Until recently, tissue diagnosis of intrathoracic lymph nodes was being done by computed tomography (CT)-guided fine needle aspiration/biopsy, mediastinoscopy, or thoracoscopy. These investigations had limitations in terms of tissue yield, safety profile, and cost. In recent years, endoscopic ultrasound (EUS)-guided tissue sampling either through the esophagus (EUS) or

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Endobronchially (endobronchial ultrasound) has come up as safe and accurate method for achieving etiological diagnosis.\(^4,5\) Endobronchial ultrasound (EBUS) is an excellent complementary test to CT and positron emission tomography (PET) scan in staging of solid tumors of the lungs, especially those which are small and do not show definitive evidence of malignancy on CT scan or even esophageal cancer (where assessing the mediastinal lymph node involvement is crucial).\(^6,7\)

Another important and clinically very useful indication of EBUS is in patient with mediastinal lymphadenopathy of unknown etiology.\(^8,9\) Apart from providing structural information about the airway wall and surrounding structures, central and peripheral lung histological specimens can be obtained under sonographic guidance improving diagnostic and patient management.\(^10-13\) EBUS in a setup already using bronchoscopy and the training is a very cost-effective, feasible, and highly effective mode to evaluate mediastinal lymphadenopathy.\(^13,14\) In this study, we assessed the usefulness and safety profile of this technique in diagnosing mediastinal lymphadenopathy of unknown cause.

### SUBJECTS AND METHODS

#### Study design

A prospective observational study was conducted on 100 consecutive patients in the department from November 2011 to January 2013. Adult inpatient and outpatient individuals with mediastinal and/or hilar lymphadenopathy of unknown cause as evident on chest radiograph/CT scan were included in the study whereas those with a history of coagulopathy and recent myocardial infarction were excluded from the study. The study was approved by the ethical committee of the institution. A written informed consent was taken from all the subjects. The study protocol included detailed history, examination findings, measurement of complete hemogram, bleeding parameters (activated partial thromboplastin time and prothrombin time), tuberculin skin test (Mantoux) using 5 purified protein derivative units, and CT of the thorax.

#### Procedure

EBUS-guided transbronchial needle aspiration (TBNA) was performed with a 22-gauge needle (NA-201 SX-4022) under ultrasound and color Doppler guidance with an EBUS bronchoscope (BF-UC 180F, Olympus, Tokyo, Japan). EBUS-TBNA was performed on an outpatient/inpatient basis under conscious sedation using midazolam (2–5 mg intravenous [IV]) and pentazocine (10–30 mg IV). Lymph nodes stations and numbers were determined according to the 7th edition of the International Association of the Study of Lung Cancer Classification. EBUS-TBNA was performed through the bronchus with at least three passes of the needle per lesion. In each patient, the largest and/or most hypoechoic lymph node was targeted. Samples obtained by needle aspiration were analyzed by smear prepared as alcohol fixed in Papanicolaou stain and air-dried in May-Grunwald Giemsa stain.

All aspirates obtained were evaluated for:
1. Cytopathological examination (granulomas, necrosis, and tumor cells – further sent for typing of tumors)
2. Mycobacterial smears and culture in normal saline.

Cell blocks were prepared in formalin and sent wherever malignancy was suspected on the basis of history, clinical, and laboratory evaluation. It underwent morphological evaluation and immunohistochemistry. Specimens were tested for thyroid transcription factor-1, p-63 and if required any specific stain or marker to track the primary site.

The usefulness was evaluated by EBUS-TBNA diagnosis based on cytopathologic and microbiological analysis. After the procedure, a patient was observed for 2 h and was instructed to contact the hospital in the case of chest pain, breathlessness, or any other complaint.

### RESULTS

#### Baseline comparisons

The median age of study population was 47.1 years, with a range of 18–83 years. Forty of our patients belonged to age group of 40–60 years. This was followed by 31/100 patients in the age group of 20–40 years and 24/100 patients in 60–80 years. Only 4/100 and 1/100 patients were in 18–20 years and >80-year age group, respectively. Patients presented with fever (most common - 64), followed by cough (48). Shortness of breath was seen in 4 patients. Associated clinical features included weight loss (n = 42) and hemoptysis (n = 10) [Table 1].

On the basis of the CT scan findings, patients underwent EBUS-TBNA. In each patient, largest and most hypoechoic node was targeted. Most commonly located and targeted lymph node in our study was subcarinal (Station No. 7), accounting for around 55.72% of the total targets followed by paratracheal (4R and 4L) (25.1%), hilar (Station No. 10) (13.7%), and pretracheal (3%) lymph nodes (Station No. 2) [Figure 1].

