

## Utility of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and additional value of cell block in the diagnosis of mediastinal lymphadenopathy: A one year study in a tertiary care hospital in north India

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### Abstract

**Background:** Endobronchial Ultrasound guided Transbronchial Needle Aspiration (EBUS-TBNA) is a technique using which mediastinal lymph nodes can be sampled under real time ultrasonographic visualisation. In addition cell block can be prepared leading to increased diagnostic yield. We conducted a retrospective study to evaluate the role of EBUS-TBNA FNA and cell block in patients of enlarged mediastinal and hilar lymph nodes.

**Methodology:** We retrospectively studied 100 patients who underwent EBUS-TBNA FNA along with cell block preparation between January 2018 to December 2018. Bronchoalveolar lavage was also done. AFB staining and routine and fungal culture and sensitivity were done in all patients. The diagnostic accuracy of the FNA and cell block was analysed.

**Results:** We found 80% sensitivity and 100% specificity of EBUS-TBNA FNA. Sensitivity and specificity of cell block were found to be 94% and 100% respectively. Diagnostic accuracy of EBUS-TBNA FNA for tuberculosis and malignancy was 66.7% and 90% respectively while that of cell block was 83.3% and 90% for tuberculosis and malignancy respectively. Both FNA and cell block had 100% diagnostic accuracy for sarcoidosis.

**Conclusions:** In patients of mediastinal and hilar lymphadenopathy, EBUS-TBNA can be used as a procedure of choice as this is a safe procedure with reasonably high diagnostic accuracy. The sensitivity increases when EBUS aspirate is used along with cell block. Additional studies like IHC can be done on the cell block in patients of malignancy.

**Keywords:** EBUS TBNA FNA; Cell block; Mediastinal lymphadenopathy; Tuberculosis; Sarcoidosis; Malignancy.

### Introduction

Endobronchial Ultrasound-guided Transbronchial needle aspiration (EBUS-TBNA) is a technique by which mediastinal lymph nodes can be sampled using fine needle aspiration under direct ultrasonographic visualisation. As the aspiration biopsy is being performed under real time monitoring of ultrasound image the safety and accuracy is significantly increased as compared to conventional TBNA [1]. EBUS-TBNA is a minimally invasive technique which finds its use as a diagnostic procedure for patients of tuberculosis, sarcoidosis, lymphoma and malignancy presenting with mediastinal lymphadenopathy [2]. This technique also finds its merit in lung cancer staging and as a diagnostic procedure for intrapulmonary tumors [3]. Endobronchial ultrasound (EBUS) enables the visualization of lymph node structure, thus allowing the pulmonologist to evaluate and sample lymph nodes. Tumor invasion of the tracheobronchial wall can be assessed more accurately with EBUS than with CT [4]. This procedure has been used successfully and safely for sampling mediastinal and hilar lymph nodes over the past decade. Because of ultrasound guidance even nodes <10 mm can be safely aspirated. In patients with lung cancer and sarcoidosis, EBUS-TBNA has been shown to increase the yield and sensitivity compared with standard bronchoscopic techniques including conventional TBNA [5]. Further it offers a less invasive and safer technique compared with mediastinoscopy to sample intrathoracic lymph nodes [6]. This procedure can be performed in the ambulatory care setting under sedation and

tissue can be obtained with less potential complications [7]. Various studies have demonstrated a high sensitivity and specificity of EBUS-TBNA in detecting benign and malignant lung diseases [8,9]. EBUS-TBNA is an important alternative of mediastinoscopy in diagnosis of granulomatous intrathoracic lymphadenopathy with a high diagnostic accuracy in intrathoracic tubercular lymphadenopathy. As compared to conventional TBNA a higher smear and culture positivity can be obtained in a tuberculosis patient using EBUS-TBNA [10]. Even though newer modalities like Positron emission tomography-CT (PET-CT) have revolutionized cancer diagnosis, still tissue sampling would be needed to achieve the correct pathological diagnosis [11]. One additional advantage of EBUS is that cell block can be prepared which not only increases the diagnostic yield but can be utilized for additional studies like IHC [12]. There have been few studies demonstrating the efficacy of EBUS-TBNA FNA and cell block in the diagnosis of mediastinal lymphadenopathy. We present a one year retrospective study demonstrating the efficacy of EBUS-TBNA FNA and the additional utility of cell block.

