Original Article

The ratio of lymph node eluate/serum cytokeratin 21-1 is a potential predictor of lymph node metastasis in lung adenocarcinoma

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Background: Lymph node metastasis (LNM) plays an important role in prognosis of lung cancer, either in preoperative TNM staging or postoperative disease recurrence or progress. This study aimed to explore the diagnostic performances of biomarkers from lymph node eluate for LNM in lung adenocarcinoma (LUAD).

Methods: A prospective analysis was conducted based on lymph node eluate specimens collected via ultrasound-guided lymph node biopsy from 48 LUAD patients with suspected LNM in the neck. According to cytopathological results, the patients were categorized into two groups: one with LNM and the other with non-LNM (NLNM). Carcinoembryonic antigen (CEA), cytokeratin 21-1 fragment (CYFRA21-1), neuron-specific enolase (NSE) and gastrin precursor releasing peptide (ProGRP) in lymph node eluate were detected by immunoassay analyzers, and tumor marker was simultaneously collected in serum of patients.

Results: The serum levels of CEA, CYFRA21-1, NSE, and the ratios of CYFRA21-1 and NSE in the lymph node eluate to serum were significantly higher in the LNM group, compared to NLNM group. The areas under curves (AUCs) for CEA, CYFRA21-1, NSE, and ratios of CYFRA21-1 and NSE were 0.79, 0.91, 0.85, 0.93, and 0.89, respectively. The ratio of CYFRA21-1 in lymph node eluate to serum (rCYFRA21-1) performed best in diagnosing, with a sensitivity of 92.3%, a specificity of 92.9%, and an AUC of 0.93.

Conclusions: The rCYFRA21-1 to that in the serum might serve as a potential predictor for LNM in LUAD.

Keywords: Lung adenocarcinoma (LUAD); lymph node eluate; tumor biomarkers; cytokeratin 21-1 fragment (CYFRA21-1); lymph node metastasis (LNM)

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Introduction

According to the global cancer statistics 2020, lung cancer remains the primary contributor to cancer-related fatalities worldwide (1). As per the 2024 Chinese cancer report, lung cancer becomes the leading cause of morbidity and mortality in malignant tumors in China (2). Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancer, while lung adenocarcinoma (LUAD) is the most common subtype of NSCLC (3). Even in patients with stage I LUAD, the recurrence rate after radical surgery

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can reach up to 30% (4). Lymph node metastasis (LNM) plays an important role in prognosis of lung cancer, either in preoperative tumor node metastasis (TNM) staging or postoperative disease recurrence or progress. So, there has been a growing interest in LNM for decades.

Current clinical methods for detecting LNM include positron emission tomography/computed tomography (PET/CT), tumor markers, and cyto- or histopathological biopsy. PET/CT predicts LNM through changes in parameters such as metabolic index within the lymph nodes (5,6), but it is radiative and costly. Tumor biomarkers are upregulated in tumor tissues, tumor patient blood, and excreta, reflecting the occurrence and development of tumors. The traditional tumor markers for lung cancer mainly include carcinoembryonic antigen (CEA), cytokeratin 21-1 fragment (CYFRA21-1), neuron-specific enolase (NSE), gastrin precursor releasing peptide (ProGRP) and squamous epithelial cell carcinoma antigen (SCCA). With the updates and iterations of detection technologies, such as next-generation sequencing and liquid biopsy, accumulating studies have shown their high sensitivity and specificity in predicting with stage II–IV lung cancer (7-9). However, there is still a long way to go from bench to bed. Cyto- or histopathological analysis, as the gold standard approach, sometimes there may be false negatives due to the low proportion of tumor cells in the mass (10), and it has a relatively long reporting time. Compared to histopathological biopsy, cytological biopsy is less invasive and more easily acceptable for patients.

