Endobronchial Ultrasound–Guided Transbronchial Needle Aspiration for the Diagnosis and Subtyping of Lymphoma

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Abstract

Background: Excisional biopsies are typically used to diagnose lymphoma, but data suggest that endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is equally effective. In this study, we determined whether EBUS-TBNA could accurately diagnose and subtype lymphoma.

Methods: The cases of patients who had undergone EBUS-TBNA for suspected lymphoma were retrospectively reviewed. EBUS-TBNA results were categorized as lymphoma, specific nonlymphoma diagnosis, granulomatous inflammation, or adequate or inadequate lymphocytes with no specific diagnosis. To quantify the ability of EBUS-TBNA to diagnose lymphoma, we used likelihood ratios. To quantify the ability of EBUS-TBNA to diagnose and subtype lymphoma, we calculated sensitivity and specificity. For this analysis, lymphoma that could be subtyped on the basis of EBUS-TBNA was classified as a true positive; lymphoma that could not be subtyped was classified as a false negative.

Results: Of the 181 patients included, 75 (41.5%) were ultimately diagnosed with lymphoma. EBUS-TBNA was able to establish a diagnosis of lymphoma in 63 patients (84%). Granulomatous inflammation diagnosed on the basis of EBUS-TBNA was associated with a low likelihood of lymphoma being present (likelihood ratio, 0.00; 95% confidence interval [CI], 0.00–0.276). Adequate lymphocytes were associated with a low likelihood of lymphoma (LR, 0.25; 95% CI, 0.14–0.49). EBUS-TBNA was able to establish a diagnosis and subtype the lymphoma in 67% (95% CI, 0.45–0.88) of patients with *de novo* lymphoma and 81% (95% CI, 0.70–0.91) of patients with relapsed lymphoma.

Conclusions: EBUS-TBNA is an effective, minimally invasive diagnostic test for patients with suspected lymphoma and can provide valuable clinical information, even with "negative" results.

Keywords: endobronchial ultrasound–guided fine needle aspiration; lymphoma; mediastinal lymphadenopathy

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Lymphoid neoplasia is diagnosed on the basis of the World Health Organization (WHO) classification system. The WHO recommends the use of multidimensional diagnostic modalities, including cytomorphologic studies, immunophenotyping, cytogenetic analyses, and molecular studies to accurately subtype lymphoma (1–4).

Surgical excision and core biopsy are the current preferred sampling techniques for diagnosing lymphoma, but there is growing interest in using less invasive endoscopic techniques with lower complication rates (5). In patients who present with intrathoracic adenopathy that is suspicious for lymphoma, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is an attractive option, given the risks of surgical or core needle sampling (6, 7). In such patients, EBUS-TBNA also may be useful for excluding alternative diagnoses, such as sarcoidosis, and may help to identify patients who require more invasive tissue sampling (8, 9).

The diagnostic utility of EBUS-TBNA in lung cancer diagnosis and staging, including biomarker analysis, has been well established (10, 11). Emerging data suggest that EBUS-TBNA, along with appropriate immunohistochemical, flow cytometric,

cytogenetic, and molecular studies, can definitively diagnose lymphoma (12, 13). Nonetheless, because disease management often hinges on the pathologic subtype and grade, there is concern that the smaller samples obtained via EBUS-TBNA compared with those obtained by surgical or core needle biopsy may be insufficient to guide treatment decisions. Even in our high-volume dedicated cancer center, EBUS-TBNA is only used in a minority of patients with suspected lymphoma.

There is a paucity of data on whether EBUS-TBNA provides adequate tissue samples for diagnosing and subtyping lymphoma (i.e., no additional biopsies are required for pathologic subtyping and grading). Additionally, sensitivity and specificity have been reported in most of the studies on EBUS-TBNA for lymphoma in the literature; however, sensitivity and specificity can have only two possible results: positive or negative. EBUS-TBNA typically has more than two meaningful possible results. For diagnostic tests in which there are more than two meaningful results, the use of likelihood ratios is more appropriate, because it reflects the information value of each of the categories separately. Therefore, in this study, we determined on the basis of likelihood ratios whether EBUS-TBNA could accurately diagnose and subtype lymphoma in patients with isolated intrathoracic adenopathy that was suspicious for lymphoma.

Methods

We performed a retrospective review of all EBUS-TBNA cases from January 2007 to March 2014 at our institution. Institutional review board approval was obtained under protocol number PA14-0320. Patients aged 18 years or older with isolated intrathoracic adenopathy suspected of lymphoma were included. We excluded patients with known history of sarcoidosis, mycobacterial disease, or fungal infections; lung masses suspicious for primary lung cancer; and nonlymphoma malignancies in the previous 5 years that had metastasized to the chest. Patient-level factors, including medical history, were abstracted from the patient charts.

Bronchoscopy Procedure and Specimen Processing

Details of the bronchoscopy procedure and specimen processing can be found in Appendix E1 in the online supplement.

