNA templates was truncated into fragment smaller than 266 bp. Because the LINE1-97 bp primer sets can amplify most fractions of plasma cfDNA, thus qPCR results obtained with LINE1-97 primer sets represent the total amount of plasma cfDNA.

Statistical analysis

The concentration and integrity values of plasma cfDNA between the NSCLC patients (stage I/IV) and healthy control groups were compared using Mann-Whitney U test. Mean values for healthy controls and NSCLC patients with early and advanced stage groups (i.e., stage I/II and stage III/IV) were compared using Dunnett's multiple comparison tests. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) analysis were used to evaluate the clinical diagnosis value of plasma cfDNA in differentiating NSCLC patients from healthy controls. The SPSS software (version 22.0) was used for all statistical analysis, and the figures were generated by using the GraphPad Prism software (version 7.0). P value < 0.05 (two-tailed) was considered statistically significant.

Results

Clinical & pathologic characteristics of patients with NSCLC

There were 71 NSCLC patients (i.e., 41 males and 30 females) and 53 healthy controls in this research. The mean age was 48.36 and 60.90 years for healthy controls and NSCLC patients, respectively. The clinical and pathologic characteristics of patients with NSCLC were shown in Table 1. According to the Tumor Node Metastasis (TNM)

Table 2. Comparison of plasma cfDNA concentration, integrity between NSCLC patients and healthy controls.

	Controls	Stage I/II	Stage III/IV	Stage I/IV
No. pts	53	37	34	71
LINE1-97 (ng/ml):				
– Mean	12.40	20.94	23.10	21.97
– Median	12.14	19.47	21.20	20.00
– p-value		<0.0001	<0.0001	<0.0001
LINE1-266 (ng/ml):				
– Mean	3.19	4.36	4.00	4.19
– Median	2.96	3.90	3.79	3.80
– p-value		0.0296	0.0510	0.009
LINE1-266/97 (ng/ml):				
– Mean	0.29	0.24	0.22	0.23
– Median	0.26	0.19	0.20	0.19
– p-value		0.0411	0.0310	0.0025

staging system, 71 NSCLC patients were defined as stage I (n = 23), stage II (n = 14), stage III (n = 14), and stage IV (n = 20), respectively. Among 71 NSCLC patients, 32 cases (i.e., 32/71; 45.07%) had regional lymph node metastases (LNM) and 20 cases (i.e., 20/71; 28.17%) had distant metastasis.

Comparison of cfDNA concentration between NSCLC patients & healthy controls

To compare the concentration levels of plasma cfDNA between NSCLC patients and healthy controls, the concentration of LINE1-97 bp and LINE1-266 bp fragments were quantified. The mean concentration of LINE1-97 fragment in NSCLC patients with stage I/II, stage III/IV, and in healthy controls were 20.94, 23.10 and 12.40 ng/ml, respectively (Table 2). The mean values of LINE1-97 fragment were significantly higher in NSCLC patients with stage I/II ($p < 0.0001$) and stage III/IV ($p < 0.0001$) than in healthy controls (Figure 1). However, there was no statistically significant difference between patients with stage I/II and stage III/IV ($p = 0.7893$). Similarly, the mean concentration of LINE1-266 fragment in NSCLC patients with stage I/II and stage III/IV, and in healthy controls were 4.36, 4.00 and 3.19 ng/ml, respectively (Table 2). The mean value of LINE1-266 fragment was significantly higher in NSCLC patients with stage I/II than in healthy controls ($p = 0.0296$, Figure 2). However, no statistically significant difference was found between patients with stage I/II and stage III/IV ($p = 0.8454$).

In addition, the area under the ROC curve (AUC) for discriminating NSCLC patients with stage I/II, stage III/IV from healthy controls by *LINE1* fragments concentrations were 0.772 (95% CI: 0.673–0.871), 0.796 (95% CI: 0.692–0.900), and 0.632 (95% CI: 0.508–0.756), 0.642 (95% CI: 0.523–0.762), respectively (Table 3). When the cut-off value >18.73 ng/ml, the sensitivity and specificity of the LINE1-97 fragment in distinguishing NSCLC patients with stage I/II from healthy controls were 0.59 and 0.87, respectively. Similarly, for *LINE1*-266 fragment, the sensitivity and specificity are 0.70 and 0.60 when the cut-off value >3.23 ng/ml. These findings indicated that quantification of *LINE1* fragments at various sizes could be used to distinguish patients with NSCLC from healthy controls.

