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Original Article

Role of fiberoptic bronchoscopy-guided needle aspiration cytology (EBNA) in diagnosing lung cancer in endobronchial lesions: A single-center experience

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ABSTRACT

Objectives: Globally, lung cancer is the leading cause of new cancer diagnosis and deaths. In spite of advancement in diagnostic modalities, lung cancer diagnosis is often delayed due to lack of bronchoscopy facility and techniques. In the present study, we have analyzed role of bronchoscopy in diagnosis of lung cancer with special emphasis on endobronchial needle aspiration (EBNA) cytology in comparison to other conventional diagnostic techniques (CDTs) such as bronchial wash (BW) and forcep biopsy (FB).

Material and Methods: Prospective and observational study screened 1496 cases with suspected lung malignancy on clinical and radiological basis. Bronchoscopy guided techniques such as EBNA, BW, and FB are used in exophytic endobronchial lesions (EEL) in confirming the diagnosis of lung cancer and to find additive yield over other techniques such as BW and FB. Rapid on-site evaluation analysis of all EBNA samples done in pathology laboratory allied center. Finally, histopathology proven 893 lung malignancy cases are included in study. Statistical analysis is done using Chi-square test.

Results: In the present study, 893 diagnosed lung cancer patients between 29 and 85 age groups; male is 60.02% (536/893) and females are 39.97% (357/893). Commoner radiological presenting features are mass lesion in 48.60% (434/893) cases, Hilar opacity in 33.37% (298/893) cases, and collapse segmental/lobar in 12.54% (112/893) cases. During bronchoscopy, anatomical location is documented on the right side of tracheobronchial in 57.33% (512/893) cases as compared to the left side of tracheobronchial wall 23.96% (214/893) and growth at carina documented in 18.70% cases (167/893) cases. Upper lobe bronchi are commoner site on both the sides as compared to other segmental bronchi. In the present study, yield of FB and FB plus BW in EEL is 84.65% (756/893) and 88.35% (789/893), respectively. Yield of EBNA, EBNA plus BW, and EBNA plus FB in EEL is 70.99% (634/893), 73.48% (656/893), and 99.66% (890/893), respectively. Overall, yield of all bronchoscopy guided techniques (EBNA + FB + BW) in our study in EEL is 100%. Additional yield of EBNA in EEL over other CDTs (CDTs such as FB plus BW) is 11.65%. Sensitivity of FB and EBNA in diagnosing lung malignancy in EEL is 84.65% and 70.99%, respectively. FB is more sensitive technique than EBNA in EEL ($P < 0.00001$). Sensitivity of FB plus BW in EEL is 88.35% (789/893). Sensitivity of EBNA plus BW in EEL is 73.48% (656/893). Sensitivity of EBNA plus FB in EEL is 99.66% (890/893) ($P < 0.00001$).

Conclusion: EBNA has documented very crucial role in diagnosing lung cancer in comparison to other CDTs. Although FB is more sensitive test then EBNA in EEL in diagnosing disease, we have documented that EBNA has significant additive yield in proportionate number of cases. EBNA is safe, sensitive and cytology samples can give comparable results to histopathology.

Keywords: Endobronchial needle aspiration cytology, Bronchoscopy, Bronchial wash, Forcep biopsy, Cytology

INTRODUCTION

Globally, lung cancer is the leading cause of new cancer diagnosis and deaths. In spite of advancement in diagnostic modalities, lung cancer diagnosis is often delayed due to lack of bronchoscopy facility and techniques. In India, lung cancer accounts for 5.9% of all cancers and 8.1% of all cancer-related deaths.[1] History of bronchoscope use to examine tracheobronchial tree was traced to the 18th century and used rigid illuminating pipes.[2] In the mid-19th century, Ikeda invented fiberoptic bronchoscopy which has revolutionized the practice of interventional pulmonary medicine.[3] Modifications and advancements in bronchoscopy has undergone in the past five decades such as autofluorescence techniques for the early diagnosis of lung cancer, real time image guided sampling for exact staging of lung cancer by mediastinal lymph.[4] In addition, bronchoscopy advancement such as cryotherapy, argon plasma coagulation, laser, and electrocautery can be utilized now for treatment of central airway lung cancers and maintaining patency of central airways.[4]

