

# Plasma D-dimer value as a predictor of malignant lymph node involvement in operable non-small cell lung cancer

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Received: 13 March 2015 / Accepted: 30 April 2015 / Published online: 19 June 2015  
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**Abstract** Fibrin deposition and remodelling of the extracellular matrix are important early steps in tumour metastasis. The D-dimer value is an indicator of intravascular fibrin formation and degradation. Thus, the D-dimer value may be a predictor of the malignant involvement of lymph nodes in operable non-small cell lung cancer (NSCLC) patients. The study comprised 142 highly suspected lung cancer patients scheduled to undergo pneumonectomy, lobectomy or wedge resection. Of the 142 patients, 124 were subsequently diagnosed as NSCLC, and 18 were subsequently diagnosed as benign lung disease by histological examination. Preoperative plasma D-dimer values were quantified, and the relationship between plasma D-dimer and clinical variables including tumour size, involvement of lymph nodes and clinical stage was examined using Spearman correlation coefficients and  $\chi^2$  tests. The median plasma D-dimer values were statistically higher in NSCLC patients with malignant lymph nodes than in those who suffered either benign lung disease or carcinoma in situ (Kruskal–Wallis test;  $P=0.001$ ). Plasma D-dimer values were significantly correlated with clinical stage (ANOVA;  $P=0.009$ ). An obvious relationship was observed between elevated D-dimer ( $>0.475$  mg/L fibrinogen equivalent units) and malignant lymph node involvement ( $\chi^2$  test;  $P=0.0000$ ). This correlation suggests that the plasma D-dimer

value is a clinically important predictor for the malignant involvement of lymph nodes in operable NSCLC.

**Keywords** D-dimer · Non-small cell lung cancer · Lymph node · Metastasis

## Introduction

The relationship between cancer and coagulation is characterised by several related mechanisms, indicating that these processes are closely linked [1]. It is now known that cancer is frequently associated with clotting activation, such as low-grade disseminated intravascular coagulation or venous thromboembolism. This connection may result from the cancer itself or the therapeutic treatments used. Gastrointestinal, lung and pancreatic cancers are considered more likely to induce hyper-coagulation in patients [2–4]. Rather than being merely a trigger of increased thromboembolic events, cancer-induced haemostatic activity has been shown to promote cancer cell dissemination [5–7].

Dissemination of a tumour from its primary location requires several key events, including invasion of the vascular or lymphatic vessel, translocation through blood or lymph circulation and colonization in distant organs. Extravascular fibrin deposition is frequently observed within and around tumour tissue [8]. And fibrin appeared to be a critical determinant of the metastatic potential of circulating tumour cells [9]. Cross-linked fibrin strands may function as a stable scaffold for tumour cell adhesion during metastasis.

D-dimer is an indicator of intravascular fibrin formation and degradation as it derives from degraded fibrin when plasmin-induced fibrinolytic system is activated. Plasma D-dimer values are elevated in patients with various solid tumours, and some studies indicate that high D-dimer values

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are correlated with poor prognosis in lung cancer patients [10, 11]. Considering the role of fibrin in tumour metastasis, elevated plasma D-dimer values may function as an effective indicator of metastasis in non-small cell lung cancer (NSCLC) patients.

Additionally, a knowledge gap remains regarding a potential quantitative correlation between plasma D-dimer values and extent of metastasis, especially for lymph node involvement in primary NSCLC. Defining the malignant involvement of lymph nodes is crucial because this factor determines treatment options [12]. In the current study, plasma D-dimer values of NSCLC patients with either lymph node involvement or carcinoma in situ were measured, and the diagnostic performance of the D-dimer values in predicting lymph node dissemination was examined.

## Patients and methods

### Ethics statement

The study was approved by the Human Research Ethics Committee of the Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, and written informed consent was provided by all patients.

### Patients

One hundred forty-four patients seen for diagnostic lung surgical procedures were admitted the Department of Thoracic Surgery, Cancer Institute and Hospital, Chinese Academy of Medical Sciences (CAMS), between January and December 2013. Patients with pre-existing comorbidities or medications affecting D-dimer values (including concomitant malignant disease, overt viral or bacterial infection, venous or arterial thromboembolism in the preceding 2 months or intake of continuous anticoagulants) were excluded from the study. The final cohort comprised 142 patients. All patients underwent a segmentectomy, lobectomy or wedge resection with systematic lymph node dissection to determine the state of suspected lung cancer. After surgical operation, diagnosis of lung cancer and benign lung disease were confirmed by the attending pathology staff at Cancer Institute and Hospital, CAMS. Nodal tissue was paraffin-embedded, hematoxylin and eosin-stained and microscopically examined for the presence of malignant cells by histological examination. The involvement of the lymph node with malignant cells was considered as primary end-point. The NSCLC diagnosis was established in accordance with the revised World Health Organization classification of lung tumours and was staged in accordance with the revised staging for lung cancer [13, 14]. Clinical, laboratory, pathological and follow-up data for these patients were acquired from electronic oncology registries.

