ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR LUNG TUMOR DIAGNOSIS AND STAGING

JOLANDA C. KUIJVENHOVEN - VARKEVISSER

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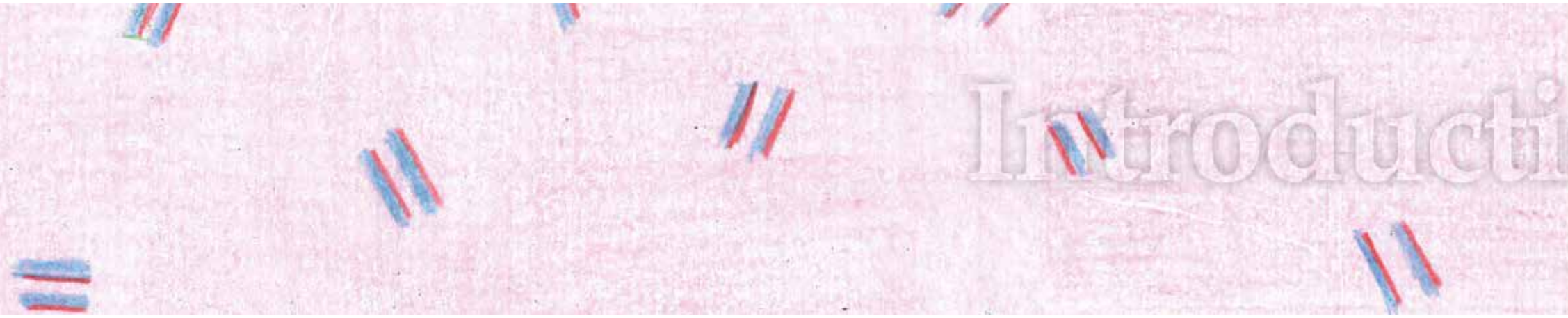


General introduction, aims and outline of this thesis

GENERAL INTRODUCTION ABOUT LUNG CANCER

Lung cancer is one of the most prevalent malignancies worldwide in both men and women and has the highest number of cancer related deaths.[1] In lung cancer, uncontrolled cell division results in changes in the normal structure and function of the tissue.[2] Lung cancer generally develops gradually. The tumor may first grow into surrounding tissues, which may cause complaints as the tumor continues to grow. Tumor cells can also spread through blood or lymph pathways to other parts of the body, resulting in metastases. Although any person can get lung cancer, inhalation of toxic substances, such as cigarette smoke or fine dust, are major risk factors.[3, 4]

The symptoms of lung cancer can vary considerably and partly depend on the location and size of the tumor and any metastases. In the early phase, when the tumor is still small, symptoms are often absent, and the tumor may be discovered by coincidence, for example when performing imaging of the chest for other indications. In later phases, typical symptoms that may point in the direction of lung cancer are: an altered coughing pattern, coughing up blood, increased shortness of breath, repeated respiratory infections, pain in the chest and pain in other parts of the body depending on possible metastasis.[5] Especially in individuals with a smoking history, such symptoms may warrant additional investigations.[6]

There are two main types of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC).[6] NSCLC accounts for approximately 85% of all lung cancers,[6] and the two most common histopathologic subtypes are adenocarcinoma and squamous cell carcinoma.[6]

STAGING AND IMAGING IN PATIENTS WITH (SUSPECTED) LUNG CANCER

The international TNM Classification of Malignant Tumors is used for classifying the extent of tumor spread.[7] In the TNM Classification, the T descriptor classifies the tumor size, location and its relation to the surrounding structures, the N descriptor classifies the involvement of hilar, mediastinal and supraclavicular lymph nodes, and the M descriptor describes the existence of intra- or extra-thoracic metastases.[7] (Table 1) Quick, safe and accurate diagnosis of the type and stage of lung cancer is crucial because prognosis and treatment options vary with cancer type and stage.[8] In patients with suspected lung cancer, the diagnostic and staging strategy commonly starts with imaging testing. Obtaining both a Computed Tomography of the chest (chest CT) and a Positron Emission Tomography and Computed Tomography (PET-CT) is generally indicated, which provides information about the tumor size, invasion in surrounding structures, regional nodal involvement and possible metastasis outside the thorax.[9-11] These imaging tests also guide the clinician in choosing the optimal site(s) for tissue sampling.

After this, (minimally) invasive techniques are required to obtain adequate tissue samples, so that a tissue-based diagnosis of the type of lung cancer can be made by means of histology or cytology. The acquisition of tissue from the primary lung lesion or suspected metastases (e.g., lung tumor, lymph nodes or distant organs) should ideally provide enough material for a timely and accurate histopathologic diagnosis with molecular characterization.[12] Multiple diagnostic tests are available for this purpose. The preferred initial site for tissue biopsy is one that could simultaneously establish a confident diagnosis including molecular and immunologic assessment and disease stage.[13]

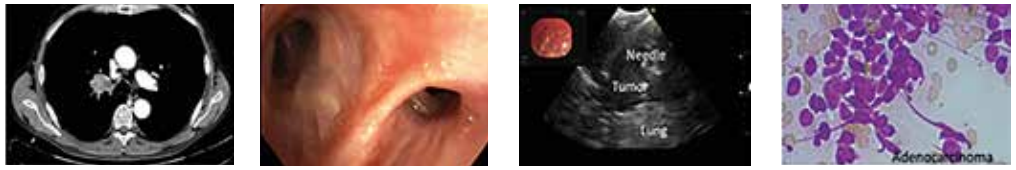
FLEXIBLE BRONCHOSCOPY AND EBUS TECHNIQUE

Historically, flexible bronchoscopy with its ancillary sampling procedures (biopsy, needle aspiration, brush and wash) is the cornerstone of lung tumor tissue acquisition, however its diagnostic yield in patients presenting without endobronchial abnormalities is low.[14, 15] Conventional flexible bronchoscopy may be even more challenging for centrally located lung tumors, due to its limited diagnostic yield[16] Alternatively, CT-guided transthoracic needle aspiration can be used to obtain a tissue diagnosis. However, centrally located lung tumors are generally not accessible through this technique and, these procedures are associated with a high risk of complications including pneumothorax and bleedings.[14, 15, 17] Recently, endobronchial endoscopic ultrasound-guided fine-needle aspiration (EBUS-TBNA) was proposed for diagnosing lung cancer that present without endobronchial abnormalities. In those lung tumors that are located adjacent to the major airways, real-time ultrasound controlled tumor sampling is feasible.[13]

The clinical use of flexible endoscopy began with the development of fiberoptic instruments in the 1960s in the gastroenterology and proctology. In 1966 flexible bronchoscopy was introduced in clinical practice by Shigeto Ikeda, a Japanese thoracic surgeon. A flexible bronchoscope, equipped with fibre optics, camera, and light source, allowed for real-time, direct visualization of the airways. It can be used to examine the respiratory tract starting from the oral or nasal cavity to the sub-segmental bronchi.[18-20]

In the 1990s, these flexible bronchoscopes were supplanted by video chip endoscopes for most purposes.[21] A Convex probe endobronchial ultrasound (CP-EBUS), in addition to a light source, with the ability to perform real-time endobronchial ultrasound-guided transbronchial needle aspiration was developed in 2002. EBUS is a bronchoscopic technique that combines optical endoluminal imaging with ultrasound to visualize structures adjacent to the airway wall enabling real-time ultrasound guided sampling of lymph nodes and parenchymal lung tumors.[12-13] EBUS scopes provides a forward oblique view, the angle and direction vary with different EBUS equipment. Color flow and Doppler features permit identification of vascular and cystic structures. The major advantage of EBUS is its ability to guide real-time sampling of lesions located beyond the airway wall. EBUS-TBNA is performed using an EBUS scope equipped with a 7.5 MHz convex ultrasound probe attached on the tip. The tip of the EBUS is placed adjacent to the area of interest. Both the ultrasound image and plain-view endoscopic image are displayed on the same monitor [22, 23] *(picture 1)*.

Picture 1

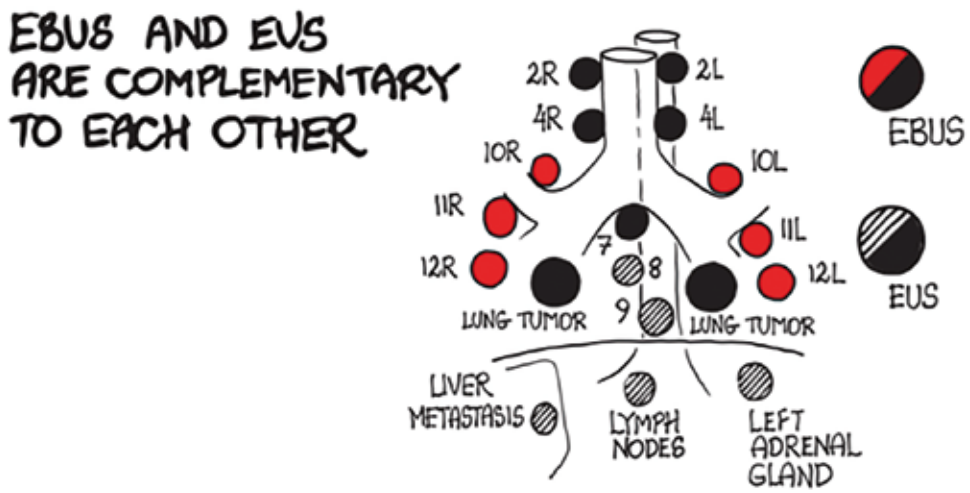


A **B** **C** **D**

A: *CT-scan of the chest with a right sided centrally located intrapulmonary lung tumor.*
B: *Bronchoscopic image demonstrating normal anatomy without endobronchial abnormalities.*
C: *Real-time EBUS guided tumor sampling. Image of the lesion with the needle in it.*
D: *TBNA samples showing adenocarcinoma.*

A flexible 19 to 25 gauge needle containing a retractable stylet is inserted through the bronchoscope's working channel and advanced just proximal to the ultrasound probe. The needle is then pushed through the bronchial wall and into the target lesion (i.e., tumor or lymph node) under direct ultrasound visualization. Suction is commonly applied using a 20 mL syringe, or alternatively the stylet is removed slowly from the inside of the needle (slow pull technique). Subsequently, the entire transbronchial needle system is removed from the bronchoscope. The aspirated specimen is removed from the needle lumen and processed for cytologic and/or histologic analysis.[24]
Most commonly, mediastinal, hilar and centrally located lung tumors are visualized and sampled from the endobronchial system. However, in case lymph nodes and lung tumors are located adjacent to the esophagus then they can be approached from the esophagus either using the EBUS or regular and larger sized gastrointestinal (GI) EUS scope [25-27] *(picture 2)*.

Picture 2 *Diagnostic reach of EBUS and EUS (B)*[52]



One of the first publications on linear EBUS is from 2003.[28] Since then, large numbers of trials evaluating the clinical role of EBUS-TBNA and EUS (B) -FNA for the diagnosis and staging of lung cancer have been performed, and their role in clinical practice has been expanding rapidly. EBUS has replaced surgical mediastinoscopy as the initial tissue sampling technique of choice and has obtained a central role in lungcancer staging guidelines.[13, 29]

For peripheral parenchymal lung lesions, guidance techniques such as radial EBUS, fluoroscopy and electromagnetic navigation have been developed and are helpful, especially in cases in which an airway leads to the tumor. Radial EBUS (R-EBUS) provides a 360° view of the lung tissue when the bronchoscope is placed in the smaller airways. Prior to the procedure, the airway of choice is selected based on the chest CT. On radial ultrasound imaging, lung tumors present with a typical ultrasound pattern. [30, 31]

When the tumor is more centrally located without endobronchial abnormalities, R-EBUS, fluoroscopy and electromagnetic navigation do not significantly contribute to the diagnostic yield.[32] Alternatively, CT-guided transthoracic needle aspiration can be used to obtain a diagnosis, but centrally located lung tumors are generally not accessible through this technique and, if accessible, there is a high risk of complications including pneumothorax and bleedings.[14, 15, 17]

LUNG CANCER STAGING

Staging of lungcancer is important because it directs treatment options and the prognosis. The international TNM Classification of Malignant Tumors is used for classifying the

extent of tumor spread.[7] For diagnosis centrally located lung tumors located adjacent to the major airways, which is now also recommended in clinical guidelines, an EBUS is advised.[13] If the tumor is adjacent to the esophagus, EUS(B) can be used for diagnosing centrally located malignancies.[27]

Staging of the T descriptor

Patients with NSCLC and tumor invasion of the mediastinum or centrally located vessels (T4 stage) have a worse prognosis in terms of survival compared to patients with stage T1-3. Five year survival rates vary between 28% and 44% in the published literature.[33, 34] Treatments options are also different for patients with T4 lung tumors, who are most often treated with multimodality treatment including (neo-adjuvant) chemotherapy and/or radiotherapy, sometimes followed by surgery.[35, 36] Therefore, accurate preoperative assessment of mediastinal tumor invasion (T4) is important for assessing prognosis and prescribing optimal treatment. However, this is challenging as imaging tests such as chest CT, FDG-PET and MRI have suboptimal sensitivity and specificity, which may result in large numbers of false negatives and false positives.[37] Accurate staging is crucial to allocated patients to the optimal therapy. Beyond their diagnostic capabilities, in selected cases, EBUS and EUS are able to visualize the anatomical relationship of lung tumors with centrally located vessels and the mediastinum,[38, 39] but there is limited evidence about its diagnostic accuracy regarding tumor staging.

Staging of the N descriptor

Staging of the nodal descriptor is important because this effects the treatment options, either surgical resection of the lung tumor or radical radiotherapy (stage I-II)[40] or treatment with chemo-radiation therapy (stage III).[36] The N descriptor describes regional lymph node involvement by the International Association for the Study of Lung Cancer (IASLC) is: N0 indicates absence of regional lymph node metastases. N1 describes a metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes. Patients with N2 disease have a metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s). N3 metastases are located in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s).[7] (table 1)

Table 1 *TNM 8th*[7]

TNM 8th - Primary tumor characteristics	
T_x	Tumor in sputum/bronchial washings but not be assessed in imaging or bronchoscopy
T_0	No evidence of tumor
T_{is}	Carcinoma in situ
T_1	≤ 3 cm surrounded by lung/visceral pleura, not involving main bronchus
$T_{1a(mi)}$	Minimally invasive carcinoma
T_{1a}	≤ 1 cm
T_{1b}	> 1 to ≤ 2 cm
T_{1c}	> 2 to ≤ 3 cm
T_2	> 3 to ≤ 5 cm *or* involvement of main bronchus without carina, regardless of distance from carina or invasion visceral pleural *or* atelectasis or post obstructive pneumonitis extending to hilum
T_{2a}	>3 to ≤4cm
T_{2b}	>4 to ≤5cm
T_3	>5 to ≤7cm in greatest dimension *or* tumor of any size that involves chest wall, pericardium, phrenic nerve *or* satellite nodules in the same lobe
T_4	> 7cm in greatest dimension *or* any tumor with invasion of mediastinum, diaphragm, heart, great vessels, recurrent laryngeal nerve, carina, trachea, oesophagus, spine *or* separate tumor in different lobe of ipsilateral lung
N_1	Ipsilateral peribronchial and/or hilar nodes and intrapulmonary nodes
2	Ipsilateral mediastinal and/or subcarinal nodes
3	Contralateral mediastinal or hilar; ipsilateral/contralateral scalene/ supraclavicular
M_1	Distant metastasis
M_{1a}	Tumor in contralateral lung or pleural/pericardial nodule/malignant effusion
M_{1b}	Single extrathoracic metastasis, including single non-regional lymphnode
M_{1c}	Multiple extrathoracic metastases in one or more organs

For more than 50 years, mediastinal lymph node staging in patients with lung cancer was performed by (cervical) mediastinoscopy. This is a surgical procedure, with a sensitivity for mediastinal metastases around 79%.[29] Mediastinoscopy provides access to both upper and lower para tracheal zones and the anterior part of the subcarinal station, however, has limitations in its diagnostic reach in the dorsal part of the subcarinal station and the lower mediastinum. Draw backs are its invasiveness including a scar in jugolo, need for general anaesthesia, an operating theatre and the risk of laryngeal nerve palsy resulting in persistent hoarseness and bleed.[41, 42]

It has been shown that mediastinal staging of lung cancer can also be done by endosonography, either by EBUS-TBNA or EUS (B)-FNA or its combination, which is of major benefit because these techniques are less costly and minimally invasive.[43] EBUS-TBNA has similar diagnostic range to cervical mediastinoscopy,[29] and EUS-FNA can reach additional lymph node stations located in the lower and posterior mediastinum and also the left adrenal gland.[25, 27] The ASTER study showed that a combined endosonography investigation (EBUS and EUS) followed by surgical staging through mediastinoscopy in absence of metastases at endosonography, resulted in greater sensitivity for mediastinal nodal metastases compared with mediastinoscopy alone (94% vs 79%).[29] Based on these findings, clinical guidelines currently recommend to perform endosonography (either EBUS and/or EUS) for mediastinal lymph node staging in patients with proven or suspected lung cancer if CT or PET-CT shows abnormal lymph nodes, in case of a centrally located lung tumor, or a lung tumor >3cm.[13] Also, systematic EBUS followed by EUS-B increased sensitivity for detection of N2/N3 disease by 9% compared to PET-CT-targeted EBUS alone.[25] Subsequent surgical nodal staging is still recommended if the endosonography is negative while the imaging is still suspect for nodal metastasis. The Mediast trial (NTR 6528), randomized patients staged nodal negative by EBUS between direct surgical tumor resection and confirmatory mediastinoscopy; will shed light on the role of surgical staging.[44]

TREATMENT OPTIONS AND PROGNOSIS FOR PATIENTS WITH LUNG CANCER

There are several treatment options for patients with lung cancer, which are commonly discussed for each individual patient in amulti-disciplinary tumor board meeting. In some cases, a single treatment is prescribed, but usually patients receive combinations of treatments. Which treatment is most useful primarily depends on the type of cancer and the stage of disease, but also the patients physical condition is taken into account.[45-47]
For patients with stage I-II disease lung tumor resection or radiation therapy are the most common options.[48] Advanced stage III disease is mostly treated with multi-modality treatment (chemotherapy/immunotherapy/radiation therapy). Surgical lung tumor treatment generally involves resection of the tumor containing lobe including lobe specific nodal dissection.
The first choice treatment for patients with stage IV lungcancer is either chemotherapy or an immune checkpoint inhibitor or the combination. Patients with specific active driver mutation are suitable for tyrosine kinase inhibitors.[49]
5 year survival of lungcancer is related to the stage of the disease. For local disease, 5 year survival rates are 52%, for mediastinal metastasis this is 24% and for stage IV disease this is only 4% respectively.[50] Survival is besides the stage of the disease also dependent on other patients' characteristics as age, performance status, gender and the social economic status.[50,51]

GOALS AND OUTLINE OF THIS THESIS

At the outset of this PhD project in 2016, there were unresolved questions about the role of endosonography in patients with a centrally located lung tumor regarding tumor diagnosis and its suitability to assess tumor invasion in the surrounding structures (stage T4). The aim of this thesis is to further explore the role of endosonography in the diagnosis and staging of lung cancer. The thesis consists of three parts.