Yield of various bronchoscopic techniques such as bronchial washing, bronchial brushing, and endobronchial and transbronchial biopsy depends on visibility of tumors endoscopically. In endobronchial pathology, yield of these techniques is satisfactory and highest for forcep biopsy (FB) 74% in comparison to bronchial brushing 59% and bronchial washing 48%. Significant increase in yield of bronchoscopy observed with combination of all these modalities up to 88%.[4] Bronchoscopic lesions or abnormalities have been classified as endobronchial, submucosal, and peribronchial types according to their visibility. Needle aspiration cytology has been validated over decades during bronchoscopy for diagnosis of lung cancer in all these three types of lesions and categorized as EBNA or endobronchial needle aspiration cytology and TBNA or transbronchial needle aspiration cytology.[5] Addition of EBNA or TBNA in these lesions enhanced diagnostic yield and sensitivity of bronchoscopy procedure in addition with other conventional diagnostic modalities.[5] TBNA is superior to all other conventional sampling modalities in peribronchial and submucosal lesions and its results are comparable with bronchoscopic FB in endobronchial tumor with an average diagnostic yield of 80%.[6] Dasgupta *et al*.[5] and Govert *et al*.[6] documented use of EBNA, along with other modalities during bronchoscopy in diagnosis of lung cancer in exophytic endobronchial lesions (EEL) and observed additive yield of EBNA and specifically mentioned EBNA that is the only positive test in proportionate number of cases.

Bronchoscopy-guided EBNA/TBNA is complementary to other conventional diagnostic techniques (CDTs) such as bronchial wash (BW), bronchial brush cytology, and FB.[7,8] Interestingly, conventional TBNA/EBNA is still underutilized in majority of bronchoscopy centers across the world.[9] Reasons for underutilization of this novel technique EBNA/TBNA would be inadequate trainings to technique, difficulty in needle handling during bronchoscopy and cytology technique, decreased yield with poor technique and inadequate laboratory backup due to lack of rapid on-site evaluation (ROSE) facility, and lastly lack of cytopathology expertise over histopathology in lung malignancy.[10] In spite of an increase in diagnostic yield by addition of EBNA/TBNA to other conventional diagnostic modalities, it is not possible to perform all techniques in the same patient.[10] In the present study, we have utilized all conventional fiberoptic bronchoscopy-guided diagnostic modalities including EBNA in diagnosing lung malignancies.

MATERIAL AND METHODS

Data source

Prospective and observational study conducted during January 2014–September 2022 in Respiratory Medicine and Critical Care Medicine Department in Venkatesh chest hospital and MIMSR Medical College, Latur. Objectives of the present study were to document the role of EBNA in EEL in confirming the diagnosis of lung cancer and to find out additive yield over other conventional techniques such as BW and FB. Total 1496 suspected lung malignancies on clinical and radiological basis were screened and finally 893 confirmed lung cancer cases were included in the study after the hospital's ethical committee approval. We have taken written informed consent of all study patients [Figure 1].

Inclusion criteria

Suspected lung malignancies on clinical and radiological basis such as-

1. Cases with unexplained paralysis of vocal cord (hoarseness of voice) or stridor
2. Chest X-ray with radiological features of malignancy (coin lesions, mass lesions, mediastinal widening, unilateral high hemidiaphragm, segmental/complete lung collapse, and non-resolving pneumonia)
3. Normal chest X-ray with high clinical suspicion, localized monophonic wheeze, endobronchial disease, or growth symptoms such as hemoptysis, persistent cough, cases with suspected recurrent post-obstructive pneumonia, suspicious sputum cytology, unexplained, and recurrent pleural effusion.