Comparison of cfDNA integrity between NSCLC patients & healthy controls

Plasma cfDNA integrity was calculated as the ratio of qPCR results (*LINE1*-266/*LINE1*-97). The mean plasma cfDNA integrity in NSCLC patients with stage I/II, stage III/IV and in healthy controls were 0.24, 0.22 and 0.29, respectively (Table 2). The mean value were significantly higher in healthy controls than in stage I/II and in stage III/IV NSCLC patients ($p = 0.0411$ and $p = 0.0310$, Figure 3). However, there was no statistically significant difference between patients with stage I/II and stage III/IV ($p = 0.999$).

Furthermore, the AUC of the ROC curve for discriminating stage I/II, stage III/IV NSCLC patients from healthy controls by plasma cfDNA integrity were 0.655 (95% CI: 0.533–0.776) and 0.661 (95% CI: 0.541–0.782) (Table 3). When the cut-off value >0.2, the sensitivity and specificity of the cfDNA integrity *LINE1*-266/97 in differentiating NSCLC patients with stage I/II from healthy controls were 0.54 and 0.77, respectively. These findings suggested that plasma cfDNA integrity could be used to distinguish NSCLC patients from healthy controls.

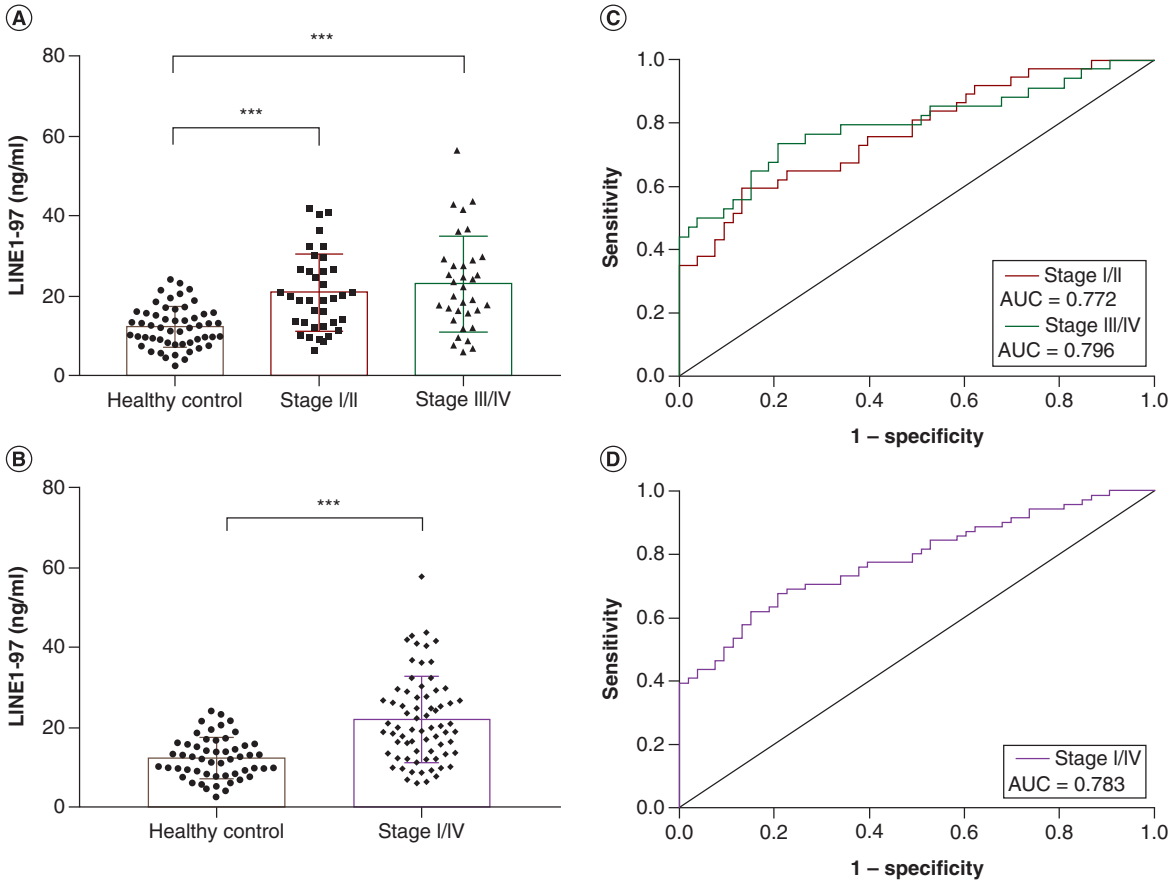

Figure 1. Comparison of plasma cfDNA concentration in NSCLC patients and healthy controls. (A) The concentration of *LINE1*-97 bp fragment was significantly higher in NSCLC patients with stage I/II and stage III/IV than that of healthy controls. **(B)** The concentration of *LINE1*-97 bp fragment was significantly higher in NSCLC patients with stage I/IV than that of healthy controls. **(C & D)** Receiver operating characteristic curves for distinguishing NSCLC patients from healthy controls.
AUC: Area under the curve.

