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The expanding role of endobronchial ultrasound in patients with centrally located intrapulmonary tumors

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ABSTRACT

Objectives: Tissue acquisition of lung tumors is crucial for diagnostic and treatment purposes. In patients with centrally located lung tumors without endobronchial abnormalities the yield of conventional bronchoscopy is poor. Objective of this study was to assess diagnostic yield of EBUS-TBNA in patients with lung tumors, located near or adjacent to the major airways.

Methods: International multicenter retrospective analysis (2013–2018) of linear EBUS databases in Bologna, Italy and Amsterdam, The Netherlands. Patients with a centrally-located lung tumor without endobronchial abnormalities who underwent lung tumor search with linear EBUS were included. Diagnostic yield, feasibility of EBUS guided tumor sampling, complication rate, adequacy of the aspirates for mutational analysis, and assessment of mediastinal/vascular invasion (T4) were evaluated.

Results and conclusion: Real-time EBUS-TBNA diagnostic yield to sample centrally located intrapulmonary tumor was 83% (136/163) and it was independent of tumor location (paratracheal, mainstem, lobar, segmental bronchus). The feasibility to sample the lung tumor was 89% (145/163). In 4 cases the tumor was not found with EBUS. In the other 14 cases, tumor sampling was not performed due to: loss of the echo window after needle insertion [n = 3], interposition of a large vessel [n = 7], switch to radial EBUS [n = 1], switch and sampling through EUS or EUS-B [n = 3]. No major complications occurred. Mutational analysis was successful in 54/63 (86%) of samples. Using surgery as reference standard, EBUS proved more reliable than CT (24/24, 100% versus 22/24, 91.7%, respectively) in the assessment of mediastinal/vascular tumor invasion (T4 status). In conclusion: Lung tumors presenting without endobronchial abnormalities and located adjacent to the major airways can be safely sampled by EBUS-TBNA resulting in high diagnostic yield irrespective of tumor location. Successful molecular profiling and reliable assessment of mediastinal/vascular invasion (T4) in patients with advanced disease provide additional value to EBUS procedures in the setting of centrally-located lung lesions.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. Obtaining a tissue sample in patients with suspected lung cancer is very important for diagnostic and staging purposes [2]. Moreover, with the clinical availability of novel treatments for advanced disease, there is an increased demand for more and high quality tissue (histology/cytology) for mutation analysis and immunotherapy application [3].

Flexible bronchoscopy with its ancillary sampling procedures (biopsy, needle aspiration, brush and wash) is the corner stone of lung tumor tissue acquisition, but its diagnostic yield in patients presenting

Abbreviations: CT, computed tomography; PET-CT, positron emission tomography and computed tomography; EBUS-TBNA, endobronchial ultrasound trans bronchial needle aspiration; EUS-B-FNA, esophageal ultrasound fine needle aspiration (using an EBUS scope); EUS-FNA, esophageal ultrasound fine needle aspiration (using a GI EUS scope); EBUS, assessment tool; ROSE, rapid on site cytological evaluation; TTNA, trans thoracic needle aspiration; TTB, trans thoracic biopsy; NSCLC, non-small cell lung cancer; NSCLC NOS, non-small cell lung cancer not otherwise specified; SCLC, small cell lung cancer

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without endobronchial abnormalities is low [2,4–7]. Guidance techniques (radial EBUS/fluoroscopy/electromagnetic navigation) can be helpful in peripheral parenchymal lesions, especially in cases in which an airway leads to the tumor. However, these techniques often do not significantly contribute to the diagnostic yield of centrally-located lesions, where their diagnostic yield is limited [8,9].

Centrally-located lung tumors adjacent to the larger airways can be identified and sampled by EBUS-TBNA [10–15]. However, the applicability of linear EBUS for lung tumor sampling in relation to the tumor location in the tracheobronchial tree, the adequacy of TBNA specimens for mutational analysis, and EBUS usefulness in T4 staging (presence/absence of mediastinal and vascular tumor invasion) are unknown. We addressed these issues in a large multinational multicenter group of patients.

2. Methods

2.1. Study design and patients selection

This is a retrospective multi center international study undertaken in Bologna, Italy and Amsterdam UMC, The Netherlands. In both centers, patient data were retrieved from the Institute endosonography databases. The search period was between Jan 1st 2013 and October 10th 2018.

Patient data used for analysis were selected based on the following:

- Presence of a centrally-located suspected intrapulmonary lung tumor, positioned near or adjacent to the airways (up to the segmental bronchi) (and therefore in reach of EBUS) AND
- The absence of endobronchial abnormalities at conventional flexible bronchoscopy AND
- Underwent an EBUS examination that aimed to lung tumor tissue sampling.

