Endobronchial Ultrasound Elastography for Evaluation of Intrathoracic Lymph Nodes: A Pilot Study

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Keywords

Elastography · Endobronchial ultrasound · Lymph nodes · Diagnostic accuracy

Abstract

Background: Endobronchial ultrasound (EBUS) elastography is a new imaging procedure for describing the elasticity of intrathoracic lesions and providing important additional diagnostic information. **Objectives:** The aim of this study was to utilize the feasibility of qualitative and quantitative methods to evaluate the ability of EBUS elastography to differentiate between benign and malignant mediastinal and hilar lymph nodes (LNs) during EBUS-guided transbronchial needle aspiration (EBUS-TBNA). **Methods:** Patients with enlarged intrathoracic LNs required for EBUS-TBNA examination at a clinical center for thoracic medicine from January 2014 to April 2014 were prospectively enrolled. EBUS sonographic characteristics on B-mode, vascular patterns and elastography, EBUS-TBNA procedures, pathological findings, and microbiological results were recorded. Further-

more, elastographic patterns (qualitative method) and the mean gray value inside the region of interest (quantitative method) were analyzed. Both methods were compared with a definitive diagnosis of the involved LNs. **Results:** Fifty-six patients including 68 LNs (33 benign and 35 malignant nodes) were prospectively enrolled into this study and retrospectively analyzed. Using qualitative and quantitative methods, we were able to differentiate between benign and malignant LNs with high sensitivity, specificity, positive and negative predictive values, and accuracy (85.71, 81.82, 83.33, 84.38, and 83.82% vs. 91.43, 72.73, 78.05, 88.89, and 82.35%, respectively). **Conclusions:** EBUS elastography is potentially capable of further differentiating between benign and malignant LNs. These proposed qualitative and quantitative methods might be useful tools for describing EBUS elastography during EBUS-TBNA.

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Introduction

Convex probe endobronchial ultrasound (CP-EBUS) with the ability to perform real-time EBUS-guided transbronchial needle aspiration (EBUS-TBNA) has increased its diagnostic possibilities for mediastinal and hilar lymphadenopathy, including lymph node (LNs) metastasis in lung cancer and other malignancies, lymphoma, sarcoidosis, and tuberculous lymphadenopathy [1–6]. Few studies have evaluated various ultrasound characteristics including gray scale and power Doppler noted on EBUS to predict the malignancy of LNs [7–9]. However, these features are limited either in sensitivity or specificity, and no single criterion for malignant LNs had a satisfactory predictive value. Although combining various factors of sonographic measurements could improve its accuracy, this largely increases the difficulties for bronchoscopists during clinical practice [10].

Elastography has been presented as a new technology for the visualization of tissue elasticity, which allows noninvasive characteristics of the stiffness difference between pathological and normal tissues [11, 12]. Recent reports have indicated that elastography has been used as a complimentary imaging technique to conventional endoscopic ultrasound (EUS) for the assessment of LNs [13]. The most significant advantage of EUS elastography is that it can be performed in real time during a diagnostic examination and provide immediate information to the endosonographer. This can affect patient management through the delineation of a lesion and more precise targeting of fine needle aspiration (FNA), without the need for extensive training or costly devices [14]. Furthermore, this is becoming an independent criterion for differentiating malignant from benign LNs during EUS-guided FNA [15, 16]. A recent meta-analysis revealed that the pooled sensitivity of EUS elastography for the differential diagnosis of benign and malignant LNs was 88%, and specificity was 85% [17]. With regard to EBUS, elastography has also been suggested to be a useful tool in differentiating the malignant involvement of intrathoracic LNs based on EBUS elastographic patterns [18–22].

The purpose of this study was to determine whether EBUS elastography characteristics in patients undergoing EBUS-TBNA could predict the malignant involvement of intrathoracic LNs, and to confirm the value of qualitative and quantitative methods in evaluating EBUS elastography for differentiating malignant from benign LNs.

