Determining Factors in Diagnosing Pulmonary Sarcoidosis by Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration

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Background. **Although the role of endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) in pulmonary sarcoidosis has previously been investigated, the determining factors in diagnosing sarcoidosis by EBUS-TBNA without rapid on-site evaluation (ROSE) are unclear.**

Methods. **Patients with clinically and radiographically suspected sarcoidosis underwent EBUS-TBNA without ROSE in a prospective study. Presence of non-caseating epithelioid cell granulomas was pathologic evidence of sarcoidosis.**

Results. **The EBUS-TBNA was performed in 120 patients, 111 of whom had confirmed sarcoidosis. For the patients with sarcoidosis (62 stage I, 49 stage II) EBUS-TBNA provided sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 93.69%, 100%, 100%, 56.25%, and 94.17%, respectively, in the diagnosis of sarcoidosis. Diagnostic yield of EBUS-TBNA for sarcoidosis was associated with disease stage, but not associated with serum angiotensin converting enzyme level, number of lymph node stations sampled per patient, or total number of passes performed per patient. At EBUS-TBNA, 284 mediastinal and hilar lymph nodes were aspirated in 111 patients. Multivariate logistic regression revealed that short-axis diameter and more than 1 needle pass per lymph node were independent risk factors associated with positive pathology. No major procedure-related complications were observed.**

Conclusions. **Endobronchial ultrasound-guided transbronchial needle aspiration is a safe procedure with high sensitivity for diagnosing sarcoidosis, having a higher diagnostic yield in stage I than stage II. To obtain a higher diagnostic yield of EBUS-TBNA in pulmonary sarcoidosis without ROSE, operators should select the largest mediastinal or hilar lymph node accessible and puncture with 3 to 5 passes.**

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Sarcoidosis is a multisystem disorder of unknown etiology that is characterized by non-caseating epithelioid cell granuloma, primarily affecting the lung and generalized lymphatic system. Enlarged paratracheal or hilar lymph nodes are present in up to 85% of patients [1]. The diagnosis is established when clinicoradiologic findings are supported by cytologic or histologic evidence of non-caseating epithelioid cell granulomas and other causes of granulomas have been excluded [2]. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a real-time, accurate, minimally invasive, and safe technique for assessing undiagnosed mediastinal and hilar adenopathy [3–6]. It has been widely used clinically in diagnosing sarcoidosis and has been one of the most important developments in the last decade [7]. While the value of EBUS-TBNA for the diagnosis of sarcoidosis has been investigated by some researchers [8–20], in a recent meta-analysis of its use in sarcoidosis the pooled sensitivity of EBUS-TBNA was 79%, ranging from 54% to 93% [21]. None the less, the accuracy of the diagnosis of EBUS-TBNA in sarcoidosis is quite variable. Thus, we have attempted to determine, by multivariate analysis, the important and related factors for predicting the diagnostic yield of EBUS-TBNA in sarcoidosis.

Patients and Methods

This prospective study reports the results of patients who underwent EBUS-TBNA in whom sarcoidosis was considered to be the leading pre-procedure diagnosis, with clinical and radiologic features suggestive of sarcoidosis. The EBUS-TBNA was conducted on consecutive patients with enlarged mediastinal or hilar lymph nodes (at least 1 node $\geq$ 1 cm in short-axis), based on computerized tomography (CT), from October 2009 to February 2012. Patients were excluded from the study if there was a significant clinical suspicion of malignancy or infection. Follow-up was conducted through February

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2014. All patients were fully informed of the procedure and written consent was obtained. The protocol was approved by the Ethics Committee of Shanghai Chest Hospital (KS10-03).

Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration

The EBUS-TBNA was performed with the patient under conscious sedation (midazolam) and local anesthesia (lidocaine), as described previously [22, 23]. After white light bronchoscopy was performed orally, target lymph nodes and peripheral vessels were examined by EBUS, using a linear array ultrasonic bronchoscope (BF-UC 260F-OL8; Olympus Ltd, Tokyo, Japan). Scanning was performed at the frequency of 7.5 MHz and images were processed by an Olympus ultrasound processor (EU-C2000; Olympus Ltd). Lymph nodes were classified based on the international staging system [24]. Diameter of target lymph nodes was measured and recorded under frozen ultrasound image. A dedicated 22-gauge needle was used for aspiration (NA-201SX-4022; Olympus Ltd). At least 2 needle aspirations were recommended for each target lymph node. However, if a visible histologic core specimen was obtained, 1 aspiration was acceptable. The bronchoscopist evaluated whether the procedure was sufficient for each sampled area. All procedures were conducted by 2 experienced bronchoscopists (S.J., T.J.). Rapid on-site evaluation (ROSE) was not performed. Cytologic smears were stained by hematoxylin and eosin by 2 pathologists blinded to subject details. 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 microbiologic assessment. Endobronchial or transbronchial lung biopsies for pathologic examination or bronchial brushings and washings for microbiologic detection (including special staining for acid-fast bacilli and fungi, as were specimens for culture for mycobacteria and fungi) were also performed according to the operator's judgment.