Of these patients, the following data were collected: demographical characteristics, CT and PET-CT imaging, bronchoscopy and EBUS reports, reports from other diagnostic evaluations, cyto-pathological reports, mutational analysis, complications and follow up data. Different location of the tumor relative to the airways and location of the probe during sampling procedure were retrieved from imaging and EBUS reports (Fig. 1).

2.2. Definition of a centrally located lung tumor

In the literature, various definitions of a centrally located lung tumor exist. Guidelines published by Silvestri et al in CHEST 2013 define as central those lesions in which the medial margin stays within the inner third of the chest [16]. For the purposes of this study, we used this definition in combination with localization of the lesions adjacent to the airways and therefore in potential reach of EBUS.

2.3. Study endpoints

The primary endpoint of this study was to assess diagnostic yield of linear EBUS for obtaining a tissue diagnosis of intrapulmonary tumors located adjacent the major airways without endobronchial abnormalities at conventional bronchoscopy.

Secondary endpoints included:

- to asses diagnostic yield of the lung tumor in relation to the tumor location (paratracheal, adjacent to the main bronchi, adjacent to lobar bronchi or adjacent to segmental airways)
- feasibility of EBUS-TBNA sampling of centrally located lung tumors detected by EBUS
- linear EBUS complication rate
- suitability of EBUS TBNA samples for molecular analysis
- feasibility and accuracy of EBUS for mediastinal/vascular tumor invasion (T4) detection.
- adequacy of EBUS-TBNA parenchymal tissue samples for lung cancer diagnosis;
- sensitivity of EBUS for the diagnosis of a centrally located lung tumor;

2.4. Procedure

Cases were performed at the endoscopic units of the two referral centers by experienced interventional pulmonologists. Procedures were mainly performed in an outpatient setting, either under conscious sedation using midazolam/fentanyl, or propofol/remifentanil sedation. Following a conventional bronchoscopy, a systematic EBUS examination (Olympus BF-UC180 F or UC 180 F, Olympus Medical Systems Europe, Ltd., Pentax EB-1970 UK or Pentax EB19-J10U, Pentax, Hamburg, Germany) was performed according to EBUS AT [17]. For sampling procedures, 19 G, 21 G, 22 G or 25 G needles were used. Once

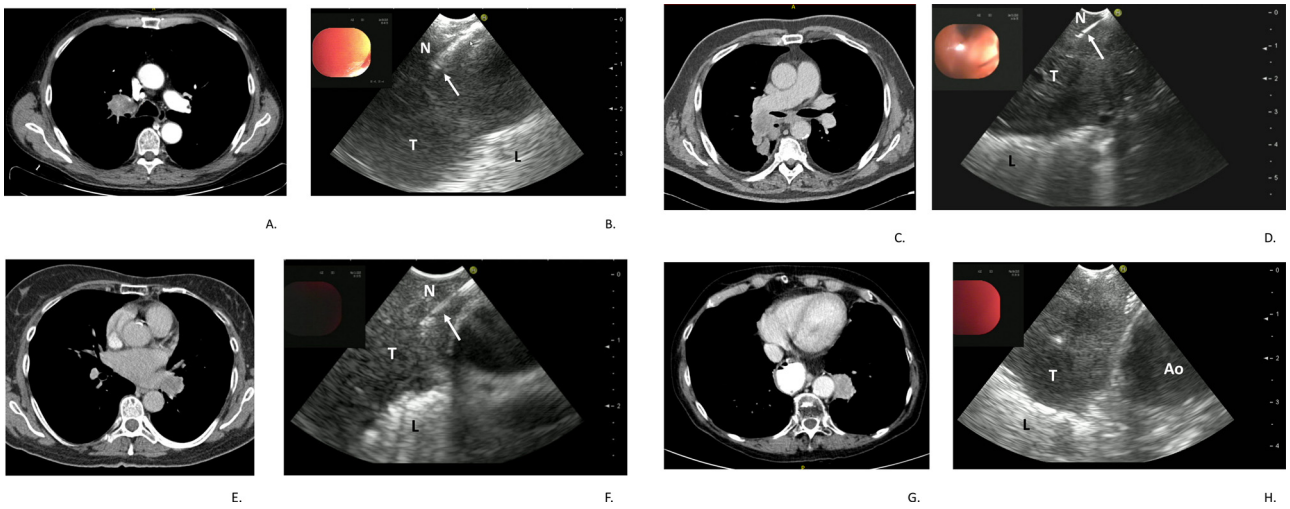


Fig. 1. EBUS-TBNA sampling stratified for tumor location relative to the tracheobronchial tree.
Pictures A, C, E and G show CT images of centrally located lung tumors (T) adjacent to trachea, main bronchi, lobar bronchi and segmental bronchi, respectively. Figures B, D, F and H show the correspondent EBUS-TBNA procedural images. The needle (N), when present in EBUS images, is signaled by arrow tips. L = compromised lung parenchyma, Ao = aorta.