![Figure 1: Location of lymph nodes targeted by EBUS-TBNA](image)

#### Table 1: Baseline characteristics of the patients

| Characteristics             | Findings |
|-----------------------------|----------|
| Age distribution (years)    | 18-83    |
| Mean age group (years)      | 47.15    |
| Sex distribution            | Male:female 63:37 |
|                            | Female: 37 |
| Symptoms profile            | Cough: 48 |
|                            | Joint pain: 34 |
|                            | Breathlessness: 4 |
|                            | Weight loss: 42 |
|                            | Hemoptysis: 10 |

| Cytology of the sample | Adequate:inadequate |
|------------------------|---------------------|
|                        | 92:8                |

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Cytopathological analyses of air-dried and alcohol-fixed smear showed adequate samples in 92/100 patients, i.e., with cytological findings suggestive of granuloma or atypical cells or reactive lymphoid tissue (rapid on-site evaluation [ROSE] was not performed due to nonavailability of on-site pathologist). Inadequate sample was 8/100 in which no definite pathology was obtained, and only scant material was aspirated. On further analysis of adequate samples, 71/92 (77.1%) were granulomas, followed by malignancy in 16/92 (17.3%). Reactive lymph nodes were 5/92 (5.4%) [Figure 2].

Out of the 71 patients with granuloma on cytology, 38 patients had necrosis, i.e., necrotizing granulomatous lymphadenitis and 33 had nonnecrotizing granulomas. Definitive diagnosis of mycobacterial disease was made when acid-fast bacilli (AFB) seen on Ziehl–Neelsen (ZN) staining and/or culture for mycobacteria was positive. The cases with necrotizing granuloma (AFB smear and culture negative) and a suggestive clinical profile were diagnosed as probable cases of TB (11/71 patients). Presumptive diagnosis of sarcoidosis and sarcoid-like granuloma was made on the basis of nonnecrotizing granuloma and AFB negative on ZN stain, with supportive clinical settings, namely, angiotensin-converting enzyme positive, tuberculin negative, and radiological evidence in 30/71 patients. One case each of Mycobacterium abscessus and Mycobacterium avium intercellulare was observed [Table 2 and Figure 3].

Malignancy was found in 16 (17.3%) patients. The most common malignancy was adenocarcinoma (6/16), followed by squamous cell (4/16) and small cell carcinoma (2/16). There were 3 patients of Hodgkin’s lymphoma and one had non-Hodgkin’ lymphoma, differentiated on the basis of immunohistochemistry.

Thus final diagnosis (out of 100 samples) was classified as follows:
1. Confirmed TB (30%)
2. Probable TB (11%) (necrotizing granuloma; AFB negative)
3. Atypical mycobacterial disease (2%)
4. Rest of nonnecrotizing granulomatous lymphadenitis (including cases of sarcoidosis and sarcoid-like reaction) (33%)
5. Malignant (16%)
6. Reactive lymphadenitis (5%)
7. Nondiagnostic sample (8%) [Table 3 and Figure 4].

On analyzing the safety profile, fever of mild to moderate in nature was seen in 26/100 patients who were relieved by taking a single dose of antipyretics. Nausea was seen in 40/100 patients. Mild bleeding and oxygen desaturation were observed in 4/100 and 2/100 patients, respectively, which were controlled by conservative measures during the procedure. Occasional complaints of bronchospasm were observed in 3/100 patients during observation period.

Table 2: Granulomatous etiology

| Etiology                        | Frequency | Percentage |
|--------------------------------|-----------|------------|
| Mycobacterial (TB and atypical) | 41        | 57.7       |
| Sarcoïd/sarcoïd-like            | 30        | 42.2       |
| Total                           | 71        | 100        |

TB: Tuberculosis

Table 3: Final diagnosis

| Diagnosis                        | Frequency | Percentage |
|----------------------------------|-----------|------------|
| Tuberculosis: Confirmed          | 30        | 30         |
| Tuberculosis: Probable           | 11        | 11         |
| Atypical mycobacterial disease   | 2         | 2          |
| Sarcoïd and sarcoïd-like         | 30        | 30         |
| Malignancy                       | 16        | 16         |
| Reactive lymphadenopathy         | 5         | 5          |
| Inadequate sample                | 8         | 8          |

Figure 1: Lymph node station targeted

Figure 2: Cytology of adequate sample

Figure 3: Mycobacterial etiology. M Tb = Mycobacterium tuberculosis, MAC = Mycobacterium avium complex, M. abscessus = Mycobacterium abscessus

Figure 4: Final diagnosis
All the patients were discharged from the hospital 3 h postprocedure, and none required any prolonged hospital stay or treatment from procedural complications.