Data Collection and Outcome

Collected data included target location and diameter, aspirations per lymph node, and complications. Pathologic diagnosis by EBUS-TBNA and the final diagnosis were also reviewed. Specimen adequacy was defined by presence of a number of epithelioid cells, histocytes, or lymphocytes. Specimens revealing non-caseating epithelioid cell granulomas were classified as "positive," or "negative" if none found. The final diagnosis of pulmonary sarcoidosis was based on clinic radiologic findings being supported by histologic or cytologic specimens, demonstrating non-caseating epithelioid cell granulomas if a negative microbiologic result was obtained from all samples (Fig 1). Other granulomatous diseases were excluded by reviewing the patient's history and microbiologic results. All patients were followed up clinically and radiographically for at least 12 months.

An EBUS-TBNA diagnosis was subsequently confirmed by results of either other pathologic or microbiologic examination involving common bronchoscopy, CT-guided transthoracic needle aspiration, thoracotomy, mediastinoscopy, or clinical follow-up. Patients' subsequent therapy was performed on the basis of their corresponding final diagnosis.

Statistical Analysis

Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy rate of EBUS-TBNA were calculated according to standard definitions. Univariate and multivariate analyses assessed the independent risk factors for positive pathology. A t test was used for comparison of continuous variables and the χ^2 test or Fisher exact test, when appropriate, was used for categoric variables. Significance was considered for a p value less than 0.05 and all analyses were 2-sided. Significant variables in univariate analysis or those deemed clinically important were then entered in a multivariable logistic regression model. For statistical analyses, SPSS 11.5 (SPSS Inc, Chicago, IL) was used.

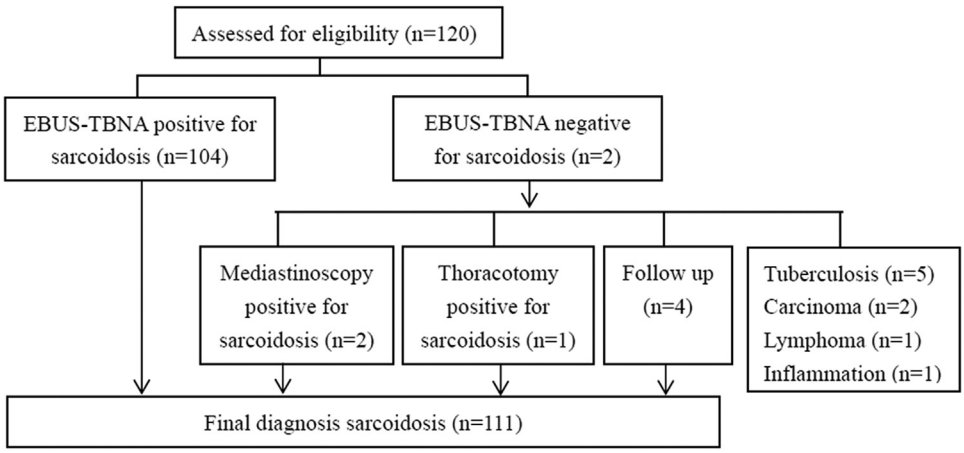

Fig 1. Patients with mediastinal or hilar lymphadenopathy and suspected sarcoidosis undergoing endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA).

Results

During the study period, 788 patients underwent EBUS-TBNA in our medical center, with 120 eligible patients being enrolled. Of the 120 patients, 36 were male and 84 female, with an average age of 50.24 years (range, 23 to 75). There were 111 patients finally diagnosed with sarcoidosis, the remaining patients having tuberculosis (n = 4), nonspecific inflammation (n = 2), lung cancer (n = 2), and lymphoma (n = 1) (Fig 1). No major procedure-related complications were observed.

The EBUS-TBNA revealed non-caseating epithelioid cell granulomas in 104 of 111 cases (93.69%). Microbiological evaluations for tuberculosis or fungal infection all gave negative results. The remaining 7 patients with sarcoidosis undiagnosed by EBUS-TBNA were eventually diagnosed by mediastinoscopy (n = 2), thoracotomy (n = 1), or clinical follow- up (n = 4), all being defined as false-negative cases (Fig 1). The EBUS-TBNA for sarcoidosis provided sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 93.69%, 100%, 100%, 56.25%, and 94.17%, respectively.

