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Suitability of EBUS-TBNA Specimens for Subtyping and Genotyping of NSCLC: A Multi-Centre Study of 774 Patients

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Abstract

Rationale—The current management of advanced non-small cell lung cancer (NSCLC) requires differentiation between squamous and non-squamous sub-types as well as epidermal growth factor receptor (EGFR) mutation status. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is increasingly used for the diagnosis and staging of lung cancer.

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VJ Performance of procedures, compilation of data, review of manuscript
MM Performance of procedures, compilation of data, review of manuscript
BJN Performance of procedures, compilation of data, review of manuscript
DMR Review of pathological specimens, review of manuscript
MF Review of pathological specimens, review of manuscript
GK Review of pathological specimens, review of manuscript
RCR Performance of procedures, compilation of data, review of manuscript
AGN Revision of manuscript for intellectual content
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However, it is unclear whether cytology specimens obtained with EBUS-TBNA are suitable for the sub-classification and genotyping of NSCLC.

Objectives—To determine whether cytology specimens obtained from EBUS-TBNA in routine practice are suitable for phenotyping and genotyping of NSCLC.

Methods—Cytological diagnoses from EBUS-TBNA were recorded from 774 patients with known or suspected lung cancer across 5 centres in the United Kingdom between 2009 and 2011.

Measurements and Main Results—The proportion of patients with a final diagnosis by EBUS-TBNA in whom subtype was classified was 77% (95% CI 73% - 80%). The rate of NSCLC not otherwise specified (NSCLC-NOS) was significantly reduced in patients who underwent immunohistochemistry (adjusted OR 0.50 95% CI 0.28 – 0.82, P=0.016). EGFR mutation analysis was possible in 107 (90%) of the 119 patients in whom mutation analysis was requested. The sensitivity, negative predictive value and diagnostic accuracy of EBUS-TBNA in patients with NSCLC was 88% (95% CI 86% - 91%), 72% (95% CI 66% - 77%) and 91% (95% CI 89% - 93%) respectively.

Conclusions—This large multi-centre pragmatic study demonstrates that cytology samples obtained from EBUS-TBNA in routine practice are suitable for sub-typing of NSCLC and EGFR mutation analysis and that use of immunohistochemistry reduces the rate of NSCLC-NOS.

Keywords

Endobronchial ultrasound; non-small cell lung cancer; adenocarcinoma; EGFR mutation; squamous cell carcinoma; NSCLC-NOS

INTRODUCTION

Traditionally, the pathology of lung cancer has been divided into non-small cell lung cancer (NSCLC) and small cell lung cancer, reflecting the different tumour biology and susceptibility to treatments. In recent years, it has become apparent that subtyping and genotyping helps to guide optimal treatment of advanced NSCLC. Late phase clinical trials have provided three major observations with regard to the efficacy or safety of specific treatments for particular subtypes or genotypes of NSCLC. First, a large randomised non-inferiority trial of Pemetrexed and Cisplatin in 1725 patients with NSCLC[1] demonstrated that Pemetrexed is only of benefit in patients with non-squamous histology, while in patients with squamous subtype Pemetrexed was inferior to the standard treatment of Cisplatin and Gemcitabine. This has been reflected in guidance from the National Institute of Health and Clinical Excellence (NICE) which recommended pemetrexed as a first line treatment for patients with adenocarcinoma or large cell carcinoma in September 2009[2].

Second, a randomised phase II trial of bevacizumab plus carboplatin and paclitaxel versus carboplatin and paclitaxel alone revealed that fatal pulmonary haemorrhage was significantly higher in patients with squamous subtype of NSCLC[3]. Consequently, bevacizumab is contra-indicated in patients with squamous cell lung cancer (SQCC). Third, phase III randomised trials in East Asia have demonstrated that the tyrosine kinase inhibitors only have improved progression-free survival (PFS) in patients with NSCLC harbouring an activating EGFR mutation. In patients without an EGFR mutation, standard chemotherapy may offer superior PFS[4;5] . Further targeted agents in patients with specific cancer genotypes are set to emerge[6].