Definitions

Suspected de novo *lymphoma* was defined as new isolated intrathoracic lymphadenopathy or a mediastinal mass found on imaging studies in patients with a clinically compatible presentation and no history of lymphoma. *Suspected recurrent lymphoma* was defined as new intrathoracic adenopathy or a mediastinal mass observed on imaging scans in patients with a clinically compatible presentation and a history of lymphoma. Clinically compatible presentation was determined by the referring physician and included any of the following: B symptoms (fever, night sweats, and/or weight loss), cytopenia, and/or peripheral lymphadenopathy.

The EBUS-TBNA results were classified according to the pathologic diagnosis, as follows:

1. *Lymphoma that could be subtyped*: EBUS-TBNA was diagnostic of lymphoma and subtyping was possible using solely the EBUS-TBNA sample; no additional tissue sampling was required.
2. *Lymphoma that could not be subtyped*: EBUS-TBNA demonstrated cells that were consistent with lymphoma on the basis of cytomorphologic or immunophenotypic features, but additional tissue sampling was required to subtype the disease.
3. *Specific nonlymphoma diagnosis*: EBUS-TBNA diagnosed a condition other than lymphoma. For example, the finding of acid-fast organisms in addition to granulomas was consistent with mycobacterial disease. The finding of other forms of cancer was also considered definitive.
4. *Granulomatous inflammation without a specific diagnosis*: EBUS-TBNA demonstrated granulomas but no specific diagnosis by direct staining. If granulomas were present and staining identified a pathogen (e.g., acid-fast organisms) in the same EBUS-TBNA sample, establishing a specific diagnosis (e.g., tuberculosis), it was classified as a specific nonlymphoma diagnosis rather than as granulomatous inflammation without a specific diagnosis.
5. *Adequate lymphocytes but no specific diagnosis*: Adequate benign lymphoid tissue was present on at least one EBUS-TBNA specimen.
6. *Inadequate lymphocytes but no specific diagnosis*: No cytologic evidence of lymph node sampling was present.

All patients were assigned a final diagnosis by the treating multidisciplinary hematology oncology and pulmonary team on the basis of a review of their clinical, pathologic, microbiologic, and radiologic data, with follow-up for at least 6 months. The final diagnostic categories were as follows:

1. De novo *lymphoma*: Final diagnosis of lymphoma in a patient with no history of lymphoma
2. *Relapsed lymphoma*: Final diagnosis of lymphoma in a patient with a history of lymphoma
3. *Other malignant diagnosis*: Nonlymphoma malignancy on final diagnosis
4. *Specific infectious disease*: Findings established by microbiologic cultures in the appropriate clinical context (e.g., histoplasmosis and tuberculosis)
5. *Sarcoidosis*: A diagnosis of sarcoidosis was based on clinicopathologic criteria if granulomas were found, there was a compatible clinical history, and other causes of granulomatous disease had been excluded.
6. *Other nonspecific benign diagnosis*: If no specific disease was found on EBUS-TBNA or another diagnostic test or biopsy and the patient was observed for a minimum of 6 months with no changes, the condition was classified as benign.

Those diagnosed with lymphoma were subclassified as having Hodgkin lymphoma (HL), high-grade non-Hodgkin lymphoma (NHL), or low-grade NHL.

Diagnostic Outcomes

We studied the ability of EBUS-TBNA to diagnose lymphoma by asking two distinct questions. The first was how effective EBUS-TBNA was in determining whether a patient had lymphoma. However, identifying that a patient has lymphoma is not always sufficient, because the precise subtype of lymphoma dictates treatment. Therefore, the second question was how effective EBUS-TBNA was in determining the subtype of lymphoma present so that treatment decisions could be made.

To determine whether EBUS-TBNA could diagnose lymphoma, we used likelihood ratios. We considered all patients with a final diagnosis of *de novo* or relapsed lymphoma to have lymphoma; this served as the reference standard of truth. Patients with any other final diagnosis were considered not to have lymphoma. A diagnosis of lymphoma included all lymphomas, even those that could not be subtyped. We used likelihood ratios to determine whether EBUS-TBNA could diagnose lymphoma because they allow the interpretation of diagnostic tests that have more than two possible results.