PART 1: ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR DIAGNOSING LUNG TUMORS

In part 1 of this thesis, we will evaluate the role of endosonography in the diagnosis of centrally located lung tumors in terms of diagnostic accuracy and safety. Specifically, the following topics will be addressed:
- The role of EBUS for diagnosing lung cancer in patients with a centrally located lung tumor (chapters 1 and 2)
- The role of EUS-B for diagnosing lung cancer in patients with a centrally located lung tumor (chapter 3)
- The role of intracardiac EUS-FNA for diagnosing cardiac tumors (chapter 4)
- A protocol of a RCT evaluating the novel Acquire needle for the diagnosis and staging of lung cancer through EBUS and EUS-B (chapter 5)

PART 2: ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR T STAGING OF LUNG TUMORS

In part 2 of this thesis, we will evaluate the role of endosonography in T4-staging of centrally located lung tumors. Specifically, the following topics will be addressed:
- The diagnostic accuracy of EBUS to assess T4 status in patients with lung cancer (chapter 6)
- The diagnostic accuracy of EUS (B) to assess T4 status in patients with lung cancer (chapter 7)

PART 3: NOVEL LUNG CANCER STAGING STRATEGIES AND ITS IMPACT ON SURVIVAL

In part 3 of this thesis, we will evaluate whether an endosonographic mediastinal lymph node centered staging strategy leads to improved survival compared to a mediastinoscopy centered staging strategy. Specifically, the following topic will be addressed:
- Five-year survival after endosonography versus mediastinoscopy for mediastinal nodal staging of lung cancer (chapter 8)

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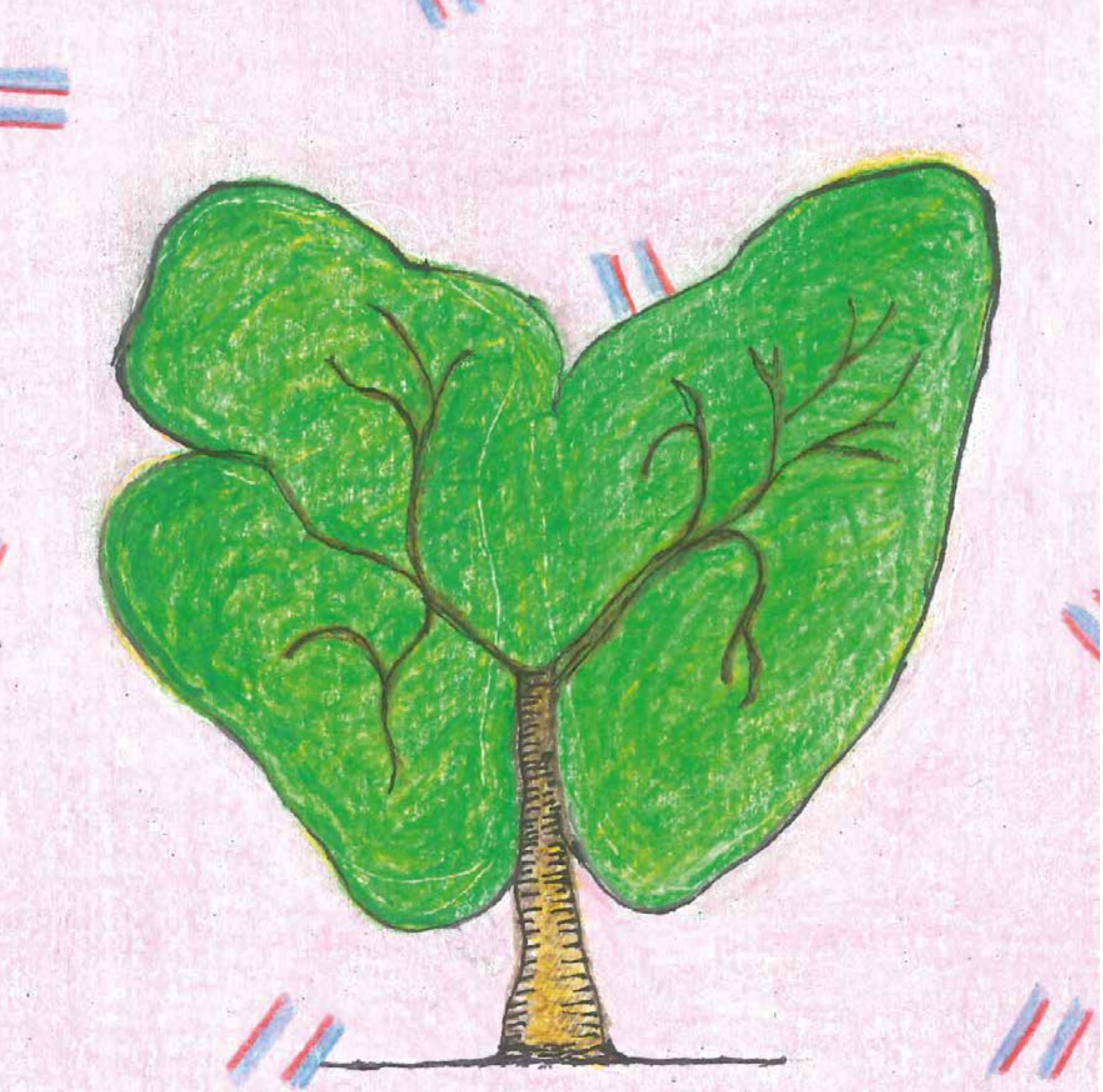
General Introduction



Part 1

Endobronchial and esophageal ultrasound for diagnosing lung tumors

PART ONE CHAPTER 1

Endobronchial ultrasound for the diagnosis of centrally located lung tumors: A systematic review and meta-analysis.

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ABSTRACT

Introduction

Obtaining a tissue diagnosis of centrally located lung tumors in patients presenting without endobronchial abnormalities is challenging and therefore a considerable diagnostic problem.

Objective

The objective of this study was to evaluate the performance of linear endobronchial ultrasound guided – transbronchial-needle aspiration (EBUS-TBNA) for the diagnosis of centrally located lung tumors.

Methods

We performed a systematic review (PROSPERO, CRD42017080968) and searched MEDLINE, Embase, BIOSIS Previews and Web of Science till November 18, 2018 for studies that evaluated the yield and/or sensitivity of EBUS-TBNA for diagnosing centrally located lung tumors. We assessed study quality using QUADAS-2 and performed random-effects meta-analysis.

Results

5,657 manuscripts were identified; of these 14 were included, including 1175 patients who underwent EBUS-TBNA for diagnosing an intrapulmonary tumor. All studies had a high risk of bias or applicability concerns, predominately regarding patient selection. Average yield of EBUS-TBNA for diagnosing centrally located lung tumors was 0.89 (95%CI 0.84-0.92) and average sensitivity for diagnosing malignant tumors was 0.91 (95%CI 0.88-0.94). Among studies reporting this information, EBUS related complications occurred in 5.4% of patients (42/721).

Conclusion

EBUS-TBNA has a high yield and sensitivity for diagnosing centrally located lung tumors and is safe, in selected patients. Prospective studies are recommended to evaluate the routine use of this procedure for diagnosing intrapulmonary tumors.

Endobronchial ultrasound for the diagnosis of centrally located lung tumors: A systematic review and meta-analysis Part 1. Chapter 1

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the world.[1] If lung cancer is suspected, a tissue diagnosis should be obtained in order to establish a definite diagnosis. In patients with centrally located lung tumors suspected for lung cancer, current clinical guidelines recommend conventional flexible bronchoscopy with biopsy or TBNA to obtain a diagnosis.[2] However, bronchoscopy is non-diagnostic in a considerable proportion of patients, especially in the absence of endobronchial abnormalities.[3]
Computed tomography (CT) guided transthoracic needle aspiration can be used to obtain a diagnosis, but for centrally located lung tumors this technique has a high risk of complications including pneumothorax and bleedings.3 Moreover, such tumors are frequently inaccessible for a transthoracic approach and the diagnostic yield is lower than for peripheral lung tumors.[4, 5]

Current staging guidelines advocate endobronchial and esophageal ultrasound (EBUS and EUS-(B)) as the techniques of choice for mediastinal nodal tissue staging of non-small cell lung cancer (NSCLC).[6-8] In patients in whom CT imaging shows a centrally located lung tumor located adjacent to the major airways, endobronchial endoscopic ultrasound-guided fine-needle aspiration (EBUS-TBNA) is suggested for diagnostic purposes following a non-diagnostic conventional bronchoscopy.[6, 9]

Although the EBUS technique for mediastinal nodal staging of lung cancer has rapidly spread, its role in obtaining an adequate tissue sample directly from intrapulmonary tumors has received much less attention. If sufficiently feasible and accurate, diagnosing lung tumors through EBUS could have major logistic advantages, as tumor and mediastinal nodal staging can be performed in the same session.[6, 10]

Various reports regarding the role of EBUS-TBNA in the diagnosis of centrally located lung tumors have been published, but its feasibility, yield, sensitivity and safety are not well-established.[6] Therefore, we conducted a systematic review and meta-analysis with the aim of obtaining summary estimates of the yield and sensitivity of EBUS-TBNA for diagnosing centrally located lung tumors in patients with suspected lung cancer.

MATERIAL AND METHODS

The protocol of this systematic review was prospectively registered at PROSPERO under registration number CRD42017080968. This review is reported following the PRISMA-DTA guidelines.[11]

Eligibility criteria

Studies were included if they evaluated the yield and/or sensitivity of EBUS-TBNA for diagnosing centrally located lung tumors located adjacent or near the major airways - with the aim of obtaining a tissue sample from the suspected lesion. Various definitions of a centrally located lung tumor exist and we followed those as reported by the authors of the primary studies. Studies were eligible for analysis regardless of whether patients were selected based on the results of previous tests. If studies aimed to obtain a tissue diagnosis

from centrally located lung tumors invading the mediastinum or central vessels, they were also included. However, we excluded studies that focused on diagnosing mediastinal tumors, studies that aimed to diagnose lung cancer by sampling mediastinal nodes, liver lesions or left adrenal gland lesions, and studies focusing on lung cancer staging rather than diagnosis. We also excluded studies using a radial instead of a linear EBUS scope, and studies including less than 10 patients with centrally located lung tumors.

Literature search strategy and selection

We searched for eligible studies in MEDLINE (Ovid), Embase (Ovid), BIOSIS Previews (Ovid) and Web of Science. Searches were developed by a medical information specialist (R.S.). No date or language restrictions were applied. The complete search strategy is provided in supplementary appendix (Table S1.1). The final search was performed on November 18, 2018. We checked reference lists of all included papers for additional studies

Two authors (J.K. and L.C.) independently reviewed the titles and abstracts of all search results for eligibility. If an article was considered potentially eligible, both authors independently examined the full article for inclusion. Disagreements were resolved after discussion with a third author (J.A.).

Data extraction and synthesis

Data were extracted from included studies by two authors (J.K. and F.L.). We extracted the first author, year of publication, journal of publication and country of patient recruitment. We also extracted whether or not patients had received previous tests to obtain a biopsy-based diagnosis of the centrally located tumor. We extracted details about age and gender, availability of rapid on-site cytological evaluation (ROSE), needle type, number of needle passes performed, procedure length, tumor size, the number of patients with endobronchial abnormalities, the reference standard, and any complications induced by EBUS-TBNA.

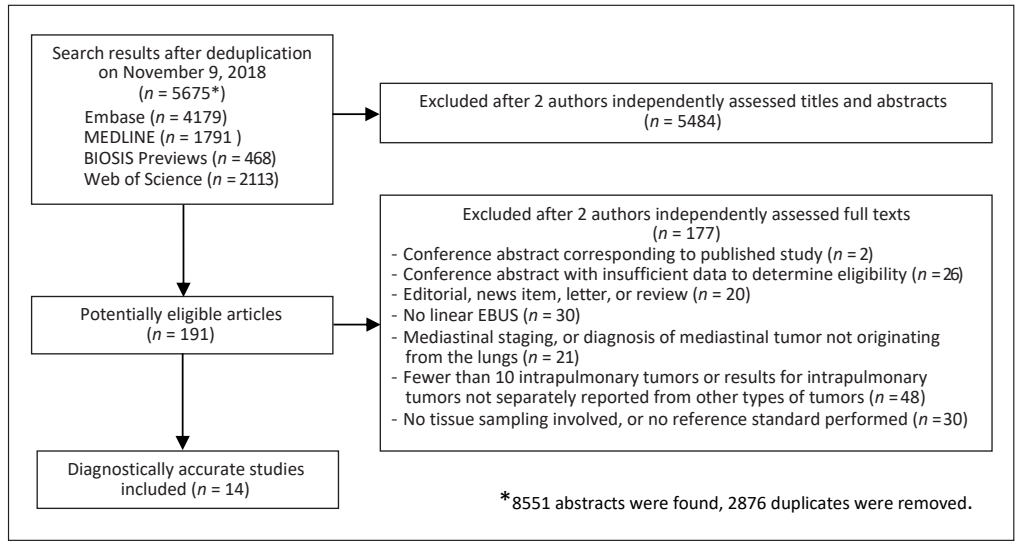
Furthermore, we extracted the total number of patients in whom EBUS-TBNA was performed with the aim of diagnosing a centrally located lung tumor, the number of patients in whom an adequate tissue sample was obtained by EBUS -TBNA, the number of patients in whom EBUS-TBNA made a correct biopsy-proven diagnosis (malignant or non-malignant), the number of patients in whom EBUS-TBNA diagnosed a malignancy, and the number of patients in whom the targeted intrapulmonary tumor turned out to be malignant, according the reference standard.

EBUS-TBNA was considered to have reached an inadequate diagnosis if additional diagnostics were needed to obtain a correct diagnosis (e.g. because the tumor could not be visualized or sampled through EBUS-TBNA), or if the reference standard reached a different diagnosis. EBUS-TBNA was considered to have reached a correct diagnosis if the reference standard resulted in the same diagnosis, or if EBUS-TBNA tissue samples contained malignant cells as in such cases a reference standard is rarely performed.

Risk of bias and applicability concerns assessment

Two authors (J.K. and F.L.) independently assessed study quality using the QUADAS-2 tool.[12] Disagreements were resolved by consensus and in difficult cases, two other authors (L.C. and D.K.) made the final decision. Study designs with a high risk of bias or applicability concerns included: 1: retrospective (nonconsecutive) inclusion of patients; 2: exclusion of patients in whom the intrapulmonary tumor could not be visualized by EBUS; 3: a case-control design; 4: exclusion of patients that did not match the review question; 5: endoscopists that were not blinded to the final diagnosis while performing EBUS; 6: a suboptimal reference standard for patients with a non-diagnostic or non-malignant EBUS-TBNA (e.g., clinical follow-up instead of surgical-pathological verification); 7: partial or 8: differential verification of patients with a non-diagnostic or non-malignant EBUS-TBNA; 9: exclusion of patients with missing reference standard results.

Figure 1.1 *Flowchart of the selection process of the included studies. EBUS, endobronchial ultrasound*



Primary outcomes

The primary outcomes of this review were: 1) the yield of EBUS-TBNA for diagnosing centrally located lung tumors, and 2) the sensitivity of EBUS-TBNA for diagnosing malignant centrally located lung tumors.

Yield was defined as the number of patients in whom EBUS-TBNA made a correct tissue diagnosis, relative to the total number of patients in whom EBUS was performed with the aim of diagnosing a centrally located lung tumor.

Sensitivity was defined as the number of patients in whom EBUS-TBNA made a correct tissue diagnosis of any malignancy, relative to the total number of patients in whom the targeted centrally located lung tumor turned out to be malignant.

Analysis

We calculated estimates of yield and sensitivity of the included studies with 95% confidence intervals, using the normal approximation. We then performed a univariate random effects meta-analysis according to DerSimonian-Laird.13 Data analyses were performed in the "meta" package in R version 3.0.

RESULTS

Study selection and study characteristics

The searches identified 5675 results. After screening titles and abstracts, 191 potentially eligible articles remained, of which 14 studies were included in the final analysis.[9,14-26] Of these, 3 were conference abstracts. Figure 1.1 provides the details of the study selection and the reasons for excluding studies.