Coupled with the emergence of personalised therapies for advanced NSCLC has been the rapid expansion of endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) which allows sampling of mediastinal and hilar lymphadenopathy under

direct vision. The technique employs a 21 or 22 gauge needle and therefore obtains smaller samples than biopsy via mediastinoscopy. Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) was initially developed for the nodal staging of lung cancer. However, it is now commonly used as an initial investigation in patients with suspected NSCLC after computed tomography scan as it may provide a tissue diagnosis and accurate nodal staging in a single investigation[7]. However, given the smaller sample size obtained it is unclear whether aspirates from EBUS-TBNA in routine practice provide sufficient material to allow subtyping and genotyping of NSCLC in order to guide treatment. We therefore conducted a large pragmatic multi-centre study to clarify whether samples from EBUS-TBNA were suitable for subtyping of NSCLC and EGFR mutation testing.

METHODS

Patients and EBUS-TBNA samples

Consecutive patients with suspected NSCLC underwent EBUS-TBNA between January 2009 and March 2011 across 5 centres in the UK (University College London Hospital, University Hospital Birmingham, University Hospital of North Tees, Lancashire Teaching Hospital and Papworth Hospital, Cambridge). The samples obtained at EBUS-TBNA were expelled from the needle using the stylet and placed into liquid fixative for cell-block processing. Needle contents were also flushed with saline into the liquid fixative. The specimen was centrifuged to form a pellet, suspended in agar, fixed in neutral buffered formalin and processed as a cell block from which a single hematoxylin and eosin (H&E) stained section was cut. Further sections were cut and used for immunohistochemical staining as required[8].

Pathological and molecular techniques

Interpretation of the EBUS-TBNA specimens was carried out by the local pathologist and there was no centralised reporting. Classification of NSCLC was based upon morphological appearances (H&E stain) and immunostaining was performed if the sample was sufficient and clinically indicated (Figure 1a-d). Antibodies to cytokeratins 5/6 (CK5/6) and p63 were deemed to be consistent with squamous cell carcinoma[9;10]. Antibodies to Thyroid transcription factor 1 (TTF-1) were also employed as TTF-1 is known to be expressed in approximately 75% of lung adenocarcinomas[11;12].

EGFR mutations were detected using DNA sequencing techniques and patients were considered to be positive for EGFR mutation if 1 of 29 EGFR mutations was detected by polymerase chain reaction based assays. Four centres employed the commercially available amplification refractory mutation system (ARMS) kit (Qiagen) which is able to detect an EGFR mutation in samples which contain 1% tumour. The remaining centre employed a matrix-assisted laser desorption/ionization mass spectroscopy system for detecting EGFR mutations (Sequenom MassARRAY).

Endpoints and statistical analysis

The primary endpoint of the study was the proportion of patients with NSCLC undergoing EBUS-TBNA in whom it was possible to subtype the lung cancer. The co-primary endpoint was the proportion of samples that was suitable for EGFR testing as determined by the local testing centre. Using regression analysis, the rate of NSCLC-NOS was determined according to age, lymph node location, size, pathological differentiation and whether immunohistochemistry was performed or not. Covariates demonstrated to be significant at the 10% level were entered into the multivariate model. The unit of analysis was the patient.

Each patient was followed up for at least 6 months duration and each EBUS-TBNA procedure was classified as a true positive, true negative or false negative result according to the final diagnosis of malignancy. Standard definitions for the calculation of the sensitivity and negative predictive value of EBUS-TBNA (secondary endpoints) in patients with NSCLC were applied. Proportions were compared using the Chi-squared test. All statistical calculations were carried out using STATA version 10 (Statacorp., USA). Ethical approval was not required given the observational nature of the study. Although the study is retrospective, all data were prospectively recorded in each centre. Results were fully disclosed to the patients and also discussed in multi-disciplinary team meetings in order to determine the treatment strategies.

RESULTS

Between 2009 and 2011, 774 consecutive patients with known or suspected NSCLC underwent EBUS-TBNA at 5 UK centres. Four hundred and fifty-five (59%) were male and the median age of patients with NSCLC was 69 (range 31 – 88) years. Baseline characteristics are summarised in Table 1. Two hundred and ninety (37%) patients had more than 1 lymph node sampled and in total 1047 lymph nodes were aspirated. The size and location of lymph nodes sampled and the diagnostic yield are shown in Table 2.