Exclusion criteria

Cases unfit for bronchoscopy were excluded such as-

1. Cases with coagulopathy which cannot be corrected and platelets
2. Cases with mechanical ventilation with high PEEP

3. Cases with refractory hypoxemia
4. Cases with recent myocardial infarction or unstable angina
5. Cases with significant dysrhythmia and hemodynamic instability
6. Cases with poor ability to cooperate with procedure.

Ethical approval

This study is approved by the Ethics Committee of Venkatesh Chest Hospital and Critical Care Center and MIMSR Medical college, Latur, India (Approval # VCC/122014; Approval date September 2, 2014).

Bronchoscopic definition of exophytic endobronchial growth (EEL)

During the bronchoscopy procedure, operators notified different types of endobronchial growth patterns. These exophytic endobronchial lesions were described as cauliflower like, pedunculated, polypoidal, nodular exophytic, multinodular ulcerated' endobronchial growth [Figure1].

Lesions with normal endobronchial mucosa with bulge (peribronchial growth) and abnormal endobronchial mucosa without oblivious growth were not included in the definition of endobronchial growth.

Bronchoscopy procedure in endoscopy suit

Bronchoscopy procedure was performed in endoscopy suit by Fujinon Epx 201H bronchoscope by two operators/teaching faculties of our institute trained in all bronchoscopy techniques including EBNA. Bronchoscopy procedure followed standard guidelines for topical analgesia during endoscopy used 10% lignocaine solution. Bronchoscope introduced transnasally in majority of cases and few cases required oral introduction whenever difficulty in nasal negotiation in the presence of nasal mucosa hypertrophy. Pre-procedure topical analgesia was given with xylocaine jelly for nasal mucosa and xylocaine spray for oropharyngeal mucosa. During bronchoscopy procedure, topical instillation of 10% xylocaine solution for analgesia of vocal cords and epiglottis.

Study design

After negotiation and entry of bronchoscope in trachea to carina, we have bronchoscopically installed small aliquots of diluted 1% lignocaine. During bronchoscopy procedure, conventional technique sequences were decided as EBNA first, BW, bronchial brush, and then FB to avoid contamination due to bleeding secondary to biopsy samplings. Following this sequence as EBNA first also helped us in avoiding false positivity and in some cases with fleshy vascular growth, we have avoided major bleeding by performing EBNA than FB. We have used 22-gauge MW 522 needle catheters (Mill-Rose Laboratories) during bronchoscopy to perform EBNA procedure. The bronchoscope was moved just proximal to endobronchial growth and the needle with catheter was moved out of scope, then needle was pushed out and introduced into endobronchial lesion. EBNA methodology as classically described in cytology technique "to and fro" used under applied suction from a 20-mL syringe. We have performed 4–5 passages of EBNA samplings during bronchoscopy and stop the EBNA only after confirmation by assistant and cytopathology technician for adequacy of specimen. We were having a ROSE facility and adequacy of samples was judged during the procedure in the majority of cases. Prepared EBNA cytology slides and fixed with 95% alcohol immediately for prevention of artefact and to increase the yield. All other bronchoscopic samples (forcep biopsy and bronchial wash) were sent for cytology and histopathology examination at the Pathology Department [Figure 1].

EBNA cytology images documented during the present study with typical cytology findings of Non-small cell carcinoma [Figure 2], Small cell carcinoma [Figure 3], Squamous cell carcinoma [Figure 4] and Dysplasia [Figure 5] were categorized.

Statistical analysis

The statistical analysis was done using Chi-square test in R-3.4 software. Significant values of χ^2 were seen from probability table for different degree of freedom required. *P* value was considered significant if it was below 0.05 and highly significant in case if it was <0.001.