the target lesion was visualized by endobronchial ultrasound, the needle was placed through the working channel of the EBUS bronchoscope. When technically feasible, the lesion was punctured through the tracheobronchial wall under real-time ultrasound guidance (Fig. 1). When EBUS sampling of the lesion was not performed, the reason (anatomical, technical or clinical) was noted. All procedures were conducted with rapid on-site evaluation (ROSE), with an expert cytologist/cyto-technician evaluating adequacy of EBUS samplings after collection through Diff Quick® staining. Specimens (smears, cell-block and/or clot-core) were sent for pathological evaluation by an expert pathologist for definitive diagnosis. After the procedure, outpatients were monitored conforming to local practice before being discharged, for adverse event detection and registration. Adverse events occurring during the procedure or afterwards were extrapolated from endoscopy reports and/or patients' dossiers.

2.5. Diagnostic issue

2.5.1. Definitions of diagnostic yield scenarios, feasibility, sample adequacy, sensitivity and specificity

Diagnostic yield was calculated considering best and the worst case scenarios.

In the best case scenario, the diagnostic yield was calculated as the number of cases in which EBUS guided TBNA of the lesion provided a correct diagnosis relative to the total number of cases in which a lesion was successfully sampled through EBUS. In the worst case scenario, it was calculated as the number of cases in which EBUS sampling provided a correct diagnosis relative to the number of patients with a centrally located intrapulmonary lesions adjacent near or adjacent to the major airways in whom the intention was to sample the lesion.

For this scenario we excluded the patients with endobronchial abnormalities and the patients where there was a decision made during the EBUS procedure not to sample (e.g. N2/3 or M1 disease).

Feasibility of tumor sampling was defined as successful tumor sampling rate in those cases in whom tumor sampling was intended. We did not include in this calculation those cases with endobronchial abnormalities and the cases in which sampling was not performed per clinical judgement (e.g. considered unnecessary, because of tissue proven nodal or distant metastasis by ROSE in the same endoscopy session).

EBUS-TBNA samples were judged as adequate when they contained sufficient material for cyto-pathological evaluation. Accordingly, samples were divided into three groups: 1) diagnostic for malignancy, when the sample allowed a diagnosis of malignancy according to WHO classification [18]; 2) diagnostic for benign disease, when sample was adequate to provide a diagnosis and no malignant cells were reported; 3) non-diagnostic, when either sample was not adequate, or when cells with atypia were reported but clear diagnosis of malignant disease could not be made.

Reports of subsequent diagnostic procedures and clinical/radiological follow-up were examined, when available, for diagnosis verification.

Sensitivity and specificity were calculated on successful EBUS-TBNA attempts (needle in the lesion). Sensitivity for malignancy detection was defined as the number of samples in which EBUS-TBNA made a diagnosis of any malignancy relative to the total number of cases in which the targeted intrapulmonary lesion turned out to be malignant. Specificity was defined as the number of non-malignant EBUS samplings relative to the number of patients in which the final diagnosis was that of a benign disease. For this purpose, non-diagnostic EBUS samples were considered as negative. PPV and NPV were also calculated.

2.5.2. Final diagnosis, true negatives and false negatives

Tumors were classified according to the 2015 WHO Classification for Lung Tumors [18]. Reference standard techniques included: 6 months clinical and radiological follow up (with CT-scan), trans-bronchial biopsy, imaging-guided TTB or TTNA, mediastinoscopy and surgical resections. Tumor positive EBUS-TBNA samples were regarded as true positive. In the absence of proven malignancy following EBUS-TBNA, other pathological data (cytology or histology obtained with other techniques) were seeked and/or a clinical/radiological follow-up of at least 6 months was retrieved from dossiers in order to assess a final diagnosis.

Cases in which a definite benign diagnosis was obtained through EBUS samplings, cases where surgical-pathological benign diagnosis was available and cases in which the lesion had remained stable after 6 months clinical/radiological follow up were considered true negatives. EBUS samples that were either negative or non-diagnostic were considered false negatives if a second diagnostic procedure (i.e., TTNA) led to a diagnosis of malignancy.

2.6. Molecular analysis and assessment of T4 staging

Pathological reports of EBUS samplings were checked for data on mutational analysis, when indicated by local guidelines recommendations. Tests included: EGFR or K-RAS mutation, ALK gene translocation, and PD-L1 expression. Molecular testing was performed at the institutes' laboratories following international guidelines [19].

International staging guidelines define as T4: a tumor which invades diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, or the carina [20]. We postulated that during EBUS procedure, after lung tumor visualization, the endoscopist would note whether the lung tumor was invading the mediastinum or centrally located vasculature (T4). To explore the role of EBUS in T4 assessment, we retrospectively checked the EBUS reports of all patients of our series who underwent tumor resection, as surgery is the best reference standard available. Furthermore, we noted all cases that were deemed suggestive of T4 involvement at EBUS even if they were not submitted to surgery. Preoperative chest CT scans of all the aforementioned patients were reviewed with the aid of a qualified and expert chest radiologist at each center, to look for correlations between EBUS and conventional imaging. The reviewing radiologist was blinded to EBUS and pathological evaluation of mediastinal/vascular invasion.