Which is superior to others? PET/CT or serum tumor markers, many studies have constructed predicting models for LNM in NSCLC (5,6,10,11). However, no matter how perfect the data are, the role is to screen and pick the patients with suspecting LNM. Confirmation of LNM depends on pathology. Pathology is irreplaceable at any time, but when the pathological results show false negative results or uncertain results, who is responsible? In this prospective study, suspected LNM-specimens were divided into two parts, one for lymph node eluent and one for cytopathological examination. When the cytopathological results show false negatives, the cross-validation effect between lymph node eluent data can be a complement to improve the accuracy of pathological results. Reviewing literatures, there are many retrospective reports on serum tumor markers for predicting LNM in NSCLC. However, research on clinical tumor marker levels in lymph node eluate to predict LNM in NSCLC is rare. This study aims to explore a simple, fast, and efficient indicator for predicting LNM in LUAD. We present this article in accordance with the TRIPOD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-410/rc).

Highlight box

Key findings

- CYFRA21-1 in lymph node demonstrated the highest diagnostic accuracy, with a sensitivity of 96.6%, a specificity of 88.9%, and an area under curve (AUC) of 0.915.
- rCYFRA21-1 (ratio of CYFRA21-1 in lymph node eluate to serum) was more efficient in picking out lymph node metastasis (LNM) in lung adenocarcinoma patients, with positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio and AUC of 12.9 (>10), 0.08 (<0.1), 155.4, and 0.93, respectively.

What is known and what is new?

- LNM plays an important role in prognosis of lung cancer, either in preoperative TNM staging or postoperative disease recurrence or progress. CYFRA21-1, a component in the epithelial cell cytoskeleton identified as a marker for lung cancer, did not change while malignant transformation. CYFRA21-1 specifically expresses within epithelial tissues, such as the small intestine, colon, exocrine pancreas, bladder, gallbladder, bronchus, and hepatic ducts. The level of serum CYFRA21-1 reflects the tumor burden in the body and the extent of cancer cell breakdown.
- First, compared with cytological pathology reports, lCYFRA21-1 (CYFRA21-1 in lymph node eluate) has a stronger sensitivity and shorter-report-time, and can complement pathological analysis in non-small cell lung cancer with LNM. Second, among tumor markers, the diagnostic efficacy of rCYFRA21-1 is higher. Third, compared with serological tumor markers, rCYFRA21-1 is more stable and eliminates the influence of different tumor burdens.

What is the implication, and what should change now?

- LNM should be suspected on the basis of lCYFRA21-1 >1.5 ng/mL or rCYFRA21-1 >0.76 (cutoff point). For cases with rCYFRA21-1 indicate LNM but cell pathology is negative, another puncture is needed or clinical empirical treatment should be performed.

Methods

Specimen collection

We prospectively collected lymph node eluate specimens from LUAD patients treated at the First Affiliated Hospital of Wenzhou Medical University, China, over a period spanning from January 2019 to December 2020. A total of 48 LUAD cases (28 males and 20 females, ages 45 to 85 years, mean 68.2±9.7 years) were included. During the B-ultrasound examination, patients' lymph nodes were