### Materials and Methods

This is a one year retrospective study from January 2018 to December 2018. An ethical committee approval and a written informed consent from study subjects was obtained. A total of hundred patients who underwent EBUS-TBNA for hilar or mediastinal lymphadenopathy at our institution

were evaluated. In all cases EBUS-TBNA FNA was done, cell blocks were prepared and Bronchoalveolar Lavage (BAL) was also done. AFB smear was done on the BAL fluid in all cases, routine bacterial and fungal culture were done in all cases. Bronchoscopy was performed in a dedicated bronchoscopy room. Continuous sedation was given using Midazolam and Fentanyl.

### Preparation of Cell Block

The aspirate was placed into a container having buffered formalin. The sample was processed as a biopsy sample. Paraffin wax embedding was done and 6µm thick sections were obtained and stained using H & E staining. The cytology smears and cell block were evaluated by expert cytopathologist. Additional findings which helped in reaching a definitive diagnosis were Bronchoalveolar lavage fluid (BAL) AFB staining and routine bacterial and fungal culture findings. The criteria for adequacy of specimen was presence of lymph nodal tissue or granulomas with or without necrosis or if atypical cells were seen or if any other specific finding was seen that contributed to a specific diagnosis. The findings of aspirate smears and cell block were compared independently.

In patients where EBUS-TBNA FNA revealed granulomatous inflammation, findings of cell block and AFB stain of BAL fluid helped in reaching a definitive diagnosis. Clinical and radiological findings which helped in reaching a definitive diagnosis were given due consideration. Patients having AFB positivity were considered as a definitive diagnosis of tuberculosis. Patient with granulomas on aspiration or cell block but with AFB negative, however clinical and radiological findings suggestive of tuberculosis were considered as patients of tuberculosis.

Definitive diagnosis of sarcoidosis was taken in patients showing non-caseating granulomas in aspirate smears or cell block with negative AFB smear but with suggestive clinical and radiological features. In cases where there was absence of any specific cytopathological finding but sufficient material was there in FNA or cell block a diagnosis of reactive lymphadenopathy was made. Findings of BAL fluid culture were taken into consideration in all cases showing reactive lymphadenitis.

Patients having atypical cells in aspirate smears or in cell block or endobronchial biopsy wherever available were further investigated for malignancy.

### Results

In our 1 year retrospective study from January 2018 to December 2018, a total of 100 patients were evaluated. These patients had presented in the pulmonology department with enlarged mediastinal lymph nodes. EBUS-TBNA FNA with cell block was done in these patients and FNA smears and cell block were sent to the Pathology and department. BAL fluid aspirate for AFB smear and routine and fungal culture for all these patients was sent to the microbiology department. There were 54 males (54%) and 46 females (46%). Mean age was 48.7±19.6 years.

Maximum patients 34(34%) were in the age group of 61-80 years (Table 1 & 2).

Adequate material on EBUS-TBNA FNA for cytopathological evaluation could be obtained in 92 out of 100 patients (92%) while cell blocks showed adequate cellularity in all 100 patients (100%). Definitive diagnosis could be made on EBUS-TBNA FNA in 80 out of 100 (80%) patients. In the 20 patients in which definitive diagnosis could not be reached with EBUS-TBNA FNAC, eight had inadequate material. In rest of the 12 patients which were AFB smear positive on BAL fluid, however no granulomas or necrosis was seen on EBUS-TBNA FNA. EBUS-TBNA cell block was diagnostic in 94 out of 100 patients (94%). In the 6 patients of tuberculosis where a definitive diagnosis could not be made on cell block, a reactive picture was seen on the cell block. Diagnoses resulting from EBUS-TBNA are shown in Table 3.