The pathological subtypes of NSCLC diagnosed by EBUS-TBNA are shown in Figure 2. In total, 503 patients had a final diagnosis of NSCLC in intra-thoracic lymph nodes. The number of patients with a final diagnosis by EBUS-TBNA of NSCLC – NOS (the primary endpoint) was 101 (23%, 95% CI 20% - 27%. Two hundred and ninety-one patients had their EBUS-TBNA specimens submitted for immunostaining and this was possible in 280 (96%, 95% CI 93% - 98%). Of the 101 patients with a diagnosis of NSCLC – NOS by EBUS-TBNA, immunostaining was performed in 53, not done in 42 and not possible in 6 patients. In univariate analysis, there was no association between NSCLC-NOS and age, lymph node size, lymph node location, number of lymph nodes aspirated and pathological differentiation. However, a significant relationship was seen on univariate and multivariate analysis between immunohistochemistry not performed and the final diagnosis of NSCLC-NOS (Table 3). When immunostaining was possible, the risk of the NSCLC tumour being unclassified was halved in the multivariate analysis (OR 0.50, 95% CI 0.28 – 0.88, P=0.016).

Five hundred and two patients had lymph nodes aspirated that were greater than 1cm in short axis. Of these, 291 had NSCLC diagnosed by EBUS-TBNA and the number of patients diagnosed with NSCLC –NOS was 55 (19%, 95% CI 15% – 24%) in this subgroup. In the 88 patients with recorded lymph node size less than or equal to 1cm in short axis, the prevalence of malignancy was 54% (48 patients) and the number of patients diagnosed with NSCLC-NOS was 6 (26%, 95% CI 12 – 47%). In 180 patients the lymph node size was not recorded. There was no statistically significant difference (P=0.41) in the NOS-NSCLC rate in nodes greater or less than 1cm in short axis. Five hundred and ninety-two patients had sampling with a 22 gauge needle while the larger 21 gauge needle was used in 107 patients and was associated with a NSCLC-NOS rate of 27% (95% CI 23% – 32%) and 11% (95% CI 5 – 21%) respectively (P=0.006). Needle size was not recorded in 75 patients.

One hundred and nineteen (27%) patients who had NSCLC diagnosed by EBUS-TBNA had EGFR mutation analysis requested on the routinely obtained sample. Of these, 68 (57%) were adenocarcinoma, 19 (16%) had squamous cell carcinoma, 10 (8%) had large cell carcinoma and 22 (18%) had NSCLC-NOS. EGFR mutation analysis was possible (the co-primary endpoint) in 107 (90%, 95% CI 82% – 94%) cases and 7 (6%) patients with EGFR mutations were identified. Of the 7 patients who had an EGFR mutation, all were Caucasian

and had adenocarcinoma. The median age of these patients was 58 years (range 53 – 71) and 5 (71%) were female. Four out of the 7 EBUS-TBNA samples which expressed an EGFR mutation were also noted to stain for TTF-1.

In the overall cohort of 774 patients, EBUS had a sensitivity of 88% (95% CI 86% - 91%), negative predictive value of 72% (95% CI 66% - 77%) and diagnostic accuracy of 91% (95% CI 89% - 93%). Of the 69 false negative EBUS-TBNA procedures, 62 patients had lymphoid cells only aspirated and subsequent surgery, mediastinoscopy or clinical follow-up confirmed malignancy (Figure 2). Seven patients with inadequate EBUS-TBNA samples were subsequently shown to harbour malignancy in the mediastinal lymph nodes. None of the 32 specimens in which granulomas only were found at EBUS-TBNA were proven to be false negative results.

The sensitivity from aspiration of hilar lymph nodes (stations 10 and 11) was 88% (95% CI 79% - 94%) and no different to the sensitivity from mediastinal lymph nodes (88%, 95% CI 85% - 91%). The median size of hilar lymph nodes was 15mm (range 7 – 40). Sensitivity in patients with lymph nodes ≤1cm in short-axis was 65% (95% CI 50% - 77%), and significantly lower than the sensitivity of 91% (95% CI 88% - 93%; P<0.0001) in patients with nodes >1cm. There was no interaction between lymph node location and size.