Table 1. Patient and clinical characteristics by final diagnosis

Characteristic	High-Grade NHL (*n* = *18*)	Low-Grade NHL (*n* = *36*)	Hodgkin Lymphoma (*n* = *21*)	Nonlymphoma (*n* = *106*)	*P* Value
Age, years					
Median (range)	66 (22–82)	68 (38–79)	35 (19–80)	55.5 (20–86)	
Mean (SD)	61.3 (16.9)	64.5 (10.6)	42.1 (17.3)	55.2 (13.8)	<0.0001*
Sex, n (%)					
Female	8 (44)	18 (50)	15 (72)	54 (51)	
Male	10 (56)	18 (50)	6 (28)	52 (49)	0.30
Race, n (%)					
White	13 (72)	31 (86)	14 (67)	79 (74)	
Black	3 (17)	3 (8)	4 (19)	15 (14)	
Hispanic	1 (6)	1 (3)	0 (0)	9 (8)	
Asian	1 (6)	1 (3)	3 (14)	3 (3)	0.24
Suspicion of lymphoma, n (%)					
De novo	10 (56)	5 (14)	3 (14)	52 (49)	
Relapsed	8 (44)	31 (86)	18 (86)	54 (51)	0.0001
Cough, n (%)					
No	12 (67)	25 (70)	14 (67)	81 (76)	
Yes	6 (33)	11 (30)	7 (33)	25 (24)	0.65
Fever of unknown origin, n (%)					
No	18 (100)	35 (97)	21 (100)	101 (95)	
Yes	0 (0)	1 (3)	0 (0)	5 (5)	0.91†
Fatigue, n (%)					
No	13 (72)	33 (21.3)	18 (86)	91 (86)	
Yes	5 (27)	3 (11.5)	3 (14)	15 (14)	0.32†
Night sweats, n (%)					
No	16 (89)	34 (94)	20 (95)	91 (86)	
Yes	2 (11)	2 (6)	1 (5)	15 (14)	0.50†
Weight loss, n (%)					
No	15 (83)	33 (92)	20 (95)	89 (84)	
Yes	3 (17)	3 (8)	1 (5)	17 (16)	0.45†
Hilar ± mediastinal lymphadenopathy, n (%)					
No	3 (17)	0 (0)	0 (0)	5 (5)	
Yes	15 (83)	36 (100)	21 (100)	101 (95)	0.05†
Bilateral hilar ± mediastinal lymphadenopathy, n (%)					
No	9 (50)	12 (33)	7 (33)	38 (33)	
Yes	9 (50)	24 (66)	14 (67)	68 (66)	0.64
Symmetrical hilar ± mediastinal lymphadenopathy, n (%)					
No	12 (67)	29 (80)	13 (62)	63 (60)	
Yes	6 (33)	7 (20)	8 (38)	43 (40)	0.15
Pulmonary infiltrates, n (%)					
No	15 (83)	25 (70)	20 (95)	94 (89)	
Yes	3 (17)	11 (30)	1 (5)	12 (11)	0.02
Mediastinal mass, n (%)					
No	12 (67)	35 (97)	19 (90)	96 (90)	
Yes	6 (33)	1 (3)	2 (10)	10 (10)	0.01†
Extrapulmonary adenopathy, n (%)					
No	18 (100)	29 (80)	21 (100)	101 (95)	
Yes	0 (0)	7 (20)	0 (0)	5 (5)	0.01†
Median lymph node size on EBUS, cm (range)	1.7 (0.8–6.3)	1.5 (0.6–3.5)	1.4 (0.7–2.5)	1.4 (0.5–3.3)	0.14*
Lymph node with FDG avidity, n (%)					
No	2 (11)	10 (28)	2 (10)	32 (30)	
Yes	16 (89)	22 (61)	18 (86)	69 (60)	0.08

Definition of abbreviations: EBUS= endobronchial ultrasound; FDG= fluorodeoxyglucose; NHL = non-Hodgkin lymphoma.
*Kruskal-Wallis test.
†Fisher's exact test.

Table 2. EBUS-TBNA likelihood ratio test for lymphoma (n = 75) and nonlymphoma (n = 106)

EBUS-TBNA Test Result	Lymphoma Absent	Lymphoma Present	Likelihood Ratio Test (*95% CI*)
Lymphoma	0	63	∞ (11.192, ∞)
Specific nonlymphoma diagnosis	12	0	0.00 (0.00–0.982)
Granulomatous inflammation	41	0	0.00 (0.00–0.276)
Adequate and inadequate lymphocytes	53	12	0.31 (0.181–0.545)

Definition of abbreviations: CI = confidence interval; EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration.

For the outcome of the ability of EBUS-TBNA to diagnose and subtype lymphoma, we calculated sensitivity and specificity. For this analysis, all EBUS-TBNA lymphoma results that could be subtyped were classified as true positives. Those that could not be subtyped were classified as negative. All other EBUS-TBNA results were also classified as negative for lymphoma. The reference standard for determining sensitivity and specificity was the final diagnosis. The patient had to be observed for a minimum of 6 months for the condition to be classified as benign on final diagnosis.

Statistical Methods

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were estimated for all patients and for patients in prespecified subgroups of interest: suspected *de novo* lymphoma versus relapsed lymphoma, HL, high-grade NHL, and low-grade NHL. We used χ^2 tests or Fisher's exact test to compare categorical variables and the nonparametric Kruskal-Wallis test to compare continuous variables.

Likelihood ratio was defined as the probability of seeing the result in those with the disease divided by the probability of seeing the result in those without that disease. As applied to this study, we were interested in likelihood ratio (lymphoma present), likelihood ratio (granulomatous inflammation), likelihood ratio (adequate lymphocytes), and likelihood ratio (inadequate lymphocytes). We applied Bayes' theorem to further refine the diagnostic utility of EBUS-TBNA for lymphoma diagnosis. Because the results are not dichotomous, we calculated posttest odds, which in turn were estimated by multiplying the pretest odds by the likelihood ratio. *P* values less than 0.05 were considered to be significant. All tests were two-sided. All statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC).