Correlation between plasma cfDNA & lymph node metastasis in NSCLC patients

Lymph node metastasis (LNM) is one of the most common forms of lung cancer metastatic and is an important factor in the staging and prognosis of lung cancer. To explore the correlation between plasma cfDNA and lymph node metastasis, we compared plasma cfDNA concentration and its integrity between LNM-positive patients and LNM-negative patients.

In 39 LNM-negative patients and 32 LNM-positive patients, the concentrations of plasma cfDNA (*LINE1*-97 and *LINE1*-266) were 22.68, 21.11 ng/ml, and 4.36, 3.97 ng/ml, respectively. The AUC of ROC curve for distinguishing LNM-positive patients from LNM-negative patients by *LINE1* fragments concentrations were 0.532 (95% CI: 0.396–0.668) and 0.506 (95% CI: 0.370–0.641). In addition, the plasma cfDNA integrity values in 39 LNM-negative patients and 32 LNM-positive patients were 0.23 and 0.23, respectively. The AUC of ROC curve for distinguishing LNM-positive patients from LNM-negative patients by plasma cfDNA integrity was 0.538 (95% CI: 0.403–0.673). However, there was no significant difference in plasma cfDNA concentration or integrity between LNM-positive patients and LNM-negative patients (Figure 4). These results demonstrated that there is no significant correlation between plasma cfDNA and lymph node metastasis in NSCLC patients.

Diagnostic value of combined plasma cfDNA concentration & integrity in NSCLC patients

To explore the diagnostic value of combined plasma cfDNA concentration and integrity for early detection of NSCLC, the sensitivity, specificity and AUC were evaluated. The sensitivity, specificity and AUC of the combination of *LINE1*-97 + *LINE1*-266, *LINE1*-97 + *LINE1*-266/97 and *LINE1*-97 + *LINE1*-266 + *LINE1*-