One patient's EBUS procedure resulted in a death. The patient was a 48 year old male who presented with stage IV adenocarcinoma of the lung. The EBUS-TBNA procedure was uncomplicated and the patient was discharged home after the procedure with normal vital observations. Twenty-four hours later the patient was admitted to hospital with clinical features of severe pneumonia and sepsis. Group A Streptococcus was isolated from blood cultures and also from a throat swab. The patient deteriorated from sepsis and respiratory failure and died within 48 hours of admission. The scenario was attributed to the carriage of organisms by the EBUS scope from the pharynx into the lungs. No other complications were reported.

DISCUSSION

While the sophistication of patient selection for treatment has increased, the size of lung cancer samples to obtain that information has reduced. The challenge for the pathologist and lung cancer multi-disciplinary team is to optimise diagnostic specimens and staging, while also supplying sufficient information to guide oncological therapy. Since at least 75% of patients have inoperable disease, the information to guide treatment algorithms must be obtained from small histology or cytology specimens.

EBUS-TBNA is an important investigation for the diagnosis of mediastinal and hilar lymphadenopathy in patients with lung cancer. It has been recommended as an initial investigation by NICE in patients with enlarged mediastinal lymph nodes as it may provide an inoperable disease stage and a pathological diagnosis in a single investigation[2]. This large multi-centre pragmatic implementation study demonstrates that routine samples from EBUS-TBNA are able to provide sufficient information to allow subtyping in 77% and EGFR mutation testing in 90% of patients with NSCLC.

The proportion of patients with NSCLC for which the final diagnosis using EBUS-TBNA specimens was NSCLC NOS was 23%. This is consistent with data from alternative biopsy techniques. An analysis of the California Cancer Registry of 175,298 patients diagnosed with lung cancer between 1989 and 2006 demonstrated a NSCLC-NOS rate of 22.1%[13]. The rate of NSCLC-NOS was higher in the patients who had a cytological diagnosis alone (37%). The United Kingdom National Lung Cancer Audit (NLCA) recently published data on 26,731 patients diagnosed with NSCLC in England and Wales in 2010[14]. These patients

underwent diagnosis and staging of lung cancer in a real world setting and the audit demonstrated an overall NSCLC-NOS rate of 24.4%. This highlights that EBUS-TBNA may be as good as other sample acquisition techniques for subtypying and that there will always be a proportion of patients with NSCLC in whom further subtyping is not possible, due to lack of differentiation. EBUS-TBNA is also able to sample central parenchymal lung lesions that would otherwise not be accessible without a considerably more invasive approach[15]. Therefore increased application of EBUS-TBNA may improve the rate of histological confirmation in patients with NSCLC, which currently stands at a mean of 72% in the NLCA[14].

Previous studies have shown that samples from cytology are valid when compared to subsequent larger samples. Indeed, the morphologic features that distinguish squamous cell carcinoma (predominantly keratinized cytoplasm and intercellular bridges) from adenocarcinoma (mucin vacuoles and gland formation) span less than the 250-μm inner diameter of a 25-gauge fine needle[16]. In a recent retrospective study of 48 patients[17], cell block samples from EBUS-TBNA were compared to histological specimens obtained by alternative procedures such as bronchoscopy and CT guided biopsy. All subtypes diagnosed by EBUS-TBNA were validated by histological samples. When immunohistochemistry was performed on cell blocks, there were six cases diagnosed as NSCLC-NOS on EBUS-TBNA samples which were diagnosed with a specific cell type on alternative histological samples (3 adenocarcinomas, 2 squamous cell carcinomas and 1 large cell undifferentiated carcinoma). A further study of 101 individuals demonstrated a 93% concordance between small biopsy and cytology specimens[18]. As in this study, lack of supporting immunohistochemistry contributed to unclassified cytology cases. In another report, 158 (85%) cases of NSCLC were typed by cytology and 28 (15%) were classified as NSCLC-NOS[19]. Utilising histological specimens from the same patients, 183 (98%) cases were subtyped by histology and only 3 (2%) cases were classified as NSCLC-NOS. There was 88% concordance between cytological and histological typing. The available data therefore confirm that cytological specimens are reliable for subtyping with no false positive results from cytological subtyping observed and that use of immunohistochemistry can reduce the NSCLC-NOS rate.