Results

We reviewed 4,070 consecutive EBUS-TBNA cases from January 2007 to March 2014. One hundred eighty-one patients had suspected lymphoma, 111 (61.3%) had suspected relapse, and 70 (38.7%) had suspected *de novo*. At final diagnosis, 75 patients (41.5%) had lymphoma.

Of the 111 patients with suspected relapsed lymphoma, 24 had undergone bone marrow transplantation before EBUS-TBNA, 86 had undergone chemotherapy alone, and 25 had undergone chemoradiation therapy. Patients with NHL tended to be older than patients with HL and patients with diagnoses other than lymphoma ($P < 0.0001$) (Table 1). Of the patients with a final diagnosis of lymphoma, 21 (28%) had HL and 54 (72%) had NHL (36 low grade and 18 high grade).

Three hundred sixty-nine lymph nodes and 19 mediastinal masses were sampled, with a median of 2 lymph nodes per patient. A median of five needle passes were performed per lymph node (range, 3–19). There was no difference in the number of passes between patients with and without lymphoma ($P = 0.27$).

Ability to Diagnose Lymphoma

Of the 75 patients who were eventually diagnosed with lymphoma, EBUS-TBNA was diagnostic in 63 (84%). The likelihood ratio of each finding is shown in Table 2. The likelihood ratio of an EBUS-TBNA demonstrating lymphoma was ∞ (95% CI, 11.192, ∞) because we considered all lymphoma results to be true positives. The likelihood ratio for granulomatous inflammation was 0.00 (95% CI, 0.00–0.276). The likelihood ratio for inadequate and adequate lymphocytes combined was 0.31 (95% CI, 0.181–0.545). Separately, the likelihood ratio for inadequate lymphocytes was 1.06 (95% CI, 0.24–4.59), and the likelihood ratio for adequate lymphocytes was 0.25 (95% CI, 0.14–0.49). Details of the likelihood ratio for *de novo* and recurrence stratified separately can be found in Table E1 in the online supplement. False-negative results were found in 24% of HL samples, 22% of high-grade NHL samples, and 8% of low-grade NHL samples ($P = 0.19$).

Table 3. EBUS-TBNA subtype versus final subtype of lymphoma

EBUS-TBNA Result for Subtyping of Lymphoma	Final Subtyping of Lymphoma				
	Nonlymphoma Diagnosis	Classical Hodgkin Lymphoma	High-Grade Non-Hodgkin Lymphoma	Low-Grade Non-Hodgkin Lymphoma	Total
Nonlymphoma diagnosis	106	9	5	3	123
Classical Hodgkin lymphoma	0	12	0	0	12
High-grade non-Hodgkin lymphoma	0	0	13	0	13
Low-grade non-Hodgkin lymphoma	0	0	0	33	33
Total	106	21	18	36	181

Definition of abbreviations: EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration.

Table 4. Sensitivity and specificity of EBUS-TBNA in diagnosing and subtyping lymphoma

Test	Estimate	95% CI	Total	True Positive	False Negative	False Positive	True Negative
Lymphoma, all patients							
Sensitivity	0.77	(0.68–0.87)	181	58	17	0	106
Specificity	1.00	(1–1)					
PPV	1.00	(1–1)					
NPV	0.86	(0.8–0.92)					
De novo							
Sensitivity	0.67	(0.45–0.88)	70	12	6	0	52
Specificity	1.00	(1–1)					
PPV	1.00	(1–1)					
NPV	0.90	(0.82–0.97)					
Relapsed							
Sensitivity	0.81	(0.7–0.91)	111	46	11	0	54
Specificity	1.00	(1–1)					
PPV	1.00	(1–1)					
NPV	0.83	(0.74–0.92)					
Hodgkin's lymphoma							
Sensitivity	0.57	(0.36–0.78)	21	12	9	0	0
PPV	1.00	(1–1)					
NPV	1.00	(1–1)					
NHL							
Sensitivity	0.85	(0.76–0.95)	54	46	8	0	0
PPV	1.00	(1–1)					
NPV	1.00	(1–1)					
High-grade NHL							
Sensitivity	0.72	(0.52–0.93)	18	13	5	0	0
PPV	1.00	(1–1)					
NPV	1.00	(1–1)					
Low-grade NHL							
Sensitivity	0.92	(0.83–1)	36	33	3	0	0
PPV	1.00	(1–1)					
NPV	1.00	(1–1)					

Definition of abbreviations: CI = confidence interval; EBUS-TBNA = endobronchial ultrasound–guided transbronchial needle aspiration; NHL = non-Hodgkin lymphoma; PPV = positive predictive value; NPV = negative predictive value.

Ability to Diagnose and Subtype Lymphoma

Of the 75 patients who were eventually diagnosed with lymphoma, EBUS-TBNA established a diagnosis that could be subtyped in 58 (12 *de novo* and 46 relapsed). The sensitivity of EBUS-TBNA in these cases was 77% (95% CI, 0.68–0.87). In 5 (7%) of the 75 patients with lymphoma, EBUS-TBNA diagnosed lymphoma, but the specific subtype could not be established. These cases were regarded as false negatives in this analysis because they required a confirmatory biopsy (Table 3). The sensitivity of EBUS-TBNA for the cohort and different subsets is shown in Table 4.