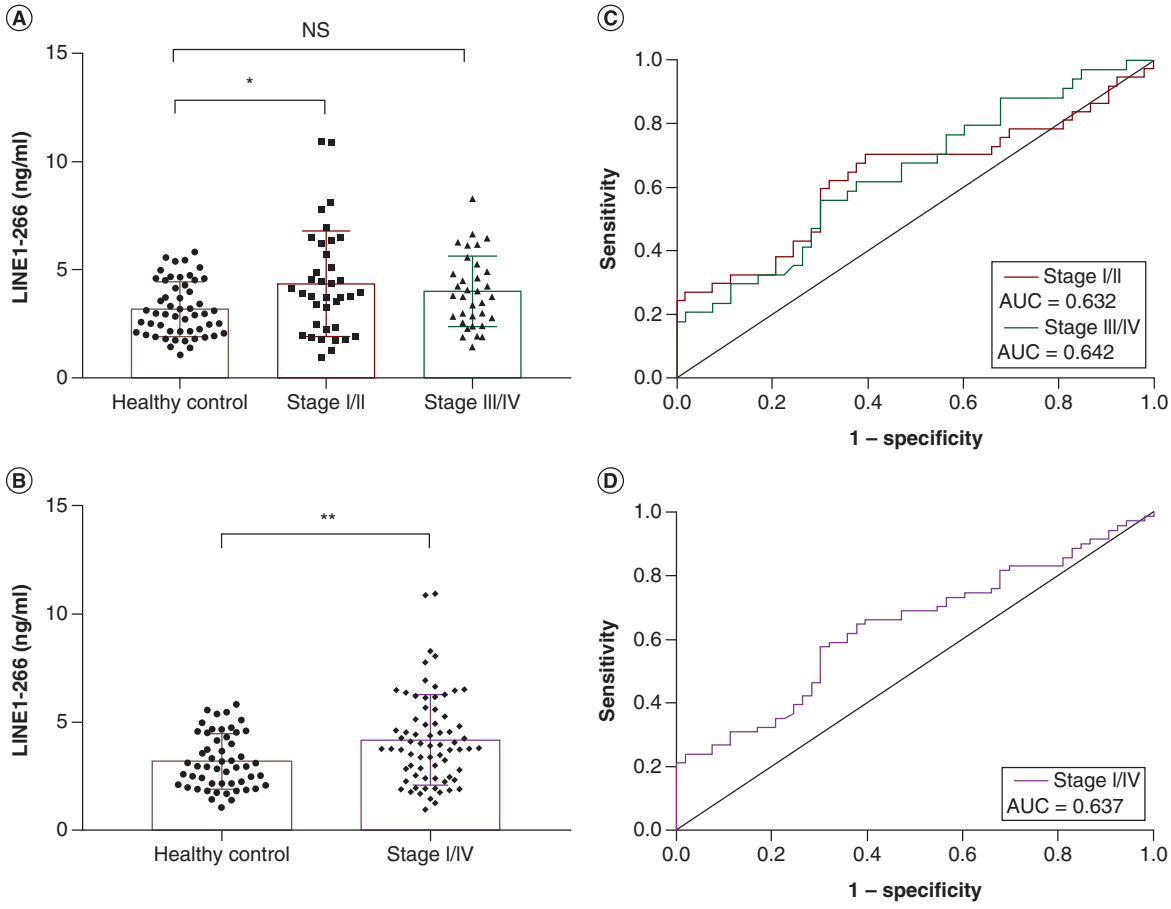

Figure 2. Comparison of plasma cfDNA concentration in NSCLC patients and healthy controls. (A) The concentration of *LINE1*-266 bp fragment was significantly higher in NSCLC patients with stage I/II than that of healthy controls. **(B)** The concentration of *LINE1*-266 bp fragment was significantly higher in NSCLC patients with stage I/IV than in healthy controls. **(C & D)** Receiver operating characteristic curves for distinguishing NSCLC patients from healthy controls. AUC: Area under the curve.

Table 3. Diagnostic value of plasma cfDNA concentration and integrity in non-small-cell lung cancer patients.

	Cut-off (ng/ml)	Sensitivity	Specificity	AUC (95% CI)
LINE1-97:				
Stage I/II	18.73	0.59	0.87	0.772 (0.673–0.871)
Stage III/IV	16.30	0.74	0.79	0.796 (0.692–0.900)
Stage I/IV	17.56	0.62	0.85	0.783 (0.705–0.862)
LINE1-266:				
Stage I/II	3.23	0.70	0.60	0.632 (0.508–0.756)
Stage III/IV	3.73	0.56	0.70	0.642 (0.523–0.762)
Stage I/IV	3.73	0.58	0.70	0.637 (0.540–0.734)
LINE1-266/97:				
Stage I/II	0.20	0.54	0.77	0.655 (0.533–0.776)
Stage III/IV	0.20	0.50	0.77	0.661 (0.541–0.782)
Stage I/IV	0.20	0.52	0.77	0.658 (0.563–0.753)

AUC: Area under the curve.