Table 1.1 *Characteristics of the included studies in which patients underwent EBUS procedure for diagnosis of a centrally located lung lesion*

Study details, *Journal [Ref.]*	Previous tests to obtain a tissue diagnosis prior to EBUS-TBNA	Mean/Median age (range) in years	Male, %	Rapid on-site cytological evaluation	Needle type	Number of needle aspirates	Procedure length, min	Tumor size, mm	Reference standard in patients with a non-diagnostic or nonmalignant EBUS-TBNA	EBUS-TBNA induced complications
Nakajima, 2008 Japan *Journal of thoracic oncology [9]*	Non diagnostic conventional bronchoscopy in 74 % of patients	63 (37-86)	83	Available	22G	NR	NR	Mean: 30, (range: 10-70)	-Surgical-pathological verification	-None
Tournoy, 2008 Belgium *Lung cancer [14]*	Non diagnostic conventional bronchoscopy in 82 % of patients	65 (43–82)	60	Available	22G	NR	Mean: 21, (range: 10-60)	Mean: 25, (range: 10-70)	-Surgical-pathological verification -CT-guided biopsy	-Patient intolerance with procedure being abandoned *n=2 (3.3 %)* -Self-limiting atrial fibrillation *n=1 (1.7 %)*
Eckhart, 2010 Denmark *World journal of xurgery [15]*	Non diagnostic conventional bronchoscopy in all (100 %) patients	67 (29-86)	56	Not available	22G	2	NR	NR	-Surgical-pathological verification -Bronchoscopy -CT-guided biopsy -Clinical follow-up	-None
Khan, 2012[a] UK *Thorax [16]*	Non diagnostic conventional bronchoscopy in all (100 %) patients	68 (NR)	47	NR	NR	NR	NR	NR	-Surgical-pathological verification -CT-guided biopsy -CT-scan follow-up	-NR
Batthi, 2013 USA *Journal of bronchology & interventional pulmonology [17]*	NR	69 (NR)	50	NR	22G	NR	Mean: 56, (SD 23)	Mean: 53, (SD 24)	-Surgical-pathological verification -CT-guided biopsy	-Moderate bleeding *n=3 (9.4%)* -Tachycardia *n=1 (3.1 %)*
Verma, 2013 South Korea *Yonsei medical journal [18]*	Non diagnostic conventional bronchoscopy in 40 % of patients	63 (40-81)	68	Not available	22G	2	NR	Mean (short axis): 27.5, (range: 8-82)	-Surgical-pathological verification -CT-guided biopsy -Clinical follow (>6 months)	-Pneumothorax *n=1 (2.7 %)* -Self-limiting moderate bleeding *n=1 (2.7 %)*
Yang, 2013 China *Chinese journal of tuberculosis and respiratory disease [19]*	NR	NR	NR	Not available	22G	NR	NR	NR	NR	-Moderate *bleeding n=1 (1.3%)*
Zhao, 2013 China *Chinese medical journal [20]*	Non diagnostic conventional bronchoscopy in all (100%) patients	56 (33-78)	64	Available	22G	3-6	NR	Mean (short axis): 34, (range: 20-100)	-Surgical pathological verification	-None
Evison, 2013 UK *British thoracic*	Non diagnostic conventional	NR	NR	NR	NR	NR	NR	NR	NR	-NR

NR= Not reported; SD = Standard deviation; EBUS-TBNA = Endobronchial ultrasound-guided transbronchial-needle aspiration; CT= Computed tomography
a Age and gender data apply to the complete cohort of 119 patients; however, 5 of these were excluded from this review because of the lack of a final diagnosis.
b Age and gender data apply to the complete cohort of 308 patients; however only 82 of these had an intrapulmonary tumor.
c Age and gender data apply to the complete cohort of 72 patients; however, 6 of these were excluded from this review because of the lack of a final diagnosis.
d Age and gender data apply to the complete cohort of 1891 patients; however, only 290 of these had an intrapulmonary tumor.

Across included studies various definitions for the targeted lung tumors were used, ranging from "central lung parenchymal lesions"[16] to "an intrapulmonary mass with the medial margin located within the inner third of the hemi thorax based on chest CT-scan imaging"[14]. Table S1.3 summarizes the different definitions used for centrally located lung tumors across the included studies.

Table 1.1 shows detailed characteristics of the 14 included studies. The first article was published in 2008 and the last in 2018. Nine studies reported the proportion of patients that underwent a previous non-diagnostic conventional bronchoscopy, ranging from 33 to 100%; information on previous bronchoscopy was not reported in the remaining 5 studies. The mean/median age of the patients ranged from 56 to 69 year, and the ratio of male patients ranged from 31% to 83%. ROSE was available in 6 studies, not available in 4 studies, and 4 studies did not report on the availability of ROSE. The type of needle that was used was a 22 Gauge needle in 11 studies, both 21- or 22-Gauge needles in 1 study, and not reported in 2 studies. Six studies reported on the number of needle aspirates, which varied from 2 to 6. Three studies reported on the mean/median procedure length: 21 minutes, 46 minutes and 56 minutes. The mean/median tumor size ranged from 25 to 53 mm. seven studies (542 patients in total) explicitly excluded patients with endobronchial abnormalities or did not encounter such patients, and three studies explicitly included patients with endobronchial abnormalities (27 patients with endobronchial abnormalities in total). The remaining four studies made no comments regarding the presence of patients with endobronchial abnormalities.

Risk of bias and applicability concerns

Detailed results of the quality assessment of included studies are available in the online supplementary appendix (Table S1.2). All studies had at least one item with a high risk of bias and/or applicability concerns. The most common source of bias was retrospective inclusion of patients, which was the case in 12 of 14 included studies. It was unclear for 11 studies whether inappropriate exclusions were avoided, which we considered the case if patients in whom the tumor could not be visualized by EBUS were excluded.
The quality of the reference standard in the absence of a specific diagnosis following EBUS, was variable ranging from surgical-pathological verification to clinical follow-up.

Table 1.2 *Yield and sensitivity for EBUS TBNA for diagnosis of centrally located intrapulmonary lesions*

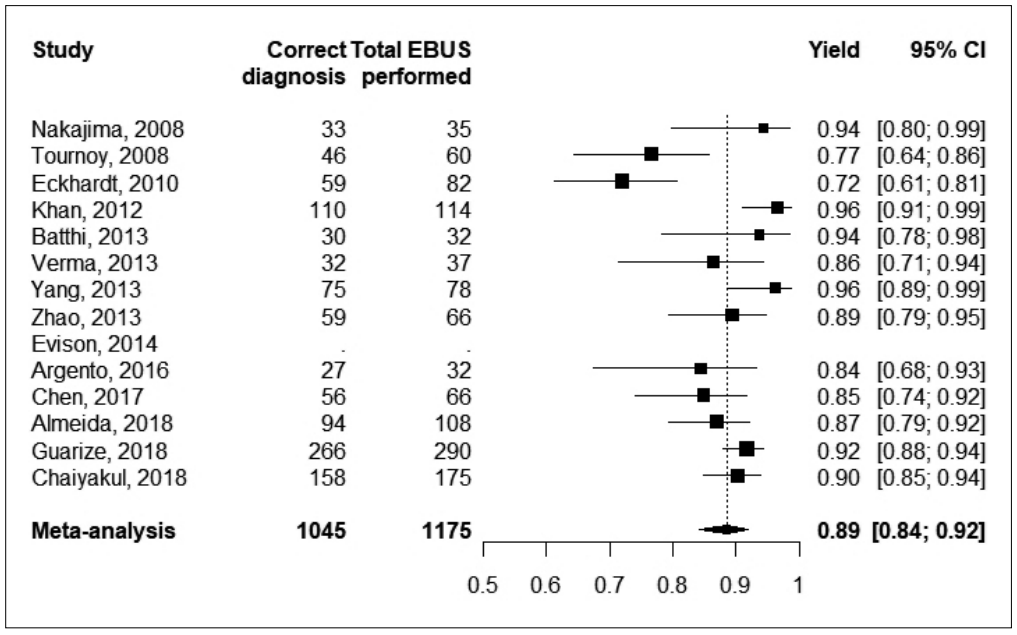
Study [Ref.], year	Total EBUS performed, n	Total with any malignancy, n (%)	Adequate tissue sample by EBUS-TBNA, n (%)	Correct diagnosis by EBUS-TBNA, n	Correct diagnosis of any malignancy by EBUS-TBNA, n	Yield for correct diagnosis[a] [95% CI]	Sensitivity for malignancy[b] [95% CI]
Nakajima [9], 2008	35	34 (97)	35 (100)	33	32	0.94 [0.80; 0.99]	0.94 [0.79; 0.99]
Tournoy [14], 2008	60	58 (97)	46 (77)	46	46	0.77 [0.64; 0.86]	0.79 [0.67; 0.88]
Eckhardt [15], 2010	82	51 (62%)	79 (96)	59	48	0.72 [0.61; 0.81]	0.94 [0.83-0.98]
Khan [16], 2012	114	111 (95)	113 (99)	110	108	0.96 [0.91; 0.99]	0.97 [0.92; 0.99]
Batthi [17], 2013	32	32 (100)	NR	30	30	0.94 [0.78; 0.98]	0.94 [0.78; 0.98]
Verma [18], 2013	37	33 (89)	NR	32	32	0.86 [0.71; 0.94]	0.97 [0.81; 1.00]
Yang [19], 2013	78	65 (83)	NR	75	62	0.96 [0.89; 0.99]	0.95 [0.87; 0.99]
Zhao [20], 2013	66	63 (95)	66 (100)	59	59	0.89 [0.79; 0.95]	0.94 [0.84; 0.98]
Evison [21], 2013	49	47 (95)	NR	NR	38	-	0.81 [0.67; 0.90]
Argento [22], 2016	32	30 (94)	NR	27	26	0.84 [0.68; 0.93]	0.87 [0.69; 0.95]
Chen [23], 2017	66	56 (85)	NR	56	48	0.85 [0.74; 0.92]	0.86 [0.74; 0.93]
Almeida [24], 2018	108	93 (86%)	NR	94	88	0.87 [0.79-0.92]	0.95 [0.88; 0.98]
Guarize [25], 2018,	290	NR	NR	266	241	0.92 [0.88-0.94]	-
Chaiyakul [26], 2018	175	147 (84%)	NR	158	135	0.90 [0.85-0.94]	0.92 [0.86; 0.95]

NR= Not reported; EBUS= Endobronchial ultrasound; EBUS-TBNA= Endobronchial ultrasound-guided transbronchial-needle aspiration.
a Yield was calculated as the number of patients in whom EBUS-TBNA made a correct tissue diagnosis (non malignant or malignant) divided by the total number of patients in whom EBUS was performed with the aim of diagnosing an intropulmonary tumor.
b Sensitivity was calculated as the number of patients in whom EBUS-TBNA made a correct tissue diag nosis of malignancy divided by the total number of patients in whom the targeted intrapulmonary tumor turned out to be malignant.

Diagnostic yield and sensitivity

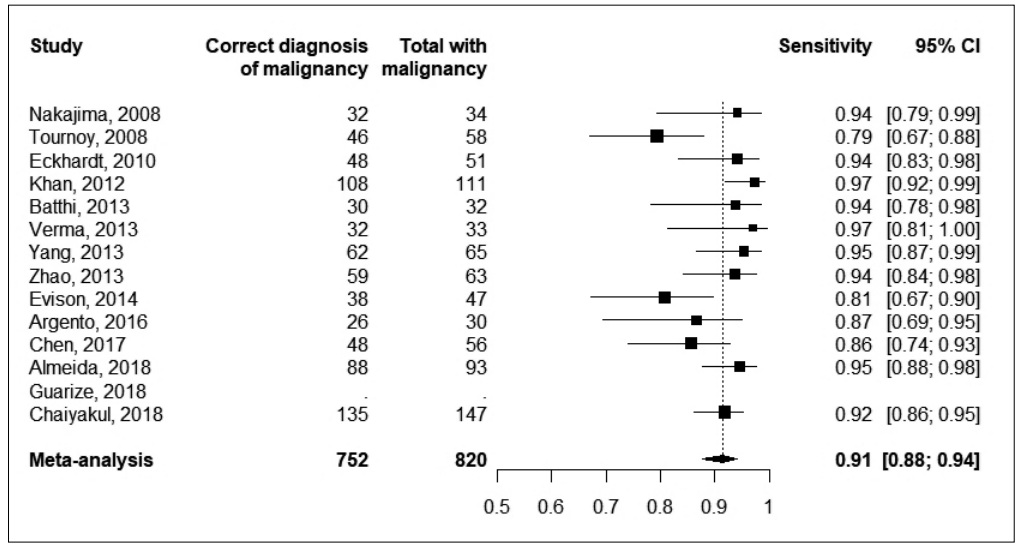
Table 1.2 shows the estimates of yield and sensitivity for the individual studies. The total number of patients included in this review is 1175; the number of patients included in the individual studies ranged from 32 to 290. The proportion of patients with a final diagnosis of malignancy varied from 62% to 100%. Final diagnosis of malignancy included NSCLC in 620 patients, SCLC in 126 patients and another malignant diagnosis in 61 among 12 studies reporting this information. Detailed information about the final diagnosis is available on the supplementary appendix (Table S1.4).

Figure 1.2 *Yield of EBUS-TBNA for diagnosing centrally located intrapulmonary lesions*



The yield of EBUS-TBNA for diagnosing intrapulmonary lesions ranged from 0.72 to 0.96 across the included studies; 1 study did not report sufficient information to calculate yield. The average yield after meta-analysis was 0.89 (95%CI 0.84-0.92) (Figure 1.2).
The sensitivity of EBUS-TBNA for diagnosing malignant intrapulmonary tumors ranged from 0.77 to 0.97 across included studies; 1 study did not report sufficient data to calculate sensitivity. The average sensitivity after meta-analysis was 0.91 (95%CI 0.88-0.94) (Figure 1.3).

Figure 1.3 *Sensitivity of EBUS-TBNA for diagnosing malignant centrally located intrapulmonary tumors*



Complications

In 5 studies (281 patients) there were no complications due to EBUS-TBNA, and in 3 studies (453 patients) this information was not reported. In the remaining 6 studies (490 patients), a total of 42 complications were reported: major bleed (n=1), moderate/self-limiting bleeding (n=17), atrial fibrillation (n=1), tachycardia (n=1), intolerance with the procedure (n=2), pneumothorax (n=2), desaturation (n=14) and a minor complication that was not specified (n=4). Overall, among studies reporting this information, the complication rate was 5.4% (42/721), although many of these can be considered as minor.

DISCUSSION

In this systematic review, we found that EBUS-TBNA has a high yield and sensitivity for diagnosing centrally located lung tumors. The findings of this study are clinically relevant as tissue acquisition of centrally located lung tumors without endobronchial abnormalities is a large clinical problem. The current analysis seems to imply that under the condition that the tumor is located adjacent to the major airways a diagnosis can be obtained through EBUS-TBNA in approximately 9 out of 10 patients with low risk of complications.

Some limitations should be discussed regarding the studies under consideration. All studies included in this systematic review had a high risk of bias or applicability concerns when assessed by QUADAS-2.12 Especially the fact that almost no prospective studies on the topic have been performed is surprising. Because of this, yield and sensitivity may have been overestimated. In addition, several different definitions of a centrally located lung tumor were used in the included studies, ranging from the inner one third (American College of Chest Physicians guidelines)[8] to the inner two thirds (European Society of Thoracic Surgery guidelines and National Comprehensive Cancer Network)[7,27]of the hemi-thorax.

Variations operator's experience, lesion size, localization in relation to the major airways and the availability of ROSE are key factors that may affect the performance of EBUS-TBNA.[28] Such heterogeneity could lead to major variation in yield and sensitivity across clinical settings, but the limited number of eligible studies and incomplete reporting in some of them, did not allow us to perform sensitivity analyses. However, average estimates of yield and sensitivity were relatively consistent across individual studies, suggesting that EBUS-TBNA may be useful in different clinical settings.

Seven studies explicitly excluded or did not encounter patients with endobronchial abnormalities and 3 studies explicitly reported to have included several patients with such abnormalities. Among these 10 studies, only 27 of 660 (4%) patients showed endobronchial abnormalities. Therefore, it is unlikely that the presence of endobronchial lesions has overestimated the yield and sensitivity of EBUS-TBNA in diagnosing centrally located lung tumors in our review.

We found a high proportion of patients with malignancy across the included studies. This may, again, be related to the retrospective nature of most studies; some may have only selected patients with a high likelihood of malignancy. The prevalence of malignant tumors is likely to be lower in practice.

Complications occurred in only 5.8% of patients with just 2 serious adverse events (a major bleed which needed an intervention and one pneumothorax). These numbers are comparable with those reported in previous studies on EBUS-TBNA related complications in sampling nodes and mediastinal masses.[29] The most common complication was self-limiting bleeding, and only 2 patients had a pneumothorax due to EBUS-TBNA, thus suggesting that a routine chest x-ray after EBUS-FNA of intrapulmonary tumors may not be indicated.

EBUS-TBNA is a cost-effective lung cancer staging procedure that can be performed in outpatients under moderate sedation.[30] Moreover, it provides the advantage that it can combine lung tumor diagnosis and loco regional mediastinal and hilar staging in a single procedure. Endosonography is very operator-dependent and should be learned and performed in a systematic way.[28]4 There is a need for learning and certification programs in endosonography such as the "ERS comprehensive training program" in order to train qualified doctors to be able to independently and competently perform EBUS.[31] Besides nodal assessment, diagnosing intrapulmonary tumors should be part of training programs.

A substantial number of studies have evaluated the performance of EBUS-TBNA in diagnosing mediastinal tumors and in mediastinal nodal staging in patients with lung cancer,[32] and this application is now recommended in most clinical guidelines.[7, 8] However, the number of evaluations on the performance of EBUS-TBNA in diagnosing intrapulmonary tumors is limited and almost all are retrospective.6 Based on our own experience, for patients with a previous non-diagnostic bronchoscopy, we believe that EBUS-TBNA should be considered for those patients who present with an intrapulmonary tumor located adjacent or near the larger airways, especially in case of the absence of endobronchial lesions or nodal metastases. Future prospective studies with clear

definitions of a centrally located lung tumor are advised to confirm the current findings. The definition of the tumor positioned within the inner one third of the hemi thorax by drawing concentric lines from the midline-may qualify best.[33]

Despite the parenchymal origin of the lesion, linear EBUS seems more useful then radial EBUS for the analysis of centrally located lung tumors without endobronchial abnormalities. Radial EBUS can be used to detect lung lesions provided an airway reaches to the lesion, however a real-time controlled aspiration is not possible.[34–36]
Also conventional TBNA- without EBUS guidance- can also be used for primary lung tumor analysis[2]. The needle can be placed on a widened carina or inserted on a specific location in the airways based on chest CT scan findings. The diagnostic yield of conventional TBNA depends on the size and the location of the lung tumor and a diagnostic yield of 56% reported[2]. A comparison study between EBUS guided TBNA and conventional TBNA has not been performed.

A recent meta-analysis of our group reported a high yield and sensitivity of EUS-(B)-FNA for diagnosing centrally located intrapulmonary tumors in case the lung mass is located adjacent the esophagus.[37] Using EUS-(B)-FNA, left sided and lower paraesophageal nodes and tumors can be reached.10 As such, it is complementary to EBUS-TBNA, which provides access to structures close to the large airways on both sides.[38]
A combined approach of EBUS-TBNA and EUS-B-FNA for mediastinal lymph node staging is increasingly performed in clinical practice.[38]Such an approach could also be useful in the diagnosis of centrally located intrapulmonary tumors.[39] A combined EBUS and EUS procedure using just the EBUS scope for both nodal and tumor diagnosis is an elegant minimally invasive diagnostic approach.

In conclusion, the present systematic review and meta-analysis implies that EBUS-TBNA is a safe procedure with a high yield and sensitivity for diagnosing centrally located lung tumors. However, caution should be taken to extrapolate these results into routine real life practice due to the lack of high-quality studies included. Future prospective studies are indicated to evaluate whether the current findings are reproducible and to further refine the criteria for recommending EBUS-TBNA in this setting.