**DISCUSSION**

In our prospective observational study, we analyzed the data of 100 consecutive adult patients presenting to the department. Previous studies on diagnosis of mediastinal and/or hilar lymphadenopathy by EBUS-TBNA have had a wide variation in total number of subjects and mean age. The range varied from as low as 18 in a study by Rintoul et al. to 502 in a study by Herth et al. In our study, the age group ranged between 18 and 83 years with mean age group of 47.1 years, implying a relatively younger age group involvement.

On the basis of CT findings, lymph nodes were targeted at least thrice which is recommended as per the current guidelines and other studies. Most common lymph nodes targeted were the mediastinal (subcarinal station 7 [55%] and paratracheal [25%]) lymph nodes followed by hilar (18%), which comprise maximum targeted in other studies as well.

In our study, there were 92 adequate samples, i.e., a high diagnostic yield of 92% with adequacy being defined as cytological findings suggestive of granuloma or atypical cells or reactive lymphoid tissue. Other international studies have reported higher diagnostic yield, ranging from 93.5% to 100%. These studies have evaluated suspected mediastinal metastasis of bronchogenic carcinoma or diagnosis of suspected malignancy. There is, however, a wide variation in recent Indian literature reports yield around 88% by Dhamija et al. and 78% by Gupta et al.

The adequacy varied over such a vast range due to variation in sample size, lymph node size (subcentimeter), calcified nodes, and learning curve of the procedure [Table 4].

Currently, thoracic CT and PET are used in the evaluation of mediastinal lymphadenopathy. Thoracic CT has a sensitivity of 55% and specificity of 81% in identifying malignant disease. PET-CT has a higher sensitivity and specificity than CT, but false positives may still occur, particularly in patients with granulomatous diseases such as TB and sarcoidosis. False negatives may also occur and are more common in lung adenocarcinoma and metastatic disease. The accuracy can be enhanced, and the false results can be reduced by combing imaging tools with EBUS.

The sensitivity and specificity of real-time EBUS-TBNA for mediastinal and hilar lymphadenopathy seem to be equivalent or even superior to that of mediastinoscopy, the “standard technique” with a diagnostic yield reported to be very high. However, mediastinoscopy can only sample nodal stations 1-4 and 7 but access to hilar nodal locations could be difficult and may require thoracoscopy and on some occasion a thoracotomy. Moreover, it cannot be repeatedly operated on the same patient.

Prior to the availability of EBUS, conventional TBNA and transbronchial lung biopsy (TBLB) have been the procedures of choice for diagnosis of intrathoracic pathology such as sarcoidosis. However, they have been mired with fears of poor yield and complications such as pneumothorax and lack of real-time guidance. EBUS came out to be as a single best procedure to obtain histological proof, especially sarcoidosis; however, according to the study by Gupta et al., it needs to be combined with TBLB for the best diagnostic yield. Further, if EBUS-TBNA is not

**Table 4: Comparative studies**

| Study          | Number of subjects | Age (years) | Location of lymph nodes | Diagnostic yield (%) | Final diagnosis | Adverse events       |
|----------------|--------------------|-------------|-------------------------|----------------------|-----------------|----------------------|
| Our study      | 100 (unknown etiology) | 47          | 7                       | 92                   | Granulomatous Malignancy | Few with fever, cough, etc. |
| International  |                    |             |                         |                      |                 |                      |
| Rintoul et al.[14] | 20 (suspected cancer) | 65          | Subcarinal=10            | 72.2                 | Staging done    | None                 |
|                |                    |             | Paratracheal (left/right)=9+2 |                      |                 |                      |
|                |                    |             | Pretracheal/vascular=3    |                      |                 |                      |
|                |                    |             | Hilar=2                  |                      |                 |                      |
| Herth et al.[15] | 502 (unknown and cancer) | 58.9       | Subcarinal=127           | 93.5                 | Malignancy=493 | None                 |
|                |                    |             | Paratracheal=93          |                      |                 |                      |
|                |                    |             | Hilar=82                 |                      |                 |                      |
| Yasufuku et al.[20] | 102 (NSCLC) | 67.8        | Mediastinal=82%          | 100                  | Staging done    | None                 |
|                |                    |             | Hilar=18%                |                      |                 |                      |
| Nakajima et al.[21] | 43 (known carcinoma) | 58.9       | Mediastinal=37           | 95.3                 | Staging done    | None                 |
|                |                    |             | Hilar=23                 |                      |                 |                      |
| Indian         |                    |             |                         |                      |                 |                      |
| Dhamija et al.[23] | 300 (unknown) | 11-88       | Paratracheal (left/right)=31/1 | 88                   | Sarcoïd=84     | None                 |
|                |                    |             | Paratracheal (left/right)=29 |                      | TB=75 Malignant=52 |                      |
|                |                    |             | Interlobar (right/left)=5/2 | 62.9                 | Sarcoïd=21 (53.8%) | None                 |
| Srinivasan et al.[24] | 39 (unknown) | 43          | Hilar=3                 | 92                   | Sarcoïd=11 (28.3%) | Malignancy=7 (17.9%) |