EBUS-TBNA had 67% (95% CI, 0.45–0.88) sensitivity for identifying *de novo* lymphoma. Its sensitivity in patients with relapsed lymphoma was 81% (95% CI, 0.70–0.91). There was no statistically significant difference between the sensitivities for *de novo* and relapsed lymphoma ($P = 0.33$).

Table 5. EBUS-TBNA result versus final diagnosis

	Final Diagnosis						
EBUS-TBNA Result	Lymphoma *De Novo*	Lymphoma Relapse	Other Malignant Diagnosis	Specific Infectious Diagnosis	Sarcoidosis	Nonspecific Benign Diagnosis	Total
Lymphoma, can be subtyped	12	46	0	0	0	0	58
Lymphoma, cannot be subtyped	2	3	0	0	0	0	5
Adequate lymphocytes	3	6	0	5	0	44	58
Inadequate lymphocytes	1	2	0	0	0	4	7
Granulomatous inflammation	0	0	0	5	18	18	41
Specific nonlymphoma diagnosis	0	0	11	1	0	0	12
Total	18	57	11	11	18	66	181

Definition of abbreviation: EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration.

False-negative results occurred more commonly with HL and high-grade NHL than with low-grade NHL (43% vs. 28% vs. 8%; $P = 0.008$). The difference between high-grade NHL and low-grade NHL was not statistically significant ($P = 0.10$).

Final Other Malignant Diagnosis

Other malignancies were ultimately diagnosed in 11 (6%) of the 181 patients. Small cell lung cancer was diagnosed in eight, and non–small cell lung cancer was diagnosed in three. EBUS-TBNA was diagnostic in all these patients.

Final Benign Diagnosis

Ninety-five patients had benign diagnoses on the basis of clinical findings, radiologic data, cultures, serologic findings, and confirmatory biopsies. These included 11 patients with a specific infection (2 mycobacterial and nine fungal), 18 with sarcoidosis, and 66 with a nonspecific benign condition.

All 66 patients with nonspecific benign diagnoses underwent clinical and imaging follow-up for a median of 18 months (range, 8–52 mo). Of the 66 patients who had a nonspecific benign diagnosis and in whom follow-up imaging was performed, 30 (45.5%) experienced a decrease in lymph node size and 36 (54.5%) experienced no change. No patients experienced an increase in lymph node size.

A comparison of the EBUS-TBNA results and the final diagnosis is shown in Table 5.

Discussion

EBUS-TBNA established a diagnosis in 84% of patients with lymphoma. In association with flow cytometry and immunohistochemical analysis, it had an overall sensitivity of 77%, specificity of 100%, and NPV of 86% in the diagnosis and subtyping of *de novo* and recurrent lymphoma. EBUS-TBNA performed the best among patients with a final diagnosis of low-grade NHL, with a 92% sensitivity rate. The sensitivity for HL was significantly lower; however, because of the small sample size, we could not demonstrate a difference between high- and low-grade NHL. Remarkably, no patients with granulomatous inflammation in our study developed or were diagnosed with lymphoma, which is contrary to the traditional belief that granulomatous inflammation is indicative of persistent or new lymphoma (14).

The findings in this study are similar to those of an earlier review of patients referred for EBUS-TBNA between August 2005 and December 2006 at our institution (15). In that study, we reported our initial experience in 25 patients, among whom lymphoma was ultimately diagnosed in 11 patients. In that study, EBUS-TBNA identified lymphoma in 10 (90.9%) of 11 patients with lymphoma, which is similar to the 84% we found in this study (15). However, in addition to the weakness inherent in any small study, one of the criticisms of our prior study was that patients in whom additional tissue was obtained for purposes of subtyping were included among the true positives. In that study, two patients identified as having lymphoma required additional biopsies for subtyping; thus, the ability of EBUS-TBNA to diagnose and subtype lymphoma was 72.7%, which is similar to our present finding.

The sensitivity of EBUS-TBNA for diagnosing and subtyping lymphoma in this study was somewhat lower than the 89% reported by Moonim and coworkers; however, their analysis included 16 patients with paired tissue samples that provided an additional confirmation of the diagnosis, and they also described immunophenotyping techniques not routinely available in the United States (16). Interestingly, they reported that an EBUS-TBNA diagnosis was adequate for clinical management in 52 (79%) of 66 lymphoma cases, a finding that is similar to our own. We found a much higher sensitivity than the 57% reported by Steinfort and colleagues (17), even after categorizing lymphoma that could not be subtyped as a false negative. That study, however, consisted of a far smaller patient population than that in our current study, with 44% of their patients suspected of sarcoidosis; in addition, the use of flow cytometry was not reported.