Mediastinal invasion was diagnosed by EBUS if there was continuous opposition of the tumor and the mediastinum without a separation of the two structures by an endosonographically visible tissue plane. The diagnosis could be further supported by dynamic maneuvers. Vascular invasion by EBUS was defined as an interruption of the intimal layer of a central extrapulmonary vessel or evidence of tumor encroachment into the vessel or left atrium (Fig. 2). In all cases, possible vascular tumor invasion was further assessed by color flow Doppler.

At chest CT scan, mediastinal tumor was documented as: replacement of mediastinal fat by soft-tissue mass, mass surrounding trachea or esophagus, obvious invasion of mediastinal structures, tumor contact of more than 3 cm with the mediastinum, obliteration of the fat planes that are normally seen adjacent to mediastinal structures, compression of mediastinal structures by a mass, mediastinal pleural or pericardial thickening. Vascular invasion was judged to be present when: the mass surrounded mediastinal vessels or clearly invaded them, the tumor was in contact with more than one fourth of the vessel's circumference, or the obliteration of fat planes that are normally seen adjacent to vessels was noticed [21].

Cases in which the EBUS evaluation was compatible with T4 and ultimately surgery confirmed vascular/mediastinal invasion were defined as true positives. Cases that were negative for T4 at EBUS but showed mediastinal/vascular invasion at surgery were defined as false negatives. True negatives were defined as cases in which both surgery and EBUS were negative for T4.

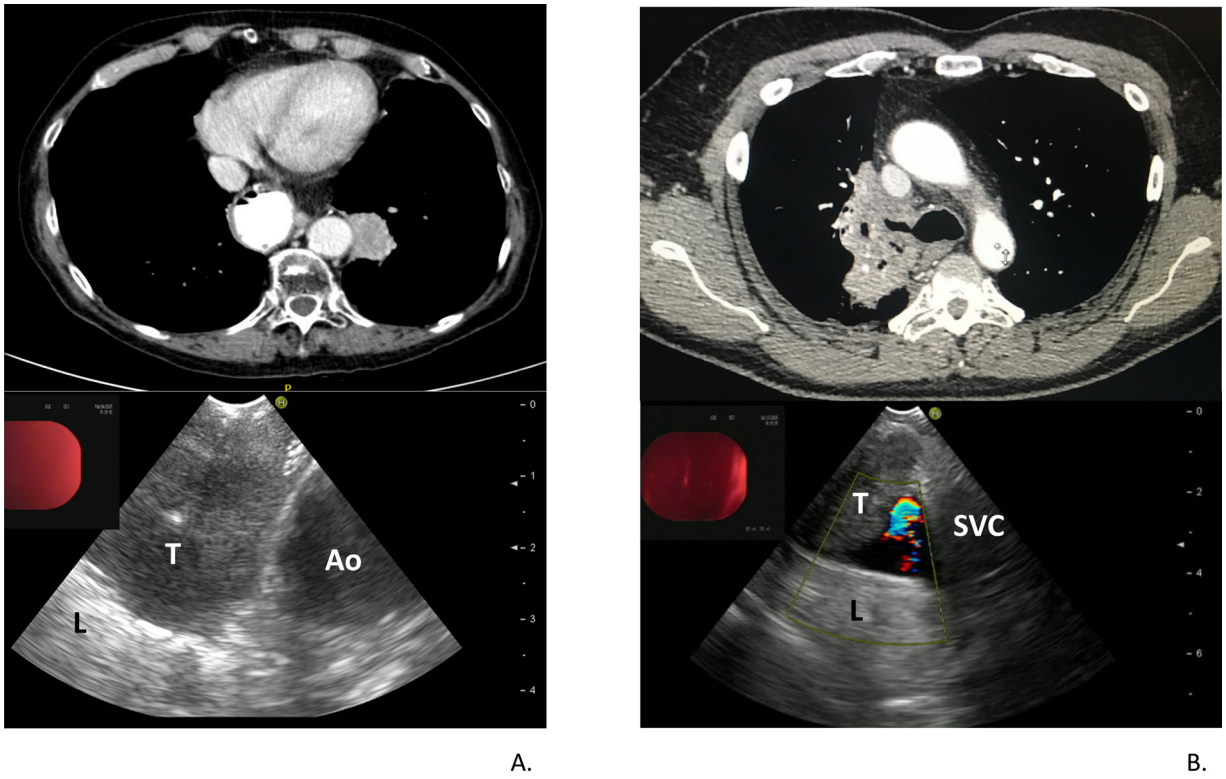


Fig. 2. EBUS evaluation of suspect T4 stage at imaging.
A. Above, chest CT image suspect for T4 (invasion of the aorta wall), below correspondent EBUS evaluation. A clear US margin is visible separating tumor (T) from lumen of the aorta (Ao), therefore EBUS evaluation is T4 negative. L = compromised lung.
B. Above, chest CT image suspect for T4 (invasion of SVC), below correspondent EBUS evaluation. EBUS imaging demonstrates tumor invasion of superior vena cava (SVC), with the aid of eco-color Doppler, therefore evaluation is T4 positive. L = compromised lung.