RESULTS

Covariates

In the present study, 893 diagnosed lung cancer patients between 29 and 85 age groups, male is 60.02% (536/893) and females are 39.97% (357/893). In addiction history, we have observed that 58.11% (519/893) cases are smoker and 39.16% cases with smoking index more than 20 pack years. Commoner symptoms are cough in 79.84% (713/893), shortness of breath in 46.13% (412/893), hemoptysis in 33.03% (295/893), and chest pain in 22.17% (198/893) cases. Clubbing on general physical examination is documented in 38.29% (342/893) cases. Commoner radiological presenting features are mass lesion in 48.60% (434/893) cases, Hilar opacity in 33.37% (298/893) cases, and collapse segmental/lobar in 12.54% (112/893) cases. During bronchoscopy, anatomical location is documented on the right side of tracheobronchial in 57.33% (512/893)

Table 1: Clinical Evaluation, radiological patterns, and anatomical sites during bronchoscopy (*n*=893).

Symptoms/signs	Number of Patients (*n*=893)	Percentage
Cough	713	79.84
SOB	412	46.13
Hemoptysis	295	33.03
Chest pain	198	22.17
Weight loss	156	17.46
Hoarseness of voice	65	7.27
Clubbing	342	38.29
SVC syndrome	49	5.48
Lymphadenopathy	39	4.36
Radiological feature		
Mass lesion	434	48.60
Hilar Opacity	298	33.37
Collapse (lobar/segmental)	112	12.54
Consolidation	98	10.97
Pleural effusion	85	9.51
Mediastinal Widening	76	8.51
Site of lesion		
Right side	512	57.33
Left side	214	23.96
Carina	167	18.70

cases as compared to left side of tracheobronchial wall 23.96% (214/893) and growth at carina documented in 18.70% cases (167/893) cases. Upper lobe bronchi are commoner site on both the sides as compared to other segmental bronchi [Table 1].

Core observations

In the present study, yield of FB and FB plus BW in EEL is 84.65% (756/893) and 88.35% (789/893), respectively. Yield of EBNA, EBNA plus BW, and EBNA plus FB in EEL is 70.99% (634/893), 73.48% (656/893), and 99.66% (890/893), respectively. Overall, yield of all bronchoscopy guided techniques (EBNA + FB + BW) in our study in EEL is 100%. Additional yield of EBNA in EEL over other CDTs (CDTs such as FB plus BW) is 11.65% [Table 2]. Sensitivity of FB and EBNA in diagnosing lung malignancy in EEL is 84.65% and 70.99%, respectively. FB is more sensitive technique than EBNA in EEL ($P < 0.00001$) [Table 2]. Sensitivity of forcep biopsy and EBNA in diagnosing lung malignancy in EEL is 84.65% and 70.99% respectively. Forcep biopsy is more sensitive technique than EBNA in EEL ($P < 0.00001$) [Table 3]. Histopathology type in the present study: We have documented adenocarcinoma in 40.76% (364/893) cases, squamous cell carcinoma in 35.72% (319/893) cases, non-small cell carcinoma in 12.43% (111//893) cases, small cell carcinoma in 7.61% (68/893) cases, and large cell carcinoma in 3.47% (31/893) cases. Adenocarcinoma trends are equally observed histological type as compared to squamous cell type irrespective of smoking trends in the study cases. We have documented that EBNA samples have given satisfactory results with histopathology specimens subjected to immunohistochemistry. In adenocarcinoma, 69% cases are EGFR positive, 6% ALK positive, and 8% ROS positive and 17% are all negative.

Table 2: Diagnostic yield of fiberoptic bronchoscopy-guided procedures in EEL (*n*=893).

Number of patients of exophytic lesions (*n*=893)	Positive results (*n*=893)	Yield (%)
EBNA positive only	634	70.99
FB positive only	756	84.65
Both EBNA and FB	890	99.66
EBNA+BW	656	73.48
FB+BW	789	88.35
EBNA+FB+BW	893	100

EEL: Exophytic endobronchial lesions, FB: Forcep biopsy, BW: Bronchial wash, EBNA: Endobronchial needle aspiration cytology

Table 3: Sensitivity of bronchoscopy guided EBNA and FB in EEL (*n*=893).