Materials and Methods

Patient Selection

The role of elastography and other sonographic features of CP-EBUS in diagnosing intrathoracic lymphadenopathy was studied at a clinical center for thoracic medicine at Shanghai Chest Hospital, Shanghai Jiao Tong University. Patient enrollment was prospectively performed from January 2014 to April 2014. Follow-up was conducted up to April 2016. EBUS elastography and EBUS-TBNA were performed according to the following guidelines: (1) enlarged mediastinal/hilar LNs (at least 1 node >1 cm in the short axis) based on computed tomography (CT) or positive intrathoracic LNs detected (defined as standardized uptake value >2.5) by positron emission tomography (PET); (2) clinical and radiological features suspicious for malignancy or benignity, and pathological confirmation was clinically required to exclude other diseases or support systemic treatment of intrathoracic disease; (3) no contraindication to the procedure. This study was approved by the local Ethics Committee of Shanghai Chest Hospital (No. KS-1422), and written informed consent was obtained from all patients before the examination.

EBUS Characteristics of B-Mode and Vascular Patterns

Based on previous studies of EBUS B-mode and vascular patterns on Doppler [7–10], malignant LNs characteristics were described according to the following characteristics: short axis with a diameter >1 cm; round shape; heterogeneous echogenicity; absence of central hilar structure (CHS); distinct margin and nonhilar perfusion of vascular pattern including central, capsular, and mixed (2 or more kinds mixed from hilar, central, and capsular vascular patterns). Digital videos and images of all LNs obtained by CP-EBUS were reviewed and analyzed independently by 2 operators and a sonographer, who were blinded to the results of EBUS-TBNA. Final characteristics of the ultrasonography findings for LNs were determined based on the agreement of the 3 reviewers.

EBUS Elastography

EBUS elastography videos were recorded with the ultrasound system (Hi-vision Avius; Hitachi, Japan), and were used in conjunction with the EB 1970 Pentax linear ultrasonic bronchoscope (EB1970, Pentax, Japan). After the ultrasound system generated stable EBUS elastography videos during the EBUS examinations, a 20-s video was recorded on the hard disk drive embedded in the ultrasound system. The bronchoscopists (J.S., X.Z.) reviewed and selected the representative image when elastic curve showed relatively consistent frequency and amplitude from the EBUS elastography video. The same parameter settings of the equipment were used throughout the whole study in order to minimize variability and increase the repeatability of acquisition. The region of interest (ROI) must be set to include the LNs and peripheral tissues, without covering the vessels. A balloon was used to improve the coupling to the wall, and the probe was attached to the wall with just sufficient pressure to obtain an optimal EBUS elastography. Usually, internal compression from movement in the vicinity of the vessel and/or the heart could automatically generate a satisfied EBUS elastography for examined intrathoracic lesions. If this could not generate elastography, the bronchoscopist might use a bronchoscope to provide an appropriate and frequent compression for the lesion.

Fig. 1. Representative appearance of the qualitative pattern analysis of EBUS elastography images. **a** Score 1 (scattered soft, mixed green-yellow-red). **b** Score 2 (homogeneous soft, predominantly green). **c** Score 3 (intermediate, mixed blue-green-yellow-red). **d** Score 4 (scattered hard, mixed blue-green). **e** Score 5 (homogeneous hard, predominantly blue).

Fig. 2. Software screenshot with the elastography image and quantitative analysis result. The mediastinal lymph node with a sarcoidosis case was defined as score 3 on EBUS elastography, and the elastography image was exported into a computer program developed by Matlab™. The region of interest (ROI) was set on a double screen display. After performing a pixel-by-pixel subtraction of the

B-mode gray image from the color elasticity image, the matrix of red, green, and blue values obtained from subtraction was transformed into a matrix of gray tone, and these values vary from 255 (all blue pixels) to 0 (all red pixels). The mean gray value inside a chosen ROI was computed as 186.87, according the formula: mean gray = sum gray/number of points.

The representative images as well as videos were sent to an independent sonographer (L.W.) for image analysis to confirm: (1) whether the image covered the maximum diameter of the LN, and (2) whether the characteristics of the representative image could be reproduced in the EBUS elastography video. The representative image led to the final results of the qualitative and quantitative analysis of EBUS elastography, without knowledge of any of the pathological results. Three reviewers should reach an agreement on the qualitative analysis and typical elastography image, which was selected from the video. In order to visualize tissue elasticity patterns, different elasticity values were marked with different colors, which were shown in real-time and superimposed on B-mode images. EBUS elastography was performed by a red-green-blue color map, where hard tissue was marked with dark blue, medium hard tissue with cyan, intermediate tissue stiffness with green, medium soft tissue with yellow, and soft tissue with red [18, 19]. Real-time elastography qualitative scores of the LNs were assessed and divided into 5 scores according to elastographic patterns as previously described (Fig. 1) [23]. Score 1 was assigned as heterogeneous soft pattern with green, yellow, and red colors mixed, and there was almost no blue color. Score 2 was a homogeneous soft pattern with predominantly green color and could also have minor blue, yellow, or red colors. Score 3 displayed a mixed color of blue,