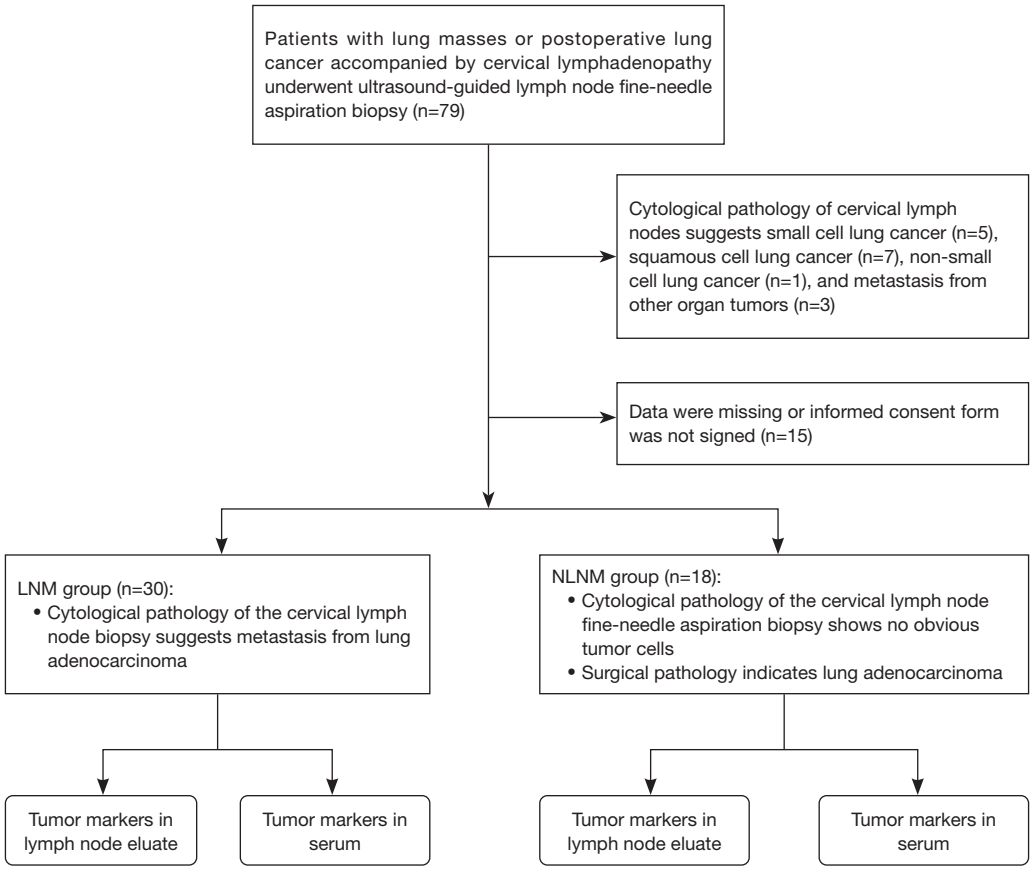

Figure 1 The flowchart of participants through the study. LNM, lymph node metastasis; NLNM, non-lymph node metastasis.

suspected to have migrated. Guided by ultrasound, three 22 gauge fine needles (outer diameter is about 0.7 mm) were punctured to obtain lymph node tissue. After withdrawing the first needle, attached the needle's end to a 5 mL syringe that has been primed with 1.0 mL of physiological saline. Then, transferred the mixture of the specimen and the saline into a sterile Eppendorf tube and centrifuged at 3,500 rpm for 5 min. The supernatant was taken and stored in the refrigerator at −80 ℃. After the second needle was taken, connected the needle's end to a 5 mL syringe filled with air, sprayed the specimen onto a clean glass slide, prepared a cell smear. The second cell smear was made following the same method, then send them for cytopathological examination. According to the results of cytopathological examination, the patients were separated into LNM group and non-lymph node metastasis (NLNM) group (see *Figure 1*). The histopathologic diagnosis of LUAD followed the World Health Organization/International Association for the Study of Lung Cancer categorization guidelines for lung cancer histology. Every 5 mL of venous blood was sampled from the patient after an overnight fasting, then centrifuged at 3,500 rpm for 5 min to separate the serum which was further stored at −80 ℃ in a refrigerator. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All patients submitted written informed consent to participate in this study. The study was approved by the Institutional Ethics Review Board of the First Affiliated Hospital of Wenzhou Medical University (No. YS2023094).

Biomarker analysis

The levels of CEA in the lymph node eluate and in the serum were detected by the DXI800 Immunoassay Analyzer (Beckman, Brea, CA, USA). NSE, CYFRA21-1 and ProGRP in the lymph node eluate and in the serum were determined by the Roche e602 Immunoassay Analyzer (Roche, Berlin, CA, Germany). Creatinine was tested by the