Technique	Positive yield	No yield	Total diagnosed cases
FB	756	137	893
EBNA	634	259	893

χ^2=48.29 P<0.00001. FB: Forcep biopsy, EBNA: Endobronchial needle aspiration cytology, EEL: Exophytic endobronchial lesions

DISCUSSION

Diagnostic yield of EBNA and EBNA plus techniques in endobronchial lesions

Total yield of EBNA in EEL is 70.99% (634/893). In our previous published studies,[11,12] we have documented 62.60% and 60.66%, respectively, in small sample sizes. Kacar *et al*.[13] observed yield in 77.9% cases. Numerous studies[5,14,15] have reported 70–96% diagnostic yield of EBNA in central airway tumors suspected of bronchogenic carcinoma.

In the present study, yield of EBNA, EBNA plus BW, and EBNA plus FB in EEL is 70.99% (634/893), 73.48% (656/893), and 99.66% (890/893), respectively. Thus, EBNA has documented complimentary role to other conventional diagnostic tests BW and FB in increasing significant yield. Similarly, studies by Salathe *et al*.[16] Caglayan *et al*.[17] reported increase in diagnostic yield after adding EBNA to CDTs were 65–79% and 79–91%, respectively ($P < 0.001$). Various authors, Hapomik *et al*.,[10] Gullón *et al*.,[18] and Gellert *et al*.,[19] observed that addition of EBNA to conventional diagnostic yield increases diagnostic sensitivity of bronchoscopy in EEL. They also mentioned that yield is significantly reduced

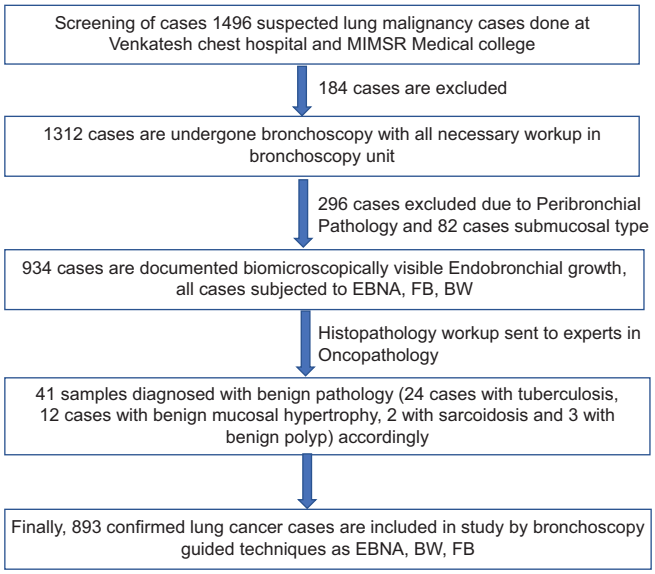

Figure 1: Image showing flow of study. EBNA: Endobronchial needle aspiration cytology, BW: Bronchial wash, FB: Forcep biopsy.

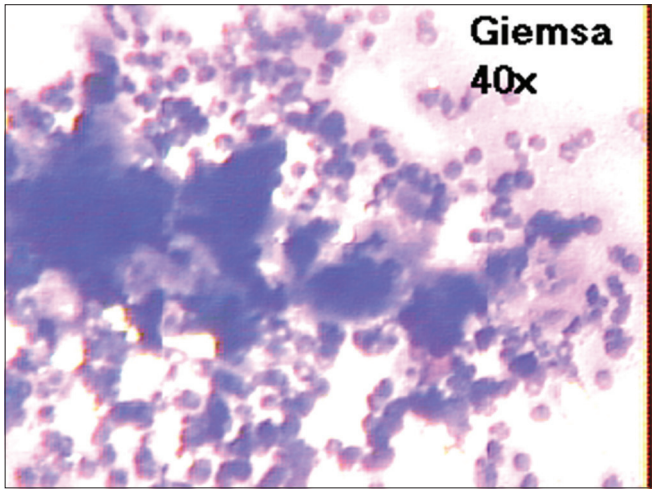

Figure 2: Non-small cell carcinoma in endobronchial needle aspiration cytology.

