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Original Article

Comparative analyses of ultrasound features, clinical indexes, and prognosis in the presence or absence of axillary lymph node metastasis in lung cancer

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Abstract: *Objective:* The aim of the current study was to investigate ultrasound features, clinical indexes, and prognoses of axillary lymph node metastasis (ALNM) in lung cancer. *Methods:* A total of 7,945 patients diagnosed with primary lung cancer were retrospectively analyzed and enrolled, including 38 axillary lymph node metastasis (ALNM) patients and 40 non-ALNM (NALNM) patients. Tumor tissues and ALNM tissues were sampled. Interventional ultrasound (IUS) imaging data and other medical data were further collected. Expression levels of epidermal growth factor receptor (EGFR), Ki67, CK5/6, TTF1, and Napsin A were detected via immunohistochemistry (IHC). *Results:* IUS analysis revealed that ALNM was not significantly associated with the amount of metastatic lymph nodes and maximum thickness of lymph node cortexes, but significantly correlated with lymph node blood flow ($P<0.05$). Expression levels of EGFR, Ki67, and CK5/6 were not statistically related to ALNM. However, there were significant differences in TTF1 and Napsin A expression levels between the NALNM group and ALNM group ($P<0.05$). Mean survival time was 21.62 months in the NALNM group, with a median survival time of 19.57 (3.16-36.0) months, significantly longer than those in the ALNM group (mean survival time: 19.25 months; median survival time: 17.35 (2.73-36.0) months). *Conclusion:* ALNM is closely related to poor prognosis of patients with lung cancer. ALNM presents remarkable features in IUS imaging signs. These may be used as indicators in evaluating ALNM.

Keywords: Lung cancer, axillary lymph node metastasis, ultrasound, prognosis

Introduction

Lung cancer ranks first among malignant tumors in the world, currently, with especially high morbidity and mortality rates in China [1]. According to data released by the National Cancer Center in 2015, there were 730,000 cases and 610,000 deaths due to lung cancer in 2015. The 5-year prevalence rate of lung cancer was 130.2 (1/100,000) in China from 2006-2011. It was 84.6 (1/100,000) for males, ranking 2nd in malignant tumors, and 45.6 (1/100,000) for females, ranking 4th in malignant tumors [2]. The overall 5-year survival rate of cancer patients is only 14% [3]. Tumor-node-metastasis (TNM) staging criteria (the 8th version) were developed by the International Association for the Study of Lung Cancer (IASLC) in 2015. According to the SEER database, distant metastasis was found in 57% patients with lung cancer at initial diagnosis. Therefore, treatment of advanced patients is an important component of the lung cancer therapy system [4]. At present, the roles of a variety of pathological and molecular markers in identifying high-risk recurrent patients have been determined. However, the stage of tumor-lymph node metastasis is still the most important factor in evaluating prognosis [5]. Current surgical and pathological evaluations have demonstrated the essential role of lymph node assessment as a prognostic variable in the first stage. Previous studies have shown that supraclavicular lymph node metastasis (SCLNM) often occurs in patients with lung cancer, but axillary lymph node metastasis (ALNM) is seldom observed in lung cancer. In 1942, It was reported that the incidence rate of ALNM was 6.6%, for 1,298 cases of bronchopulmonary cancer [6]. ALNM is classified as a different type of distant lymph node metastasis. However, there is a lack of reporting concerning

Table 1. General clinical data of between NALNM and ALNM patients with lung cancer

Characteristic	n (78)	Percentage
Gender		
Male	42	53.8%
Female	36	46.2%
Smoking History		
Yes	33	42.3%
No	45	57.7%
Pathology		
Adenocarcinoma	19	24.4%
Squamous	15	19.2%
Adenosquamous	12	15.4%
Small cell	7	9.0%
Non-small cell	25	32.0%
Pathological grade		
Poor	28	35.9%
Moderate	40	51.3%
Unclear	10	12.8%
Primary tumor position		
Left	38	48.7%
Right	40	51.3%
Type		
Central	36	46.2%
Peripheral	42	43.8%

clinical diagnosis, pathological features, and prognostic analysis of patients with ALNM.