Out of 100 patients a definitive diagnosis of tuberculosis was given in 36 patients (36%). Out of these 36 patients, 34 (94%) were positive for Acid Fast Bacilli on ZN stain. Sixteen of these 36 patients (44.4%) showed necrotizing granulomatous inflammation in EBUS aspirate while 8(22.3%) patients showed non necrotizing granulomas. In 10(27.8%) patients no granulomas or necrosis was seen. So diagnosis of reactive lymphadenitis was given on EBUS aspirate. In two clinical suspects with negative AFB smear and reactive EBUS-TBNA cytology but positive cell block (granulomatous), patients were treated as a case of tuberculosis and responded well to ATT. In cell block necrotizing granulomatous inflammation was seen in 18 out of 36 patients (50%). Non necrotizing granulomas were seen in 10 patients (27.8%). In 6 patients (18%) no necrosis or granulomas were seen. However AFB smear was positive in these 6 patients. So in 6 patients diagnosis of tuberculosis could not be made on cell block.

In 6 patients with clinical history, radiological findings and lab results (ACE levels) suggestive of sarcoidosis, non-necrotizing granulomas were seen in both EBUS aspirate and cell block. AFB smear was negative in these 6 patients. In 36 (36%) patients both EBUS-TBNA FNA and cell block did not show granulomas/necrosis. In these patients a diagnosis of reactive lymphadenitis was given. AFB smear was also negative. In 6 (16.7%) of these patients EBUS-TBNA FNA smears showed inadequate material. However adequate cellularity was seen on cell block. In patients in which diagnosis of reactive lymphadenitis was given, bronchoalveolar lavage (BAL) fluid culture findings were taken into consideration. Out of 36 patients, BAL fluid culture was sterile in 20 patients (55.5%). *Aspergillus spp* was grown in 10 patients (28%), *Candida spp* in 2 patients (5.5%), *Klebsiella pneumoniae* in 2 patients (5.5%) and *Pseudomonas aeruginosa* in 2 patients (5.5%) (Table 4).

Twenty two patients (22%) in our study were diagnosed positive for malignancy. Incidence of malignancy was higher in age group >40 years. EBUS-TBNA FNA smears were inadequate in 2 of these patients. However cell block showed adequate cellularity in all of these patients. There were 8 patients (36.4%) of Small Cell Carcinoma, 6 patients

(27.2%) each of Adenocarcinoma and Squamous Cell Carcinoma. In addition there were 2 patients (9.2%) of Non Hodgkin Lymphoma. In 20 patients (90%) we could give a diagnosis as suggestive of malignancy on EBUS-TBNA FNA including exact typing as Small Cell Carcinoma in one case. In both cases of Lymphoma correct diagnosis could be made on FNA and cell block. However further typing was done on Immunohistochemistry (IHC).

EBUS-TBNA FNA was diagnostic in 66.7%, 100% and 90% of cases of tuberculosis, sarcoidosis and malignancy

respectively. EBUS-TBNA cell block was diagnostic in 83.3%, 100% and 100% of cases of tuberculosis, sarcoidosis and malignancy respectively. Sensitivity and specificity of EBUS-TBNA FNA were 80% and 100% respectively with a positive predictive value of 100% and a negative predictive value of 64.29%. Sensitivity and specificity of EBUS-TBNA cell block were 94% and 100% respectively with a positive predictive value of 100% and a negative predictive value of 85.7%.

**Table 1:** Age wise distribution of patients in different diagnostic categories on EBUS-TBNA (N=100)

Age Group (years)	Tuberculous Lymphadenitis No. (%)	Sarcoidosis No. (%)	Reactive No. (%)	Malignancy No. (%)	Total No. (%)
0-20	6 (6%)	0 (0%)	6 (6%)	0 (0%)	12 (12%)
21-40	10 (10%)	4 (4%)	6 (6%)	0 (0%)	20 (20%)
41-60	10 (10%)	2 (2%)	10 (10%)	10 (10%)	32 (32%)
61-80	10 (10%)	0 (0%)	14 (14%)	10 (10%)	34 (34%)
>80	0 (0%)	0 (0%)	0 (0%)	2 (2%)	2 (2%)
<b>Total</b>	<b>36 (36%)</b>	<b>6 (6%)</b>	<b>36 (36%)</b>	<b>22 (22%)</b>	<b>100 (100%)</b>