Endobronchial ultrasound for the diagnosis of centrally located lung tumors: A systematic review and meta-analysis Part 1. Chapter 1

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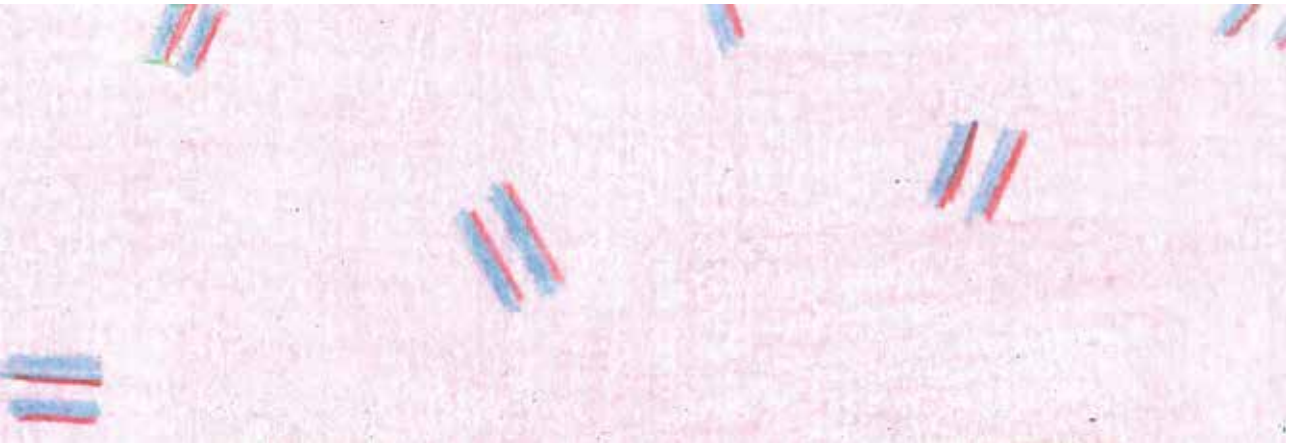

Supplemental Content

Table S1.1 *Search strategy*

Ovid MEDLINE(R) ALL <1946 to November 09, 2018>

#	Searches	Results
1	exp lung Neoplasms/us	312475
2	exp lung cancer/ or exp Carcinoma, Non-Small-Cell Lung/ or exp Carcinoma, Small Cell/ or Lung Neoplasms/ or ((lung$ or pulmon$) adj5 (tumor$ or tumour$ or cancer$ or onco$ or carcinoma or malign* or lesion* or mass* or nodule* or neoplas$)).ti,ab,kf.	2576
3	(EBUS or "transbronchial ultraso*" or "endobronchial ultraso*" or "transbronchial needle aspiration").ti,ab,kw.	742
4	2 or 3	2687
5	1 and 4	1791

Embase Classic+Embase <1947 to 2018 November 03>

#	Searches	Results
1	exp lung tumor/ or ((lung$ or pulmon$) adj5 (tumor$ or tumour$ or cancer$ or onco$ or carcinoma or malign* or lesion* or mass* or nodule* or neoplas$)).ti,ab,kw.	484138
2	exp endobronchial ultrasonography/ or ("transbronchial needle aspiration" or EBUS or transbronchial ultraso* or endobronchial ultraso*).ti,ab,kw.	6382
3	1 and 2	4392
4	(embase or elsevier or canadian).cr.	25742810
5	3 and 4	4179

Web of Science(Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI)

#	Searches	Results
1	TS=(EBUS or "transbronchial ultraso*" or "endobronchial ultraso*" or "transbronchial needle aspiration")	3661
2	TS=((lung$ or pulmon$) NEAR/5 (tumor$ or tumour$ or cancer$ or onco$ or carcinoma or malign* or lesion* or mass* or nodule* or neoplas$))	28810
3	#1 AND #2	2113

BIOSIS Previews <1993 to 2015>

#	Searches	Results
1	((lung$ or pulmon$) adj5 (tumor$ or tumour$ or cancer$ or onco$ or carcinoma or malign* or lesion* or mass* or nodule* or neoplas$)).mp.	151302
2	(EBUS or "transbronchial ultraso*" or "endobronchial ultraso*" or "transbronchial needle aspiration").mp.	681
3	1 and 2	468

Literature searches were performed on November 9th, 2018
Total number of search results after deduplication: 5675

Endobronchial Ultrasound for the Diagnosis of Centrally Located Lung Tumors: A Systematic Review and Meta-Analysis *Supplemental* Part 1. Chapter 1

Table S1.2 *QUADAS-2 assessment of risk of bias and applicability concerns*

Study [Ref], year	Patient selection				Index test	Reference standard	Flow and timing		
	1. Was a retrospective (non-consecutive) inclusion of patients avoided?	2. Were inappropriate exclusions avoided?	3. Was a case-control design avoided?	4. Did the included patients match the review question?	5. Were endoscopists blinded to the final diagnosis?	6. Was the reference standard likely to correctly classify the target condition?	7. Was partial verification of patients with a non-diagnostic or non-malignant EBUS avoided?	8. Was differential verification of patients with a non-diagnostic or non-malignant EBUS avoided?	9. Were exclusions due to missing reference standard results avoided?
Nakajima [9], 2008	☹	?	☺	☺	☺	☺	☺	☺	☺
Tournoy [14], 2008	☹	☺	☺	☺	☺	☹	☺	☹	☺
Eckhardt [15], 2010	☹	?	☺	?	☺	☹	☹	☹	☺
Khan [16], 2012	☹	?	☺	?	☺	☹	☹	☹	☹
Batthi [17], 2013	☹	☺	☺	☹	☺	☹	☺	☺	☺
Verma [18], 2013	☹	?	☺	☹	☺	☹	☹	☹	☺
Yang [19], 2013	☹	?	☺	?	☺	?	?	?	?
Zhao [20], 2013	☹	?	☺	☺	☺	☺	☺	☺	☺
Evison [21], 2013	☺	?	☺	☹	☺	?	?	?	☺
Argento [22], 2016	☹	?	☺	☺	☺	☹	☹	☹	☺
Chen [23], 2017	☹	☺	☺	☺	☺	☹	☺	☹	☹
Almeida [24], 2018	☹	☺	☺	?	☺	☹	☺	☹	☺
Guarize [25], 2018	☹	?	☺	?	☺	?	?	?	☺
Chaiyakul [26], 2018	☺	?	☺	☺	☺	☹	☺	☹	☺

☺ *Low risk of bias/applicability concerns*
☹ *High risk of bias/applicability concerns*
? *Unclear risk of bias/applicability concerns*

Table S1.3 *Definitions of centrally located lung tumors*

Study, year	Targeted intrapulmonary tumors were defined as:
Nakajima, 2008	"Pulmonary masses whose drainage bronchus is difficult to be reached such as mediastinal type lung cancer adjacent to the trachea, lesions adjacent to the main bronchus or the segmental bronchus"
Tournoy , 2008	"The centrally located lung lesions were defined as an intrapulmonary mass with the medial margin located within the inner third of the hemi thorax based on chest CT-scan imaging"
Khan, 2012	"Central lung parenchymal lesions"
Batthi , 2013	"Centrally located peribronchial lung lesions"
Verma 2013	"Centrally located lung lesions were defined as an intrapulmonary nodule or mass located adjacent to the tracheobronchial tree as visualized on chest CT scan"
Yang, 2013	"Parabrachial or parabronchial intrapulmonary lesions proved by CT scan"
Zhao, 2013	"Intrapulmonary lesions located near the central airway"
Evison, 2013	"Intra-parenchymal lung lesions"
Argento, 2016	"Centrally located intraparenchymal lesions" "Lesions completely surrounded by lung parenchyma were included"
Chen, 2017	"Peribronchial lung lesions"

Table S1.4 *Final diagnosis of intrapulmonary lesions*

Study [ref], year	**Total EBUS performed, n**	**Final diagnosis: any malignancy, n**	**Final diagnosis: benign, n**
Nakajima [9], 2008	35	34 (97%) *-NSCLC (n=26)* *-SCLC (n=1)* *-Lung metastasis (n=6)* *-BALT lymphoma (n=1)*	1 (3%) *-Focal fibrosis (n=1)*
Tournoy [14], 2008	60	58 (97%) *-NSCLC (n=42)* *-SCLC (n=14)* *-Lymphoma (n=1)* *-Atypical Carcinoid (n=1)*	1 (2%) *-Hamartoma (n=1)*
Eckhardt [15], 2010	82	51 (62%) *-Metastasis (n=1)* *-Lymphoma (n=2)* *-Neuro-endocrien carcinoma (n=1)* *-NSCLC (n=36)* *-SCLC (n=11)*	31 (38%) *-Sarcoidosis (n=7)* *-Cysts (n=1)* *-Infection (n=3)* *-Amyloidsis (n=1)* *-Struma (n=1)* *-NR (n=18)*
Khan [16], 2012	114	111 (97%) *-Squamous cell carcinoma (n=35)* *-Adenocarcinoma (n=32)* *-SCLC (n=25),* *-NSCLC-NOS (n=12)* *-Malignant cells – NOS (n=3)* *-Extra pulmonary metastases (n=2)* *-Lymphoma (n=1)* *-Not suitable for further invasive tests (follow up CT suggestive of lung malignancy) (n=1)*	3 (3%) *-Bronchial cysts (n=2)* *-Benign at follow-up (n=1)*
Batthi [17], 2013	32	32 (100%) *-Squamous cell carcinoma (n=14)* *-Adenocarcinoma (n=9)* *-SCLC (n=5)* *-Undifferentiated NSCLC (n=2)* *-Large cell carcinoma (n=1)* *-Features of both squamous cell and adenocarcinoma (n=1)*	0
Verma [18], 2013	37	33 (89%) *-NSCLC (n=24)* *-SCLC (n=7)* *-Lymphoma (n=1)* *-Malignant fibrous histiocytoma (n=1)*	3 (8%) *-Tuberculosis (n=1)* *-Pneumonia (n=1)* *-EBV related lymphoproliferative disorder (n=1)*
Yang [19], 2013	78	65 (83%) *-Adenocarcinoma (n=36)* *-Squamous cell carcinoma (n=8)* *-Poorly-differentiated carcinoma (n=6)* *-Unknown type carcinoma (n=3)* *-SCLC (n=9)* *-Pulmonary Sarcomatoid carcinoma*	13 (17%) *-Pulmonary inflammation (n=7)* *-Pulmonary tuberculosis (n=5)* *-Fibrosis (n=1)*

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: 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 lungtumor was 89% (145/163). In 4 cases the tumor was not found with EBUS and. 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). So 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.

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 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.

METHODS

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 1 st 2013 and October 10 th 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 (Figure 2.1).

The expanding role of endobronchial ultrasound in patients with centrally located intrapulmonary tumors Part 1. Chapter 2

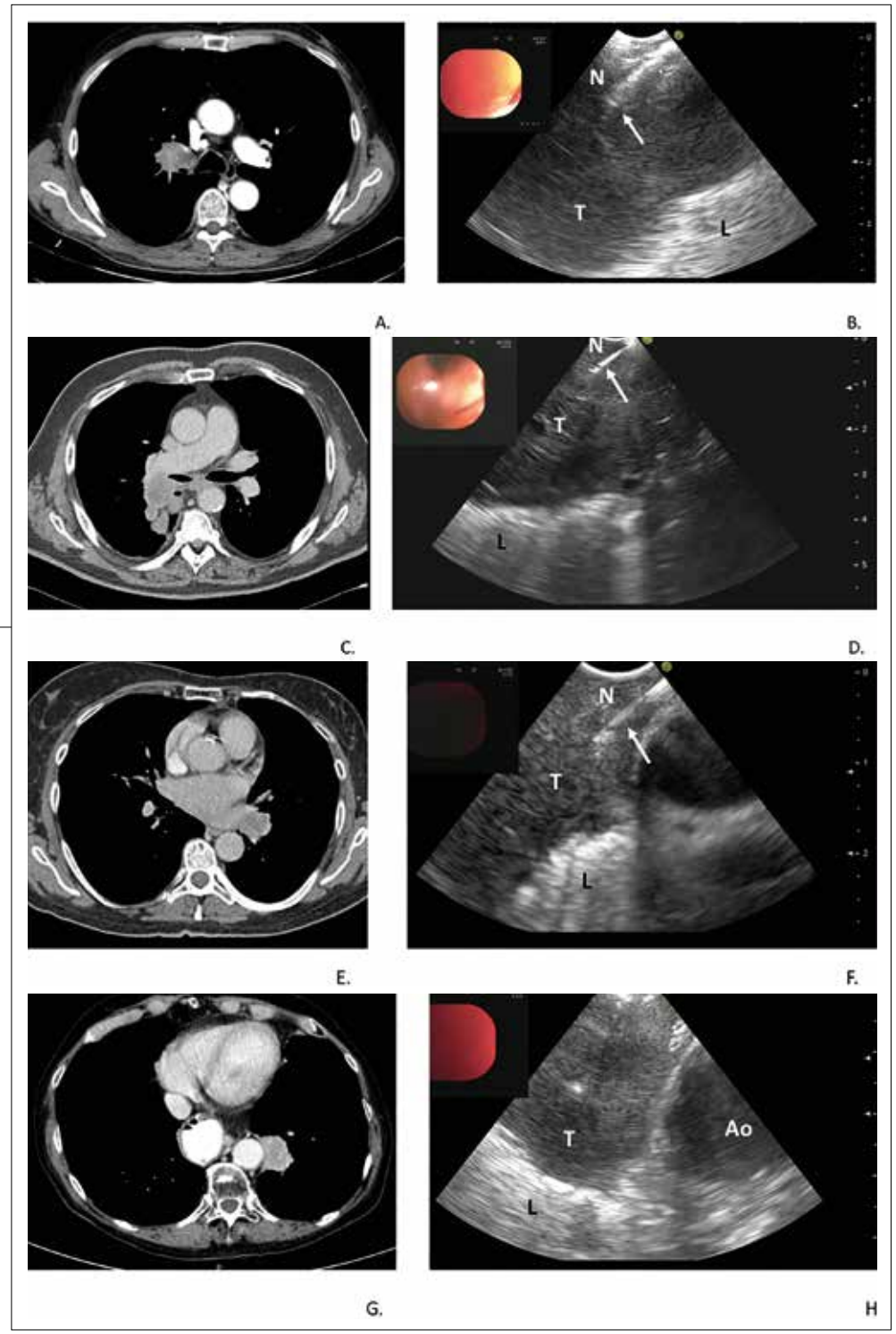


Figure 2.1 *EBUS-TBNA sampling stratified for tumor location relative to the tracheobronchial tree. Pictures A, C, E and G show CT images of centrally located lung tumors (T) adjacent to trachea, main bronchi, lobar bronchi and segmental bronchi, respectively. Figures B, D, F and H show the correspondent EBUS-TBNA procedural images. The needle (N), when present in EBUS images, is signaled by arrow tips. L = compromised lung parenchyma, Ao = aorta.*

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.

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;

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-UC180F or UC 180F, 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 19G, 21G, 22G or 25G needles were used.
Once 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 (Figure 2.1). 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.

DIAGNOSTIC ISSUE

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 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.

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

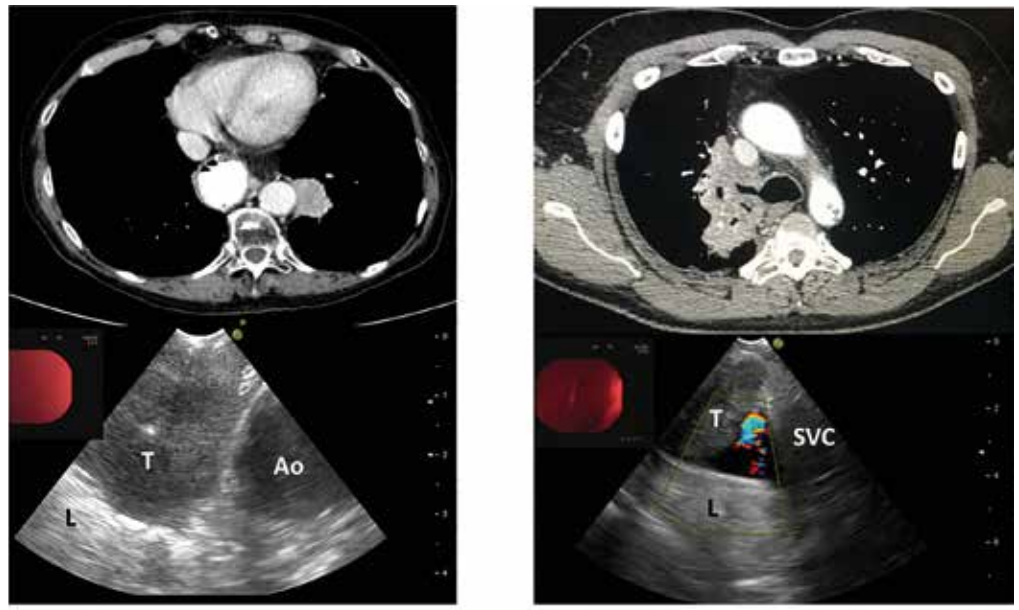
positive. In the absence of proven malignancy following EBUS-TBNA, other pathological data (cytology or histology obtained with other techniques) were seeked and/or a clinical/radiological follow-up of at least 6 months was retrieved from dossiers in order to assess a final diagnosis.
Cases in which 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.

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 that 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 (Figure 2.2). In all cases, possible vascular tumor invasion was further assessed by color flow Doppler.

Figure 2.2 *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.

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.

Statistics

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

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.

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 lungtumor was detected by EBUS. In 159 cases a sampling attempt was attempted (See Flowchart, 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 2.1. 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.

Table 2.1 *Baseline characteristics 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.

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.