### D-dimer value measurement

Blood samples were collected upon initial (pre-treatment) diagnosis prior to surgical treatment. Whole blood was collected by taking a 3-mL venous blood sample into a blood collection tube containing sodium citrate as an anticoagulant. Quantitative D-dimer values were obtained using the commercially available D-dimer assay kit INNOVANCE D-dimer (Germany).

### Statistical analysis

As the D-dimer levels were not normally distributed, the results of the D-dimer level tests are reported as median. D-dimer values were statistically analysed as a continuous and dichotomous variable ( $\leq 0.475$  mg/L fibrinogen equivalent units (FEU) or  $> 0.475$  mg/L FEU). Lymph node involvement was also statistically analysed as a continuous and dichotomous variable (either positive or negative). The association between pairs of variables was assessed with Spearman correlation coefficient.  $\chi^2$  tests were used to assess the relationships between categorical variables. Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences) 21.0 software.

## Results

### Patient characteristics

Patient characteristics are shown in Table 1. Of the 142 patients enrolled, 18 patients (12.7 %) had a diagnosis of benign lung disease, and 124 patients were diagnosed as NSCLC. Fifty patients (35.2 %) were diagnosed as NSCLC with involved lymph nodes, with a mean number of involved lymph nodes of 2.2 (range, 1 to 17 involved lymph nodes). Six NSCLC patients (4.2 %) had ten or more malignant lymph nodes. Twenty cancer patients (14.1 %) had five to nine malignant lymph nodes. Twenty-four cancer patients (16.9 %) had one to four malignant lymph nodes. NSCLC patients had a mean tumour size of 3.6 cm (range, 0.6 to 9.5 cm). Ten cancer patients (7.0 %) had tumour size larger than 5.0 cm. The other 114 cancer patients (80.3 %) had tumour size smaller than 5.0 cm.

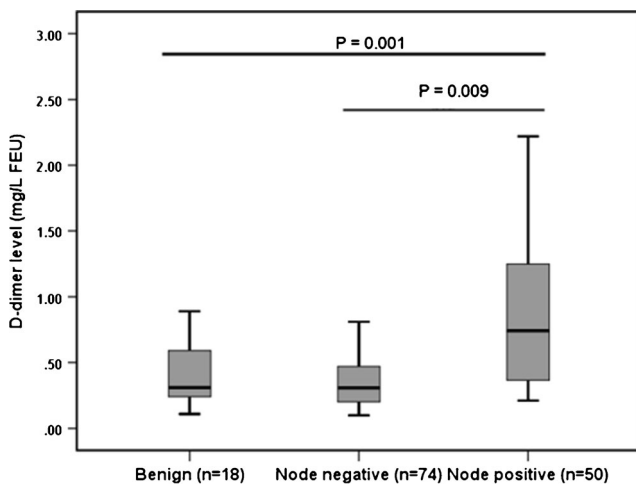
### Median D-dimer values were higher in patients with malignant lymph nodes than patients without lymph node involvement

The median D-dimer values of patients with different types of lung disease are presented in Fig. 1. D-dimer values were statistically different in those patients with benign lung disease, node-negative carcinoma and node-positive carcinoma

**Table 1** Characteristics of the study population

	Benign lung disease ( <i>n</i> =18)	NCLC without lymph node involvement ( <i>n</i> =74)	NCLC with lymph node involvement ( <i>n</i> =50)
Age (years)			
Median	55	60	62
Range	41–70	39–81	39–76
Sex ( <i>n</i> = 142)			
Male	8	44	34
Female	10	30	16
D-dimer value (mg/L FEU)			
Mean	0.41	0.42	0.99
Range	0.11–0.89	0.10–2.10	0.21–4.93
Number of positive lymph nodes			
Mean			2.2
Range			1–17
Tumour size (cm)			
Mean		3.10	4.43
Range		0.60–6.50	1.20–9.50
Smoking history			
Yes	8	40	30
No	10	34	20
Histological types of lung cancer			
Adenocarcinoma		16	18
Squamous cell carcinoma		57	31
Large cell carcinoma		1	1