Our findings are in contrast to those reported by Iqbal and coworkers, who found an overall diagnostic sensitivity of 29% and a surprising nondiagnostic rate of 52% for EBUS-TBNA (18). Our EBUS-TBNA technique is different from that reported by those authors. We typically use a 22-gauge needle, perform an average of five passes per lymph node and perform cell counts to obtain 1 million cells. We also tend not to rely on subjective estimates of visible cores. Notably, on the basis of prior experience at our institution reported by Caraway and colleagues (19), immediate on-site assessment is necessary for an appropriate diagnostic workup of lymphoproliferative disorders in patients with mediastinal lymphadenopathy. We always triage material to ensure that adequate material is available for cytologic or ancillary studies, especially for immunophenotyping at the time of biopsy (19).

EBUS-TBNA was less sensitive for diagnosing HL in our study, which is consistent with the findings of other investigators and may reflect the scarcity of the characteristic Reed-Sternberg cells that are needed for the diagnosis (Figure 1) (16).

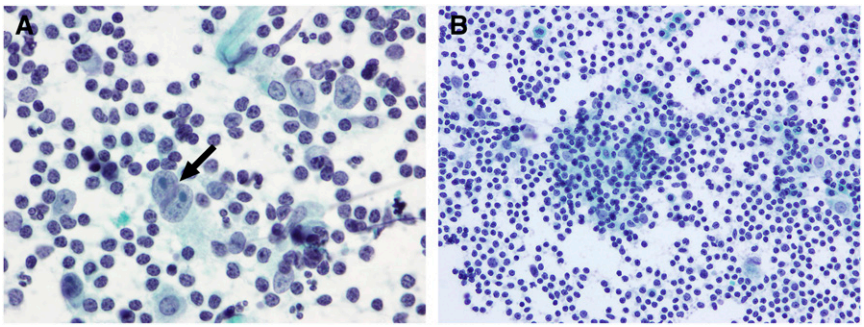

Figure 1. (*A*) Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) aspirate smear of Hodgkin lymphoma containing a large, atypical, binucleated (Reed-Sternberg) cell (*arrow*) on a background of lymphocytes (Papanicolaou stain). (*B*) EBUS-TBNA aspirate smear showing a dispersed population of small, cleaved lymphocytes with scattered large cells. Flow cytometric analysis demonstrated a κ light chain–restricted population with pan B cells expressing CD10, supporting a diagnosis of low-grade follicular lymphoma (Papanicolaou stain).

Additional tissue was needed in 19% of HL cases versus 2% of NHL cases ($P = 0.009$).

In our study, EBUS-TBNA had many gradations of positive and negative results. As we observed, a positive diagnosis of lymphoma that can be subtyped is different from a positive diagnosis of lymphoma that cannot be subtyped, because treatment depends on the subtype. In some studies, a diagnosis of lymphoma in which the specific subtype was not determined by EBUS-TBNA was considered a negative result (16). However, this result is not the same as having inadequate lymphocytes. An initial diagnosis of lymphoma justifies more invasive testing to determine the subtype. Similarly, a specific nonlymphoma diagnosis is informative, even if it is negative for lymphoma. Although this is clinically obvious, the likelihood ratio provides subtle insights into these other categories of results. In this study, we found that the negative result of granulomatous inflammation reduced the probability of lymphoma to almost zero. This is different from a negative result of inadequate lymphocytes. However, when complex histologic interpretations are simplified as either positive or negative, this information is lost, as is the ability to discriminate.

The clinical implications of our findings depend not only on the likelihood ratio but also on the pretest probability of disease. In patients with suspected *de novo* lymphoma, the prevalence of lymphoma was 26%. As Figure 2 shows, if the pretest probability of lymphoma is 26% and EBUS-TBNA demonstrates adequate lymphocytes, the posterior probability will be 8%. In contrast, patients with suspected recurrent lymphoma have a higher pretest probability of disease, as would be expected. In this study, the prevalence of recurrent lymphoma was 51%. If the pretest probability of lymphoma is 51% and EBUS-TBNA demonstrates adequate lymphocytes, the posterior probability will be 21%. In contrast, the posterior probability of disease will be much lower if EBUS-TBNA demonstrates granulomas, even in patients with a history of lymphoma.

We have integrated the concepts of pretest probability and likelihood ratios for EBUS-TBNA into a new diagnostic algorithm for patients with suspected lymphoma (Figure 3). If a patient has suspected *de novo* lymphoma and EBUS-TBNA shows granulomatous inflammation or adequate lymphocytes, careful observation with serial computed tomography (CT) and clinical follow-up is indicated, provided that the pretest probability is fairly low (as it was in our study and others) (15, 17, 20). On the one hand, if a patient has suspected recurrent lymphoma and EBUS-TBNA shows granulomatous inflammation, observation and serial CT are required. On the other hand, if a patient has suspected recurrent lymphoma and EBUS-TBNA shows adequate lymphocytes, the approach may depend on the initial subtype of lymphoma. As in other studies, EBUS-TBNA appears to be more sensitive for diagnosing low-grade NHL than HL and high-grade NHL. Observation is indicated in patients with a history of low-grade NHL if the EBUS-TBNA reveals adequate lymphocytes. However, in patients with HL or high-grade NHL, the use of another biopsy technique may be warranted.