2.7. Statistics

Data were nonparametric and presented with median, mean and range. Data were processed using SPSS (IBM SPSS Statistics, version 22. Chicago, IL).

3. Ethics

This retrospective analysis was conducted in accordance with the amended Declaration of Helsinki and publication of the data was approved by the Medical Ethics Committee in The Netherlands and Italy.

4. Results

Between January 2013 and October 2018, 2007 patients with a known or suspected lung cancer underwent bronchoscopy and EBUS for diagnostic and/or staging purposes. 226 (11.2%) had a suspected centrally located lung tumor and 183 (81%) were located adjacent to the major airways without endobronchial abnormalities. In 179 patients, the lung tumor was detected by EBUS. In 159 cases a sampling attempt was carried out (See Flowchart 1, with complete search strategy and exclusion information).

Lung tumor sampling by EBUS was feasible in 145/163 (89%). In 4 cases the lung tumor was not found with EBUS and in the other 14 cases sampling was not performed due to: loss of the echo window after needle insertion [n = 3], interposition of a large vessel [n = 7], switch to radial EBUS [n = 1], switch and sampling through EUS or EUS-B [n = 3].

The baseline characteristics of the 145 patients in whom EBUS-TBNA was successfully performed are described in Table 1. The mean age was 66.26 years and 50.3% were males. Mean size of the lesions was 29.25 mm on the short axis (median 25.3) and 38.47 mm on the long axis (median 25.0). Most lesions were located on the right side: 35.9% were in the right upper lobe (RUL), 38.6% in the right lower lobe (RLL) and 0.7% in the middle lobe (RML). 24.8% of the lesions were adjacent to the trachea, 24.1% to the mainstem bronchus or bronchus intermedius, 40.7% to a lobar bronchus, and 10.3% to a segmental bronchus.

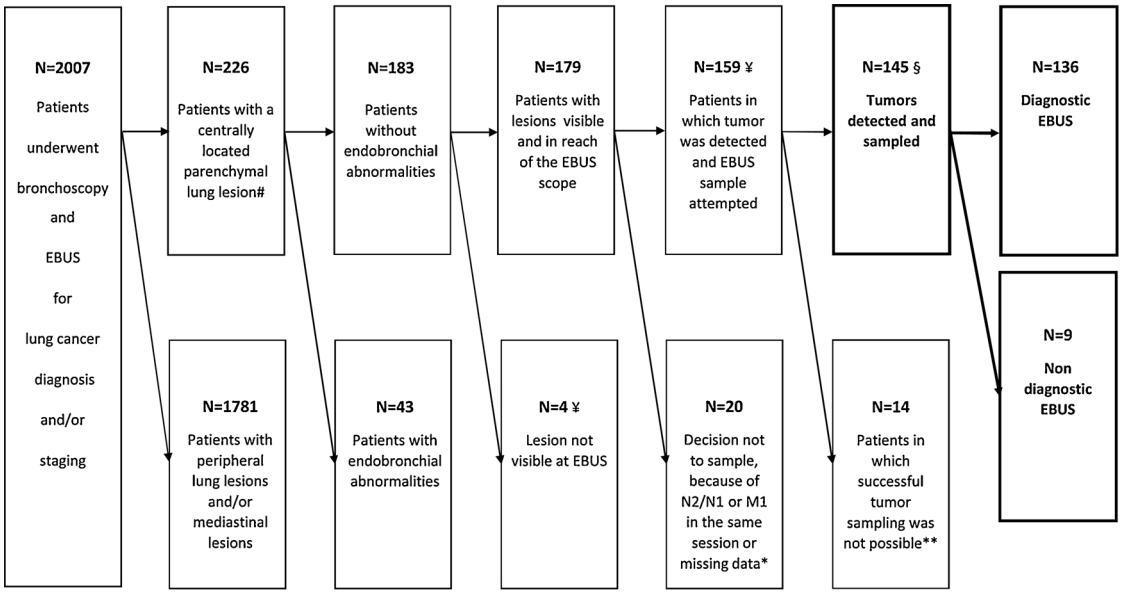
A definite diagnosis was achieved by EBUS-TBNA in 136/145 (94%) samplings: adenocarcinoma n = 69 (47.6%), squamous cell carcinoma n = 20 (13.8%), NSCLC-NOS n = 14 (9.7%), SCLC n = 9 (6.2%), metastasis n = 10 (6.9%), other tumor n = 7 (4.8%) and benign lesion n = 7 (4.8%). In 9 cases (6.2%) the procedure was non-diagnostic.