Immunohistochemistry profiles do not feature in the diagnostic criteria for squamous cell or adenocarcinoma in the current WHO classification of NSCLCs which is based on resected surgical specimens[20]. However when morphological criteria are unable to distinguish subtypes in smaller samples, a panel of antibodies including TTF-1, p63 and CK5/6 as well as a mucin stain has been recommended in order to minimise the proportion of NSCLC tumours that remain unclassified and to make the key distinction between squamous and non-squamous subtypes[21]. The current large pragmatic study shows that samples obtained by EBUS-TBNA are suitable for this approach from any accessible lymph node station and even when sampling lymph nodes less than 1cm in size.

The EGFR-tyrosine kinase inhibitors erlotinib and gefitinib have become established as first-line treatments for patients with advanced lung cancer that harbour an EGFR mutation. Current European Society of Medical Oncology guidelines recommend that all never or former light smokers (<15 pack years) or patients with non-squamous histology should be tested for EGFR mutation status regardless of performance status[22]. Cytological samples in alcohol based fixatives may preserve nucleic acids better than formalin[23] and molecular profiling of cytology samples has been shown to be reliable when compared with histological samples from the same patient[24]. In this study, EGFR mutation testing was requested in 119 patients and the test was possible and deemed reliable in 107 (90%) cases. In the remaining cases, there was insufficient tumour sample to perform the investigation. Previous studies have assessed the utility of EBUS-TBNA samples for EGFR testing with

variable results. In one study EGFR mutation testing was possible in 27 out of 35 patients (77%) undergoing EUS-FNA or EBUS-TBNA[25]. Another study of 36 patients in Spain undergoing EBUS-TBNA suggested EGFR mutation analysis was feasible in 26 (72%) cases[26]. Billah demonstrated that 96% of specimens from EBUS-TBNA in a cancer centre were able to undergo EGFR mutation testing[27]. Similarly high rates of reliable EGFR mutation testing of EBUS-TBNA samples have been observed by Nakajima and colleagues[28;29]. A recent study, in which cell blocks were prepared from 128 lung cancer cytology specimens, demonstrated that molecular analysis was possible in 98% of specimens[30].

It is widely accepted that NSCLC may contain areas of mixed adenocarcinoma, large and squamous cell carcinoma. Up to 25% of small cell carcinomas are thought to contain areas of NSCLC differentiation[31]. This pathological heterogeneity implies that smaller cytological samples may not be representative of the entire lesion. Another potential area of controversy in NSCLC is that of genetic tumour heterogeneity. Conflicting evidence exists. Three studies comparing EGFR mutation status in primary tumour and local lymph node metastases demonstrated significant discrepancies between the sites[32-34]. However a recent study showed that when highly sensitive techniques for mutation detection are employed, no discordant mutation patterns were detected among 77 paired primary and metastatic tumours[35]. These authors suggested that weak EGFR mutation signals in an area without EGFR amplification may not reach the threshold of detection because of the mixture with normal cells resulting in pseudoheterogeneity. The authors concluded that true genetic heterogeneity is rare[35]. This latter view would support EGFR mutation status being assessed in the most accessible tissue only, rather than multiple sites being sampled.

This study confirms the high yield from EBUS-TBNA of detecting malignancy in intra-thoracic lymph nodes in a real world setting. A sensitivity of 88% in 774 patients was observed which is similar to a sensitivity of 93% observed in a meta-analysis of 1299 patients[36]. This study contains the first reported death attributed to EBUS-TBNA. The patient was likely immunosuppressed due to widely metastatic malignancy and succumbed to sepsis within 72 hours of the procedure. Sepsis may be attributed to the process of introducing pharyngeal micro-organisms into the lower respiratory tract. The large number of patients included in this study renders subgroup analyses powerful. EBUS-TBNA of lymph nodes less than 1cm has a significantly lower sensitivity than when the procedure is performed in nodes greater than 1cm. This may be due to the increased technical difficulty of sampling smaller lymph nodes. However, when small lymph nodes were sampled successfully (regardless of lymph node location), the samples were still suitable for NSCLC sub-typing and EGFR mutation analysis.