green, yellow, and red, which comprised both hard and soft patterns and was considered to be an intermediate hardness pattern. Score 4 was a heterogeneous hard pattern with blue and green colors mixed and could have minor yellow or red colors mixed as well. Score 5 displayed a homogeneous hard pattern with predominantly blue colors and had a small number of green, yellow, or red colors sometimes.

In order to approach a quantitative analysis for better describing the stiffness of LNs, the previously selected EBUS elastography image was exported into the software developed by Matlab™. The software could transform the image from the 3 principal components of red-green-blue color to gray scale, in which these values varied from 255 (all blue pixels) to 0 (all red pixels); and the target was contoured later. The mean gray value inside the target was calculated (Fig. 2) [24].

EBUS-TBNA and Final Diagnosis

EBUS-TBNA was performed on patients under moderate sedation (midazolam) and local anesthesia (lidocaine), as previously described [25, 26]. After oral white light bronchoscopy, target lesions and peripheral vessels were examined by EBUS (Pentax). Scanning was always performed at a frequency of 7.5 MHz during EBUS-TBNA. In all cases, EBUS-TBNA was carried out with a

22-gauge needle (Wilson-Cook Medical, USA) on targeted lesions under the guidance of real-time ultrasound. LN classification was based on the newest international staging system [27]. Three needle aspirations were performed for each target lesion. However, if a visible histology specimen was obtained, 2 aspirations were acceptable. On-site cytological evaluation was not performed. Cytology smears were stained by hematoxylin and eosin by cytopathologists. Macroscopic tissue fragments were formalin-fixed and paraffin-embedded before being examined by other pathologists under light microscopy. Flush specimens were placed in saline solution for microbiological assessment, according to the judgment of the doctors [6, 28].

The final diagnosis for LNs was based on the histology/cytology or microbiological specimens obtained by EBUS-TBNA and/or other means. An EBUS-TBNA diagnosis was subsequently confirmed by results of either other pathological or microbiological examinations involving white light bronchoscopy, CT-guided transthoracic needle aspiration, thoracotomy/thoracoscopy, mediastinoscopy, or clinical follow-up. The subsequent therapy of patients was performed based on their corresponding final diagnosis. A follow-up of at least 12 months was used to exclude malignancy in the patients with benign disease or nonmetastatic LNs in malignant disease.

Statistical Analysis

Observed data from different LNs were statistically independent, even if some of the data may have been chosen from the same patient. Frequency, percentage, and mean (range) were used for descriptive statistics. Comparisons of continuous variables among the multiple groups were performed by one-way analysis of variance procedures, followed by the Bonferroni post hoc test for intergroup comparisons, and the *t* test was used for comparison between 2 groups. In both qualitative and quantitative methods, receiver operating characteristic (ROC) analysis was performed in order to display the range of trade-offs between true-positive and false-positive rates possible with EBUS elastography. The intraobserver and interobserver variability of EBUS sonographic features (B-mode and color Doppler) and the qualitative scores of elastography were calculated by Cohen's kappa and Fleiss's methods, respectively. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for the differential diagnosis between benign and malignant LNs were calculated with the final diagnosis. A *p* value <0.05 was considered statistically significant. SPSS version 20.0 (IBM, New York, NY, USA) was used for statistical analyses.

Results

Patients and LNs

A total cohort of 56 patients (32 males, 24 females; mean age, 56.07 years) was retrospectively analyzed for this study, which included 33 benign and 35 malignant nodes. Baseline characteristics of all nodes evaluated in this study are summarized in Table 1. All EBUS videos and images obtained for all patients were of good quality. No patients had complications related to the EBUS-TBNA procedure.