Interventional ultrasound (IUS), characterized by non-invasiveness and high resolution, has become a principal examination method for tumors. This method can not only describe the conditions of lung cancer tissues, but also provide further details on the enlargement of axillary lymph nodes. The presence or absence of lymph node metastasis is an important factor affecting the prognosis of lung cancer patients. The present study retrospectively investigated pathological and IUS data of 78 patients that were diagnosed with lung cancer, including 38 cases with ALNM. Furthermore, this study analyzed ultrasonographic characteristics of axillary lymph nodes in lung cancer, examining the clinical significance of ultrasound examinations.

Materials and methods

Clinical data

Evaluating the clinicopathological information of lung cancer patients with ALNM, medical records and pathological reports of 7,945 patients, diagnosed with primary lung cancer, from May 2004 to November 2014, were reviewed. A total of 38 patients with ALNM and 40 patients with NALNM were randomly selected. Data were collected, including gender, age at diagnosis of lung cancer, smoking history, histological and clinical stage of lung cancer, treatment type, and survival rate from the date of diagnosis of lung cancer. Tumor tissues and ALNM paraffin-embedded tissues, excised surgically, were also obtained from the Department of Pathology. All patients were pathologically diagnosed with lung cancer. The histological type of primary lung cancer was determined based on WHO classification criteria. Of the 38 patients with ALNM, there were 12 cases on the right side, 19 on the left side, and 16 cases on both sides. The median long diameter of axillary lymph node was 17.80 (10.4-61.7) mm. Other clinical characteristics are shown in **Table 1**.

This study was approved by the Ethics Review Committee and informed consent was obtained from all patients. The patients were followed up via telephone interviews. The last follow-up was on November 28, 2017. A total of 7 cases were lost during follow-up. Survival times were recorded from the start of treatment. Survival and loss to follow-up was recorded as censored data and survival times were measured.

Ultrasound examination of ALN

PhilipsHD11XE (L12-3 probe and probe frequency of 8-12 MHz) and GELogiq-7 (L10 probe and probe frequency of 8-10 MHz) color Doppler diagnostic equipment were used. Patients were examined in the supine position, with the chest and bilateral axillae fully exposed. First, locations, sizes, shapes, edge, and blood flow grades of lung cancer were examined by two-dimensional image and color Doppler ultrasound. Tumor flow grade was determined based on Adler standard. Next, they were asked to raise their hands to their heads. Bilateral axillae were scanned, and the number of lymph nodes observed was recorded. The axillary lymph node with the most typical morphological changes was selected to measure the long diameter and anteroposterior diameter on the maximum section, calculating the aspect ratio, according to the methods previously described in the literature. Axillary lymph nodes were

observed on multiple sections and the maximum thickness of cortex was measured. Lymph nodes with uneven cortex thickening and disappearance of medulla were observed. Finally, the flow of lymph nodes was observed by color Doppler imaging and divided into the following types: Type I (non-flow type): obvious flow signals were not detected; Type II (hilar type): flow signals of hilar lymph node in lymph nodes were detected; and Type III (mixed type): flow signals were detected in both perimeter and interior of lymph nodes.

Hematoxylin-eosin (HE) and immunohistochemistry (IHC)

Paraffin-embedded tissues of lung cancer were sliced into 4 μm-thick sections, followed by H&E staining for histopathologic examinations.

S-P staining was used for IHC analysis. After dewaxing with xylene and dehydration with gradient ethanol, sodium citrate buffer was used for antigen retrieval. This was followed by blocking the peroxidase with 3% H_2O_2. After the slide was blocked with 10% donkey serum, the phosphate buffered solution (PBS) (negative control) and primary antibody was added for incubation at 4°C overnight. Afterward, the sections were washed with PBS three times and incubated with the ready-to-use general secondary antibody. This was followed by color development using diaminobenzidine (DAB) for further observation and photography under the microscope. Cells with brown and tan nuclei were defined as positive cells and the number of positive cells was counted. Ratios of the number of positive cells to the number of total cells in a visual field of greater than 5% were defined as positive expression.