(Kruskal–Wallis test,  $P=0.001$ ). A statistically significant difference of median D-dimer values was observed between node-positive cancer patients and those patients without



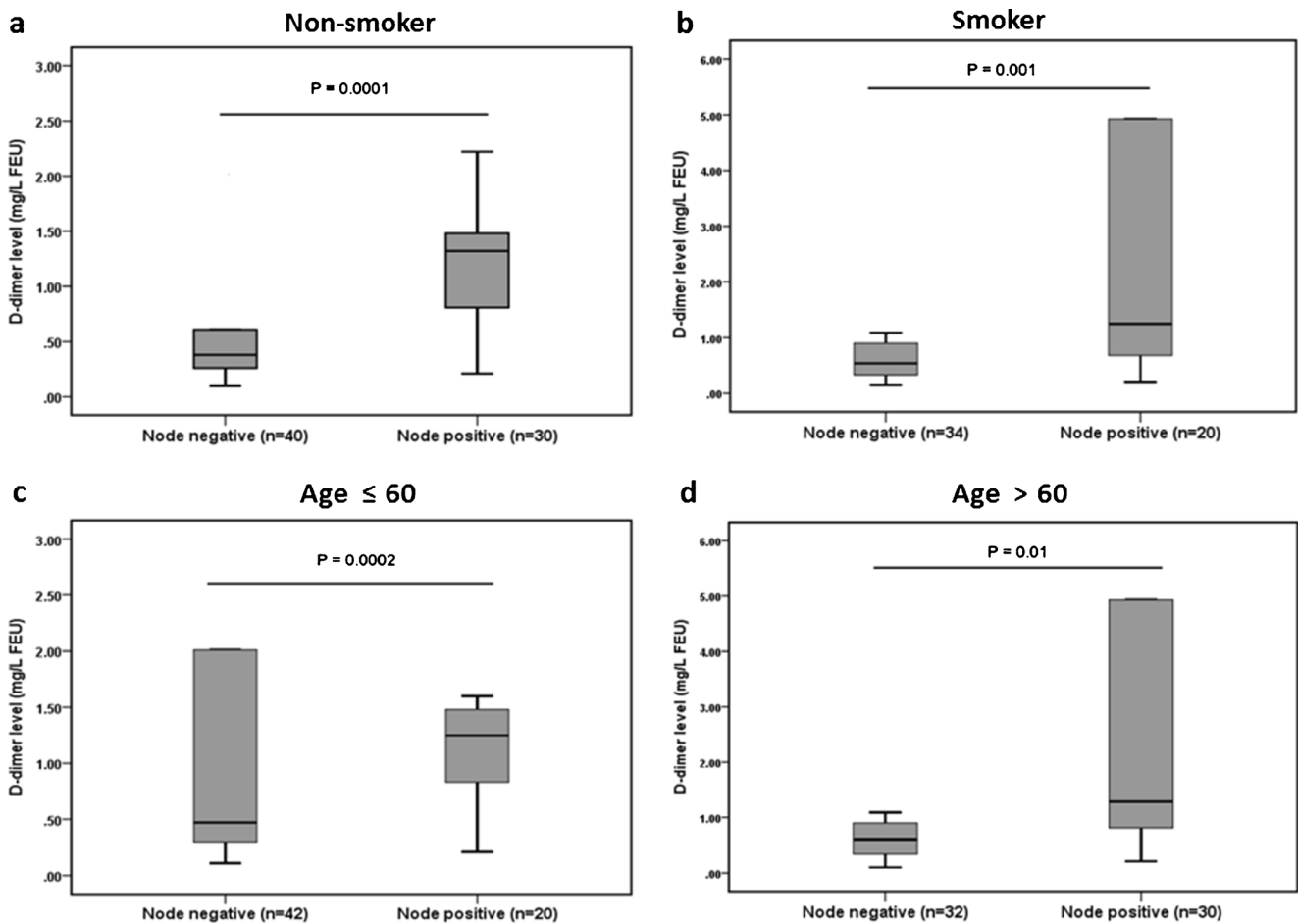
**Fig. 1** Median D-dimer values were higher in lymph node-involved patients compared with those with negative lymph node. Kruskal–Wallis test was used to compare the plasma D-dimer values among the benign group, lymph node-negative group and lymph node-positive group.  $P$  values  $<0.05$  were considered as statistically significant. The Wilcoxon rank sum test was used to compare the plasma D-dimer values between the lymph node-negative and lymph node-positive patients.  $P$  values  $<0.05$  were considered as statistically significant