The diagnosis yield of EBUS-TBNA for sarcoidosis was associated with disease stage, but not with number of lymph node stations sampled (Table 1), serum angiotensin converting enzyme (SACE) level, or number of passes performed per patient. The SACE level and number of passes performed per patient of sarcoidosis patients diagnosed by EBUS-TBNA and those not diagnosed were 69.26 ± 30.45 U/L versus 60.41 ± 27.08 U/L, $p = 0.46$ and 7.17 ± 2.56 versus 7.14 ± 2.67, $p = 0.98$, respectively.

At EBUS-TBNA, 284 mediastinal and hilar lymph nodes were aspirated in the 111 patients. On univariate analysis, positive pathology was associated with short-axis diameter, long-axis diameter, more than 1 needle passes per lymph node, and disease stage. Lymph node region was not associated with positive pathology on univariate analysis (Table 2). Multivariate logistic regression revealed that short-axis diameter, more than 1 needle passes per lymph node, and disease stage

Table 1. Definitive Diagnosis by Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration (EBUS-TBNA) in 111 Patients

Characteristics	Diagnosis Yield	*p* Value
Disease stage		0.04
I	98.39% (61/62)	
II	87.76% (43/49)	
Stations per patient		0.53
1	86.67% (13/15)	
2	97.37% (37/38)	
3	95.00% (38/40)	
4	88.24% (15/17)	
5	100.00% (1/1)	
Total	93.69% (104/111)	

Table 2. Univariate and Multivariate Analysis of Factors Predicting Positive Pathology for Sarcoidosis in Specimens Undergoing Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration

Characteristic	Pathology Positive Lymph Nodes (n = 227)	Pathology Negative Lymph Nodes (n = 57)	Total (n = 284)	Univariate *p* Value	Multivariate *p* Value	Adjusted OR of Positive (95% CI)
Mean lymph nodes size by EBUS (range), cm						
Short-axis diameter	1.65 (0.8–3.28)	1.47 (0.64–2.17)	1.61 (0.64–3.28)	1.15E-03	0.01	3.02 (1.27–7.19)
Long-axis diameter	2.02 (1.06–4.28)	1.77 (1.08–3.25)	1.96 (1.06–4.28)	1.75E-03		
Disease stage (II/I)	90/137	37/20	127/157	9.34E-04	5.03E-03	0.41 (0.22–0.76)
Passes per node (≥2 passes/1 pass)	218/9	46/11	264/20	3.39E-04	5.67E-03	3.91 (1.49–10.28)
Region				0.54		
Superior mediastinal nodes (2R, 4L, 4R)	57	14	71			
Subcarinal nodes (7)	91	19	110			
N1 nodes (10L, 10R, 11L, 11Ri, 11Rs, 12L, 12R)	79	24	103			

CI = confidence interval; EBUS = endobronchial ultrasound; OR = odds ratio.

were independent risk factors associated with positive pathology (Table 2).

A diagnosis was established with 1 to 7 needle aspirations for 45% (9 of 20), 79.09% (87 of 110), 85.87% (79 of 92), 85.37% (35 of 41), 92.86% (13 of 14), 66.67% (2 of 3), and 50% (2 of 4) of sarcoidosis, respectively. The diagnostic yield of 3 biopsy specimens nearly reached a plateau, while with more than 5 needle aspirations per lymph node the diagnostic yield decreased (Fig 2).

Comment

A recent meta-analysis has suggested that future studies on EBUS-TBNA in sarcoidosis should employ a uniform methodology with regard to number of lymph nodes aspirated and number of passes per lymph node [21]. The objective of our study was to form such a methodology. Determination of factors related to diagnostic yield of EBUS-TBNA in sarcoidosis is important in improving diagnostic yield. Compared with published papers [8–20] concerning the diagnostic yield of sarcoidosis by EBUS-TBNA, our prospective study enrolled the largest number of patients with pulmonary sarcoidosis to date to determine the factors related to a positive diagnosis.

The utility of EBUS-TBNA in the diagnosis of sarcoidosis in our study was satisfactory. The sensitivity was 93.69%, higher than many studies [8-16, 18, 19]. The reasons may be the following. First, patients in our study were all stage I or stage II. While Garwood and colleagues [8] included patients with all stages, their diagnostic yield would have been much higher if they had only selected patients who had stage I or II disease. Second, our diagnosis was based on both cytologic and histologic results. Although histologic results may offer more information for a definitive diagnosis, cytology can be complementary. However, this requires an experienced cytologist and probably cannot be undertaken in a community hospital. Third, as a prospective exploration we punctured more passes per patient than most studies. The average number of passes per patient in our study was 7.17. There were 99.1% patients who had punctures for at least 3 passes, which may be another reason for our high diagnostic yield.