Table 1. Characteristics of LNs included in the study

Total LNs	68
Malignant LNs	35
Adenocarcinoma	12
Squamous carcinoma	5
NSCLC-NOS	4
Small cell lung cancer	13
Lymphoma	1
Benign LNs	33
Tuberculosis	7
Sarcoidosis	12
Nonspecific lymphadenitis	14
Mean LN size by EBUS-TBNA, mm	
Long-axis diameter	25.17±9.42
Short-axis diameter	19.92±9.09
LN station	
3P	3
4L	6
4R	16
7	27
11L	5
11Ri	4
11Rs	6
12L	1
Needle passes/nodes, <i>n</i>	2.66±0.74 (1–5) ^a

NSCLC-NOS, non-small cell lung cancer not otherwise specified; LNs, lymph nodes. ^a Mean ± SD (range).

Thirty-one of 35 malignant LNs (88.57%) were diagnosed based on EBUS-TBNA, and this was later confirmed by other pathology and clinical follow-up. The remaining 4 LNs were EBUS-TBNA pathology negative and were proven as false-negative cases by EBUS-TBNA, including 1 node of Hodgkin lymphoma in a patient with a definitive diagnosis through a mediastinoscope, 1 non-small cell lung cancer (NSCLC) patient, and 1 adenocarcinoma patient with PET-CT that revealed an increased FDG uptake of LNs, and 1 small cell lung cancer (SCLC) patient with CT that revealed enlarged LNs of the tumor draining zone and that LNs became smaller after 2 cycles of chemotherapy. Thirty-three LNs were diagnosed as benign (tuberculosis, 7; sarcoidosis, 12; nonspecific lymphadenitis, 14) based on EBUS-TBNA, and diagnosed later by other pathological confirmation and clinical follow-up. Seven tuberculous LNs were diagnosed by pathology or microbiology of EBUS-TBNA samples, 10 sarcoid LNs were diagnosed by EBUS-TBNA pathology results, and 2 nodes were verified by clinical follow-up. Among the 14 cases of reactive lymphadenitis, 9 nodes were caused by intrathoracic inflammatory diseases and 5 nodes came

Table 2. Diagnostic test parameters for EBUS B-mode, Doppler, qualitative score and mean gray value to predict metastatic LNs

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
EBUS characteristics (B mode and Doppler)					
Distinct margins	74.19	28.57	47.92	55.56	50.00
Short axis >1 cm	96.77	14.29	50.00	83.33	53.03
Shape: round	83.87	28.57	50.98	66.67	54.55
Echogenicity: inhomogeneous	64.52	48.57	52.63	60.71	56.06
CHS: absence	80.65	54.29	60.98	76.00	66.67
Nonhilar perfusion	72.41	68.57	65.63	75.00	70.31
RTE qualitative score: 4–5	85.71	81.82	83.33	84.38	83.82
RTE mean gray value >192.32	91.43	72.73	78.05	88.89	82.35
EBUS-TBNA	88.57	100.00	100.00	89.19	94.12

PPV, positive predictive value; NPV, negative predictive value; CHS, central hilar structure; RTE, real-time elastography; LNs, lymph nodes.

from nonmetastatic LNs of lung cancer; 3 of these nodes were confirmed by thoracotomy, and 2 LNs from 2 patients did not have surgical pathology. One LN came from a patient with SCLC, and the other LN came from a patient with poorly differentiated lung cancer. The PET-CT of the patient with SCLC revealed that FDG uptake of the target LN station did not increase. The pathology of EBUS-TBNA revealed lymphoid tissues. The primary tumor decreased after treatment, but the target LN did not have any changes. Thus, the LN of the patient with SCLC was considered to be true negative. The patient with poorly differentiated lung cancer had extensive metastasis that involved the bilateral lungs, chest wall and bones; it was considered to be a hematogenous metastasis of lung cancer. The pathology of EBUS-TBNA revealed some collagen fibers and calcified tissues in the inflammatory exudate. The enlarged LN was caused by chronic inflammation and was not considered to be metastasized when combined with clinical experience.

Intraobserver and Interobserver Agreement of EBUS Sonographic Findings

The Cohen's kappa values of EBUS findings were 0.83 (heterogeneous echogenicity), 0.93 (CHS), 0.85 (distinct margin), and 0.88 (nonhilar perfusion of vascular pattern) for the intraobserver agreement; and 0.82 (heterogeneous echogenicity), 0.90 (CHS), 0.84 (distinct margin), and 0.86 (nonhilar perfusion of vascular pattern) for the interobserver agreement. Fleiss's kappa values of qualitative scores of EBUS elastography were 0.97 for the intraobserver agreement and 0.95 for the interobserver agreement.