lymph node involvement (Wilcoxon rank sum test,  $P=0.009$ ). As shown in Fig. 1, the median D-dimer value of patients with benign lung disease, node-negative carcinoma and node-positive carcinoma was 0.310, 0.305 and 0.740 mg/L FEU, respectively. This result indicated that the median D-dimer values were higher in patients with nodal involvement than in patients without lymph node involvement.

Some studies have shown that D-dimer values increase with age, and exposure to cigarette smoke can cause D-dimer to increase [15, 16]. To exclude the interference of these two factors, the difference of D-dimer value between cancer patients 60 years and younger versus older than 60 years and cigarette smoker versus non-smoker was compared. We found that the median D-dimer values were higher in patients with nodal involvement than in patients without lymph node involvement regardless of being a smoker or non-smoker, and the same result was also observed in patients of age 60 years and younger versus older than 60 years (Fig. 2).

### D-dimer values were higher in patients with late-stage lung cancer than in those with early-stage lung cancer

Of the histopathological variables examined, D-dimer values correlated strongest with the number of positive lymph nodes



**Fig. 2** Median D-dimer values were higher in lymph node-involved patients than those with negative lymph node regardless of exposure to cigarette smoke and increasing age. The Wilcoxon rank sum test was used to compare the plasma D-dimer values between the lymph node-negative

and lymph node-positive patients. *P* values <0.05 were considered as statistically significant. **a** NSCLC patients without cigarette exposure, **b** NSCLC patients with cigarette-exposure, **c** NSCLC patients of age 60 years and younger, **d** NSCLC patients age older than 60 years

(Spearman correlation=0.483, *P*=0.00046; Fig. 3a). D-dimer values were not directly correlated with tumour size (Spearman correlation=0.148, *P*=0.125; Fig. 3b).

Because the involvement of lymph nodes affects the clinical stage, we reasoned that D-dimer values may also correlate with the clinical stage. Analysis of variance test was used to analyse the correlation between D-dimer values and clinical stage. The results are shown in Table 2. There were no stage IV patients. A statistically significant difference of D-dimer values was observed among different clinical stages (analysis of variance, *P*=0.009; Table 2). The highest plasma D-dimer value was obtained in stage III patients (Table 2).

### Plasma D-dimer value as a predictor of malignant lymph node involvement in operable lung cancer patients

Median D-dimer values were markedly increased in patients with malignant lymph nodes compared with patients without lymph node involvement, as shown in Fig. 1. And D-dimer values showed the highest correlation with the number of

positive lymph nodes. Therefore, we hypothesized that plasma D-dimer levels may be used to detect the metastasis of lymph node.