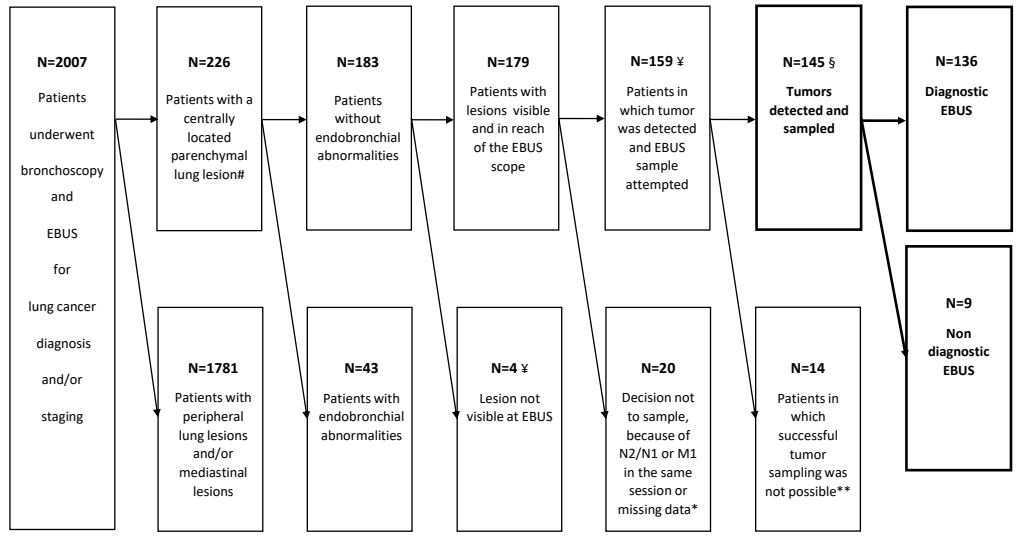
The diagnostic yield for EBUS-TBNA in the best case scenario was 94% (136/145), whereas sensitivity, specificity, NPV and PPV values were 96.3%, 77.8%, 56.2% and 100%, respectively. The diagnostic yield according to the location was 94% for lesions adjacent to the trachea, 91% for tumors close to mainstem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

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 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 further investigations led to a better definition of the histology of the tumor (See supplementary Table S2.1).
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.

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).

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.

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), is 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 sensitivity 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 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 lungtumors [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].

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.

Table 2.2 *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* (%)	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*		**96.3%, 100%**
EBUS Specificity for malignancy, NPV*		**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 should be 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. *numbers of the best case scenario. Worst case scenario is explained in the text.*

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 can 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.

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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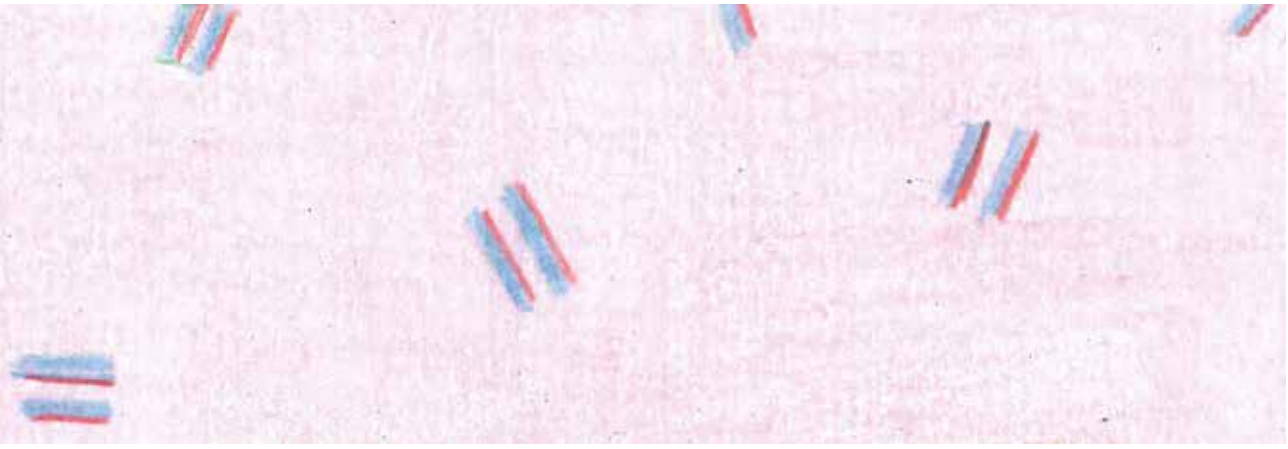
The expanding role of endobronchial ultrasound in patients with centrally located intrapulmonary tumors Part 1. Chapter 2



Supplemental Content

Table S2.1 *Cases in which EBUS diagnosis was changed by further analyses*

EBUS diagnosis	Final diagnosis	Technique which led to Final Diagnosis
Non diagnostic sample	Adenocarcinoma	TTNA
Non diagnostic sample	Squamous Cell carcinoma	TTNA
Non diagnostic sample	Organizing pneumonia	Surgery
Non diagnostic sample	Not conclusive	Clinical Radiological follow up
Non diagnostic sample	Benign disease	Clinical radiological FUP
Non diagnostic sample	Adenocarcinoma	2nd EBUS+TBB
Non diagnostic sample	NSCLC NOS	2nd EBUS procedure
Non diagnostic sample	NSCS NOS	Eco guided TTB
Non diagnostic sample	Not conclusive	Clinical radiological follow up
NSCLC NOS	Squamous Cell carcinoma	TTB
NSCLC NOS	Adenocarcinoma	2nd EBUS procedure
NSCLC NOS	Adenocarcinoma	TBB, same procedure
NSCLC, squamous cell carcinoma	Sarcomatoid Carcinoma with squamous differentiation	VATS lobectomy
Adenocarcinoma, suspicious metastatic breast cancer	Adenocarcinoma of unclear origin	2nd EBUS procedure

In this table description is provided of the cases in which the EBUS driven diagnosis was ultimately changed by other diagnostic techniques or clinical/radiological follow up. Eventually two patients (one for each centre involved) lack a definitive diagnosis and are still under follow up.

EUS-B FNA for the diagnosis of centrally located lung lesions.

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ABSTRACT

Background

Diagnosing centrally located lung tumors without endobronchial abnormalities and not located near the major airways is a diagnostic challenge. Tumors located near or adjacent to the esophagus can be aspirated and detected with EUS using GI endoscopes.

Objective

To assess the feasibility and diagnostic yield of EUS-B-FNA in para-esophageal located lung tumors and its added value to bronchoscopy and EBUS.

Methods

Retrospective, multi center international study (from 01-2015 until 01-01-2018) of patients with suspected lung cancer, undergoing a bronchoscopy, EBUS and EUS-B in one session by a single operator (pulmonologist), in which the primary lung tumor was detected and aspirated by EUS-B. In the absence of malignancy following endoscopy, transthoracic ultrasound needle aspiration, clinical and radiological follow-up of at least 6 months was performed. The yield and sensitivity of EUS-B-FNA and its added value to bronchscopy and EBUS was assessed.

Results

58 patients were identified with the following diagnosis: NSCLC (n=43), SCLC (n=6), Mesothelioma (n=2), metastasis (n=1), non-malignant (n=6). The yield and sensitivity of EUS-B-FNA for detecting lung cancer was 90%. In 26 patients (45%), the intrapulmonary tumor was exclusively detected by EUS-B. Adding EUS-B to conventional bronchoscopy and EBUS increased the diagnostic yield for diagnosing lung cancer in paraesophageally located lung tumors from 51% to 91%. No EUS-B related complications were observed.

Conclusion

EUS-B-FNA is a feasible and safe technique for diagnosing centrally located intrapulmonary tumors that are located near or adjacent to the esophagus. EUS-B should be considered in the same endoscopy session following a non-diagnostic bronchoscopy and EBUS.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. In patients with suspected lung cancer, a tissue diagnosis is crucial to establish a definite diagnosis. However, for patients presenting with a centrally located lung tumor without endobronchial abnormalities and not located near the major airways, obtaining a tissue diagnosis is a diagnostic challenge.

In routine practice, flexible bronchoscopy with its associated procedures (endobronchial biopsy, brushing and washing) will be performed, especially in case of a visible endobronchial tumor. Frequently however, no endobronchial abnormalities are visible and in these situations the diagnostic yield by standard bronchoscopic techniques is low [2–6]. Guidance techniques (radial EBUS/ fluoroscopy, navigation) can be helpful in peripherally located lung lesions in case an airway leads to the tumor but often do not contribute to the diagnostic yield of these specific central lesions [7,8].

If the tumor is located near or adjacent to the large airways EBUS-TBNA is a useful and safe procedure to obtain a tissue diagnosis [9,10].

With GI endoscopes it has been proven that lung tumors located near or adjacent to the esophagus can be detected and aspirated with EUS [11]. But this technique is commonly not available in most pulmonary practices. Current lung cancer staging guidelines recommend EUS-B (using the EBUS scope in the esophagus) for mediastinal staging, because this is a complementary to EBUS for mediastinal nodal staging [12–15]. EBUS and EUS-B are also suggested in this guideline for the analysis of lung tumors in patients with a centrally located lung tumor that are not visible with conventional bronchoscopy, provided that the tumor is located immediately adjacent to the larger airways (endobronchial ultrasound EBUS-TBNA) or esophagus (EUS-B/EUS) [16].

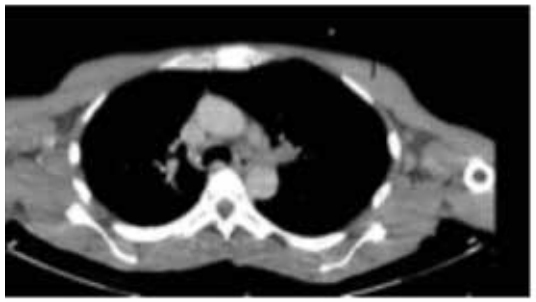
To date, however there is only limited evidence about the value of EUS-B FNA for obtaining a tissue diagnosis in centrally located lung tumors [17,18].
Therefore, we conducted this study to assess the feasibility and diagnostic yield of EUS-B in paraesophageal located lung tumors and its added value to bronchoscopy and EBUS.

METHODS

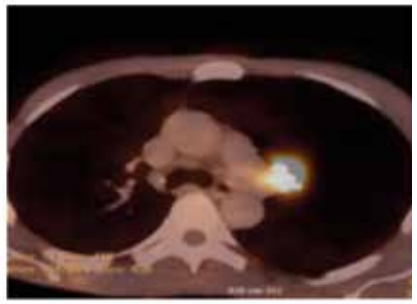
Study design and patients

This is a retrospective multi center international study undertaken in Naestved Hospital Denmark, Naestved (department of internal medicine), Zealand University Hospital, Roskilde ((department of internal medicine) and in Academic Medical Center, Department of Respiratory Medicine,University of Amsterdam, The Netherlands. In the period of January 1 st 2015 untill January 1st 2018. Patient data were retrieved for the various endosonography data bases in the 3 hospitals based on the following criteria: Patients who underwent an EUS-B-FNA to obtain a tissue diagnosis of an intrapulmonary lesion for suspected lung cancer and also routinely underwent bronchoscopy and EBUS.

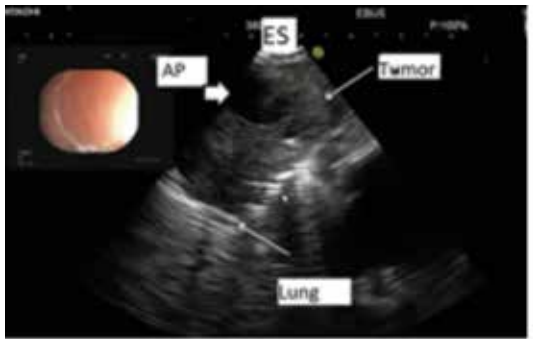
Patient selection
All patients in whom the paraesophageal located lung tumor was detected by EUS-B and sampled were identified. The presented case is an example of patient selection.
All CT and PET-CT imaging, bronchoscopy, EBUS, EUS-B reports, cytopathological reports and follow up data were collected. Also, complications of EUS-B were retrieved.



A: CT scan



B: PET-CT scan



C: EUS-B

68-year-old male, with a centrally located left upper lobe tumor located near the oesophagus. (Panel A). PET-CT scan shows FDG uptake in the tumor but not in the hilar and mediastinal lymph nodes. (Panel B). At conventional bronchoscopy there were no endobronchial abnormalities and the tumor was not visible with EBUS. With EUS-B – with the EBUS scope positioned in the flexible esophagus- an inhomogeneous solid appearing lesion with a close relation to the pulmonary artery was detected (Panel C). Fine needle aspiration showed an adenocarcinoma of the lung.

Study Endpoints
The primary endpoints of this study were to asses diagnostic yield of EUS-B and its added value to conventional bronchoscopy and EBUS for obtaining a tissue diagnosis of centrally located lung tumors located near or adjacent to the esophagus.
The secondary endpoints were the adequacy of the tissue samples and sensitivity of EUS-B for the diagnosis of a centrally located lung tumor, the feasibility of EUS-B and EUS-B related complication for diagnosing an intrapulmonary tumor.

Definitions of sample adequacy, yield and sensitivity
Biopsies were judged to be adequate when containing material sufficient for cytopathological evaluation. Samples in which cytopathological evaluation showed malignancy were considered to be true positive.
When cytopathological evaluation of EUS-B-FNA samples showed no malignancy the EUS-B diagnoses of the lung lesion was confirmed with at least 6 months follow up with clinical course and/ or CT. For calculation of yield and sensitivity of malignancy samples with a non-malignant diagnosis without follow up were assumed to be false negative in the analyses (worst case scenario).

The diagnostic yield was defined as the number of samples in which EUS-B-FNA provided a correct diagnosis in relative to the total number of samples performed with EUS-B-FNA [11].

Sensitivity of malignancy was defined as the number of samples in which EUS-B-FNA made a diagnosis of any malignancy relative to the total number of samples in which the targeted intrapulmonary tumor turned out to be malignant [11].

The EUS-B procedure
Procedures were performed under conscious sedation using midazolam/fentanyl or propofol sedation. Following a conventional bronchoscopy - systematic EBUS was performed according EBUS-STAT [19].
Following EBUS, the EUS-B procedure will be discussed in more detail: For EUS-B-FNA a flexible EBUS endoscope (Olympus BF-UC180F or UC 180F, Olympus Medical Systems Europe, Ltd., Hamburg, Germany. Or Pentax EB-1970 UK, Olympus BF-UC180F) was used. The EBUS endoscope was introduced into the esophagus by retracting the EBUS scope from the trachea to a level just above the vocal cords and from this position turn it slightly to the left and the back of the patient and advance it into the esophagus under gentle pressure while the patient was encouraged to swallow (in case of mild sedation).
The endoscope was advanced carefully till the liver was visualized on ultrasound imaging. A structured EUS assessment was performed using the esophageal assessment tool (EUS-AT) with six landmarks identified in this order: the liver, the abdominal aorta, the left adrenal gland, lymph node station 7, station 4L and 4R. This validated and systematic assessment tool is specifically developed for the examination of lung cancer patients [20].
Following identification of the intrapulmonary tumor, aspirates were performed using a 21G or 22G needle (22 Gauge Olympus ViziShot and ViziShot 2. Olympus Medical Systems Europe, Ltd., Hamburg, Germany, or 21-22 Gauge COOK needle). When the needle was placed in the lesion under ultrasonic guidance the stylet was removed, and suction was applied, or the stylet was removed using the slow pull technique. At least two samples were taken. The aspirates were processed for both cytological smears and cellblock analysis. A Chest-X-ray was not performed routinely after the procedure.

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

Ethics
This retrospective analysis was conducted in accordance with the amended Declaration of Helsinki and publication of the data was approved by the Data Protection Agency in Denmark and the medical ethics committee in the Netherlands.

RESULTS
58 patients were identified who underwent EUS-B-FNA for diagnosing an intrapulmonary tumor from January 1, 2015 until January 1, 2018. Patient characteristics are shown in Table 3.1. Of the 58 patients, 34 were female (59%) with a median age of 78 years. The aspirated lesions were localized in all lobes, with a median size of 55 mm. No EUS-B related complications were observed.

Final diagnoses were: non-small cell lung cancer (n=43) (adenocarcinoma n=26; squamous cell carcinoma n=12; non-small cell lung cancer not otherwise specified n=5), small cell lung cancer (n=6), malignant mesothelioma (n=2) and metastasis from extra-pulmonary cancer (n=1, anal squamous cell carcinoma). In 6 patients the diagnosis was non-malignant (unspecific lesion; n=1, infectious cause n=5). See table 3.1.

Table 3.1 *Characteristics of the patients included in the analysis and final diagnosis after complete work up*

Number of patients, n	58
Median age,years, range	78 (41-90)
Gender, n (%) Male female	 24 (41) 34 (59)
Localisation of lungtumor, n (%) LUL LLL RUL RML RLL	 20 (34) 6 (10) 18 (31) 1 (2) 13 (22)
Median Tumor size long axis, mm,range	55 mm (7 - 120)
Final Diagnosis after complete work up	NSCLC, N=43 (Adenocarcinoma N=26, Squamous cell carcinoma N=12, NSCLC-NOS N=5) SCLC, N=6 Malignant mesothelioma, N=2 Metastasis from an extra pulmonary tumor, N=1 Non-malignant, N=6 (Unspecific lesion N=1, Infection N=5)
EUS-B related complications	none

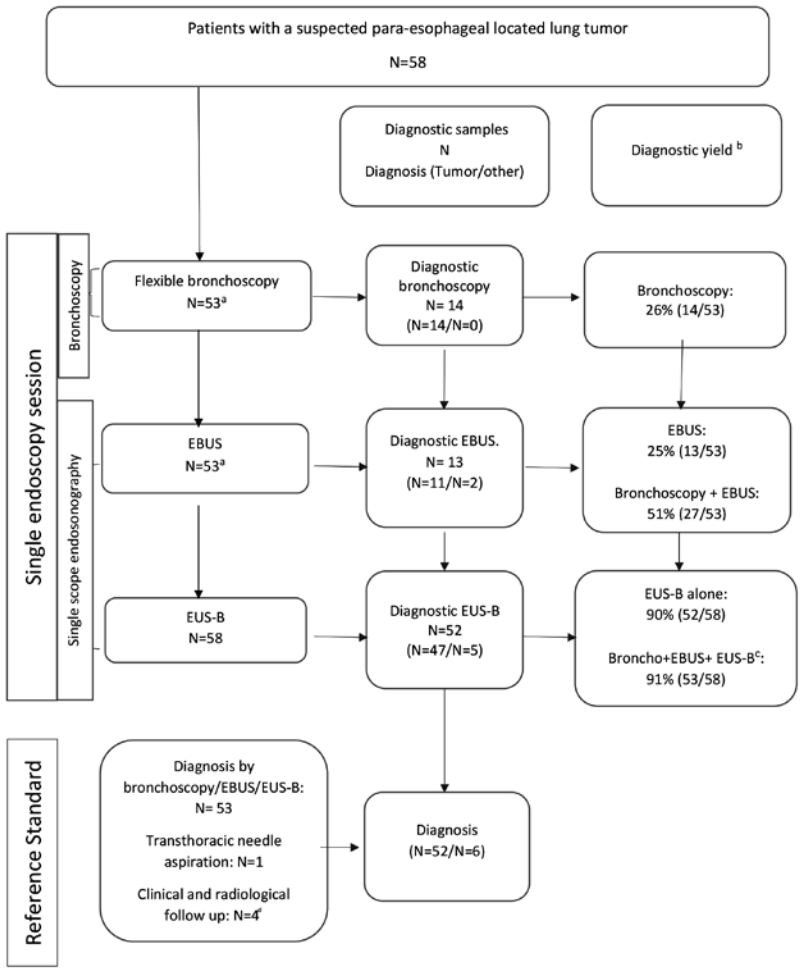
53 patients (91%) had a bronchoscopy and an EBUS-TBNA performed in the same session as the EUS-B. Five patients did not undergo bronchoscopy or EBUS due to respiratory problems (n=2) or the endobronchial procedure was expected to be of little consequence in obtaining a tissue diagnosis (n=3) due to the anatomical position of the lung lesion.