Diagnostic Yield of EBUS Characteristics in B-Mode and Vascular Patterns

Significant variables of the univariate analysis revealed CHS and vascular pattern as significant predictors of malignant nodes. The other 4 sonographic features did not show statistical significance (data not shown). Separate diagnostic test parameters are summarized in Table 2. The accuracy of predicting metastatic property was as high as 50.00–70.31% with 50.00% for distinct margins, 53.03% for short axis, 54.55% for round shape, 56.06% for in homogeneous echogenicity, 66.67% for CHS absent, and 70.31% for nonhilar perfusion vascular pattern. It could be concluded that this individual EBUS sonographic feature (B-mode or color Doppler) is suggestive of a malignancy that could not be sufficiently distinguished between benign and malignant LNs in this study.

Diagnostic Yield of EBUS Elastography

Real-time elastography qualitative scores were 4.29 ± 0.71 and 2.82 ± 1.18 ($p < 0.05$) in malignant and benign LNs, respectively, after consensus evaluation. By ROC analysis, the cutoff value of the qualitative elastographic score was 3, and yielded an area below the curve of 0.85 for differentiating benign and malignant nodes (Fig. 3). A score within 1–3 indicates benign LNs and a score within 4–5 indicates malignant nodes. Among the 33 benign LNs, 27 were colored as softness (score 1–3). Among the 35 malignant LNs, 30 were colored as stiffness (score 4–5) (Fig. 4a). Using qualitative methods in distinguishing benign from malignant LNs, sensitivity, specificity, and accuracy were 85.71, 81.82, and 83.82%, respectively (Ta-

Fig. 3. Receiver operating characteristic (ROC) curves for qualitative and quantitative methods. ROC analysis used for the differentiation between benign and malignant lymph nodes, based on the qualitative score and mean gray value of EBUS elastography images. The area under the curve was 0.85 ($p < 0.0001$, 95% CI of area 0.75–0.95) and 0.82 ($p < 0.0001$, 95% CI of area 0.70–0.93) for the qualitative score and mean gray value, respectively.

ble 2). However, 5 malignant nodes (4 SCLC and 1 lymphoma) were classified by pattern 3, while 6 benign nodes (3 tuberculosis nodes, 2 nonspecific inflammatory lymphadenitis nodes, and 1 sarcoid node) were classified by patterns 4 and 5. Typical false benign and malignant LNs of representative EBUS elastography scores are shown in Figure 4b–e.

In the quantitative analysis, the mean gray values for malignant LNs were 199.11 ± 5.64 and 169.52 ± 47.31 for benign nodes, respectively ($p = 1.13 \text{ E-}03$). The best cutoff value for the mean gray value was 192.32, which yielded an area below the curve of 0.82 by ROC analysis for differentiating benign from malignant nodes (Fig. 3). Using quantitative methods, we were able to differentiate between benign and malignant LNs with high sensitivity, specificity, and accuracy (91.43, 72.73, and 82.35%, respectively) (Table 2).

By qualitative and quantitative analyses, representative diseases (NSCLC, SCLC, tuberculosis, sarcoidosis, and nonspecific lymphadenitis) of EBUS elastography revealed different scores (Fig. 5). Particularly, nonspecific lymphadenitis revealed a significant difference when

compared with lung cancer (NSCLC and SCLC) (each $p < 0.01$). This suggests that EBUS elastography has the potential of differentiating nonspecific lymphadenitis from NSCLC and SCLC.

Discussion

It has been established that EBUS-TBNA sampling for malignancy gives a very high yield, and tissue diagnosis is paramount in such cases. Although EBUS imaging of conventional B-mode and power Doppler are very useful for the detection of intrathoracic LNs, the differential diagnosis between benign and malignant lesions remains difficult and has its limitations [8–10]. In our study, the most commonly used criteria for differential diagnosis in the decreasing order of importance according to diagnostic accuracy are as follows: nonhilar perfusion of vascular pattern (70.31%), absence of CHS (66.67%), echogenicity (56.06%), round shape (54.55%), short axis >1 cm (53.03%), and distinct margin (50.00%). It appears that nonhilar vascular perfusion is the best predictor. However, it has a limited sensitivity and specificity of 72.41 and 68.57%, respectively.