Limitations of this study are recognised. Pathological samples in this study did not undergo central review, however this reflects the pragmatic nature of the study and results in strong external validity. The centres included in the study carry out a high volume of EBUS-TBNA procedures with experienced operators and pathologists. Despite the observational design of this study, data were collected prospectively in each centre. A final issue is that not all negative EBUS-TBNA cases underwent mediastinoscopy. All patients did, however, undergo at least 6 months clinical follow-up to allow a clinical diagnosis to be made.

Recent guidance has suggested a novel algorithm for the diagnosis of adenocarcinoma in small biopsies and cytological samples[37]. In patients with positive cytology and classic morphology for adenocarcinoma or squamous cell carcinoma no further markers are required and those with adenocarcinoma can be submitted directly for EGFR mutation testing. Samples which are classified as NSCLC-NOS on morphology are recommended to undergo a panel of Immunohistochemistry that includes one squamous cell carcinoma

marker and one adenocarcinoma +/− mucin staining. If the NSCLC tumour still remains unclassified then molecular analysis is still recommended. This multi-centre study clearly demonstrates that samples from EBUS-TBNA obtained in routine practice are suitable for entry into this new diagnostic algorithm and provides further impetus for the use of EBUS-TBNA as an initial diagnostic procedure in patients with suspected lung cancer.

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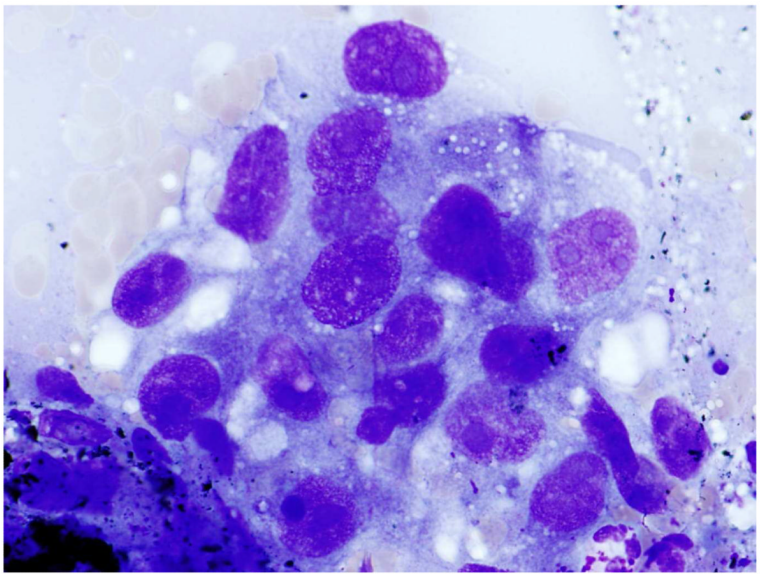
At A Glance Commentary

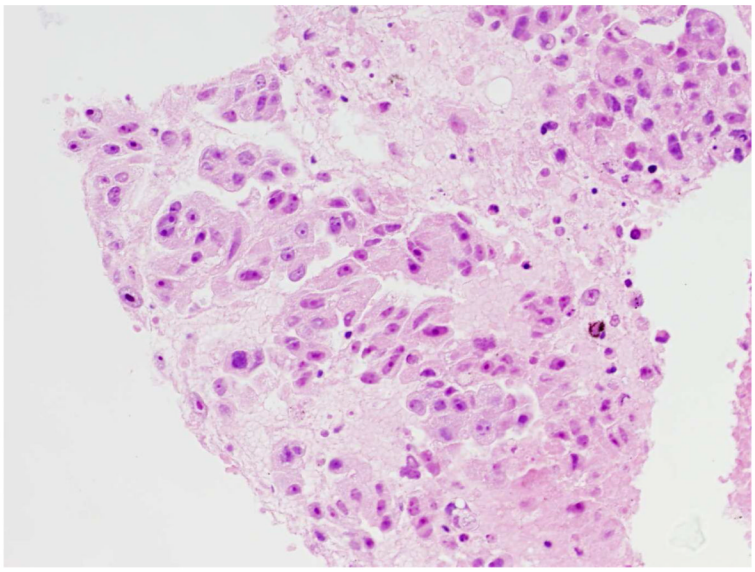
Scientific Knowledge on the Subject

Sub-typing and genotyping of the tumour is central to the modern management of patients with advanced non-small cell lung cancer. EBUS-TBNA has emerged as an important procedure for the diagnosis and staging of non-small cell cancer. However, it is unclear whether the cytological specimens obtained from EBUS-TBNA are suitable for the sub-classification and genotyping of non-small cell lung cancer.