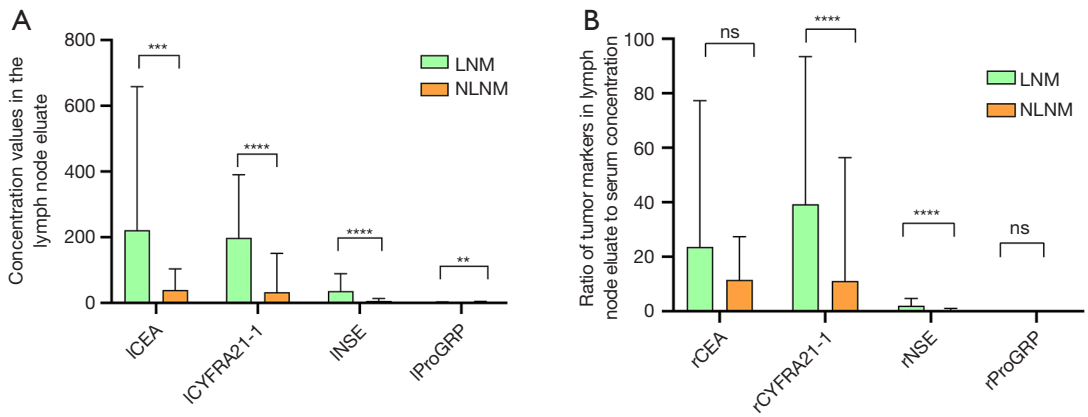

Figure 2 Comparison of tumor marker levels in the lymph node eluate in the two groups. (A) The lCEA, lCYFRA21-1, lNSE and lProGRP levels were significantly higher in the LNM group than in the NLNM group. The concentration unit of lCEA is μg/L, lCYFRA21-1 is ng/mL, lNSE is mg/mL, and lProGRP is ng/L. (B) The rCYFRA21-1 and rNSE levels in the LNM group were significantly higher than those in the NLNM group, while the rCEA and rProGRP levels in the LNM group were not significantly different from those in the NLNM group. ns, no significance; **, P<0.01; ***, P<0.001; ****, P<0.0001. LNM, lymph node metastasis; NLNM, non-lymph node metastasis; lCEA, CEA in lymph node eluate; lCYFRA21-1, CYFRA21-1 in lymph node eluate; lNSE, NSE in lymph node eluate; lProGRP, ProGRP in lymph node eluate; rCEA, ratio of CEA in lymph node eluate to serum; rCYFRA21-1, ratio of CYFRA21-1 in lymph node eluate to serum; rNSE, ratio of NSE in lymph node eluate to serum; rProGRP, ratio of ProGRP in lymph node eluate to serum; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 21-1 fragment; NSE, neuron-specific enolase; ProGRP, gastrin precursor releasing peptide.

Beckman Coulter Chemistry Analyzer AU5800 (Beckman). The doctors analyzed the biomarkers were blinded to the results of diagnosis and disease status.

Statistical analysis

The Shapiro-Wilk test was performed to evaluate data distribution. The Mann-Whitney *U* test or the *t*-test was used to compare the two groups. P<0.05 was considered statistically significant. The area under the receiver operating characteristic (ROC) curve (AUC) was used to assess predictive abilities. SPSS 24.0 (Social Science Corporation Statistical Package, Chicago, IL, USA) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA) were used for all statistical analyses.

Results

Biomarkers' profiles in LNM and NLNM groups

In this study, lCEA, lCYFRA21-1, lNSE and lProGRP represent the level of CEA, CYFRA21-1, NSE and ProGRP in the lymph node eluate, and rCEA, rCYFRA21-1, rNSE and rProGRP stands for the ratio of CEA, CYFRA21-1, NSE and ProGRP in the lymph node eluate to their counterparts in the serum. LCEA, lCYFRA21-1, lNSE, lProGRP, rCYFRA21-1 and rNSE were notably higher in the LNM group than in the NLNM group (Mann-Whitney *U* =429, 477.5, 446, 133, 482.5, 413, respectively; P=0.001, <0.001, <0.001, 0.004, <0.001, <0.001, respectively). There was no significant difference in rCEA and rProGRP between LNM and NLNM groups (*Figure 2*, *Table 1*).

Sensitivity and specificity of biomarkers in diagnosing LNM

The ROC analysis showed that the sensitivities of lCEA, lCYFRA21-1, lNSE, lProGRP, rCYFRA21-1 and rNSE for LNM were 72.4%, 96.6%, 82.8%, 100%, 92.3%, and 88.5%; their specificities were 77.8%, 88.9%, 77.8%, 11.1%, 92.9%, and 71.4%; their AUCs were 0.791, 0.915, 0.854, 0.255, 0.934, and 0.821, respectively. Notably, rCYFRA21-1 (cutoff =0.76) exhibited the best predictive performance (*Figure 3*, *Table 2*). The cutoff value is derived from the ROC curve, representing the tumor biomarker value where the Youden index (sensitivity + specificity – 1) achieves its maximum. At this point, diagnostic accuracy is