The diagnostic yield for EBUS-TBNA in the best case scenario was 94% (136/145), whereas sensitivity, specificity, NPV and PPV values were 96.3%, 77.8%, 56.2% and 100%, respectively. The diagnostic yield according to the location was 94% for lesions adjacent to the trachea, 91% for tumors close to mainstem bronchi or bronchus intermedius, 95% and 93% for lesions adjacent to lobar or segmental bronchi, respectively (See Table 2).

The diagnostic yield for EBUS in the worst case scenario was 83% (136/163). In 4 cases the primary tumor could not be visualized by EBUS and in 14 cases sampling was not performed due to technical/anatomical reasons. Sensitivity and specificity in this scenario are 84,9%, and 77,8% respectively.

The diagnosis obtained with EBUS was changed by subsequent diagnostic techniques in 14/145 (9.6%) patients. The final diagnoses for the 9 cases in which EBUS was non-diagnostic were: adenocarcinoma (n = 2), squamous cell lung carcinoma (n = 1), NSCLC NOS (n = 2) and benign disease (n = 2). For the remaining two patients with non-diagnostic EBUS result it was not possible to establish a definite final diagnosis, and they are still undergoing a clinical and radiological follow-up.

In the 5 remaining cases, EBUS was diagnostic for malignancy but



Flowchart 1. Patients selection flowchart.
Patients with a centrally located lung lesion who underwent an active search to detect the lung tumor with linear EBUS.
*Lymph node metastasis at ROSE [N2 station, n = 17; N1 station, n = 1], and left adrenal gland metastasis by EUS-B [n = 1], missing data [n = 1].
**Limiting factors included loss of the echo window after needle insertion [n = 3], sample not performed due to interposition of large vessels [n = 7], switch to radial EBUS [n = 1], switch and sampling through EUS or EUS-B [n = 3].
¥ These cases are the population of the diagnostic yield worst case scenario (N = 163).
§ These cases are the population of the diagnostic yield best case scenario(N = 145).

further investigations led to a better definition of the histology of the tumor (See Supplementary Table 1).

Twenty-six (26) patients out of the 145 with a successful sampling eventually underwent surgical resection of the lesion including final pathology assessment and T staging.

Three EBUS related complications were reported (2.1% of all EBUS sampled lesions). Two deep desaturations occurred under propofol sedation. Both resolved rapidly, one requiring short term (few minutes) assisted mask ventilation. An episode of epistaxis during high flow oxygen through nasal cannula also occurred. No pneumothorax nor clinically significant bleeding were reported.

Molecular analysis was indicated in 63/145 (43.4%) sampled patients and was successfully carried out in 54/63 (85.7%) of EBUS-TBNA tumor samples.

In the EBUS-T4 analysis, 30 patients were assessed. Of those 30 patients, 24 underwent surgical-pathological staging. In the other 6 cases EBUS evaluation suggested mediastinal/vascular tumor invasion (T4) These patients were not surgically treated due to clinical conditions/advanced disease. Among the 24 patients who underwent surgical lung tumor resection, there were no cases with a surgical-pathological proof of T4 (vascular/mediastinal invasion). EBUS assessment was negative for T4 among all operated patients. After re-evaluation by an expert radiologist, 2/24 (8.3%) operated patients had a suspicion of T4 based on chest CT-scan (one for mediastinal and one for vascular invasion), which were therefore T4 false positive at CT, but not at EBUS.

EBUS identified T4 based on mediastinal/vascular tumor invasion in 6 patients. After re-evaluation of the pre-procedural chest CT scan, 6/6 patients had confirmed T4 imaging. Since they did not undergo surgical treatment, no formal pathological confirmation was available. So, in 2/30 patients, EBUS added useful information to chest CT findings.

5. Discussion

We showed that EBUS is a safe technique that has high diagnostic yield (at least 83%) for diagnosing centrally-located lung cancers presenting without endobronchial abnormalities – provided the tumor is located adjacent or close to the major airways. The high diagnostic yield was independent of proximal (para tracheal) or distal (segmental bronchi) location in the airways. EBUS obtained tumor samples were adequate for molecular analysis in most patients. Of importance, EBUS assessment of mediastinal/vascular tumor invasion (T4) was feasible and the data suggest that EBUS has added value to chest CT in the assessment of T4 staging.

Strong points of the study besides the large number of evaluable patients (n = 145), are its multicenter international setting, careful description of the tumor lesions, reasons for sampling failure following tumor detection, molecular testing feasibility, suitability for T4 assessment and good quality of reference standard.