What This Study Adds to the Field

In patients with non-small cell lung cancer, specimens obtained from EBUS-TBNA in routine practice are sufficient to allow sub-classification in 77% and genotyping in 90%. Use of immunohistochemistry on EBUS-TBNA samples can reduce the rate of unclassified non-small cell lung cancer.





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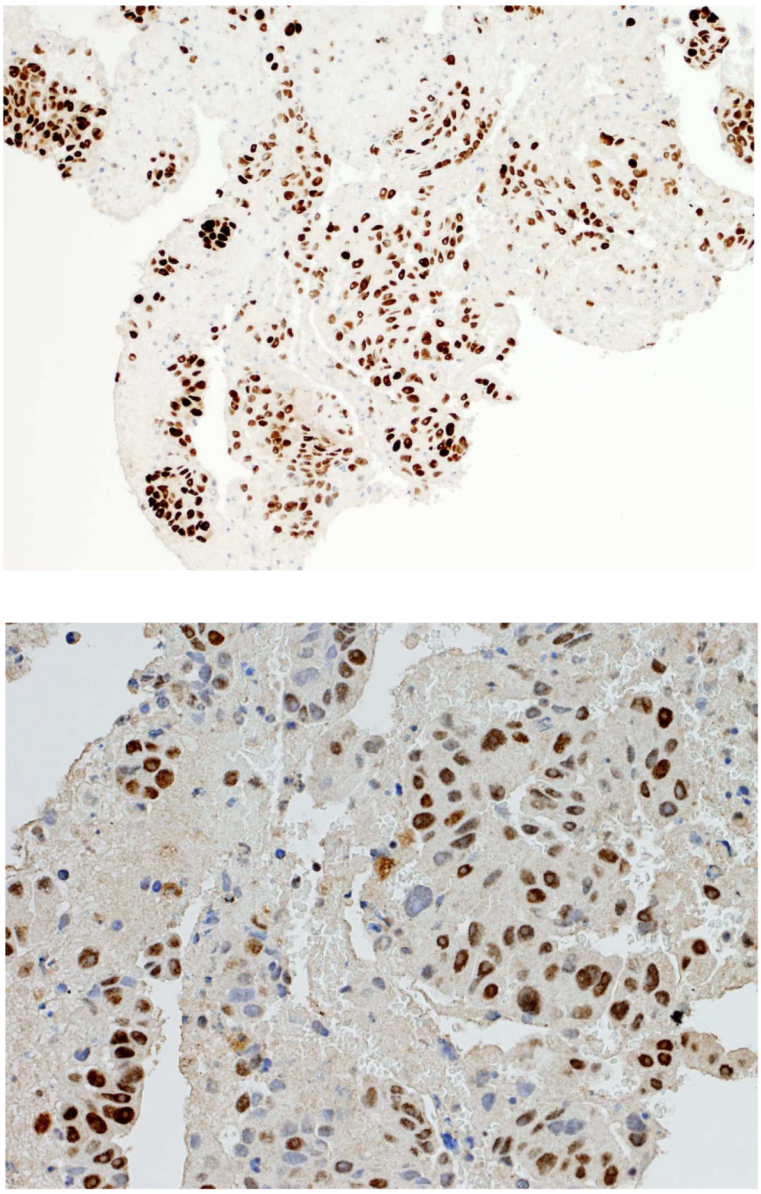

Figure 1.

a: EBUS-TBNA smear demonstrating adenocarcinoma (May–Grunwald-Giemsa stain).
b: Cell block obtained from EBUS-TBNA demonstrating adenocarcinoma
c: Adenocarcinoma from EBUS-TBNA cell block, positive for TTF-1, confirming lung origin TTF-1 - thyroid transcription factor -1
d: EBUS-TBNA cell block demonstrating adenocarcinoma to be ERCC1 positive, suggesting resistance to platinum-based chemotherapies ERCC1 - excision repair cross-complementing group 1