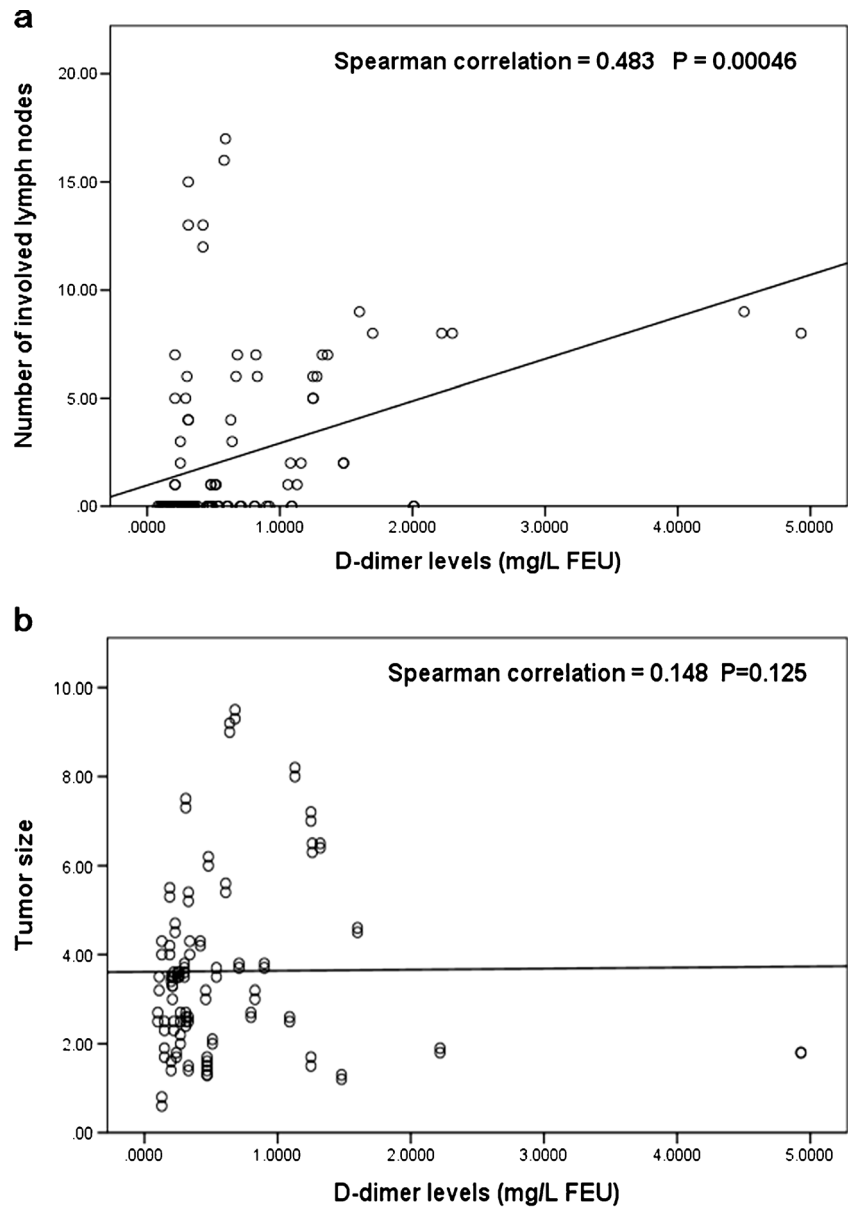
Area under the receiver operating characteristic curve value was used for predicting lymph node involvement (Fig. 4). The area under the curve (AUC) was 0.779. When setting the cutoff value at 0.475 mg/L FEU, plasma D-dimer values had a positive predictive value of 0.72 and a negative predictive value of 0.81. Likewise, the elevated plasma D-dimer value conferred moderate sensitivity of 0.72 and high specificity of 0.81.

As a dichotomous variable ( $\leq 0.475$  or  $> 0.475$  mg/L FEU), a significant correlation was observed between elevated D-dimer values and involvement of lymph nodes ( $\chi^2$  test, *P*=0.0000; Table 3).

### Discussion

The blood coagulation and fibrinolytic system plays an important role in tumour angiogenesis and metastasis.

**Fig. 3** Relationship between D-dimer values and number of positive lymph nodes. Spearman correlation was used to assess the association between plasma D-dimer value and the number of positive lymph nodes (a) and between plasma D-dimer value and tumour size (b). *P* values < 0.05 were considered as statistically significant



Indicators of fibrinolytic pathway activation, such as D-dimer, have a prognostic value for lung cancer patients [10, 17, 18]. However, these studies were mainly

focused on the relationship between the D-dimer value and the survival rate of patients with operable lung cancer.

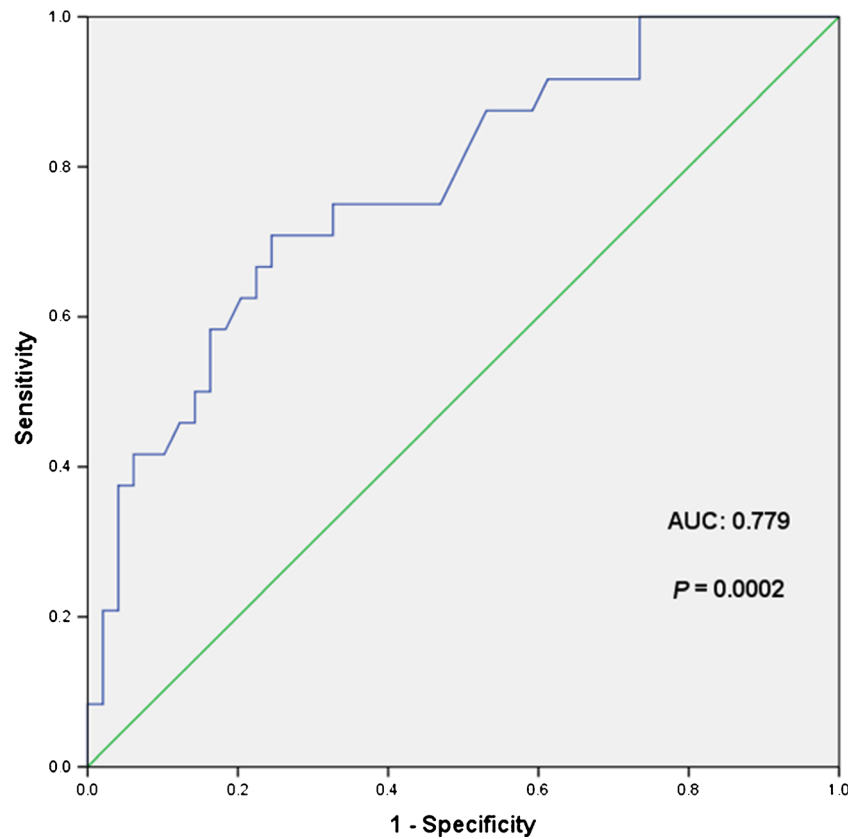
**Table 2** Relationship between D-dimer values and clinical stage