Diagnostic yield for lung tumor sampling – following tumor detection by EBUS - was described for two scenarios. The worst case scenario, consisting of cases in which EBUS could not identify the parenchymal tumor despite the located adjacent to the major airways and those in whom technical limitations (e.g. loss of echo window after needle insertion) or anatomical concerns (e.g. interposition of great vessels) prevented successful sampling of the lesion. In this context, a diagnostic yield of 83% was still achieved, with sensitivity and specificity of 84,9%, and 77,8 respectively. Considering that not all lesions will be easily visualized and/or sampled in clinical practice, this scenario is very much like what clinicians will experience in real life setting. We also reported a best case scenario, analyzing the data of all patients in which a needle was successfully placed in the tumor. In this scenario, EBUS showed very high diagnostic yield (94%) and sensitivity (96%). It

Table 1
Baseline characteristics and final diagnoses of patients with a centrally located lung lesion without endobronchial abnormalities who underwent EBUS sampling for diagnostic purposes.

Number of patients		145
Age	mean, years (range)	66.26 (18-84)
Gender	n (%)	
Male		73 (50.3%)
Female		72 (49.7%)
Size of the lesion	mean, mm (range)	
Short axis		29.25 mm (7-81)
Long axis		38.47 mm (8-91)
Location of the lesion in the lung	n (%)	
RUL		52 (35.9%)
RML		1 (0.7%)
RLL		56 (38.6%)
LUL		8 (5.5%)
LLL		28 (19.3%)
Localization of the lesion relative to the airways	n (%)	
1 Close or adjacent to the trachea		36 (24.8%)
2 Close or adjacent to mainstem bronchus or bronchus intermedius		35 (24.1%)
3 Close or adjacent to the lobar bronchus		59 (40.7%)
4 Close or adjacent to the segmental bronchus		15 (10.3%)
Sedation type	n (%)	
Propofol/Fentanyl sedation		124/145 (85.5%)
Midazolam/Fentanyl sedation		13/145 (9.0%)
General Anaesthesia		8/145 (5.5%)
ROSE	n (%)	145/145 (100%)
Final diagnosis	n (%)	
Diagnosis obtained		143/145 (98.6%)
Adenocarcinoma		73 (50.3%)
Squamous cell carcinoma		21 (14.5%)
NSCLC NOS		14 (9.7%)
SCLC		9 (6.2%)
Metastasis		9 (6.2%)
Other tumors		8 (5.5%)
Benign		9 (6.2%)
No diagnosis obtained		2/145 (1.4%)
Complications	n (%)	3/145 (2.1%)

In this Table the final diagnoses are reported that were ultimately obtained for each patient. Details about EBUS driven diagnosis are reported in the text. Eventually, 134/145 (92.4%) of patients with successful EBUS TBNA sampling were diagnosed with malignancy.

is important to note that virtually all data published so far described a "best case scenario" [10–15].

The first descriptions of EBUS sampling of parenchymal lesions were published 10 years ago by Nakajima et al. and Tournoy et al., and reported diagnostic yields ranging from 77% to 94%. In the case series from Nakajima et al., > 50% of the lesions were paratracheal, while Tournoy et al. did not detail the location of the target lesions [10,11]. More recently, similar results have been reported in larger monocentric [14] or multicentric [15] cohorts of patients submitted to EBUS-TBNA for the diagnosis of intrapulmonary lesions.

Our study confirms previous findings that EBUS-TNBA has a high diagnostic yield for diagnosing centrally located lung tumors [10,11,14,15]. However, all these studies were retrospective and calculated the diagnostic yield only based on successfully sampled lesions, corresponding to our best case scenario. We are the first to present diagnostic yield calculation taking into account lesions which could not be sampled despite visualization through EBUS. Furthermore, previous studies did not analyze the relationship between lesion location relative to the airways and sampling efficacy. The high diagnostic yield we found even for more peripheral airways is particularly promising in view of new thinner linear EBUS endoscopes that have become clinically available and will likely allow to access reliably more peripherally-located lung lesions [22].

Table 2
EBUS sampling performance and location analysis.

Feasibility of tumor sampling with EBUS (%)	n (%)	145/159 (91.2%)
EBUS Diagnostic yield (worst case scenario) (%)	n (%)	136/163 (83.4%)
EBUS Diagnostic yield (best case scenario) (%)	n (%)	136/145 (93.8%)
Diagnostic yield per sampling location[a] (%)	n (%)	
1 Trachea		34/36 (94.4%)
2 Mainstem bronchus or bronchus intermedius		32/35 (91.4%)
3 Lobar bronchus		56/59 (94.9%)
4 Segmental bronchus		14/15 (93.3%)
EBUS Sensitivity for malignancy, PPV[a]		96.3%, 100%
EBUS Specificity for malignancy, NPV[a]		77.8%, 56.2%

EBUS feasibility was calculated as those cases in which lesions were visible and sampling was through EBUS considered and attempted. Factors negatively affecting feasibility included: loss of echo-window after needle inserted in the working channel (n = 3), interposition of large vessels (n = 7), switch to another technique (n = 4). EBUS diagnostic yield was calculated in two scenarios: the worst-case scenario, i.e. considering all cases in which lesion was visualized by EBUS and sampling was considered and attempted (n = 163), and the best-case scenario, i.e. only including those cases in which a sample was successfully obtained (n = 145). See the text for further details.