At bronchoscopy, in 14 patients (24%) the tumor was visualized and biopsied. All these samples showed a malignancy. The diagnostic yield of the bronchoscopy was 26%. Another 14 patients (22%) underwent an EBUS. Thirteen of the samples obtained with EBUS were adequate for cytopathological evaluation; in 11 cases the samples showed malignancy, in 2 patients the aspirates were non-malignant (1 showed a necrotizing granulomatous inflammation consistent with tuberculosis and one showed a reactive and inflammatory changes at cytopathology with clinically picture of pneumonia and full regression on antibiotic treatment). The diagnostic yield of EBUS alone was 25%. Adding EBUS to the bronchoscopy raised the diagnostic yield from 26% to 51%. In one (2%) case the tumor could be visualized but not biopsied with EBUS-TBNA.

All 58 patients underwent an EUS-B of which 52 were diagnostic of these 47 were malignant. In 26 patients (45%) the tumor was exclusively visualized and biopsied with EUS-B-FNA. The diagnostic yield for EUS-B alone was 90%. Combining bronchoscopy, EBUS and EUS-B resulted in a diagnostic yield of 91%. See flow chart.

Of all the 58 samples included in the analysis, 55 (95%) samples were adequate. Of these 85% (N=47) were malignant and 15% (N=8) were non-malignant. Of these, four cases had a clinical and radiological follow up for at least six months, the causes were infectious, and one case had the results confirmed in repeated EUS-B-FNA and follow up CT (2 samples with reactive and inflammatory changes at cytopathology with confirmed microbiologic agents or full regression on antibiotic treatment, 2 samples showed necrotizing granulomatous inflammation consistent with tuberculosis and one sample showed inflammatory cells confirmed at re-examination). These five cases are considered true negative. In the three other cases follow up was not clinically relevant as the patients had the procedure performed on suspicion of relapse of lung cancer, relapse in mediastinal or neck lymph nodes were found. These cases were considered false negative.

Figure 3.1: *Flowchart*



Flowchart of patients with a centrally located lung tumor who underwent in a single session bronchoscopy, EBUS and EUS-B to obtain a diagnosis of the tumor.

Of the 3 (5%) inadequate samples, two cases had diagnostic adequate samples performed with bronchoscopy or ultrasound guided transthoracic needle aspiration in the same session as the EUS-B that showed malignancy. One lesion was followed with CT and showed regression of the lesion in 6 months. All three cases were considered false negative. Thus, a total of 52 lesions were considered as malignant.
Transthoracic needle aspiration biopsy (TTNAB) performed ultrasound guided was performed in the same session as endoscopic procedures in 4 (7%) cases to obtain the final diagnosis, in one of these, the diagnosis was only established with TTNAB.
The sensitivity of EUS-B of diagnosing any malignancy was 90% in this very selected population.

DISCUSSION

In this study we evaluated the largest series (n=58) of patients who underwent an EUS-B-FNA for diagnosing a centrally located lung tumor adjacent to the esophagus. We found that EUS-B is safe and has a high diagnostic yield and sensitivity (90%) for diagnosing malignancy. Adding EUS-B to a previous non-diagnostic bronchoscopy and EBUS raised the diagnostic yield from 51% to 91%.

Only one report described EUS-B for diagnosing centrally located lung tumors. Steinfort et al[21] showed in a small selected cohort study that in 26 out of 27 patients EUS-B was diagnostic. In this study, 10 lesions were inaccessible for bronchoscopic sampling and 9 lesions were inaccessible for EBUS-TBNA. Diagnoses were obtained in predominantly upper lobes and one pneumothorax occurred. The current study shows that lung tumors located in all different lobes can be visualized and biopsied safely with EUS-B.

Korevaar et al[11] showed in a systematic review and meta-analysis for diagnosing intrapulmonary lung tumors by EUS using the GI endoscope an average yield of 0.90 and an average sensitivity of 0.92. The complication rate was low with 2%. Our findings show similar results, indicating that tumor sampling by EUS-B results in similar results as the conventional EUS scope. The potential advantages of the GI EUS scope are the following: the larger overview (120-180 degrees depended of the scope manufacturer versus 60 degrees visualization of EBUS), the needle length (10 versus 6 cm), the slightly superior ultrasound quality (due to the increased amount of ultrasound crystals of the EUs transducer) and the increased stiffness of the scope. However, in clinical practice all of the above mentioned items were rated not significant and not a limiting factor for the diagnostic yield of the EUS-B approach.

An advantage of using EUS-B-instead of convention GI EUS-scope- for diagnosing lung tumors is that the whole diagnostic and staging procedure can be performed in a single endoscopy session performed by one operator. In our study 91% of patients also underwent a conventional bronchoscopy and EBUS in the same session and mediastinal staging was performed in this single session. But in 45% of patients the lung tumor was only detected and biopsied by EUS-B showed the benefit of this transesophageal approach. Additional advantage of EUS-(B) is that it can be helpful in assessing mediastinal tumor invasion (T4). We have shown that in patients with paraesophageally located lung tumor the EUS assessment (presents of absences of mediastinal tumor invasion) has important added value to CT scan of the Chest [22].

Although CT-guided transthoracic needle aspirations for centrally located parabronchial lesions can be technically undertaken, the significant draw backs are a high risk of pneumothorax and hemoptysis [16]. In addition, the diagnostic yield is lower than for peripheral lesions [10,23,24].

It should be noted that several limitations apply to this study. First the retrospective character of this study means that there is a large bias of patient selection and the data should be interpreted accordingly. Second, this study analyzed data from 3 centers with expert EUS-B operators. It remains unclear if less experienced endoscopists can achieve similar results. And third visualization of intrapulmonary tumors from the esophagus is only possible if the tumor is located near or adjacent to the esophagus. The maximum distance from the tumor to the esophagus is unknown. In the present study virtually all tumor were located adjacent to the esophagus. Whether a specific air space between the wall of the esophagus and the lung tumor (as seen on the CT) still allows lungtumor detection by EUS-B needs to be investigated. As the esophagus is located in the left posterior chest, this most often applies to central located left sided tumors.
Future studies should include larger cohorts in a prospective consecutive design.

Our results provide further support that pulmonologists staging lung cancer should be trained in EUS-B-FNA[10].

CONCLUSION

EUS-B-FNA is a feasible and safe technique for diagnosing centrally located intrapulmonary tumors that are located near or adjacent to the esophagus. EUS-B should be considered in the same endoscopy session following a non-diagnostic bronchoscopy and EBUS.

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EUS-B FNA for the diagnosis of centrally located lung lesions Part 1. Chapter 3

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Intracardiac EUS guided FNA for diagnosing cardiac tumors.

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ABSTRACT

Primary cardiac tumors are extremely rare. Obtaining a tissue diagnosis is difficult and commonly requires open-heart surgery with associated morbidity.

Esophageal endoscopic ultrasound (EUS) and Esophageal Endoscopic Ultrasound with the EBUS scope (EUS-B) provide real-time sampling of centrally located lung tumors and mediastinal lymph nodes. They also provide an excellent view of the left atrium, since it is located adjacent to the esophagus. To date, left atrium tumor diagnostics by endosonography is poorly explored.

We describe two exceptional diagnostic cases of left atrium tumors in which cardiac surgery was hazardous due to the clinical condition or previous surgical interventions. During EUS(B) guided fine needle aspiration (FNA) the left atrial masses were successfully and safely sampled, revealing a Burkitt lymphoma and a synovial sarcoma. FNA including cell block analysis enabled specific tumor diagnosis and molecular subtyping.

Our findings suggest that in selected cases linear endosonography qualifies as a minimally invasive technique for intracardiac tumor diagnostics.

INTRODUCTION

Malignant primary cardiac tumors (MPCT) are extremely rare entities with a reported incidence below 0,1% of all cancers[1]. Left atrium tumors commonly obstruct the blood flow or create mitral regurgitation, simulating mitral valve diseases. The diagnosis of a suspected MCPT should be approached from different perspectives. First, imaging techniques are mandatory to determine the exact localization and extension of the lesion. For this CT, PET-CT, cardiac MRI and echocardiography are commonly used techniques [2-5]. Second, intracardiac tissue sampling is challenging and the histological diagnosis generally requires open-heart surgery with associated morbidity and mortality. However, patients are reguraly not physically fit to undergo open-heart surgery or complex intravascular techniques 6 for diagnostic purposes.

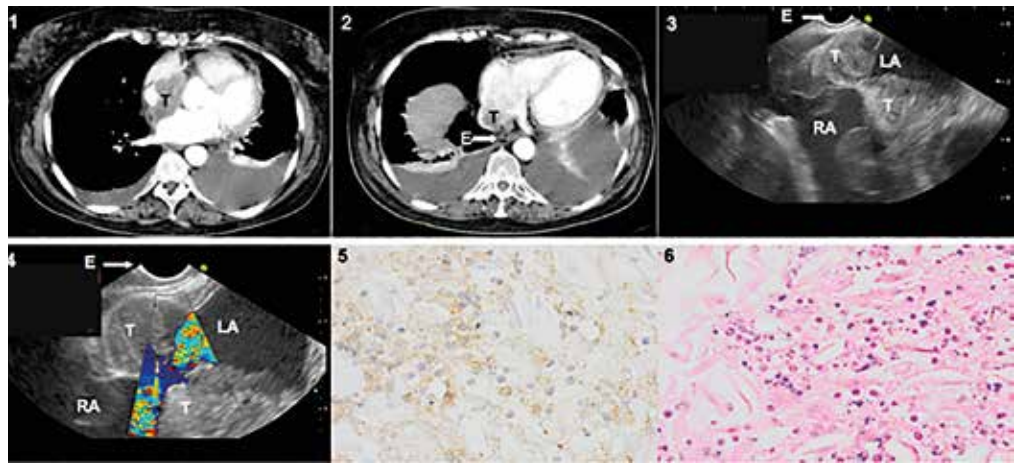
Linear endosonography is the first line technique for mediastinal nodal sampling and it is been increasingly used for centrally located lung tumor diagnosis due to the high accuracy and safety profile [7]. Altough endosonography provides an excellent overview of the left atrium, its use for left atrium tumor diagnosis has barely been explored.

We present two cases of patients with suspected MCPT in which the diagnosis was obtained in an outpatient setting by EUS(B).

Case 1

A 77 year-old female with a previous history of B cell NHL was referred to our hospital with a solitary FDG avid mass in the left atrium on PET-CT and pericardial and pleural effusion *(Figure 4.1, Panel 1 and 2).*

Figure 4.1



1 and 2. *CT scan of the thorax showing a tumor (T) inside the left atrium, adjacent to the esophagus (E) and a bilateral pleural effusion.* ***3 and 4.*** *EUS images showing the tumor (T) located inside the left atrium (LA) near to the entrance of the right atrium (RA).* ***5 and 6.*** *19G FNA cytology samples showing cells with enlarged nuclei, little cytoplasm and some clear nucleolus with blastair aspect, positive for CD20 strain, compatible with Burkitt lymphoma.*

Pleural thoracocentesis was performed twice to obtain pleural fluid for cytology, but it was described as nonspecific. Pericardial cytology showed atypical cells, requiring tissue sampling for diagnosis and possible therapeutic options. Open-heart surgery for left atrium mass biopsy was judged to be very hazardous. After obtaining consent of the patient and family it was decided to attempt an EUS guided FNA of the suspected left atrium mass. In an outpatient setting under 5 mg midazolam an EUS was performed. Systematic mediastinal evaluation did not show any enlarged or sonographically suspicious lymph nodes. The intra atrial mass was identified located in the roof of the left atrium near the atrium septum and at the inlet of the vena cava to the right atrium *(Figure 4.1, panel 3 and 4).* The mass was sampled with a 19G Cook® (Indiana, USA) needle under real- time ultrasound guidance, and the aspirate was sent for cytology, cell block and flow cytometry.

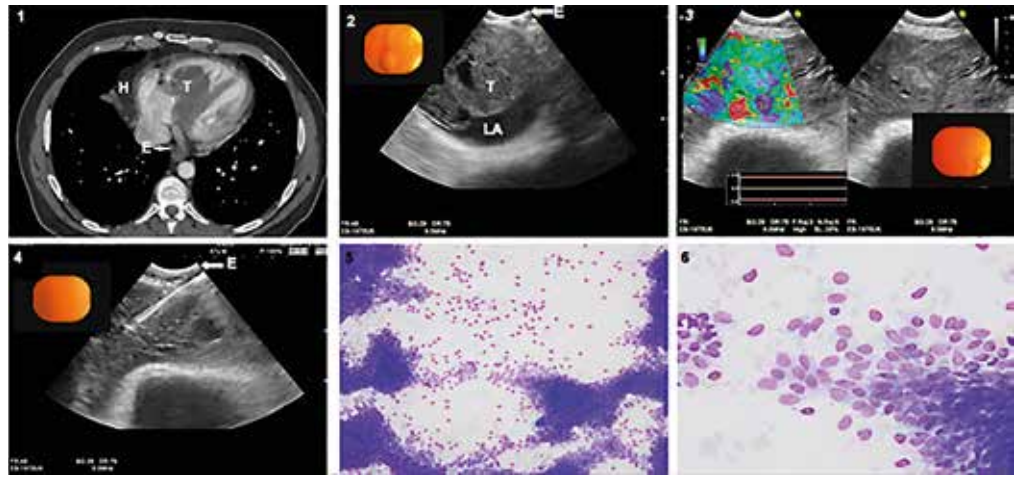
No signs of arrhythmia, bleeding, extravasate nor pericardial tamponade or other adverse events occurend following FNA. The final diagnosis based on EUS guided intra atrial sampling was a malignant Burkitt Lymphoma, a malignancy never before diagnosed by this diagnostic approach. Subsequently, a chemotherapy treatment consisting of R-CHOP was initiated. 7 years after diagnosis the patient is stable, enjoying good quality of life and there are no signs of disease recurrence at clinical and imaging following up.

Case 2

A 47 year-old male was referred to the respiratory endoscopy service after a irradical resection of a synovial sarcoma arising from tricuspidalis annulus with ingrowth in the atrium septum. Previously, a tricuspid valve plastia and annuloplasty with ring was performed. A CT scan of the chest and a MRI of the heart showed abnormalities inside and around both atrium *(Figure 4.2 panel 1 and 2).* Obtaining a tissue was indicated to differentiate between a post surgical hematoma or tumor recurrence. However, performing a repeated thoracotomy and open-heart surgery was judged to be extremely complicated due to the prior surgery.

As alternative of a re- thoracotomy, bronchoscopy + EBUS/TBNA and EUS(B) were performed under propofol sedation. At endobronchila ultrasound, a lesion in the hilium of the right lung was indentified compatibel with – and confirmed by aspiration - a post surgical hematoma (Figure 4.2, panel 1 and 2 (H)). Following EBUS, the scope was introduced into the esophagus (EUS-B) since the intracardiac mass was not visible from the airways. From the esophagus, a large inhomogeneous mass was visualized on the ultrasound image, occluding over half of the left atrium. On strain elastography imaging the mass seemed to be consisted of different rigidities. Under real- time ultrasound, guidance 4 FNA samples were taken with a 22 G Cook® (Indiana, USA) needle for cytology, cell block and culture. There were no signs of arrythmia, bleedin, extravasate or other complications. On site cytology showed malignant tumor cells, and the final diagnosis demonstrated the recurrence of the previous synovial sarcoma in the left atrium.

Figure 4.2



1. *CT scan of the thorax showing a hematoma (H)adjacent to the right atrium and a tumor (T) in the left atrium, adjacent to the esophagus (E).* **2.** *EUS-B image (EBUS scope) showing the tumor (T) inside the left atrium (LA).* **3.** *Elastography pattern of the tumor located in the left atrium showing the different densities of the tumor* **4.** *EUS-B- FNA of a 22G needle inside the tumor.* **5 and 6.** *22G FNA cytology samples demonstrating high cellularity with loose and atypical cells located in a bundle group connection with spool cellular aspect and naked cores, compatible with synovial sarcoma.*

DISCUSSION

We demonstrated that endosonography, both with the regular EUS and smaller EBUS scope, with real-time sampling of left atrium tumors was feasible and safe. In both cases, cytology and cell block analysis provided a clear diagnosis, including the necessary immunohistochemistry.

In the first case, an intracardiac Burkitt lymphoma was diagnosed after an EUS procedure. A few cases of intracardiac Burkitt lymphomas are descrined[8]. , however this is the first diagnosed by endosonography. EUS-guided diagnosis of digestive Burkitt lyphomas has been described before[9,10,] In the the described case, the minimally invasie endosonographic approach was extremely important as the patient was judgded to be to fragile for diagnostic surgery.

In the second case, intra atrial recurrence of a synovial sarcoma, was made due to the EUS(B) FNA approach and the help of elastography. Elastography shows the rigidity pattern of lesions helping to choose the optimal place to take the samples and avoid necrotic areas, and it has been demonstrated that is helpful predicting lymph node malignancy in lung cancer patients[11]. Elastography was helpful to reassure the suspicion of a malignant lesion in the left atrium and to take the tissue sample in the most suspicious place. Also, in this case a re- surgical approach with tissue sampling was not a feasible option due to the previous surgery. As synovial sarcomas have a poor prognosis, the prompt diagnosis lead to a rapid chemotherapy treatment an the patient is still alive

after a 2-year follow up. There are extremelly few reports in the literature about synovial sarcomas sampled by EUS[12], and at our knowledge, this is the only patient that has survived after the procedure with a good quality of life.

There are very few articles in the literature reporting EUS or EUS-B procedures of cardiac tumors [18-20], specially in the left atrium [21,22].