Statistical methods

Data were analyzed using SPSS10.0 software. Student's t tests and Chi-squared tests were used to compare differences in IUS parameters between patients in the NALNM group and patients in the ALNM group. Kaplan-Meier method was used to analyze the effects of ALNM on survival of patients and the log-rank test was conducted. Cox's regression analysis was performed to analyze survival risks. $P<0.05$ suggests that differences are statistically significant.

Results

Clinical data of 78 patients with lung cancer

A total of 7,945 lung cancer patients were diagnosed and treated from May 2004 to November 2014. There were 78 cases of ALNM, with an incidence rate of 0.982%. Moreover, 59.13% of ALNM were found at initial diagnosis, while 41.87% were found during treatment. The median long diameter of axillary lymph nodes was 17.20 (13.5-42.4) mm. Other clinical features are shown in **Table 1**.

Histomorphology of NALNM and ALNM

According to H&E staining, there was no tissue atypia in the NALNM group. In contrast, tissue atypia, nuclear degeneration, cancerous tissue regeneration reaction, and fibrosis in axillary lymph nodes were observed in the ALNM group (**Figure 1**).

IUS examinations

In the NALNM group, IUS examinations showed a regular shape and a relatively clear structure of the cortex and medulla. There was a stronger echo found, compared to superficial lymph nodes in other sites, and a thinner cortex. The average size of lymph nodes was 0.8 cm × 0.4 cm-1.5 cm × 0.7 cm. Blood flow was not abundant, without blood flow or short strip-like blood flow. In the ALNM group, there were 17 cases of unilateral lymph node metastasis and 21 cases of bilateral lymph node metastasis. The size of lymph nodes was 0.5 cm × 0.3 cm-2.8 cm × 1.2 cm. IUS examinations displayed cortex thickening, an unclear structure of the cortex and medulla, and punctate calcification in a small number of medullar structures. There were diversified distributions of blood flow, including less blood flow, single-branch blood flow, tree-like blood flow, and mass-like blood flow. Maximum flow velocity was 5.2-30 cm/s and the resistance index (RI) was 0.45-0.74 (**Figure 2**).

Ultrasound parameters of ALNM patients

Correlation of ALNM with the number of metastatic lymph nodes, maximum thickness of lymph node cortex, and blood flow displayed in IUS examinations was analyzed. ALNM was not statistically associated with the number of metastatic lymph nodes and maximum thickness

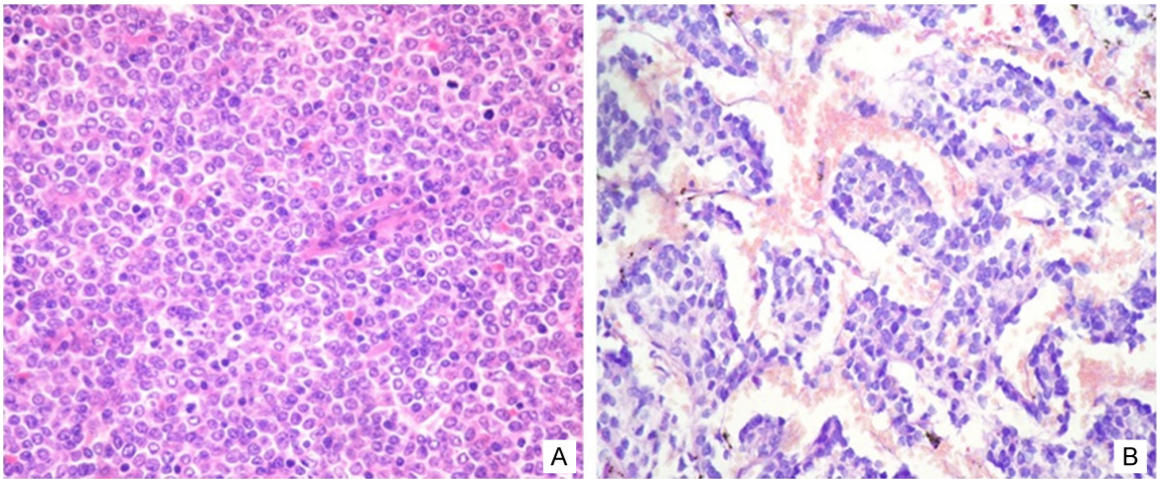

Figure 1. H&E staining of NALNM and ALNM. (A) Normal histomorphology of lymph nodes, (B) There are no normal lymph node structures, metastatic tumor cells are round and arranged in a pseudo-glandular or rosette-like shape with abundant blood vessels, showing blood sinus. (magnification 200 ×).