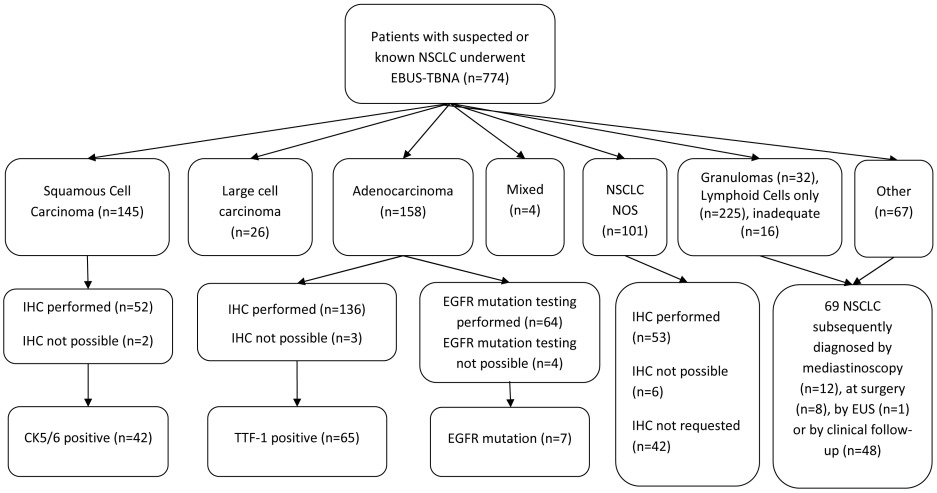

Figure 2.

Flowchart of patients

NSCLC: Non-small cell lung cancer; EBUS-TBNA: Endobronchial ultrasound-guided transbronchial needle aspiration; NOS: Not otherwise specified; IHC: immunohistochemistry; EGFR: Epidermal growth factor receptor; CK: cytokeratin; TTF-1: Thyroid transcription factor 1; EUS: Endoscopic ultrasound;

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Table 1

Baseline characteristics of patients with suspected lung cancer who underwent EBUS-TBNA

	Number (%)
Gender	
Male	455 (59%)
Female	319 (41%)
Age	
<50	43 (5%)
50 – 75	540 (70%)
>75	191 (25%)
Ethnicity	
Caucasian	683 (88%)
South Asian	21 (3%)
East Asian	5 (1%)
African	3 (<1%)
Caribbean	2 (<1%)
Other	1 (<1%)
Unknown	59 (8%)
Total	774

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Table 2

Yield according to lymph node stations sampled in 774 patients undergoing EBUS-TBNA

Lymph node station	Number of nodes sampled	Mean size of lymph node (mm)	Prevalence of NSCLC	Sensitivity	Negative Predicitve Value	Diagnostic accuracy
2R	13	17	60%	83%	80%	90%
2L	3	15	33%	100%	100%	100%
3P	3	25	33%	100%	100%	100%
4R	282	21	84%	90%	65%	92%
4L	113	18	73%	80%	64%	85%
7	436	23	74%	90%	77%	92%
10R	104	18	76%	87%	71%	90%
10L	46	18	81%	90%	71%	93%
11R	41	16	82%	93%	75%	94%
11L	6	13	100%	50%	0%	50%
Overall	**1047**	**21**	**77%**	**88%**	**72%**	**91%**

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Table 3

Univariate and multivariate analyses of factors to predict NSCLC-NOS in patients undergoing EBUS-TBNA. On the basis of univariate results, only pathological differentiation and performance of immunohistochemistry were included in the multivariate model. Performing immunohistochemistry significantly reduced the odds of obtaining a diagnosis of NSCLC-NOS.

Covariate	Unadjusted OR of NSCLC-NOS (95% CI)	Univariate P value	Adjusted OR of NSCLC-NOS (95% CI)	Multivariate P value
Age	0.99 (0.97 – 1.01)	0.53		
Lymph node location (mediastinal vs hilar)	0.64 (0.34 – 1.19)	0.159		
Lymph node size	1.0 (0.96 – 1.05)	0.92		
Pathological differentiation	1.66 (0.92 – 3.00)	0.09	1.44 (0.79 – 2.62)	0.24
Immunohistochemistry performed	0.47 (0.27 – 0.82)	0.008	0.50 (0.28 – 0.88)	0.016