Linear endosonography by EBUS/ EUS-B is incorporated the guidelines for the diagnosing and staging of lung cancer 13 as the initail diagnostic test for mediastinal tissue acquisition. It has also been shown that lung tumor sampling from the esophagus and the airways is feasible and safe [7,14]. The diagnostic utility of endosonography is constantly growing, as it has demonstrated its utility for the assessment of the mediastinal/vascular invasion (T4) in patients with centrally-located lung lesions [15].

Even if it is not a common procedure, there are reports in the literature showing the possibility of using endosonography for transvascular access biopsies of intrathoracic lesions that couldn't be reachable other way [16]. Trans-aortic approaches are feasible in selected cases[17] for para - aortic tumors or lymph nodes.

It should be clear that only very selected cases might me eligible for this kind of diagnostic approaches. Operators should be experienced and alternative diagnostic approaches should be carefully reviewed.

CONCLUSION

In very selected cases, skilled operators can use EUS(B)FNA as a minimally invasive procedure for the diagnosis of intracardiac/ left atrial tumors.

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Endobronchial ultrasound in diagnosing and staging of lung cancer using 22 G TBNB vs 22 G TBNA needles: a randomized controlled trial.

2021, Protocol submitted for publication

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ABSTRACT

Introduction

Accurate diagnosis and staging of lung cancer is crucial because it directs treatment and prognosis. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound fine needle aspiration (EUS-(B)-FNA) are important in this process, both through sampling of hilar/mediastinal lymph nodes, as well as centrally located lung tumors. With the upcoming of immunotherapy and targeted therapies, assessment of PD-L1 expression and molecular profiling of malignancies has become important but is often not possible in cytological samples obtained through standard 22G TBNA needles. Recently, a 22G needle was developed with a three-pronged cutting edge that allows for needle biopsy (TBNB). Our objective is to determine if EBUS/EUS-B guided nodal/lung tumor sampling with these Acquire™ 22G TBNB needles results in improved suitability rate for the assessment of PD-L1 expression in comparison to standard 22G TBNA needles.

Methods and analysis

This is an investigator-initiated, parallel group randomized clinical trial. Patients are recruited at outpatient clinics of respiratory medicine o NA based on current clinical guidelines. Web-based randomization between the two needles will be performed. Samples will be obtained from mediastinal lymph nodes, as well as the primary tumor, if possible. Aspirates will be processed for cytology smears and cell block analysis and will be reviewed by blinded reference pathologists. The primary outcome is the suitability rate for the assessment of PD-L1 expression in patients with a final diagnosis of lung cancer. This study that is Financially supported is by Boston Scientific Corporation.

Ethics and dissemination

The study was approved by the local Ethics Committee. Dissemination will involve publication in a peer-reviewed biomedical journal.

Registration

Netherlands Trial Register (NL7701).

INTRODUCTION

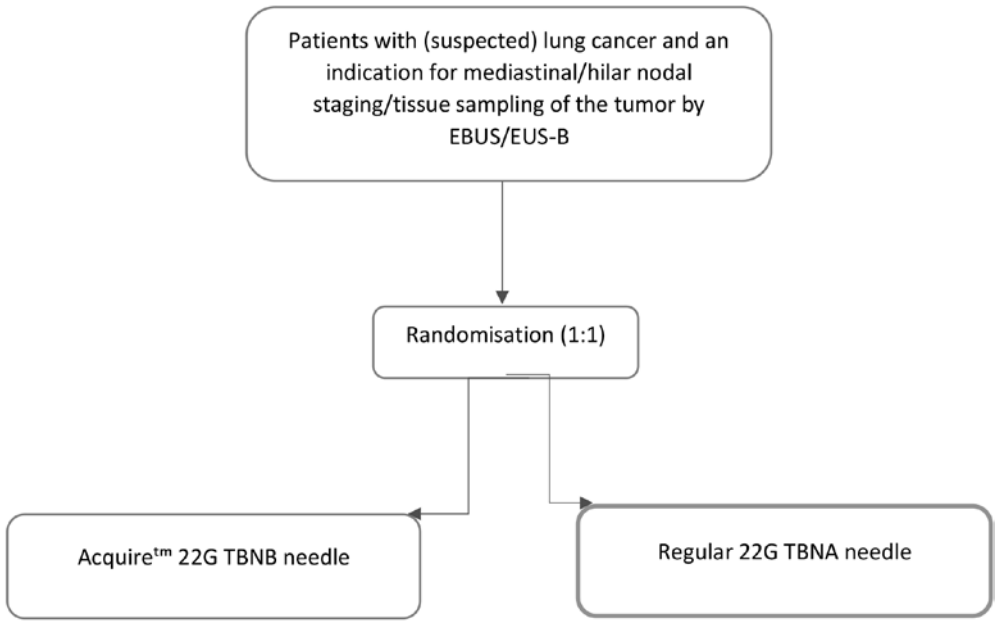
Lung cancer is the most commonly diagnosed cancer worldwide, with the highest mortality of all cancers [1]. Accurate staging is important because it directs treatment and prognosis [2, 3]. Mediastinal and hilar staging is key in this process. For decades, this was done by (cervical) mediastinoscopy, which is a costly and invasive procedure, with suboptimal sensitivity for mediastinal metastases [4]. Over the past 15 years, clinicians have started to use endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for this purpose, which is less invasive and has a similar diagnostic range to cervical mediastinoscopy [5]. Bronchial EBUS-TBNA can be combined with endoscopic ultrasound fine needle aspiration (EUS-FNA) from the oesophagus, which can reach additional lymph node stations located in the lower and posterior mediastinum [6]. It has been shown that a combined endosonography investigation (EBUS and EUS) followed by mediastinoscopy in the absence of metastases at endosonography, results in greater sensitivity for mediastinal nodal metastases compared with mediastinoscopy alone [5]. Therefore, current guidelines on lung cancer staging recommend endosonography (EBUS and/or EUS) as the initial tissue sampling technique for mediastinal nodal staging [2]. Additionally, EBUS and EUS can be used to sample centrally located lung tumors [7-9].

Over the past years, the treatment options for patients with advanced lung cancer have been expanding rapidly. To be able to assign a patient to optimal treatment, assessment of PD-L1 expression and molecular profiling of the tumor is crucial. With the advent of EBUS-TBNA and EUS-FNA, the ability to accurately assess the PD-L1 expression and molecular profiling on small, cytological tissue samples has become of great importance. Although the use of cell blocks and improves sequencing techniques have expanded the possibilities for PD-L1 assessment [10-14], the success rate of PD-L1 expression and molecular profiling remains suboptimal at cytology compared to histology [15-17].

Recently, a three-plane symmetric needle with Franseen geometry (Acquire™ 22G transbronchial needle biopsy (TBNB)) was developed *(Figure 5.1)*. In pancreatic cysts the Acquire™ 22G needle allowed improved, true histological core tissue acquisition in pancreatic cysts [18, 19]. To date, there is no literature about the Franseen biopsy needle for diagnostic and staging purposes during EBUS/EUS-B procedures in patients with (suspected) lung cancer.

We hypothesize that EBUS/EUS-B sampling of mediastinal/hilar lymph nodes and/or primary lung tumors with the Acquire™ 22G TBNB needle has a higher suitability rate for the assessment of PD-L1 expression in comparison to the regular 22G TBNA needle in patients with lung cancer.

Figure 5.1 *Flowchart of study design*



METHODS AND ANALYSIS

Design and study dates

This is a protocol of an investigator initiated parallel group randomised clinical trial, performed in both university and general hospitals in The Netherlands (Amsterdam University Medical Centers and Leiden University Medical Center), Italy (Fondazione Policlinico Universitario Agostino Gemelli, Rome) and Poland (Pulmonary Hospital Zakopane). We expect that we will complete inclusion in June 2022. The study was registered at Netherlands Trial Register (registration ID NL7701).

Study population

Patients will be recruited at outpatient clinics of respiratory medicine of the participating hospitals by their treating physicians. If the patient is willing to participate in the study, information about the study will be provided by the local investigator, who will then gain written informed consent. Eligible are patients with proven or suspected lung cancer (either non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC)), who have an indication for mediastinal/hilar lymph node or lung tumor sampling by EBUS-TBNA and/or EUS-B FNA according to current clinical guidelines [2]. Indications for mediastinal/hilar lymph node staging are: 1) suspicion of mediastinal or hilar lymph node metastases based either on size (short axis >10mm on CT) or increased FDG uptake, 2) a primary tumor without FDG uptake, 3) a primary tumor with a size of ≥3cm, or 4) a centrally

located primary tumor. Sampling of centrally located lung tumors is attempted if the intrapulmonary mass is located adjacent to the oesophagus or airway wall but not endobronchially visible with conventional bronchoscopy. The following exclusion criteria will be applied: 1) mediastinal re-staging after neo-adjuvant treatment, 2) contra-indication for EBUS or EUS-B (e.g., severe respiratory insufficiency), 3) a non-correctable coagulation disorder, 4) pregnancy, or 5) inability to consent.

Study withdrawal

Subjects can leave the study at any time for any reason if they wish to do so. The investigator can decide to withdraw a subject from the study for unanticipated urgent medical reasons. Such withdrawals will be monitored and reported in the final study report.

Randomisation and needle allocation

Randomisation will be performed prior to endosonography. Participants will be randomised to either the Boston Scientific Acquire™ 22G TBNB needle or the regular Boston Scientific 22G TBNA needle *(Figure 5.2).* Both needles have a CE mark. We will use a web-based block-randomisation (using Castor Electronic Data Capture (EDC) software, Amsterdam, the Netherlands), stratified by participating hospital to ensure that both groups are of equal size for every hospital. Endoscopists will not be blinded to needle randomisation, but the reference pathologists will be blinded for the procurement of the tissue.

Figure 5.2

A: *Acquiretm 22G TBNB needle with a three-pronged cutting edge*

B: *Regular 22G TBNA needle*

Endosonographic procedure

Endosonography will be performed at the department of pulmonary medicine of participating hospitals by experienced chest physicians who are specifically trained in EBUS and EUS-B [20]. Procedures will be performed according to institutional practice, mostly on an outpatient basis. Topical anaesthesia is applied to the pharynx, larynx, trachea and bronchi. Sedation will be administered according to institutional practice, mostly conscious sedation by midazolam 2.5-5mg iv with or without fentanyl or propofol sedation.

Vital parameters will be monitored during and after the procedure. Patients will be observed for one hour after the endosonography procedure.

Procedures will be performed using an ultrasound bronchoscope with a linear scanning transducer (EBUS scope). A dedicated ultrasound processor is used for imaging processing with Doppler flow imaging for the detection of blood vessels and that enables real-time ultrasound guidance for sampling. All patients will undergo a systematic endosonographic evaluation of all the accessible mediastinal/hilar lymph nodes and the tumor itself.[2, 21] Following the endosonographic inspection, lymph nodes will be sampled with the randomized needle from N3 to N2 to N1 to the tumor itself. A minimum of two needle passes will be performed for each lymph node and tumor. After insertion of the needle in the target lymph node or tumor, the stylet will be pulled out slowly (slow pull technique). In case of no or a limited aspirate after one pass, suction using a 10 ml syringe during the following aspirations will be applied.

Endoscopist satisfaction
After the endoscopic procedure the endoscopists satisfaction score (scale 0-10, where 0 is the lowest score and 10 the highest) will be scored.

Sample processing
Handling of the aspirates will be performed according to institutional practice. Nodal aspirates will be assessed by the local pathologist for clinical decision making. Rapid on-site evaluation (ROSE) is optional. When ROSE is performed, smears of the fine needle aspirate obtained by EBUS-TBNA or EUS-B-FNA of the intrathoracic lymph nodes are performed in situ. All aspirates (both from lymph nodes as well as the tumor) will be processed for both cytology smears and cell block analysis following local practice.
The local pathologist will analyse the nodal aspirates, including assessment of PD-L1 expression and molecular analysis for treatment. In case of N0/N1 disease based on the TBNB/TBNA specimens, surgical pathological staging or clinical radiological follow-up at six months serves as reference standard.

Independent cytopathology
The local pathologist will assess the sampling material, including cell blocks and cytological smears, for clinical purposes. After completion of the study, all cell blocks will be digitalized and reviewed by two reference pathologists for independent cytopathological review. In case less than two cell blocks of a single patient are available, cytological smears will also be included in the independent cytopathological evaluation. The reference pathologists are blinded to needle randomization but supplied with the clinical information, endosonography route (EBUS or EUS-B), results of the other tests and are aware of the differential diagnosis prior to endoscopy.

The pathologists will evaluate each cell block for the suitability of PD-L1 expression. Cell block specimens will be considered suitable if more than 100 tumor cells are present in the specimen. Additionally, the suitability for molecular analysis will be evaluated

based on the presence/absence of >1000 tumor cells in all the cell block material combined. Also, the cumulative length of tissue core will be measured, the sample adequacy will be evaluated based on the presence of lymphocytes or atypical cells or other pathognomic characteristics (e.g. granulomas), the quality of the samples will be evaluated using a Mair's objective scoring system[22] and the bloodiness of the samples will be categorized based on the percentage of blood in the microscopic field. In case the two independent pathologists do not fully agree on one of the above mentioned parameters, the pathologists will conduct a consensus review of discrepant cases in order to have a clear final judgement.

OUTCOMES
The primary outcome is the suitability rate for the assessment of PD-L1 expression on mediastinal/hilar nodal or tumor aspirates in patients with a final diagnosis of lung cancer.

The secondary outcomes are:
- Cumulative length tissue core
- Suitability for molecular analysis/next-generation sequencing in patients with a final diagnosis of lung cancer
- Sample adequacy (defined as the presence of lymphocytes or atypical cells or other pathognomic characteristics (e.g. granulomas))
- Sample quality using the Mair's objective scoring system
- Sample bloodiness
- Diagnostic sensitivity for mediastinal/hilar nodal staging (defined as the proportion of patients that have N2/N3 disease diagnosed by EBUS/EUS-B, relative to the total number of patients with a final diagnosis of N2/N3 disease as determined by the refe rence standard)
- Diagnostic sensitivity for malignancy (defined as the proportion of patients that have malignancy diagnosed by EBUS/EUS-B, relative to the total number of patients with a final diagnosis of malignancy as determined by the reference standard)
- Yield for diagnosing malignancy in the subgroup of patients with a centrally located lung tumor (defined as the proportion of patients that have malignancy diagnosed by EBUS/EUS-B, relative to the total number of patients with a final diagnosis of malignancy)
- Complication rate
- Procedure duration
- Endoscopists satisfaction of needle use

Sample size calculation
Based on results on the success rate of PD-L1 assessment in the Amsterdam UMC and published literature, we expect the acquisition rate of the regular 22G TBNA needle for the assessment of PD-L1 expression in patients with lung cancer is around 64%.[23, 24.]
We expect that this will be 86% for the Acquire ™ 22G TBNB needle. In total, 120 patients with lung cancer and tumor positive samples (e.g. lymph node metastasis/ primary tumor sample) are needed to show, with alfa=0.05 and power=0.80, that the Acquire™ 22G needle is 22% superior to the regular 22G TBNA needle. Taking into account that

80% of included patients will have a final diagnosis of lung cancer and a tumor positive aspirate, and a 5% study drop out, 158 patients will be included. In our opinion the expected increase in suitability rate for PDL-1 assessment (from 64 to at least 86%) will result in a clinically relevant improvement of lung cancer diagnosis and staging.

Statistical analysis
Results for continuous variables will be expressed as means and standard deviations, or as medians with interquartile ranges. Categorical variables will be expressed as frequencies and percentages. Chi-squared testing will be used to compare dichotomous outcomes, including the primary outcome. Continuous variables will be compared using Student's t-tests or Mann-Whitney-U tests. A two-tailed P-value <0.05 will be considered statistically significant.

Funding
This is an investigator initiated study with financial and material support from Boston Scientific Corporation.

ETHICS AND DISSEMINATION

The study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

Data handling
Data collection will be performed in the participating centres. Electronic patient record forms will be provided through web-based software (Castor Electronic Data Capture (EDC) software, Amsterdam, the Netherlands), Data collection and analysis will be monitored according to good clinical practice. Clinical monitoring will be organized in a cross over mode where CRF files will undergo a quality check. Inclusion rate will be monitored on a monthly basis. The investigators will maintain adequate records, including signed patients informed consent forms and information on adverse events. These documents need to be kept in a secured area with limited access. All records will be signed and dated by the investigators. All records are to be retained for a period of 15 years following the date the entire clinical investigation is completed, terminated or discontinued. The anonymity and confidentiality will be guaranteed and patients identification will be coded. The code will start with the abbreviation of the hospital followed by 100 with the number of inclusion (e.g. AMC1001). Patients data will be centralized by the coordinating investigator and kept under strict confidentiality according the General Data Protection Regulation (GDPR). The data of the patients included in the Netherlands will stay in the Dutch database.

Adverse events
EBUS for staging and diagnosing lung cancer is a routine clinical procedure that is considered safe with a low complication rate of less than 1% [25]. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the endosonographic procedure. All adverse events reported spontaneously by the subject or observed by the endoscopist or his staff will be recorded, as well as those occurring up to 1 week after the procedure. The investigators in the participating centres will report all serious adverse events to the coordinating investigator and principal investigator. They will report the serious adverse event to the sponsor without undue delay after obtaining knowledge of the events, as well as to the accredited medical ethics committee that approved the protocol. Only the serious adverse events with a causal association with study participation will be reported in the final study report following study completion.

Annual progress report
The sponsor/investigator will submit a summary of the progress of the trial to the accredited medical ethics committee once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

Dissemination
We anticipate the report our findings in an article that will be submitted to a peer-reviewed biomedical journal.

DISCUSSION

Lung cancer remains a major source of mortality worldwide. Diagnosis and staging procedures have improved considerably over the past decades and endosonography (either through EBUS or EUS) now plays a major role in this process. With the upcoming of immunotherapy and targeted therapies, assessment of PD-L1 expression and molecular profiling of lung malignancies has become crucial. Although endosonography is minimally invasive and adverse events are rare, a disadvantage is that cytology has a suboptimal success rate for assessment of PD-L1 expression and molecular profiling, resulting in additional procedures and treatment delay. Generally, histological samples are required for such profiling, but this may require (additional) invasive surgical procedures.
We anticipate that the Acquire™ 22G TBNB will result in a much higher rate of assessment of PDL-1 expression as compared to traditional needles, which would be a considerable clinical advantage in terms of treatment selection.