**Table 2:** Sex wise distribution of patients in different diagnostic categories on EBUS-TBNA (N=100)

Age Group (years)	Tuberculous Lymphadenitis No. (%)	Sarcoidosis No. (%)	Reactive No. (%)	Malignancy No. (%)	Total No. (%)
Male	16 (16%)	2 (2%)	22 (22%)	14 (14%)	54 (54%)
Female	20 (20%)	4(4%)	14 (14%)	8 (8%)	46 (46%)
Total	36 (36%)	6 (6%)	36 (36%)	22 (22%)	100 (100%)

**Table 3:** Distribution of diagnoses on EBUS-TBNA FNAC and Cell Block (N=100)

S. No.	Final Diagnosis (N=50) No. (%)	Diagnosis on Cytology Smear No. (%)	Diagnosis on Cell Block No. (%)
1	<b>Tuberculosis- 36 (36%)</b>		
	A) Microbiologically confirmed (AFB positive) tuberculosis- 34 (34%)	(N=36) NGL- 16 (44.4%) GL- 8 (22.3%) Reactive- 10 (27.8%)	(N=36) NGL- 18 (50%) GL- 10 (27.8%) Reactive- 6 (16.7%)
	B) AFB negative with Granulomatous Lymphadenitis with clinical history/radiology suggestive of tuberculosis- 2 (2%)	(N=36) Reactive- 2 (5.5%)	(N=36) GL- 2 (5.5%)
2	<b>Non necrotizing Granulomatous Lymphadenitis (Sarcoidosis) with suggestive clinical history- 6 (6%)</b>	(N=6) GL- 6 (100%)	(N=6) GL- 6 (100%)
3	<b>Reactive- 36 (36%)</b>	(N=36) Inadequate - 6 (16.7%) Reactive- 30 (83.3%)	(N=36) Reactive- 36 (100%)
	<b>Malignancy- 22 (22%)</b>		
	A) Squamous Cell Carcinoma- 6 (27.2%)	(N=22) Suggestive of malignancy- 6 (27.2%)	(N=22) Squamous Cell Carcinoma- 6 (27.2%)

4	B) Small Cell Carcinoma- 8 (36.4%)	(N=22) Inadequate- 2 (9.1%) Suggestive of malignancy- 4 (18.2%) Small Cell Carcinoma- 2 (9.1%)	(N=22) Small Cell Carcinoma- 8 (36.4%)
	C) Adenocarcinoma- 6 (27.2%)	(N=22) Suggestive of malignancy- 6 (27.5%)	(N=22) Adenocarcinoma- 6 (27.2%)
	D) Non Hodgkin Lymphoma- 2 (9.2%)	(N=22) Non Hodgkin Lymphoma- 2 (9.2%)	(N=22) Non Hodgkin Lymphoma- 2 (9.2%)

\* GL= Granulomatous lymphadenitis, NGL=Necrotizing Granulomatous Lymphadenitis, AFB=Acid Fast Bacilli

**Table 4:** Correlation of reactive cases with Bronchoalveolar (BAL) fluid culture (N=36)

S. No	BAL fluid culture results	No (%) (N=36)
1.	<i>Aspergillus spp</i>	10 (28%)
2.	<i>Candida spp</i>	2 (5.5%)
3.	<i>Klebsiella pneumoniae</i>	2 (5.5%)
4.	<i>Pseudomonas aeruginosa</i>	2 (5.5%)
5.	Sterile	20 (55.5%)

**Discussion**

EBUS-TBNA has evolved into an indispensable tool for the pulmonologists in the evaluation of the mediastinal lymphadenopathy [13]. Conventional TBNA without direct visualization of the targeted mediastinal lymph node has a diagnostic yield varying from 47% to 87%. EBUS-TBNA can obtain larger samples than conventional TBNA and is safer [3]. Compared with mediastinoscopy, the procedure is less invasive and safer, requires no general anaesthesia and is simpler. Even nodes unreachable by mediastinoscopy can be accessed [1]. Additional advantage is that not only tissue diagnosis can be obtained using cell block but also IHC can be applied on the cell block in case of malignancies.