Table 1 Comparisons of the tumor markers in lymph node eluate between the two groups

Parameters	LNM	NLNM	*U*	P
lCEA (μg/L)	96.0 (221.7±436.4)	16.7 (39.37±64.5)	429.0	0.001
lCYFRA21-1 (ng/mL)	163.5 (205.3±191.2)	0.5 (32.93±118.2)	477.5	<0.001
lNSE (mg/mL)	16.8 (36.5±52.6)	2.4 (6.0±7.9)	446.0	<0.001
lProGRP (ng/L)	3.0 (3.3±0.5)	3.7 (3.6±1.2)	133.0	0.004
rCEA	4.8 (23.6±53.7)	4.8 (11.6±15.9)	277.0	0.63
rCYFRA21-1	13.0 (39.3±54.1)	0.1 (11.1±45.2)	482.5	<0.001
rNSE	1.0 (2.0±2.7)	0.2 (0.43±0.62)	413.0	<0.001
rProGRP	0.06 (0.06±0.03)	0.08 (0.07±0.035)	157.0	0.31

Data are presented as median (mean ± standard deviation). LNM, lymph node metastasis; NLNM, non-lymph node metastasis; lCEA, CEA in lymph node eluate; lCYFRA21-1, CYFRA21-1 in lymph node eluate; lNSE, NSE in lymph node eluate; lProGRP, ProGRP in lymph node eluate; rCEA, ratio of CEA in lymph node eluate to serum; rCYFRA21-1, ratio of CYFRA21-1 in lymph node eluate to serum; rNSE, ratio of NSE in lymph node eluate to serum; rProGRP, ratio of ProGRP in lymph node eluate to serum; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 21-1 fragment; NSE, neuron-specific enolase; ProGRP, gastrin precursor releasing peptide.

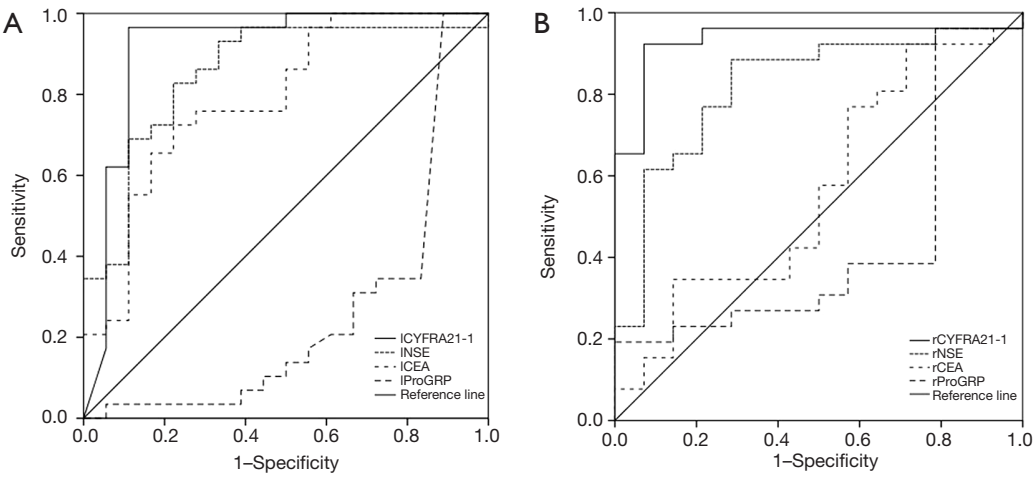

Figure 3 ROC curves of biomarkers in the lymph node eluate for LNM of LUAD. (A) ROC curves for lCEA, lCYFRA21-1, lNSE, lProGRP in differentiating metastasis in lymph node. (B) ROC curves for rCEA, rCYFRA21-1, rNSE, rProGRP in differentiating metastasis in lymph node. ROC, receiver operating characteristic; LNM, lymph node metastasis; LUAD, lung adenocarcinoma; lCEA, CEA in lymph node eluate; lCYFRA21-1, CYFRA21-1 in lymph node eluate; lNSE, NSE in lymph node eluate; lProGRP, ProGRP in lymph node eluate; rCEA, ratio of CEA in lymph node eluate to serum; rCYFRA21-1, ratio of CYFRA21-1 in lymph node eluate to serum; rNSE, ratio of NSE in lymph node eluate to serum; rProGRP, ratio of ProGRP in lymph node eluate to serum; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 21-1 fragment; NSE, neuron-specific enolase; ProGRP, gastrin precursor releasing peptide.