Clinical stage group	No. of patients	Mean D-dimer (mg/L FEU)
Stage I	70	0.4016 <sup>a</sup>
Stage II	22	0.6871 <sup>a</sup>
Stage III	32	1.1000 <sup>a</sup>

<sup>a</sup> Analysis of variance results for differences between groups; *P*=0.009. *P* values < 0.05 were considered as statistically significant. There were no stage IV patients

Our study represents the first investigation of plasma D-dimer values as a predictor for lymph node involvement in operable NSCLC. Being carried out in a specialized oncology hospital, our study comprised a large percentage of lung cancer patients in highly suspected malignant patients. Our data show that there is an obvious difference of median D-dimer values between patients with involved lymph nodes and patients without lymph node involvement. Therefore, elevated plasma D-dimer value is a good predictor of the malignant involvement of lymph nodes.

**Fig. 4** Area under the receiver operating characteristic curve values used for predicting lymph node involvement. AUC, 0.779 (95 % CI, 0.660–0.888),  $P=0.0002$



Treatment options for lung cancer patients were determined by lymph node status. Determining the involvement of lymph node via standard lymph node dissection increases operative time, blood loss and post-operative chest drainage. Some studies have explored some markers to predict lymph node status for avoiding a full lymph node clearance. Tumour size has been used as a marker for predicting lymph node status in some studies [19]. However, our result did not show a relationship between tumour size and lymph node status. We believe that this is partly due to the large variance

of tumour size and relatively small number of patients in our study.

Considering the sensitivity of using D-dimer values as predictors of positive lymph node involvement, D-dimer values in combination with other predictive factors would create a powerful assessment of whether lymph node dissection is necessary, particularly in the dissection of mediastinal lymph nodes. Currently, mediastinal lymph node status is usually determined by CT scan. The sensitivity and specificity of CT scanning for identifying mediastinal lymph node metastasis are approximately 0.55 and 0.81, respectively, confirming that CT scanning has limited ability to predict mediastinal metastasis [12]. Our study showed that a cutoff value of 0.475 mg/L FEU resulted in high specificity of 0.81 and medium sensitivity of 0.72 for elevated plasma D-dimer values predicting lymph node involvement. Although D-dimer values cannot define anatomic location, the combination of CT scanning and plasma D-dimer value determination may increase the sensitivity and specificity of mediastinal lymph node status predictions.

In conclusion, our study indicates that plasma D-dimer value is a useful tool for predicting lymph node status in operable NSCLC. However, the D-dimer value alone cannot predict lymph node status due to its low negative predictive

**Table 3** Relationship between elevated D-dimer values and involved lymph nodes

D-dimer value	No. of patients with involved lymph nodes		
	Negative	Positive	Total
≤0.475 mg/L FEU	60	14	74
>0.475 mg/L FEU	14	36	50
Total	74	50	124

$\chi^2$  test was used to assess the difference between the D-dimer ≤0.475 mg/L FEU group and the >0.475 mg/L FEU group;  $P=0.0000$ .  $P$  values < 0.05 were considered as statistically significant

value. Given the ease with which plasma D-dimer levels can be obtained, we are looking into combining plasma D-dimer levels with other methods for accurate prediction of lymph node involvement in a perspective study.

**Conflicts of interest** None

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