In our study we found out that EBUS-TBNA is a relatively safe procedure which can be done on day care basis. An additional advantage is that cell block can be prepared leading to an increase in diagnostic accuracy. Choi et al in their study reported an overall diagnostic accuracy of 83.9% for EBUS-TBNA FNA and 50% for tuberculosis [14]. The results of our study are at par with those of Choi et al. The overall diagnostic accuracy of EBUS-TBNA FNA in our study was 80%, while for tuberculosis it was 66.7%. Our findings are also at par with those of Su Ying et al who reported in their study a diagnostic accuracy of 69% for tuberculosis [9].

The overall diagnostic accuracy of EBUS-TBNA increased in our study when cell blocks were used. There was a 14% increase in the diagnostic accuracy. The diagnostic accuracy for tuberculosis was also higher with cell blocks as compared to aspirate smears (83.3% vs 66.7%). Sun et al and Cetinkaya et al reported the sensitivity of EBUS-TBNA in tuberculosis to be 85% and 79% respectively [15,16]. Our results support the use of cell block as an adjunct to EBUS-TBNA FNA. It is easier to

recognize granulomas in cell block as compared to aspirate smears because of better preservation of morphology in the clotted specimen of cell block. Sedimentation and paraffin embedding further aid in the preservation of histological structure of the granulomas. Also because of spreading and smearing of samples between two slides in FNA smears, disturbance of architecture of epithelioid cell groups occurs. For these reasons recognition of granulomas becomes easier in a cell block leading to an increase in the diagnostic yield [11].

In our study we found a high diagnostic accuracy of EBUS-TBNA FNA and cell block for sarcoidosis which was 100%. This was greater as compared to the diagnostic accuracy for tuberculosis in our study. Our findings were corroborated by the study of Mediha et al., who also found greater diagnostic accuracy of EBUS-TBNA for sarcoidosis as compared to tuberculosis [4]. Sensitivity of EBUS-TBNA in detecting noncaseating granulomas was found to be 85% in a study by Navani et al [17]. We found a high diagnostic accuracy of EBUS TBNA FNA for malignancies which was 90%. Our finding is at par with that of Choi et al who reported 93% diagnostic accuracy of EBUS-TBNA FNA for malignancy. They also reported that the diagnostic accuracy of EBUS-TBNA was higher for malignant diseases than for benign diseases which holds true for our study as well [14]. In our study also we found that the diagnostic accuracy of EBUS-FNA was higher for malignancy as compared to benign diseases (90% vs 76.9%). Our results for malignancy are almost at par with those of Yasufuku et al., who found 93% diagnostic accuracy of EBUS-TBNA FNA for malignancy [18]. Herth et al., reported a diagnostic accuracy of 97.1% of EBUS-TBNA FNA for malignancy in their study [19]. The diagnostic accuracy of EBUS-TBNA cell block for malignancy was found to be 100% in our study. In

all 22 patients definitive typing could be done on the cell block which was further confirmed on IHC.

Cell block not only increases the diagnostic yield and accuracy but also provide material for Immunohistochemistry, genetic and molecular studies. In the era of targeted therapies role of genetic and molecular analysis of tumors cannot be overemphasized. Current guidelines advocate the sub-classification and genetic analysis particularly for non-small cell lung cancer [4].

### Conclusions

Our study emphasizes that there is a definite role of EBUS-TBNA FNA and cell block in diagnosing mediastinal and hilar lymphadenopathy. This procedure is fairly safe and can be done on a day care basis, with a high diagnostic accuracy for sarcoidosis, tuberculosis and primary lung malignancies, lymphomas as well as metastatic disease involving mediastinal lymph nodes. The sensitivity increases when EBUS aspirate is used along with cell block. Typing of malignancy can be done using the cell block as additional studies like IHC can be performed on the cell block. Thus EBUS-TBNA along with cell block should be used as a procedure of choice for mediastinal and hilar lymphadenopathy as it is a safe, minimally invasive procedure with a reasonably high diagnostic accuracy.

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