at its peak, and both the rates of missed and false diagnoses are minimal, making it ideal for discerning the actual state of clinical diseases. lCYFRA21-1 achieved its largest AUC (0.915) at a threshold value of 1.5 ng/mL (*Figure 3A*). rCYFRA21-1 achieved its largest AUC (0.934) at a threshold value of 0.76 (*Figure 3B*), compared to those of lCEA (0.791), lCYFRA21-1 (0.915), lNSE (0.854), lProGRP (0.255) and rNSE (0.821). The specificity of rCYFRA21-1 in diagnosing LNM was significantly higher than those of lCEA (0.778), lCYFRA21-1 (0.889), lNSE (0.778), lProGRP (0.111) and rNSE (0.714). The positive likelihood ratio (PLR) and negative likelihood ratio (NLR) of rCYFRA21-1 were 12.9 (>10) and 0.08 (<0.1), respectively, indicating its excellent diagnostic efficacy (*Table 2*).

Table 2 The AUC, cutoff, sensitivity, and specificity for diagnosing LNM of LUAD, and the Youden index, PLR, NLR, and DOR of effective biomarkers

Parameters	AUC	95% CI	P	Cutoff	Sensitivity (%)	Specificity (%)	Youden index	PLR	NLR	DOR
lCEA (μg/L)	0.791	0.655–0.927	0.001	34.3	72.4	77.8	0.50	3.3	0.36	9.2
lCYFRA21-1 (ng/mL)	0.915	0.808–1.000	<0.001	1.5	96.6	88.9	0.85	8.7	0.04	217.5
lNSE (mg/mL)	0.854	0.738–0.970	<0.001	5.7	82.8	77.8	0.61	3.8	0.22	17.3
lProGRP (ng/L)*	0.255	0.092–0.417	0.005	1.99	100.0	11.1	0.111			
rCEA#	0.566	0.374–0.758	0.50	0.50	92.3	28.6	0.21			
rCYFRA21-1	0.934	0.848–1.000	<0.001	0.76	92.3	92.9	0.85	12.9	0.08	155.4
rNSE	0.821	0.682–0.961	0.001	0.28	88.5	71.4	0.60	3.1	0.15	20.7
rProGRP#	0.429	0.232–0.625	0.46	0.09	19.0	100.0	0.19			

*, PLR, NLR and DOR were not calculated for AUC <0.5; #, PLR, NLR and DOR were not calculated for P>0.05. AUC, area under the curve; LNM, lymph node metastasis; LUAD, lung adenocarcinoma; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; CI, confidence interval; lCEA, CEA in lymph node eluate; lCYFRA21-1, CYFRA21-1 in lymph node eluate; lNSE, NSE in lymph node eluate; lProGRP, ProGRP in lymph node eluate; rCEA, ratio of CEA in lymph node eluate to serum; rCYFRA21-1, ratio of CYFRA21-1 in lymph node eluate to serum; rNSE, ratio of NSE in lymph node eluate to serum; rProGRP, ratio of ProGRP in lymph node eluate to serum; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 21-1 fragment; NSE, neuron-specific enolase; ProGRP, gastrin precursor releasing peptide.

Demographic and clinical factors influencing CYFRA21-1

The clinical characteristics of LUAD patients are presented in *Table 3*. No statistically significant association was observed between CYFRA21-1 and creatinine (P=0.34). In the LNM group, lCYFRA21-1 level was higher in stage IV than in stage III (P=0.01), while rCYFRA21-1 level remained stable in both stages (P=0.10).

Discussion

LUAD, the most prevalent histological subtype of lung cancer, is highly invasive and metastatic. Its metastasis in the neck lymph node, always in stage III, indicates a poor prognosis. Early and precise diagnosis of LUAD-associated LNM is of great significance for improving patients' prognosis.