Elastography might provide image information to help distinguish benign from malignant tissues based on stiffness difference [22, 29]. Qualitative and quantitative methods were already used in the characterization of EBUS elastography, which exhibits better predictive accuracy than in conventional B-mode and color Doppler features, and is suggestive of malignancy [19, 21]. Our study revealed that EBUS elastography could be useful in predicting malignant LNs with a high sensitivity of 85.71 and 91.43% and a diagnosis accuracy of 83.82 and 82.35% based on qualitative and quantitative methods, respectively. Although EBUS elastography could hardly replace EBUS-TBNA in providing a pathological diagnosis, it could be a valuable supplemental method to EBUS-TBNA. How can these current results be integrated into clinical practice? When LNs were selected for the diagnosis and systemic staging of suspected lung cancer patients by EBUS, it suggested that we could sample the most likely malignant part within an LN or the LN in the same station with the highest probability of malignancy for TBNA according to EBUS elastography. Additionally, when EBUS elastography revealed a metastasis, and pathology was negative, EBUS-TBNA was recommended to be repeated or other further surgical methods were performed. On the other hand, if EBUS-TBNA returned as a normal lymphocyte or lymphoid tissue sample and EBUS elastogra-

Fig. 4. Qualitative results, representative false-negative and false-positive lymph nodes (LNs) of EBUS elastography. **a** The distribution of benign and malignant LNs according to qualitative score. **b** False-negative case of small cell lung cancer (SCLC) with an EBUS elastography score of 3. The histological specimen from EBUS-TBNA was diagnosed with SCLC with local obvious crush injury. HE. $\times 20$. **c** False-negative case of Hodgkin lymphoma with an EBUS elastography score of 3. The histological specimen from EBUS-TBNA revealed a local obvious crush injury. HE. $\times 20$. **d** False-positive case of non-specific lymphadenitis with an EBUS elastography score of 5. The histological specimen from EBUS-TBNA revealed hyperplasia of epithelioid and fiber cells. HE. $\times 20$. **e** False-positive case of tuberculosis with an EBUS elastography score of 5. The histological specimen from EBUS-TBNA revealed fiber cell hyperplasia with carbon deposition. HE. $\times 20$.

Fig. 5. Qualitative and quantitative results of different diseases with corresponding representative EBUS elastography images. Qualitative scores (**a**) and mean gray values (**b**) for different diseases including NSCLC, SCLC, tuberculosis, sarcoidosis and non-

specific lymphadenitis. **c-g** Representative EBUS elastography images of NSCLC, SCLC, tuberculosis, sarcoidosis and nonspecific lymphadenitis, respectively.

phy revealed benign features, confirmation by further invasive methods might not have been necessary. In conclusion, EBUS elastography could provide important additional diagnostic information for differentiating enlarged LNs when EBUS-TBNA provides a negative result or when used in patients not indicated for TBNA, and it can help support clinical suspicion of a benign or malignant diagnosis when lesion sampling is inconclusive.

The qualitative method of elastography is the simplest assessment for the evaluation of lesions, since color pattern is a visualized diagnostic standard that is easy to perform. A different team described only 3 elastographic patterns (predominantly non-blue, predominantly blue, and part blue, part non-blue). Their findings revealed that non-blue nodes were benign with 100% probability, while blue ones had a 94.6% chance of being malignant. However, indeterminate mixed-pattern nodes were just as likely to be benign as malignant [19]. He et al. [30] divided the scoring system into 4 grades and gave very clear-cut definitions of the different grades using the percentage of blue and non-blue areas, which was not appropriate in our opinion, because the color boundary of 50 and 80% was difficult to judge just with our eyes. Therefore, we introduced 5 scores of EBUS elastography, which could show a good agreement between these 3 observers regarding LNs belonging to a specific pattern. Our data suggest that elastography dominated by colors of green, yellow, and red or mixed partial blue areas is almost characteristic for benign LNs (scores 1, 2, and 3), and that elastography dominated by a blue color (scores 4 and 5) indicates malignancy. Benign LNs including physiological and reactive LNs showed heterogeneous or homogeneous soft patterns of mixed green-yellow-red or predominantly green. Particularly, the vascular structures of the LN hilum displayed soft patterns. Benign LNs like sarcoidosis, tuberculosis, and some chronic inflammation often showed intermediate hardness patterns with colors of blue, green, yellow, and red mixed. Malignant LNs may show inhomogeneous patterns for the focal necrosis or incomplete malignant infiltration, but predominantly hard patterns. Malignant LNs with diffuse metastatic infiltration often displayed homogeneous hard patterns [13]. The quantitative method was also introduced in our study to provide a more objective method of quantifying tissue stiffness in a defined ROI through computing the mean gray value from developed software. In our data, for the cutoff level of 192.32, this method has been shown to have a good sensitivity of 91.43% and a specificity of 72.73%. Other studies had used strain ratio as the quantitative method to characterize EBUS