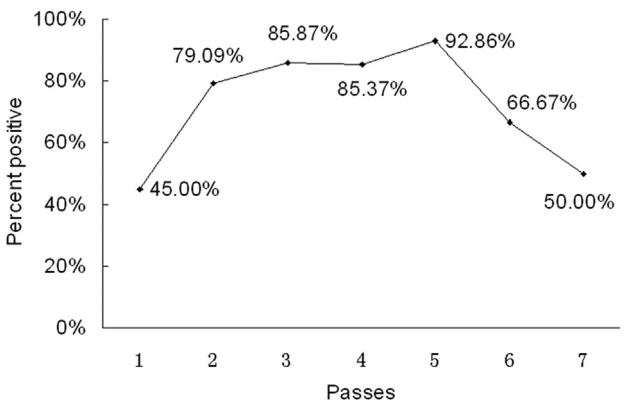

Fig 2. Yield per pass with endobronchial ultrasound-guided transbronchial needle aspiration per lymph node in patients with sarcoidosis.

We found that the diagnostic yield in stage I was significantly higher than that in stage II, similar to some studies [8, 12, 17] but not others [11, 14–16, 19]. With progressive stages of intrathoracic sarcoidosis, epithelioid cells from infiltrating intrathoracic lymph nodes infiltrate into lung parenchyma [2, 25, 26]. Thus, there may be more granulomas in intrathoracic lymph nodes of patients with sarcoidosis in stage I than that in stage II. The SACE levels are elevated in 60% of patients with sarcoidosis [2]. However, as a diagnostic tool measurement of SACE levels lacks sensitivity and specificity [27, 28]. Sensitivity of our study was 57.66% and we did not find that SACE level impacted the diagnosis yield of EBUS-TBNA. In addition, in our study the diagnostic yield was 87% when only 1 lymph node station was punctured, and there was no statistic difference between 1 station and more than 1 station being punctured. We found that more lymph node stations being biopsied did not increase diagnostic yield.

How to get a higher diagnostic yield of pulmonary sarcoidosis by EBUS-TBNA without ROSE? Garwood and colleagues [8] and Çağlayan and colleagues [16] reported more passes performed for each patient increased the sensitivity. While keeping the total number of passes per patient low, whether by performing more aspirations in fewer nodes or fewer aspirations in more nodes would increase the diagnostic yield has been unclear. Our study seems to have answered this question. Multivariate analysis based on lymph nodes revealed that the short axis and more than 1 needle pass per lymph node were independent risk factors associated with diagnostic yield. Larger lymph nodes resulted in a higher positive pathology rate as there is more active epithelioid cell hyperplasia in larger lymph nodes, making it easier to obtain the characteristic pathology of sarcoidosis. The diagnostic yield was higher when aspirating with more than 1 pass than aspirating with only 1 pass. However, there seemed to be no obvious increase in yield when more than 3 nodes were biopsied and decreased when more than 5 were biopsied. This means that when a satisfactory histology specimen was not obtained with 5 aspirations, the operator should consider aspirating other enlarged lymph nodes. Our study did not find a statistical difference in diagnostic yield among superior mediastinal, subcarinal, or hilar lymph nodes. Bilateral hilar lymphadenopathy has been noted in 50% to 85% of sarcoidosis patients [1], which means there is an advantage for EBUS-TBNA compared with other techniques such as mediastinoscopy, in which lower posterior carinal and hilar nodes stations are generally inaccessible [29].

Limitations

There are several limitations to our study. First, ROSE was not employed due to limitations of manpower and economy. Plit and colleagues [18] reported EBUS-TBNA with ROSE had high diagnostic accuracy and interobserver agreement, thus informing the bronchoscopist in real-time whether additional diagnostic procedures are needed. The ROSE also probably reduces the number of

punctured lymph nodes. Of note, a recent meta-analysis [21] suggested that the yield was not statistically different in studies employing on-site cytologic evaluation (80.1%) versus those without (81.3%). Second, because the ultrasonic function of scanning ranges is 50 degrees, when the observation target of the transverse diameter is too long, beyond the range that can be measured by ultrasound, lesions diameter measurement by EBUS is not always reliable for such nodes.

Conclusions

Endobronchial ultrasound-guided transbronchial needle aspiration is a safe procedure with high sensitivity for diagnosing sarcoidosis, having a higher diagnostic yield in stage I than stage II. To obtain a higher diagnostic yield of EBUS-TBNA in pulmonary sarcoidosis without ROSE, operators should select the largest mediastinal or hilar lymph node accessible and puncture with preferably 3 but up to 5 passes.

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