In this study, we found that the levels of all biomarkers in lymph node eluate, except ProGRP, showed a significant increase in the LNM group, compared to those in the NLNM group (all P<0.05), indicating that the lymph nodes had been infiltrated by LUAD lesions. Out of the four tumor markers in lymph node eluate, CYFRA21-1 demonstrated the highest diagnostic accuracy, with a sensitivity of 96.6%, a specificity of 88.9%, and an AUC of 0.915. Further observation found that rCYFRA21-1 was more efficient in picking out LNM in LUAD patients, with PLR, NLR, diagnostic odds ratio (DOR) and AUC of 12.9 (>10), 0.08 (<0.1), 155.4, and 0.93, respectively.

Wang *et al.* (11) established a predictive model for the combined diagnosis of lung cancer metastasis using seven serum biomarkers. In NSCLC patients, compared with patients without metastasis, CYFRA21-1 was notably higher in metastatic patients (P<0.05). Tang *et al.* (10) found that CEA and CYFRA21-1 were independent risk factors for mediastinal LNM in lung cancer (P<0.001, P=0.002, respectively), and the AUCs were 0.533 and 0.596, respectively. In esophageal cancer, Mei *et al.* (12) found that serum CYFRA21-1 and squamous cell carcinoma (SCC) levels were associated with LNM (P<0.001, 0.045), with the AUCs of 0.731 and 0.651, respectively. Especially in the early tumor stage, preoperative CYFRA21-1 was an independent risk factor for LNM. In a report by Bugalho *et al.* (13) in 2013, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect biomarkers in EBUS-TBNA lymph node biopsy specimens. Compared with patients with benign lesions, CK-19 (CYFRA21-1 is its fragment), CEA, and epithelial cell adhesion molecule (EPCAM) transcripts were significantly higher in NSCLC. A positive correlation was also found between the primary tumor size and EPCAM (r=0.476; P=0.005), CK-19 (r=0.594; P=0.001), and CEA (r=0.394; P=0.023). Hur *et al.* (14) conducted CT-guided percutaneous lung tumor biopsy and detected the

Table 3 Factors influencing CYFRA21-1 levels in patients

Clinical variables	N	sCYFRA21-1			lCYFRA21-1			rCYFRA21-1		
		Median (P25, P75)	*U*	P	Median (P25, P75)	*U*	P	Median (P25, P75)	*U*	P
Age (years)			143.0	0.62		91.0	0.29		91.0	0.29
≥60	42	4.3 (2.8–10.5)			16.6 (0.5–216.6)			2.7 (0.1–27.0)		
<60	6	4.1 (2.4–8.2)			199.2 (0.7–278.0)			26.8 (0.3–98.3)		
Gender			329.5	0.30		337.5	0.23		318.0	0.43
Male	28	4.3 (2.7–13.7)			82.6 (0.5–348.5)			9.2 (0.1–33.2)		
Female	20	4.2 (2.7–5.3)			4.6 (0.5–152.3)			1.3 (0.2–34.0)		
Creatinine elevation			70.5	0.22		81.0	0.39		84.0	0.45
Yes	5	2.8 (2.2–9.0)			2.3 (0.5–55.2)			1.2 (0.2–8.5)		
No	43	4.6 (2.8–10.3)			27.3 (0.5–280.7)			3.9 (0.1–40.7)		
Distant metastases			148.5	0.09		166	0.01		147.0	0.10
Stage III	12	3.7 (2.7–6.2)			26.4 (9.7–152.3)			5.9 (1.9–36.5)		
Stage IV	18	6.0 (3.0–15.1)			247.7 (97.0–489.6)			28.2 (9.3–62.7)		

sCYFRA21-1, CYFRA21-1 in the serum; lCYFRA21-1, CYFRA21-1 in lymph node eluate; rCYFRA21-1, ratio of CYFRA21-1 in lymph node eluate to serum; CYFRA21-1, cytokeratin 21-1 fragment.

concentration of relevant tumor markers in tumor cell fluid. They found CYFRA21-1 significantly improved the diagnostic sensitivity of NSCLC in biopsy pathology (a sensitivity of 95%, P<0.001). Yoon *et al.* and Liscia *et al.* (15,16) found that detecting the level of CYFRA21-1 in the axillary sentinel lymph nodes of breast cancer is an accurate and highly sensitive detection method for predicting LNM, which is suitable for a supplementary to cytopathology and CK-19 immunohistochemistry.