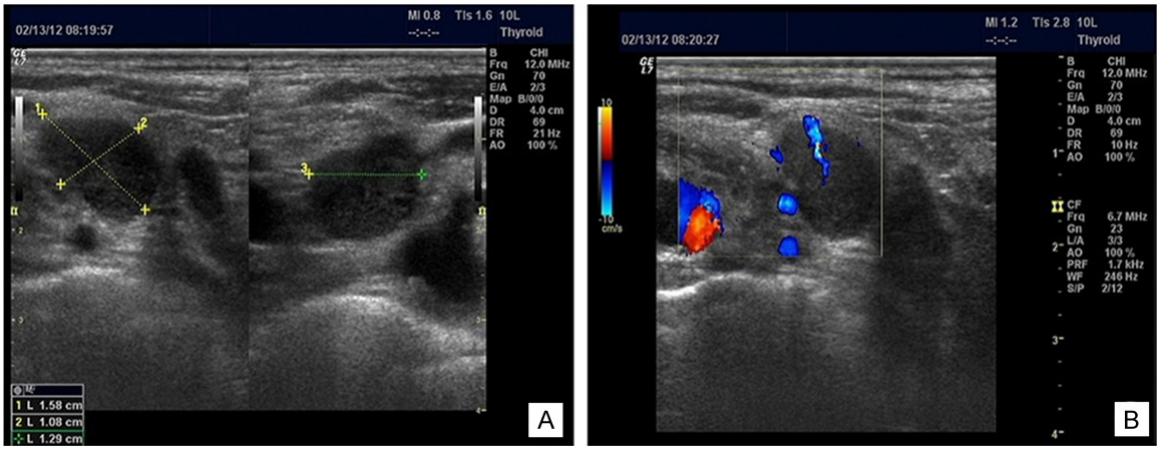

Figure 2. Ultrasound image of lymph nodes in ALNM group. A. Ultrasound gray-scale image: The ultrasound shows multiple hypoechoic nodules, unclear boundary of cortex and medulla and enlargement of lymph nodes (as large as about 1.6 × 1.3 × 1.1 cm, with an aspect ratio of 1.3). B. Blood flow signals can be seen in CDFI.

of lymph node cortexes, but significantly correlated with lymph node blood flow (P<0.05) (**Table 2**).

Differences in clinical pathological indexes of patients

Furthermore, correlation of ALNM with expression levels of relevant indexes was explored in 40 cases of NALNM and 38 cases of ALNM. Results indicated that expression levels of EGFR, Ki67, and CK5/6 in primary sites of lung cancer were not related to ALNM. However, there were significant differences in expression levels of TTF1 and Napsin A between the NALNM group and ALNM group (**Table 3**).

Differences in survival times of patients between the NALNM group and ALNM group

Patients in the NALNM group and ALNM group were followed up. The last date for follow-ups was November 28, 2017. A total of 7 patients were lost during follow-up. In lung cancer patients with ALNM, the mean survival time was 19.25 months and median survival time was 17.35 (2.73-36.0) months. In lung cancer patients without ALNM, the mean survival time was 21.62 months and median survival time was 19.57 (3.16-36.0) months, with significant differences between the two groups (P=0.004 in log-rank test) (**Figure 3**). Cox's regression analysis showed that the relative risk of death

Table 2. Ultrasound parameters of patients

Factor		n (78)	ALNM (38)	ALNM metastatic rates	χ^2/t	*P*
Number of lymph nodes	<3	59	24	40.68% (24/59)	1.44	0.057
	≥3	19	14	73.68% (14/19)		
Maximum thickness of lymph Node cortex (mm)	<3	35	20	57.14% (20/35)	0.83	0.069
	≥3	43	18	41.86% (18/43)		
Lymph node blood flow classification	I	28	5	17.86% (5/28)	3.57	0.024
	II	35	20	57.14% (20/35)		
	III	15	13	86.67% (13/15)		