The limitations of this study include its retrospective design, rather than being

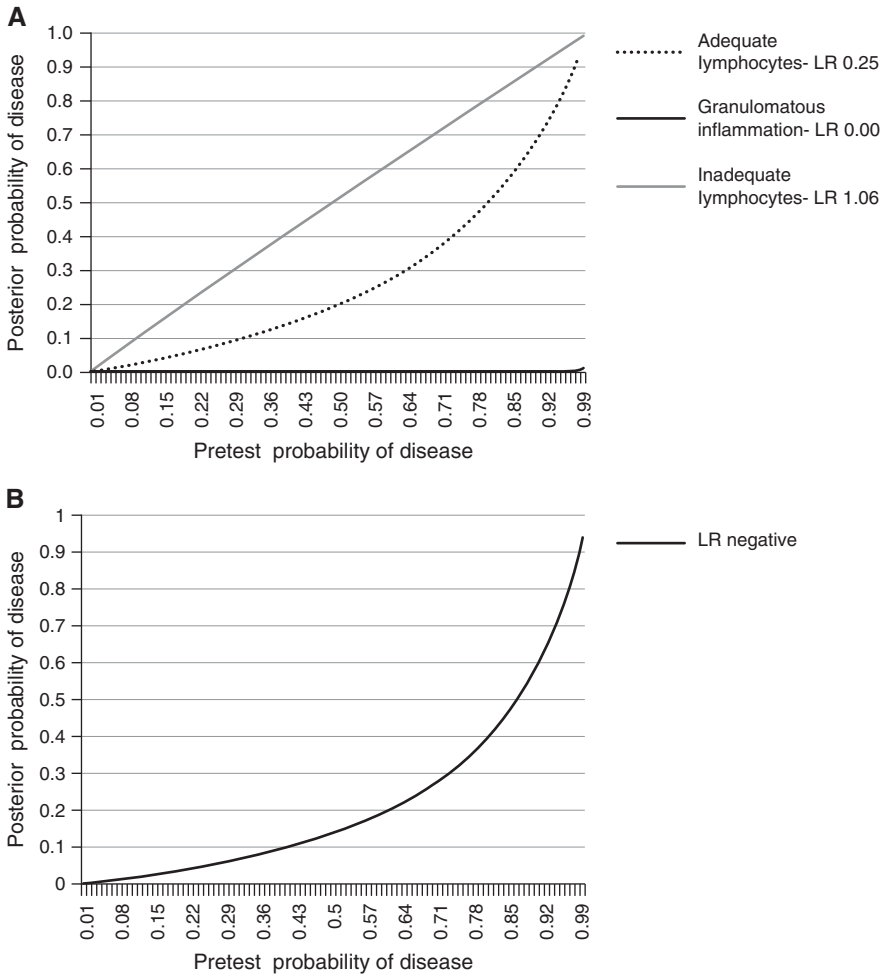

Figure 2. Pretest and posttest probability for different results of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). (*A*) Using the likelihood ratio (LR) method, patients with inadequate lymphocytes, adequate lymphocytes, and granulomatous inflammation each have different posterior probabilities for any given pretest probability. Note how a patient with granulomatous inflammation does not have the same posttest probability as one with adequate lymphocytes. (*B*) Simplified two-category test using conventional sensitivity and specificity; that is, patients with inadequate lymphocytes, adequate lymphocytes, and granulomatous inflammation are all considered to have negative results. Folding patients into a single category of negative LR will overestimate the probability of disease in those who have another specific diagnosis or granulomatous inflammation, and it will underestimate it in those with adequate lymphocytes or inadequate lymphocytes. "LR-negative" refers to the LR associated with a negative result when there are only two test categories.