CYFRA21-1, a component in the epithelial cell cytoskeleton identified as a marker for lung cancer (17), does not change while malignant transformation happens. CYFRA21-1 specifically expresses within epithelial tissues, such as the small intestine, colon, exocrine pancreas, bladder, gallbladder, bronchus, and hepatic ducts. The level of serum CYFRA21-1 reflects the tumor burden in the body and the extent of cancer cell breakdown. CEA, an oncofetal protein, is generated in gastrointestinal tissue during fetal development, but is not usually found in adults. CEA is not usually used for lung cancer screening, but for evaluating treatment response, monitoring cancer recurrence and predicting prognosis (18). NSE, initially discovered in neuronal cells, and ProGRP, a biochemically more stable precursor of GRP, are both used to detect neuroendocrine tumors, including small cell lung carcinoma, large cell neuroendocrine carcinoma, and carcinoid tumors (19).

Cancer metastasis is a multi-stage and multi-step process influenced by cancer biology, physical immune status and local microcirculation. New techniques, such as liquid biopsy, have been developed to detect tumor cells in the blood, namely circulating tumor cells (CTCs). When more than 3 CTCs are detected in the circulation and evidence is presented in the chest imaging, lung cancer can be diagnosed (20). A large number of CTCs is highly likely to tell the metastases.

In order to reduce the impact of serum CYFRA21-1 baseline with various tumor burdens, we introduced in rCYFRA21-1, representing the ratio of CYFRA21-1 in the lymph node eluate to serum. Characterized with rapid proliferation and easy necrosis, tumor cells can produce cytokeratin filaments by activating caspase 3, a regulator of the apoptosis cascade. The level of serum CYFRA21-1 is positively correlated with tumor size and stage (21). In patients with renal failure or compromised renal function, there is a falsely positive elevation in CYFRA21-1 for excretion reduction (22). Therefore, rCYFRA21-1 is better and more stable than lCYFRA21-1.

In the initial stage of LNM, micrometastases, clusters of tumor cells less than 0.2 mm in the lymph node, are sometimes difficult to be detected by H&E (Hematoxylin and Eosin staining). Although the World Health Organization classification (2015) (23) and 8th lung cancer

stage classification (24) do not include micro LNM as evidence for TNM staging, studies (4,25) have shown it is a key factor for postoperative recurrence and disease progression. In the advanced stage of LNM, biopsies often sample the necrotic tissue, also leading to false negative results. Regarding above situations, the indicator in our research can compensate the false negative results, served as the "goalkeeper" behind cytopathological gold standard diagnosis used in this study.

The strengths of our study are clear. First, compared with cytological pathology reports, it has stronger sensitivity, shorter report waiting time, and can complement pathological testing. Second, among tumor markers, the diagnostic efficacy of rCYFRA21-1 is higher. Third, compared with serological tumor markers, rCYFRA21-1 is more stable and eliminates the influence of different tumor burdens.

LNM should be suspected on the basis of lCYFRA21-1 >1.5 ng/mL or rCYFRA21-1 >0.76 (cutoff point). For cases with rCYFRA21-1 indicating LNM but cell pathology is negative, another puncture is needed or clinical empirical treatment should be performed. And it will be more meaningful to apply in preoperative staging of mediastinal lymph nodes.

There are several limitations to this study. First, it was a small-scale study conducted at a single center. To confirm our findings, larger-scale prospective studies should be performed. Second, the patients involved in the LUAD-associated LNM group in this study were all in advanced stages, thus potentially enhancing the sensitivity of the tumor markers. Third, the low proportion of the control group with NLNM (18/48) might have increased the sensitivity and constituted a spectrum bias.

Conclusions

The ratio of the CYFRA21-1 in the lymph node eluate to serum might serve as a better predictor for LNM in LUAD.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-410/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-410/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All patients submitted written informed consent to participate in this study. The study was approved by the Institutional Ethics Review Board of the First Affiliated Hospital of Wenzhou Medical University (No. YS2023094).

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