and chances of repeat procedure increased with CDTs without EBNA in the presence of endobronchial growth. Authors[5,14,15,17-19] have observed that rationale for decreased yield with CDTs is chances of inadequate sampling during FB due to superficial necrosis resulting in to negative yield, the presence of blood clot over lesions giving negative biopsy results. Authors[5,14,15,17-19] have mentioned chances of crush artifacts formation during FB technique resulted into inadequate sampling processed during histopathology especially during "serrated edges forcep type" in their studies.

We have used alligator forcep with needle and rat tooth type to prevent crush artifacts issue in our study. These hurdles of FB resulting into deceased bronchoscopy can be easily tackled with

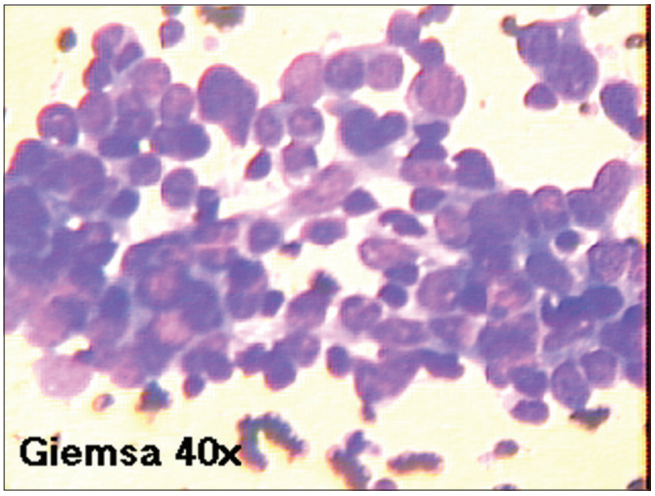

Figure 3: Small cell carcinoma in endobronchial needle aspiration cytology.

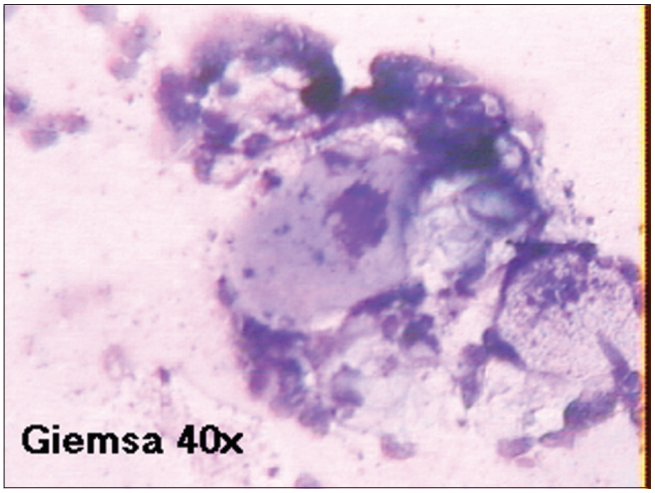

Figure 4: Squamous cell carcinoma in endobronchial needle aspiration cytology.

addition of EBNA to CDTs in diagnostic techniques during bronchoscopy procedure.[5,14,15,17-19] American College of Chest Physicians (ACCPs)[20] guidelines mentioned and recommended TBNA in endobronchial lesions to increase diagnostic yield due to chances of necrotic samplings in cauliflower type endobronchial growth and to prevent major bleeding with conventional FB use in fleshy hypervascular growth. TBNA will have more value in these two scenarios with EEL. In the literature search, we have found one study by Karahalli *et al*.[21] which is not inline to our observations mentioning no added benefit of EBNA to CDTs in increasing diagnostic yield in EEL.