[a] Numbers of the best case scenario. Worst case scenario is explained in the text.

We found a low NPV (56%) for EBUS-TBNA in centrally located lung lesions. These data are in line with other case series, which reported values ranging between 23% and 75% [10–15]. This value, however, is likely to be influenced by the high prevalence of malignancy (92.4%) in the population we analyzed. Accordingly, Almeida et al attributed their high NPV (75%) to the lower prevalence of malignancy in their study population, as compared to other studies [14].

To asses feasibility and diagnostic yield of EBUS for lung tumor sampling, ideally a prospective trial is needed. Key in such a study would be carefully description of the included patients based on CT findings.

Only 3 minor adverse events were noticed in our study, all of which were sedation-related. This confirms the excellent safety profile of EBUS as a diagnostic tool for the diagnosis of lung cancer and is in line with data collected so far [23,24]. Safety data combined with the high visualization rate of central lesions by EBUS are important, as CT guided sampling in this setting as compared to peripheral lung lesions is more challenging, with a higher complication rate [25,26].

In recent years, new effective therapeutic options such as targeted therapy and immunotherapy have been introduced for the treatment of patients with advanced and locally advanced disease [3,27,28]. Previous studies demonstrated the suitability of EBUS specimens obtained from lymphadenopathy for the molecular profiling of lung cancer [29–32]. Our series demonstrates that also EBUS-derived samples from intrapulmonary tumors can be successfully used to test all the clinically indicated molecular biomarkers in most patients (86%). To date, only Almeida et al. had provided preliminary evidence of the suitability of EBUS samples retrieved from intrapulmonary lesions for EGFR and ALK testing [14].

We are the first to investigate the potential usefulness of EBUS for the assessment of mediastinal/vascular involvement (T4) from the primary tumor. We found that, in selected cases, EBUS provides insights regarding vascular or mediastinal invasion (T4) that can be of added value to chest CT. The high resolution imaging with ultrasound, so close to the area of interest, in combination with the dynamic evaluation, are important assets for this indication.

Among operated patients with possible T4 at CT scan, no false negatives at EBUS were found, suggesting that in expert hands, EBUS can

offer valuable information in confirming or excluding locally advanced disease. Of high importance, EBUS gave additional information in 2/30 cases we included in T4 evaluation, demonstrating its relevant contribution in cases in which chest CT interpretation is uncertain. Accurate evaluation of the primary tumor (T parameter) is important in the decision making process that leads to management of lung neoplasms as tumor invasion of mediastinal structures, as well as of the large vessels (T4/stage IIIB), limits therapeutic options for patients [33]. T4 evaluation through radiological techniques such as CT and PET/CT is challenging, with variable sensitivity reported [34]. MRI of the chest has been shown not to significantly improve evaluation of mediastinal invasion [35]. Esophageal ultrasound (EUS) is useful in detecting and diagnosing lung cancer which is adjacent to the esophagus [36,37]. A recent retrospective analysis on 426 patients with NSCLC found a good specificity for EUS in evaluating local invasion, with a higher accuracy when combined with chest CT [38]. Adding T4 evaluation to EBUS applications would allow clinicians to achieve information on diagnosis, mediastinal staging and local invasion in a single procedure and exploratory thoracotomies could be prevented.

Some limitations apply to this study. Its retrospective design makes the interpretation of the results limited by flaws associated with such studies. Furthermore, we analyzed data from very experienced centers in endobronchial endoscopy, and it is uncertain whether less experienced centers and endoscopists would achieve comparable results. This especially applies to those centers in which anesthesiology assistance is not available, as most of the procedures in this study where conducted under deep sedation with propofol (85.5%). The very high prevalence of lung tumors among the study population must also be taken into account, as it is likely to have influenced our results, as discussed above.

As EBUS is indicated by guidelines for nodal staging in centrally located lung tumors [39], sampling and T4 assessment should be considered when clinically indicated. Future prospective trials, with careful inclusion criteria are needed to further refine the role of EBUS in diagnosis and T staging of lung cancer.

6. Conclusion

In patients with centrally located lung tumors located adjacent the major airways, without endobronchial abnormalities EBUS-TBNA is safe and has high diagnostic yield and sensitivity for diagnosing (lung) cancer. EBUS-TBNA samples are mostly suitable for subsequent molecular analysis. Mediastinal/vascular tumor invasion (T4) can be assessed in selected cases, and seems of added value to chest CT.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.06.006.

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