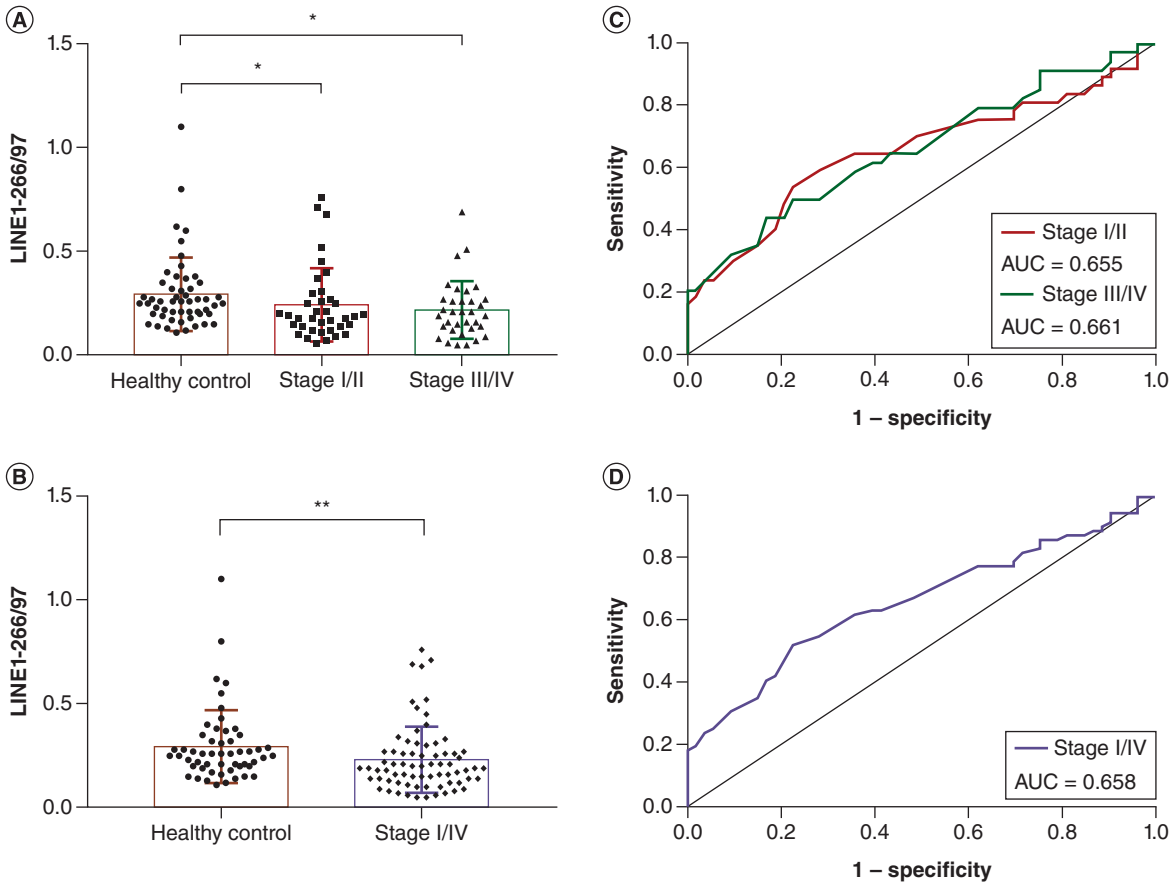

Figure 3. Comparison of plasma cfDNA integrity in NSCLC patients and healthy controls. (A) The plasma cfDNA integrity *LINE1*-266/97 was significantly lower in NSCLC patients with stage I/II and stage III/IV than in healthy controls. **(B)** The integrity of *LINE1*-266/97 was significantly lower in NSCLC patients with stage I/IV than in healthy controls. **(C & D)** Receiver operating characteristic curves for distinguishing NSCLC patients from healthy controls. AUC: Area under the curve.

266/97 were the same, namely 0.62, 0.85 and 0.781, respectively (Table 4). Furthermore, the sensitivity, specificity and AUC of the combination of *LINE1*-266 + *LINE1*-266/97 were 0.73, 0.62 and 0.733, respectively (Table 4). Particularly, the combinations of *LINE1*-97 + *LINE1*-266, *LINE1*-97 + *LINE1*-266/97 and *LINE1*-97 + *LINE1*-266 + *LINE1*-266/97 had higher AUC values than that of *LINE1*-97 alone, *LINE1*-266 alone, and *LINE1*-266/97 alone (Figure 5). These results demonstrated that the combined detection of plasma cfDNA concentration and integrity can improve the accuracy of early diagnosis of NSCLC.

Discussion

cfDNA is a degraded double-stranded DNA fragment, which present in the plasma/serum, urine and other bodily fluids of humans. It's well known that cfDNA has significant clinical value in the early diagnosis and treatment of many malignant tumors [9–12,30–32]. Plasma cfDNA concentration and integrity may represent a rapid and noninvasive biomarker, which provides important complementary information for diagnosis, monitoring and prognosis of cancer patients. Currently, there were many studies on the diagnostic value of cfDNA concentration or integrity alone in cancer patients, little is known about the diagnostic value of combined detection of plasma cfDNA concentration and integrity in cancer patients [16–24]. Therefore, to assess the diagnostic value of combined plasma cfDNA concentration and integrity for the early detection of NSCLC, the sensitivity, specificity and AUC were evaluated.

In this present study, the *LINE1* sequence was chosen as the target due to its high abundance in the human genome, accounting for 17% of the genome, therefore, quantitative real-time PCR (qPCR) of *LINE1* elements can significantly improve the cfDNA detection accuracy [29]. Our findings suggested that the concentration and