Yield of FB and FB plus techniques in EEL

In the present study, yield of FB and FB plus BW in EEL is 84.65% (756/893) and 88.35% (789/893), respectively. In our previous

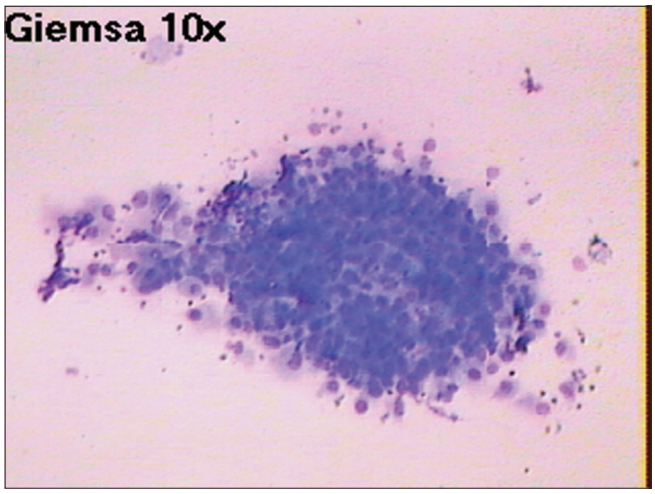

Figure 5: Dysplasia in endobronchial needle aspiration cytology.

published studies,[11,12] we have documented 79.67% and 88.18%, respectively, in small sample sizes. Kacar *et al.*[13] observed yield in 86.4% cases. Popovich *et al.*[22] mentioned that FB is the preferred test during bronchoscopy in EEL with 67–100% yield and EBNA will not replace FB in these types of lesions.

Sensitivity of FB and EBNA in EEL

Sensitivity of forcep biopsy & EBNA in diagnosing lung malignancy in EEL is 84.65% and 70.99% respectively. Thus, forcep biopsy is considered as gold standard diagnostic technique during bronchoscopy in Exophytic endobronchial lesions. We have compared our observations with our previously published data and authors with similar results in their respective studies [Table 5].

Various authors[24] have reported diagnostic sensitivity of FB in endobronchial lesions in absence of EBNA. These authors have studies FB with other CDTs and observed sensitivity of FB by Verma *et al*., Funhasi *et al*., Kulpati *et al*., Zavala *et al*., and Martin *et al*. as 81.6%, 83%, 85.7%, 97%, and 98%, respectively, in endobronchial lesions.[24]

Additional yield of EBNA over other methods

Additional yield of EBNA in Exophytic lesions over other CDTs (Conventional Diagnostic Techniques such as forcep biopsy plus bronchial wash) is 11.65%. We have compared our observations with our previously published data and authors with similar results in their respective studies [Table 6].

Shure and Fedullo[27] studied additional increase in yield of EBNA to conventional FB. They have reported addition of EBNA to FB has increased diagnostic yield from 55% to 87%.[27] Numerous authors, Dasgupta *et al.*[5] and Bilaceroglu *et al*.,[8] observed increased diagnostic sensitivity of addition of EBNA to conventional techniques. They have specifically mentioned that EBNA plus CDTs is superior to CDTs alone.[5,8]

Table 4: Sensitivity of Bronchoscopy guided conventional techniques (EBNA, BW and FB in EEL (*n*=893).

Technique	Positive yield	No yield	Total diagnosed cases
FB plus BW	789	104	893
EBNA plus BW	656	237	893
Both EBNA and FB	890	3	893

χ^2=275.64 *P*<0.00001. FB: Forcep Biopsy, BW: Bronchial wash, EBNA: Endobronchial needle aspiration cytology, EEL: Exophytic endobronchial lesions

Table 5: Diagnostic sensitivity of FB and EBNA in EEL.