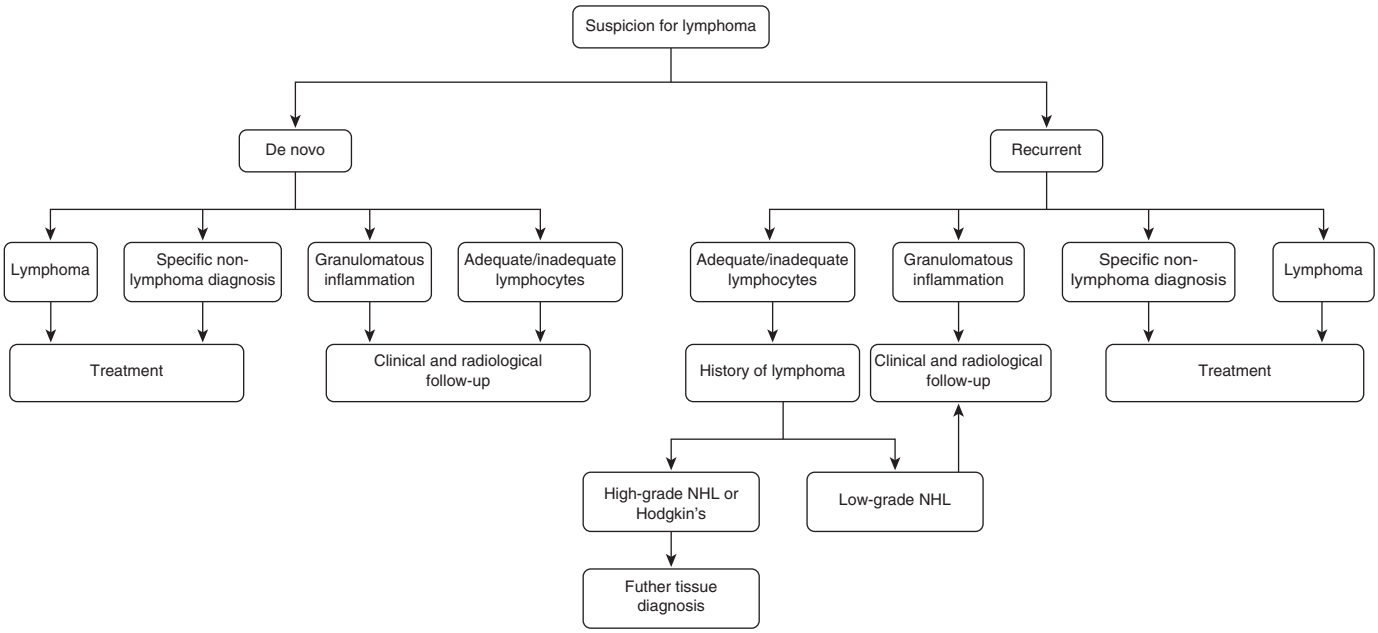

Figure 3. Algorithm for patients undergoing endobronchial ultrasound-guided transbronchial needle aspiration for suspected lymphoma. NHL = non-Hodgkin lymphoma.

a randomized trial or a study of multicenter registries. It involves a relatively small number of patients, and it is prone to selection bias because only patients referred for EBUS-TBNA were included; patients referred directly for surgical sampling were not included. In addition, as the posterior probabilities in our study are contingent on the prior probabilities, our results may not be generalizable to other patient populations, owing to differing lymphoma prevalence. Our patients were evaluated in a dedicated cancer center, so a referral bias compared with other centers cannot be excluded. Among patients with no prior history of lymphoma, we made every effort to clinically exclude patients in whom granulomatous disease such as sarcoidosis or mycobacterial disease was suspected.

Even after clinicoradiographic workup, however, there is inevitably a group of patients that require invasive tissue sampling. Because isolated mediastinal lymphoma is a relatively rare disease, it is also inevitable that a majority of such patients will end up with diagnoses other than lymphoma. In studies of isolated intrathoracic adenopathy that was not particularly suspicious for *de novo* lymphoma, lymphoma prevalence was as low as 4% (21). The prevalence of *de novo* lymphoma in our study was 26%, which is very similar to other published studies in this patient population, in whom lymphoma prevalence has ranged from 21% to 60% (15–17, 20).

Patients with a history of lymphoma constitute an important group in whom the appearance of new intrathoracic adenopathy clearly raises the specter of recurrent lymphoma and usually warrants invasive testing. This level of suspicion is borne out by our findings that fully 51% of these patients were ultimately diagnosed with lymphoma. This is very similar to prior published studies where the prevalence of lymphoma ranged between 43% and 61% for relapsed lymphoma, and our findings suggest that EBUS-TBNA can be very useful in this setting (15, 18, 19). For illustration purposes, we equated prevalence with pretest probability; however, clinical decision making should be driven by the pretest probability for an individual patient (based on the medical history, physical, and other diagnostic tests) rather than by the prevalence in a population. Also, the number for *de novo* lymphoma cases is small in our study; the confidence intervals are wide; and the generalizability of the findings may be limited because of the small sample size for this particular subgroup of patients.

The generalizability of our results also depends on the amount of pathology resources available and the specimen handling methods used. Our study population received care at a dedicated cancer center with a multidisciplinary team approach that included on-site cytopathology support and dedicated hematopathologists. Particularly among patients with suspected lymphoma relapse, the pathologists were not blinded to the prior history, and this could have influenced the results. All our samples were screened on site for cellularity, and quantitative cell counts were performed to confirm the samples' adequacy for ancillary testing, including flow cytometry. This screening allowed us to make procedural decisions, such as increasing the number of passes to maximize the yield. In addition, when granulomas or lymphocytes were found on rapid on-site evaluation, cultures were obtained to provide a definitive diagnosis. If these resources are not available and used in conjunction with EBUS-TBNA, EBUS-TBNA may not perform as well.

In summary, EBUS-TBNA is effective at diagnosing suspected lymphoma in patients with mediastinal lymphadenopathy. The present study, with 75 lymphoma cases, is the largest case series thus far describing the

performance characteristics of EBUS-TBNA in this population. We also demonstrated that EBUS-TBNA not only can diagnose the presence of lymphoma but also can provide sufficient information for subtyping in most cases. Also, by using likelihood ratios rather than conventional sensitivity and specificity, we demonstrated that certain EBUS-TBNA findings, such as granulomas, are associated with a low posterior probability of lymphoma. Given the higher discriminatory function and superior ability to predict posterior probability of disease, LRs may be more useful for clinical decision making than conventional sensitivity and specificity. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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