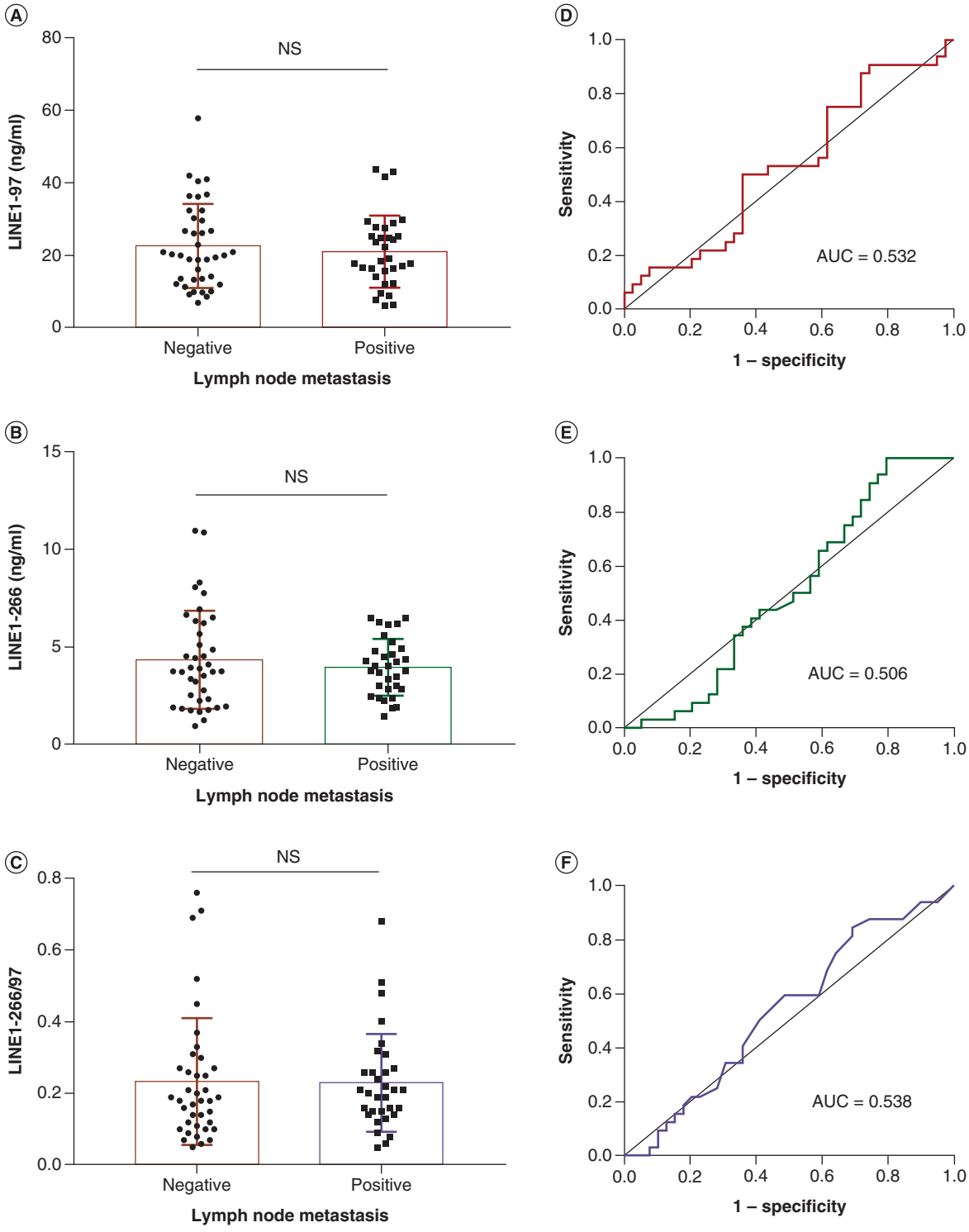

Figure 4. Correlation between plasma cfDNA and lymph node metastasis in NSCLC patients. (A–C) There was no significant difference in plasma cfDNA concentration and integrity between lymph node metastasis (LNM)-positive and LNM-negative patients. **(D–F)** Receiver operating characteristic curves for distinguishing LNM-positive patients from LNM-negative patients.
AUC: Area under the curve.

Table 4. Diagnostic value of combined plasma cfDNA concentration and integrity for NSCLC patients.

Marker	AUC	Sensitivity	Specificity	p-value
LINE1-97	0.772	0.59	0.87	<0.0001
LINE1-266	0.632	0.70	0.60	0.0296
LINE1-266/97	0.655	0.54	0.77	0.0411
LINE1-97 + *LINE1*-266	0.781	0.62	0.85	<0.0001
LINE1-97 + *LINE1*-266/97	0.781	0.62	0.85	<0.0001
LINE1-266 + *LINE1*-266/97	0.733	0.73	0.62	<0.0001
LINE1-97 + *LINE1*-266 + *LINE1*-266/97	0.781	0.62	0.85	<0.0001