Table 3. Comparison of immunohistochemical indexes between NALNM patients and ALNM patients with lung cancer

Factor		N (78)	NALNM (40)	ALNM (38)	χ^2	*P*
EGFR	-	29	32.5% (13/40)	42.1% (16/38)	0.45	0.54
	+	49	67.5% (27/40)	57.9% (22/38)		
Ki67	-	43	55.0% (22/40)	55.3% (21/38)	0.67	0.67
	+	35	45.0% (18/40)	44.7% (17/38)		
TTF1	-	15	22.5% (9/40)	15.8% (6/38)	2.51	0.012
	+	63	77.5% (31/40)	84.2% (32/38)		
Napsin A	-	19	30.0% (12/40)	18.4% (7/38)	1.67	0.028
	+	59	70.0% (28/40)	81.6% (31/38)		
CK5/6	-	25	37.5% (15/40)	26.3% (10/38)	0.72	0.75
	+	53	62.5% (25/40)	47.4% (18/38)		

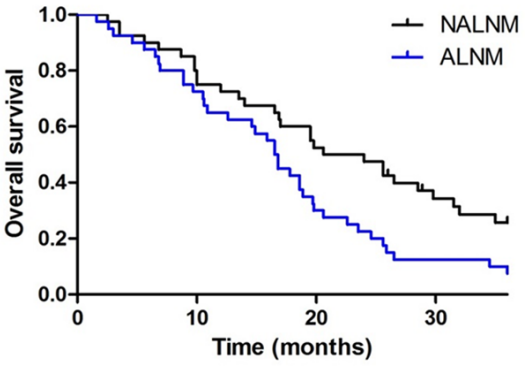

Figure 3. Survival curves of patients in the NALNM group and ALNM group.

in patients with ALNM was elevated 2.23 times (95% CI: 1.253-3.478, *P*=0.003).

Discussion

Early detection of lung cancer and timely assessment of the necessity of surgery are critical in improving therapeutic effects for cancer patients. According to the TNM staging system, non-regional lymph node metastases, such as ALNM, are classified as distant metastases. All solid cancers with distant lymph node metastasis are considered systemic diseases. Pro gnosis of patients with non-regional metastasis is also affected by the high risk of distant metastasis. Studies have demonstrated that systemic chemotherapy is predominantly applied for treatment of non-regional lymph node metastasis, with poor prognosis and a survival time of only 1-10 months [7]. However, there are few reports concerning pathological indexes and prognoses of lung cancer patients with ALNM.

The importance of systematic lymph node dissection in stage-I lung cancer has been reported. Both, 5-year and 10-year survival rates of a small number of patients without receiving systematic lymph node dissections were significantly declined, compared with those of patients receiving dissections [8]. Previous studies have shown the scope of lymph node dissection in 14 cases of non-small cell lung cancer in the pathological stage, evaluating its significance in prognosis [9]. Anatomic and pathological evaluations of patients with fewer than 15 lymph nodes showed that lymph node metastasis had statistical significance and overall survival decreased. Moreover, a retrospective study was performed on the lymphatic pathway of lymph node metastasis in bronchopulmonary cancer [10]. According to CT diagnostic criteria, metastatic lesions exist in lymph nodes with a short axis >10 mm or a long axis >15 mm. Although the axilla is often visualized on chest CT, metastasis from lung cancer to ALN has rarely attracted the attention of clinicians. Evaluating the clinicopathological features of

patients with lung cancer, the current study described survival conditions of patients with and without ALNM. These were compared. In lung cancer patients with ALNM, the mean survival time was 19.25 months and median survival time was 17.35 (2.73-36.0) months, significantly shortened, compared with those of NALNM patients.