Authors	Sensitivity of FB	Sensitivity of EBNA
Present study	84.65%	70.99%
Dasgupta *et al.*[5]	85.00%	76.00%
Shital and Rujuta[11]	79.67%	62.60%
Shital *et al.*[12]	88.18%	71.65%
Kacar *et al.*[13]	86.4%	77.9%
Caglayan *et al.*[17]	85.00%	92.00%
Siddiqui *et al.*[23]	88.3%	69.2%
Karahalli *et al.*[21]	82.7%	68.6%

FB: Forcep Biopsy, EBNA: Endobronchial needle aspiration cytology, EEL: Exophytic endobronchial lesions

Table 6: Additional yield of EBNA over CDTs in EEL.

Author	Additional yield of EBNA over CDTs
Present study	11.65%
Shital and Rujuta[11]	4.19%
Shital *et al.*[12]	4.87%
Kaçar *et al.*[13]	1%
Gullon *et al.*[17]	9.5%
Roth *et al.*[25]	8.04%
Lundgren *et al.*[26]	No additional yield of EBNA over CDTs

EBNA: Endobronchial needle aspiration cytology, CDTs: Conventional diagnostic techniques, EEL: Exophytic endobronchial lesions

Sole yield of EBNA in EEL, safety of EBNA technique, and importance of rose in increasing yield

EBNA was the sole positive test in 71 of total 893, that is, 7.95% of confirmed lung cancer cases. Although FB has diagnosed 756/893 (84.65%) cases and EBNA 70.99% (634/893) cases, only EBNA was the positive test in 71 cases. In 71 cases diagnosed by cytopathologist in EBNA, samples are further processed to immunohistochemistry analysis. All EBNA samples are processed on site as we are having ROSE facility in our center and this may be the reason for superior diagnostic yield. First published non-

randomized study documenting role of ROSE-EBNA performed by Govert *et al.*[6] in central neoplasms and they especially mentioned benefits of ROSE facility in EBNA specimens in increasing yield in endobronchial lesions. Randomized controlled trail done by Mondoni *et al.*[28] documented that addition of EBNA with ROSA facility will increase diagnostic sensitivity of bronchoscopy in EEL. Author observed.

Bronchoscopy procedure and techniques related complications including EBNA in the present study: Fiberoptic video bronchoscopy-related hypoxia documented in 36 cases and minor bleeding in 42 cases. Other complications such as significant bleeding, pneumothorax, and death were not seen. Minor bleeding was seen with FB mainly in 10.41% (93/893) cases. EBNA was very well tolerated in nearly all cases without any side effects except minor bleeding in few. Shure and Fedullo,[27] Bollinger *et al*.,[29] Jin *et al*.,[30] and ACCP Guidelines on Interventional Pulmonology[20] reported mortality rate of 0.01% and complication rate 0.7% during procedure and techniques which are comparable with our study. Other potentially life-threatening complications during procedure such as respiratory depression, airway obstruction, arrhythmias, and infections were also not observed in our study.

CONCLUSION

EBNA has documented very "crucial role" and should be considered as "complimentary" to CDTs in diagnosing lung cancer in comparison to other CDTs during bronchoscopy in presence of EEL. Importantly, EBNA samples can give rapid results and decrease chance for repeat procedure by guiding adequacy of samples before end of bronchoscopy procedure.

EBNA considered safe, especially when fleshy vascular endobronchial growth is present and risk of bleeding is high with FB. EBNA cytology samples can give comparable results to histopathology. EBNA samples are equally processed for immunohistochemistry analysis as histopathology samples. Thus, EBNA is a beneficial, safe, and minimally invasive bronchoscopic technique with insignificant side effect in the diagnosis bronchogenic carcinoma.

Although FB is more sensitive test then EBNA in EEL in diagnosing disease, we have documented that EBNA has significant additive yield in proportionate number of cases. Rationale for decreased yield with CDTs in comparison with EBNA is chances of inadequate sampling during FB due to superficial necrosis, blood clot, or crush artifacts resulting in to negative yield.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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