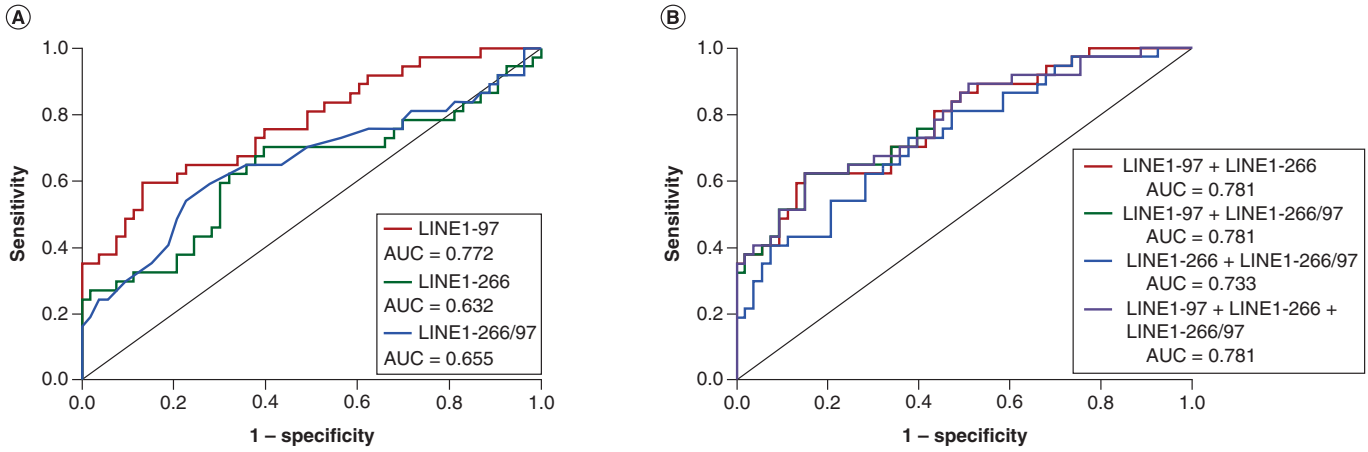

Figure 5. Diagnostic values of combined plasma cfDNA concentration and integrity in NSCLC patients. (A) ROC curves of *LINE1*-97, *LINE1*-266 and *LINE1*-266/97 for distinguishing NSCLC patients with stage I/II from healthy controls. **(B)** ROC curves of the combination of plasma cfDNA concentration (*LINE1*-97, *LINE1*-266) and integrity (*LINE1*-266/97) for distinguishing NSCLC patients with stage I/II from healthy controls.
AUC: Area under the curve.

integrity of plasma cfDNA were not associated with demographic characteristics (i.e., age and gender) in healthy control group. Meanwhile, the concentration of plasma cfDNA was also not related to age or gender in NSCLC patients group. These results were consistent with previous studies [33], namely plasma cfDNA concentration was not associated with age and gender in healthy controls group and in NSCLC patients group. However, there were some opposite opinions. For instance, Jylhava's study found significant age-related differences in the women, namely, older women had higher plasma cfDNA level than younger controls [34]. Moreover, Sozzi's study revealed that plasma cfDNA concentration had a significant age-related difference in NSCLC patients and in healthy controls [35]. These results suggested that the potential effects of age or gender on plasma cfDNA level have not been consistently demonstrated, thus larger cohort studies involving cancer patients and matched case-control subjects will need to be conducted.

Several studies have demonstrated that the concentration of plasma cfDNA was increased in various cancers including prostate cancer [16], colorectal cancer [17], breast cancer [18], lung cancer [19] and hepatocellular carcinoma [20]. In this study, the concentration of plasma cfDNA in patients with NSCLC was markedly higher than in healthy controls, which was consistent with these previous findings. Meanwhile, we observed that *LINE1*-97 fragment had higher specificity, sensitivity and AUC values in distinguishing NSCLC patients from healthy controls compared with *LINE1*-266 fragment (0.85 vs 0.70, 0.62 vs 0.58, and 0.783 vs 0.637). Specifically, *LINE1*-97 fragment had higher specificity and AUC values in distinguishing patients with stage I/II NSCLC from healthy controls than *LINE1*-266 fragment (0.87 vs 0.60, 0.772 vs 0.632). These findings indicated that quantification of *LINE1*-97 fragment is more effective in the early detection of NSCLC.

Furthermore, the integrity of plasma cfDNA *LINE1*-266/97 was lower in patients with stage I/II and stage III/IV than in healthy individuals, which was consistent with previous research [36,37]. Impressively, plasma cfDNA integrity *LINE1*-266/97 had lower specificity, sensitivity and AUC values than plasma cfDNA concentration

(*LINE1*-97) in differentiating patients with stage I/II NSCLC from healthy controls (0.77 vs 0.87, 0.54 vs 0.59, and 0.655 vs 0.772). Therefore, plasma cfDNA integrity was not competitive enough in the early detection of NSCLC patients. However, we observed that the integrity of plasma/serum cfDNA was significantly higher in most cancers than healthy controls and benign disease groups. The reasons for this discrepancy are as follows: one possible explanation is that the type and length of target fragment vary in different studies; another possible explanation is that cfDNA was degraded at different rates in various kinds of cancers, resulting in the value of cfDNA integrity varies; the final possible explanation is that the sample size of the current studies is too small to draw a unified conclusion.