elastography [21, 30]. Strain ratio is widely used in the evaluation of elastography, which is determined between lesions and its surrounding tissue. However, strain ratio measurement needs further evaluation in terms of the standardization of size and depth of the target of interest, as well as enough reference area. Since enlarged LNs always take most of the region with less area left for the surrounding tissues in a visual field of EBUS, the strain ratio method might have limitations in EBUS elastography evaluation [31]. The 2 current studies on the EBUS elastography-strain ratio evaluation had shown a significant variation in the strain ratio measurements for the LNs, since the best cutoff points of the strain ratio for differentiating malignant from benign LNs were 8 and 32.07, respectively [21, 30].

In our study, we found that some malignant tumors were very soft, while some benign lesions were very hard. There were some false-negative cases that may be explained in a similar way: the presence of abundant necrotic or vascular tissue resulted in an elastographic pattern that mainly consists of soft tissue. By contrast, there were some false-positive cases, which might represent patients with nonspecific inflammation, having areas of hard fibrotic or anthracotic tissues. This confirms again that EBUS elastography reveals tissue stiffness, and not malignancy or benignity. In addition, this study revealed that the common diseases of intrathoracic lymphadenopathy were, in the decreasing order, NSCLC, SCLC, tuberculosis, sarcoidosis, and nonspecific lymphadenitis, according to qualitative and quantitative scores of EBUS elastography. The qualitative scores of tuberculosis and sarcoidosis were approximately 3 and were relatively harder than nonspecific lymphadenitis, since epithelioid granulomas were always found in tuberculosis and sarcoidosis. This explains that a qualitative score of 3 was considered as a benign disease in this study. However, qualitative pattern analysis of EUS elastography images with 3 was usually regarded as malignant LNs. For example, in the study of Săftoiu et al. [23], a total of 31 patients including 42 LNs were enrolled; there were only 3 patients with lung cancer, 22 with gastrointestinal tumors, 2 with breast cancer and 3 with digestive tract inflammation, while no thoracic LNs were due to tuberculosis or sarcoidosis, which was common in the clinical practice of EBUS-TBNA. The softest lymphadenopathy was nonspecific lymphadenitis according to EBUS elastography, and it showed an obvious statistical difference from metastatic LNs. This implies that EBUS elastography could potentially predict metastatic LNs and achieve good diagnostic accuracy for the staging of lung cancer.

There are several limitations to this study. First, qualitative scores of EBUS elastography are a limitation due to their subjectivity. However, the associated intra- and interobserver variability was not significant. In addition, the quantitative method was also introduced to characterize EBUS elastography. Second, we only used representative static images from EBUS elastography videos, which was possibly associated with a significant selection bias induced by the examiners. Although 3 examiners were introduced to reach an agreement on this selected image in our study, it strongly suggests that quantitative analysis based on the average images computed from elastography videos is a more objective way to obtain a diagnosis, even for less experienced doctors [32, 33]. Third, the diagnostic assignment to benign or malignant LNs was not all based on the pathological diagnosis of EBUS-TBNA or other pathologies. In particular, 2 LNs from 2 lung cancer patients were judged to be benign only through clinical follow-up and experience when EBUS-TBNA did not show malignant pathology evidence. Fourth, this study mainly focused on the differential diagnosis of benign and malignant LNs using EBUS elastography, rather than on the staging of lung cancer. Therefore, we did not evaluate normal LNs on imaging, which might lead to some limitations for the application

in lung cancer LNs staging. The last limitation was due to the limited number of patients and single-institution retrospective nature of this study. More patients and clinical centers should be validated further in prospective studies.

In conclusion, the qualitative and quantitative methods introduced in this study were effective in characterizing EBUS elastography. We conclude that EBUS elastography is a promising method that allows for the differentiation of benign and malignant LNs with high sensitivity, specificity, and predictive accuracy, which may offer important supplementary information in EBUS-TBNA cases. However, the qualitative and quantitative results of this study should be validated further in prospective studies that include larger numbers of patients.

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