ALN usually receives lymphatic drainage from the upper limbs and chest wall, rather than from the lungs [11]. Therefore, metastases to these sites may be systemic. There have been many hypotheses explaining the abnormal mode of ALNM. Another possible lymphatic channel proposed is the metastasis of intercostal lymphatic ducts from anterior/posterior mediastinal lymph nodes to the chest wall and axillary lymph nodes [6]. Afterward, the study evaluated CT and clinicopathologic data of 17 ALNM patients. It was found that bronchopulmonary cancer involves ipsilateral ALN through chest wall infiltration or retrograde diffusion of supraclavicular lymph nodes. In 105 cases of left lung cancer, it was found that ipsilateral supraclavicular lymph node involvement accounted for 9.5%, while contralateral supraclavicular lymph node involvement accounted for 3.8%. There were 161 cases of ipsilateral supraclavicular lymph nodes and the lymph node involvement rate was 8.7%. The left supraclavicular lymph node involvement rate was 1.8% [13]. In addition, another study showed that ALNM occurs in the lymphatic channel or pleural adhesion newly formed on the chest wall [14]. Therefore, it is recommended that routine palpations should be performed for the axilla if chest wall invasion and mediastinal and/or supraclavicular lymph node metastasis are found.

Due to the complexity of lymph node metastasis in lung cancer, there are diversified sonographic changes and comprehensive judgment of these sonographic features is required. According to previous studies, the echo characteristics in lymph nodes and whether the lymph node boundary is clear are the most important sonographic features. However, the accuracy of determining the lymph node property through one of the characteristics is around 61.7-74.2%. This can be increased to 80.4-84.0% after comprehensive judgment of these characteristics [15]. In the ultrasonogram, lymph nodes display single or multiple quasi-circular or irregular hypoechoic or iso-echoic shadows. Lymph nodes can be easily identified by IUS. However, the detection rate of lymph nodes in different sites and sizes is different [16]. Previous evidence used the sum of the maximum and minimum diameters of lymph nodes as an index, identifying the size and boundary of benign and malignant lymph nodes according to different pathological types and sites [17]. This method reaches a sensitivity of 82.4% and specificity of 67.3%, which can improve diagnostic accuracy. The present study found that ultrasound manifestations of benign lymph nodes included the basic structure of normal lymph nodes, with mostly an oval shape. There were hyperechoic lymph node cortexes in the center formed by the arteries, veins, fat and lymph node sinus, along with peripheral uniform hypoechoic lymph node cortexes. Color Doppler ultrasound manifestations included short rod-like blood flow signals in one-way direction in lymph nodes. The concentration of blood flow signals mostly appeared in the central part, seldom in the cortex. The structural layer of metastatic lymph nodes changed or even disappeared due to tumor cell infiltration. IUS analysis showed that lymph nodes displayed a quasi-circle or irregular shape with a low and uniform internal echo. This study observed an unclear or disappeared medullary area and changed eccentricity of the hilum of lymph node. The original blood vessels were damaged due to tumor invasion. Thus, there are diversified distributions of blood flow, such as less blood flow, single-branch blood flow, tree-like blood flow, and mass-like blood flow. The maximum flow velocity was 5.2-30 cm/s and RI was 0.45-0.74. Furthermore, correlation of ALNM with the number of metastatic lymph nodes, maximum thickness of lymph node cortex, and blood flow displayed in IUS examinations was analyzed. Results showed that ALNM was not correlated with the number of metastatic lymph nodes and maximum thickness of lymph node cortex but correlated with lymph node blood flow.

After pathological sections of tumor tissues were collected from patients in both groups, expression levels of EGFR, Ki67, CK5/6, TTF1, and Napsin A were considered as pathological indexes for clinical diagnosis of lung cancer [18-20]. The current study evaluated and found that EGFR, Ki67, and CK5/6 expression levels

were not related to ALNM. However, there were significant differences in TTF1 and Napsin A expression levels between the NALNM group and ALNM group, suggesting that TTF1 and Napsin A expression levels in lung cancer tissues may be potential indicators in predicting ALNM. However, due to a limited number of patients enrolled in the present study, future large cohort clinicals studies are required to validate present findings.

Conclusion

In conclusion, two-dimensional images, combined with color Doppler blood flow characteristics, can assist in the identification of benign and malignant lymph nodes. It is conducive to the clinical differential diagnosis of metastatic lymph nodes, providing important reference value for accurate preoperative evaluations, selection of operation methods, prognosis estimation, and development of adjuvant therapeutic regimens.

Disclosure of conflict of interest

None.

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