What's more, our findings revealed that plasma cfDNA concentration and integrity were not associated with lymph node metastasis, which was inconsistent with our previous results [38,39]. There are many factors accounting for this phenomenon, the following are the most typical ones. Firstly, the number of LNM-negative patients in the blood group was higher than that in the urine group, which may affect the correlation between cfDNA concentration and lymph node metastasis. Secondly, the component of blood samples is more complex than that of urine, which may result in different results. Finally, cfDNA in urine is degraded more rapidly than cfDNA in plasma, which may affect the results. Therefore, more studies are needed to determine whether the plasma cfDNA concentration was associated with lymph node metastasis.

Although plasma cfDNA concentration or integrity has been extensively studied in cancers, little is known about the combined diagnostic effect of plasma cfDNA concentration and its integrity in the early of cancers. To investigate the diagnostic value of combined plasma cfDNA concentration and integrity for early detection of NSCLC, the sensitivity, specificity and AUC values were assessed. Our results revealed that the combination of *LINE1*-97 + *LINE1*-266 had higher AUC values compared with *LINE1*-97 or *LINE1*-266 fragment (Table 4). Moreover, the combination of *LINE1*-97 + *LINE1*-266/97, *LINE1*-266 + *LINE1*-266/97 had higher AUC values compared with *LINE1*-266/97 (Table 4). Similarly, the combination of *LINE1*-97 + *LINE1*-266 + *LINE1*-266/97 had higher AUC value compared with *LINE1*-97, *LINE1*-266 or *LINE1*-266/97. These results demonstrated that the combined detection of plasma cfDNA concentration and integrity can significantly improve the accuracy of early NSCLC diagnosis.

To our knowledge, there have been no studies evaluating the diagnostic value of the combination of plasma cfDNA concentration and integrity for early detection of NSCLC. Few studies on the concentration and integrity of plasma cfDNA in the diagnosis of NSCLC were observed. For instance, Leng's research showed that the AUC of the plasma cfDNA concentration (*ALU*-115) and integrity (*ALU*-247/115) in differentiating NSCLC patients from healthy individuals were 0.747 and 0.759, respectively. Compared with Leng's research [33], our study has slightly higher AUC values (0.781 vs 0.747, 0.781 vs 0.759). Soliman's study revealed that the AUC of the plasma cfDNA integrity (*ALU*-247/115) in distinguishing NSCLC patients from healthy individuals was 0.65 [40]. Our results have a higher AUC value (0.781 vs 0.65) compared with plasma cfDNA integrity. Szpechcinski's research showed that the plasma cfDNA concentration and integrity had slightly higher AUC value than our rseults (0.80 vs 0.781) [19]. Therefore, based on these results, we considered that the combined detection of plasma cfDNA concentration and integrity is more competitive than either plasma cfDNA concentration alone or integrity alone.

Conclusion

This study shows that plasma cfDNA *LINE1* fragments concentration were significantly higher in NSCLC patients than that of healthy controls. Plasma cfDNA integrity was significantly lower in NSCLC patients than that of healthy controls. In addition, the combined detection of plasma cfDNA concentration and integrity may be a promising biomarker for early diagnosis of NSCLC.

Author contributions

Q Huang was responsible for study conception and design; S Ren and C Yu were responsible for specimen collection; S Ren and C Yu were responsible for conducting experiments and data analysis, S Ren and Q Huang were responsible for drafting and revision of the manuscript.

Financial disclosure

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financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was approved by the Medical Ethics Committee of Daping Hospital of Army Medical University and was carried out according to the relevant guidelines and regulations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Summary points

- The concentration of *LINE1*-97 fragment was significantly higher in NSCLC patients than that of healthy controls.
- The concentration of *LINE1*-266 fragment was significantly higher in NSCLC patients than that of healthy controls.
- The AUC of ROC curve for distinguishing NSCLC patients with stage I/II from healthy controls by the combination of *LINE1*-97 + *LINE1*-266 was 0.781.
- Plasma cfDNA integrity *LINE1*-266/97 were significantly lower in NSCLC patients with stage I/II and stage III/IV than in healthy controls.
- The AUC of ROC curve for distinguishing NSCLC patients with stage I/II from healthy controls by *LINE1*-97 + *LINE1*-266 + *LINE1*-266/97 was 0.781.
- Combined detection of plasma cfDNA concentration and integrity can significantly improve the accuracy of NSCLC early detection.

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