TB: Tuberculosis, NSCLC: Nonsmall cell lung cancer
available, conventional TBNA (with endobronchial biopsy and TBLB) may be used with equal efficacy.\textsuperscript{[24]}

A recent study from India described the transesophageal use of the EBUS scope for diagnosis of mediastinal lymphadenopathy in adults. It found the technique to be simple and feasible and when combined with EBUS-TBNA. This approach covers the main limitation of EBUS-TBNA to visualize posterior nodes. Hence, EBUS-TBNA, when combined with EUS, can sample all the key nodal stations and also can be performed repeatedly.\textsuperscript{[29,30]}

Out of these 92 cases, majority was constituted by granulomatous pathology (77%). The next common pathology was malignancy (17%) followed by reactive pathology seen in 5.4 cases. In India, studies so far show a similar trend. An Indian study by Srinivasan \textit{et al.} reported 37 cases, in which sarcoidosis was 53.8%, TB was 23.3%, and malignancy was 17.9%.\textsuperscript{[31]} However, western literature reported malignancy as the main etiology. Herth \textit{et al.} reported malignancy in 82% and granulomatous in 12% (sarcoidosis 9% and TB 3%).\textsuperscript{[32]} Navani \textit{et al} also evaluated role of EBUS TBNA in diagnosing tuberculosis.\textsuperscript{[33]}

Various studies have classified granulomatous etiology into mycobacterial and sarcoid. Studies by Lee \textit{et al.} and Tremblay \textit{et al.} also used cytological criteria, wherein they included only cases of sarcoid hence were biased. In our study,\textsuperscript{[31]} we divided granulomatous etiology on the basis of cytopathology in addition to the clinical and laboratory data on the lines of the studies by Asano and Costabel and Hunninghake.\textsuperscript{[34,35]} On the basis of these criteria, TB was observed in 41 cases (57.7% of granulomatous) and sarcoidosis in thirty cases (42.4% of granulomatous).

Recent studies have also been conducted on using EBUS-guided morphological characteristics of lymph nodes in diagnosis of intrathoracic lymphadenopathy such as size, shape, margins, echogenic appearance, and the presence of a central blood vessel.\textsuperscript{[36]}

Malignancy was seen in 17.3% cases. When we compare our data with studies in western countries, we found that malignancy constitutes a lesser proportion of cases. Another important observation was sending cell blocks prepared in formalin increased the yield. It allows pathologist to carry out immunohistochemistry and cell markers to evaluate atypical cells in specimens.

There were 5.43% ($n=5$) patients with cytological diagnosis of reactive lymphoid tissue requiring confirmation of diagnosis via more invasive procedures such as thoracoscopy and mediastinoscopy. These patients, however, refused further investigation and workup.

All the patients were observed for any intraprocedural and postprocedural complications. Fever was seen in 26 patients which was a higher number as compared to previous studies. Mild self-limiting bleeding and nausea were observed in ten and twenty patients, respectively. None of the studies reported serious complications. Only three studies reported having observed agitation, cough, and presence of blood at the puncture site. Some studies have reported mediastinal abscess and inflammatory polyp as a sequel to EBUS.\textsuperscript{[37-39]} However, in our study, no major complication was observed. EBUS-guided fine needle aspiration is not only a safe procedure but also helps us getting tissue diagnosis with minimal invasion.

Limitations of the study include (1) lack of follow-up of all cases with not confirmed diagnosis and (2) comparison of the tool with the gold standard, i.e., the mediastinoscopy. ROSE was also not performed as per the institutional nonavailability of an on-site cytopathologist.\textsuperscript{[19]}

**CONCLUSIONS**

Our data show that EBUS TBNA is a safe procedure with high diagnostic yield.

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**Conflicts of interest**

There are no conflicts of interest.

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