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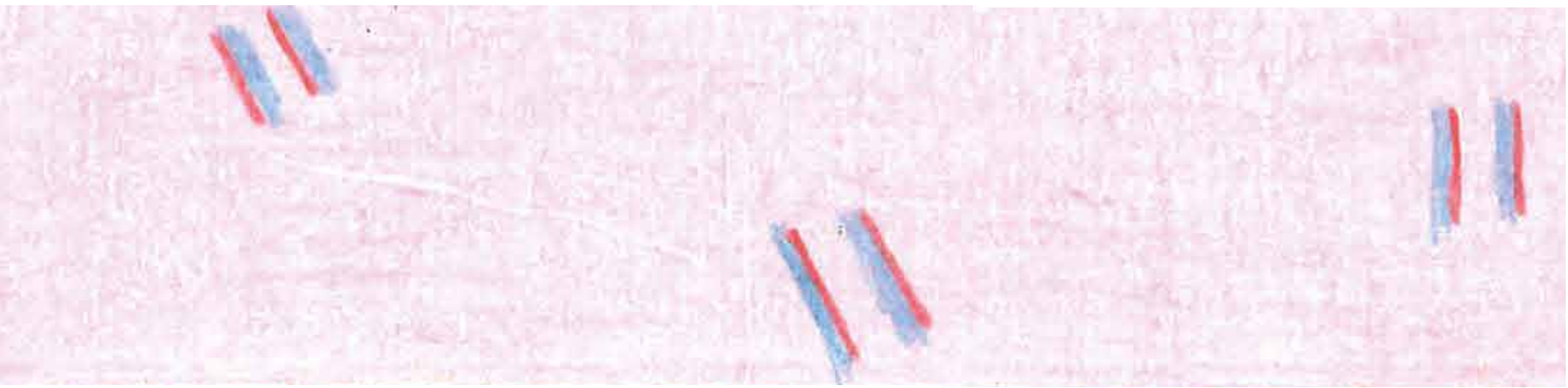
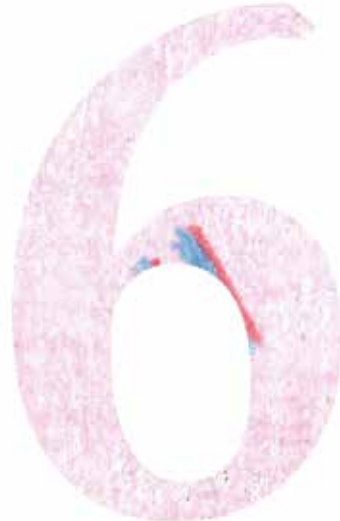
Part 2

Endobronchial and esophageal ultrasound for T staging of lung tumors





Endobronchial ultrasound for T4 staging in patients with resectable NSCLC.

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ABSTRACT

Background

In lungcancer patients, accurate assessment of mediastinal and vascular tumor invasion (stage T4) is crucial for optimal treatment allocation and to prevent exploratory thoracotomies. We assessed the diagnostic accuracy of linear endobronchial ultrasound (EBUS) for T4-status in patients with centrally located lung cancer.

Methods

This is a retrospective study among consecutive patients who underwent EBUS for diagnosis and staging of lung cancer in four hospitals in The Netherlands (Amsterdam, Leiden), Italy (Bologna) and Poland (Zakopane) between 04-2012 and 04-2019. Patients were included if the primary tumor was detected by EBUS and subsequent surgical-pathological staging was performed. T4-status was extracted from EBUS and pathology reports. Chest CT's were re-reviewed for T4-status.

Results

104 patients with lung cancer in whom EBUS detected the primary tumour, and who underwent subsequent surgical-pathological staging were included. 36 patients (35%) had T4, based on vascular (n=17), mediastinal (n=15), both vascular and mediastinal (n=3), or oesophageal invasion (n=1). For EBUS, sensitivity, specificity, PPV and NPV for T4-status were (n=104): 63.9% (95%CI 46.2-79.2%), 92.6% (83.7-97.6%), 82.1% (65.6-91.7%), and 82.9% (75.7-88.2%), respectively. For chest CT (n=72): 61.5% (95%CI 40.6-79.8%), 37.0% (23.2-52.5%), 35.6% (27.5-44.6%), and 63.0% (47.9-75.9%), respectively. When combining CT and EBUS with concordant T4 status (n=33): 90.9% (95%CI 58.7-99.8%), 77.3% (54.6-92.20%), 66.7% (47.5-81.6%), and 94.4% (721-99.1%), respectively.

Conclusion

Both EBUS and CT alone are inaccurate for assessing T4-status as standalone test. However, combining a negative EBUS with a negative CT may rules-out T4-status with high certainty.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide. (1) Patients with non-small cell lung cancer (NSCLC) invading the mediastinum or large vessels (T4 stage) have a five-year survival rate that ranges from 44% to less than 28%.[2, 3] T4 can be defined according to the 8th TNM classification as a tumor >7 cm in greatest dimension or associated with separate tumor nodule(s) in a different ipsilateral lobe than that of the primary tumor, or invading any of the following structures: diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, or vertebral body.[4, 5]

Accurate staging is crucial to ensure patients receive optimal therapy. Patients with T4 lung tumors are most commonly treated with multimodality treatment including (neo-adjuvant) chemotherapy and/or radiotherapy, sometimes followed by surgery [10, 11]. However, accurate preoperative assessment of mediastinal tumor invasion is challenging as computed tomography (CT) scanning of the chest is of limited value, with sensitivity and specificity varying from 40-84% and 57-94%, respectively.[6, 7] FDG positron emission tomography (PET) offers minimal additional information due to its poor anatomical and spatial resolution,[8] and chest MRI has low specificity for T4 assessment.[9] As such, patients with suspected mediastinal or vascular tumor invasion are still at risk for futile thoracotomy or missed surgical opportunities.[10]

Current lung cancer staging guidelines advocate the use of endosonography (endobronchial (EBUS) and/or esophageal (EUS(-B)) for regional nodal staging in patients with centrally located intrapulmonary tumors.[11] In cases where a tumor presents along the major airways, EBUS and EUS(-B) can also be used for diagnostic purposes.[12-14] However, the value of EBUS for assessing tumor invasion in the mediastinum and related structures has not yet been explored. The aim of this study was to evaluate the diagnostic accuracy of EBUS for assessing mediastinal or large vessel invasion (T4-status).

METHODS

Study design and patient selection

We undertook a retrospective international multicentre study in the Netherlands (Amsterdam University Medical Centre (location Academic Medical Centre) and Leiden University Medical Centre, Leiden), Italy (Policlinico S. Orsola-Malpighi, Bologna) and Poland (Pulmonary Hospital Zakopane, Zakopane). Patients were selected from institutional endosonography databases. Records from 1-4-2012 until 1-4-2019 were analysed. Patients were eligible for enrolment in the study if all the following criteria were present: 1) EBUS was performed for the diagnosis and/or staging of (suspected) lung cancer; 2) the primary lung tumor was detected by EBUS; 3) surgical-pathological staging including verification of tumor status was performed within 6 weeks following EBUS. Patients were excluded if they turned out not to have NSCLC, if neo-adjuvant therapy had been administered prior to surgical exploration and if the T4-status was not mentioned in the EBUS report.

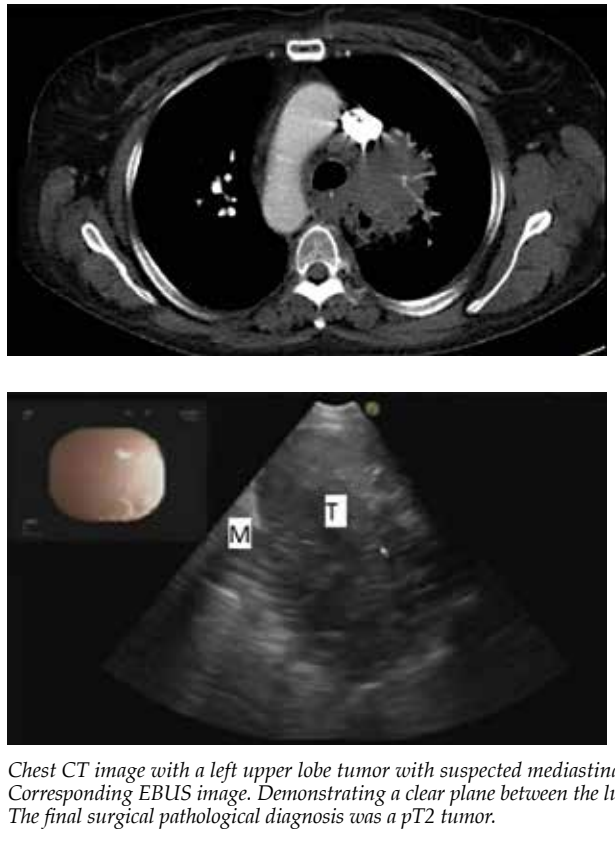
For each included patient, we collected the reports from staging modalities, including chest CT imaging, EBUS and corresponding cytopathology, surgery and corresponding histopathology. In this study, T4-status was defined according to the international staging guidelines as a tumor invading the diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, or carina.[4, 5]

EBUS procedure
All procedures were performed at the endoscopic units of the four referral centers by experienced interventional pulmonologists, who were not blinded to chest CT findings. Procedures were mainly performed in an outpatient setting, either under conscious sedation using midazolam/fentanyl, or propofol/remifentanil sedation. A systematic EBUS examination (Olympus BF-UC180F or UC 180F, Olympus Medical Systems Europe, Ltd., or Pentax EB-1970 UK or Pentax EB19-J10U, Pentax, Hamburg, Germany) was performed according to EBUS assessment tool in all centers.[15]

After visualizing a lung tumor by linear EBUS, the endoscopist evaluated the area for signs of mediastinal or vascular tumor invasion. The T4-status as reported by the endoscopist in the EBUS report was recorded and used for analysis. Mediastinal invasion was considered diagnosed by EBUS if there was continuity of the tumor and the mediastinum, i.e. without a separation of the two structures by an endosonographically identifiable tissue plane. This evaluation could be further supported by dynamic maneuvers. Vascular invasion was considered diagnosed by EBUS when the tumor interrupted the intimal layer of a central extrapulmonary vessel or if there was evidence of tumor invasion into the vessel or atrium. In all cases, possible vascular tumor invasion was further assessed by color flow Doppler *(figure 6.1)*. T4 status was extracted from EBUS reports.

Chest CT scan
Chest CT-scans were collected for review. CT-scans of insufficient quality (i.e., absence of intravenous contrast administration, slice thickness >3mm or low-dose CT for attenuation correction purposes) were excluded for final analysis. All available CT-scans were independently re-reviewed for T4-status by one board certified chest radiologist, who was blinded to the initial CT report, the EBUS report, and the intraoperative and pathology findings. These findings were used in the analysis of the diagnostic accuracy of chest CT for T4-status. Additionally, to assess inter-reviewer agreement, a second board certified chest radiologist who was also blinded to all earlier investigations, re-reviewed the chest CT-scans.

Figure 6.1 *EBUS evaluation of suspect T4 stage*



Chest CT image with a left upper lobe tumor with suspected mediastinal invasion (T4) note the right descending aorta. Corresponding EBUS image. Demonstrating a clear plane between the lung tumor (T) and the mediastinum (M) (no T4). The final surgical pathological diagnosis was a pT2 tumor.

At chest CT scan, mediastinal invasion was documented as: replacement of mediastinal fat by soft-tissue mass, mass surrounding the trachea or esophagus, obvious invasion of mediastinal structures, tumor contact of >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, or 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.[6, 16, 17]

Surgical pathological T4 assessment
All cases were reviewed in multi-disciplinary tumor board meetings as part of clinical practice, taking all available diagnostic tests into account. During these meetings, a decision was made whether there was an indication for lobectomy or pneumonectomy according to the current standards and guidelines at that time.[18] T4-status based on surgical-pathological staging after thoracotomy was the reference standard. In the pathological reports, T4 was defined in accordance with the 8th TNM classification.[5]

Study endpoints and statistical analysis

The primary endpoint is the diagnostic accuracy of EBUS for the assessment of T4-status of lung malignancy. Secondary endpoints are the diagnostic accuracy of chest CT scan and of the combined CT/EBUS approach.

True positives were cases in which the test (EBUS or CT) was compatible with T4, and vascular/mediastinal invasion was confirmed by surgical pathological staging. True negatives were cases in which the test (EBUS or CT) showed no signs of T4, and this was confirmed by surgical pathological staging. False negatives were cases where the test (EBUS or CT) showed no signs of T4, but surgical pathological staging showed mediastinal/vascular invasion. False positives were cases where the test (EBUS or CT) was compatible with T4, but surgical pathological staging showed no mediastinal/vascular invasion.

When assessing the diagnostic accuracy of the combined CT/EBUS approach, we only included patients in whom both EBUS and CT findings were concordant regarding the T4 stage (i.e. both CT and EBUS showed T4, or both showed no T4). Accuracy estimates were calculated along with 95% confidence intervals. Interobserver variability calculates for chest CT was assessed using the Kappa-statistic.

Ethics

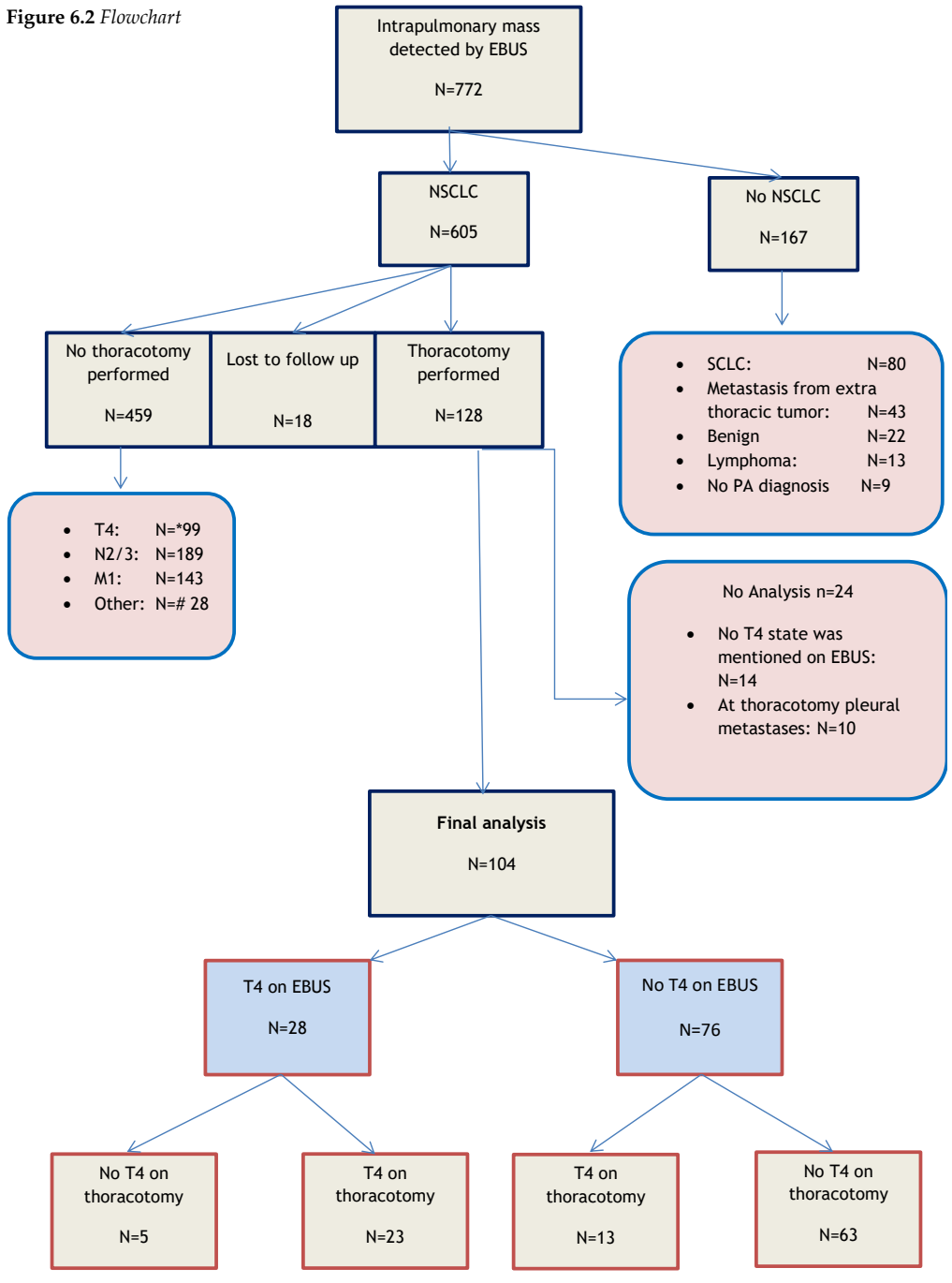
This retrospective analysis was conducted in accordance with the amended Declaration of Helsinki, and collection and publication of the data was approved by the local medical ethics committees.

RESULTS

Patient selection

In 772 consecutive patients with known or suspected lung cancer undergoing EBUS in one of the participating centres, a primary lung lesion was identified by EBUS. Of these, 167 (22%) patients had a final diagnosis other than NSCLC. Of the remaining 605 patients with NSCLC, 459 (76%) individuals were excluded because they did not undergo thoracotomy, mostly due to N2/N3 disease or distant metastases, where 18 (3%) were lost to follow-up. In total, 128 patients with NSCLC underwent thoracotomy within 6 weeks of EBUS evaluation. In 14 cases, the EBUS report did not describe presence or absence of mediastinal tumor invasion, and these were excluded. An additional 10 patients were excluded for per operative detection of pleural metastasis and subsequent abrogation of the procedure. An overview of patient selection is represented in Figure 6.2.

Figure 6.2 *Flowchart*



Overall, 104 cases were included. Patient characteristics are presented in Table 6.1. In summary, the median age of patients was 67.4 years (range 48-85) and 77 (74%) were male. Primary tumors were located in the RUL (n=44, 43%), RLL (n=10, 10%), LUL (n=20, 19%), LLL (n=19, 18%), left hilum(n=7, 7%), and right hilum (n=4, 4%). The final histological diagnoses were adenocarcinoma (n=30, 28%), squamous cell carcinoma (n=63, 61%), large cell neuro-endocrine carcinoma (n=4, 4%), and NSCLC-NOS (n=7, 6%).

Table 6.1 *Patient characteristics of the patients included in de final analysis*

Number of patients	104
Median age (Range)	67.4 years (48-85 years)
Sex male	77 (74%)
female	27 (26%)
Median long axis of the lesion on CT (Range)	54.0 mm (16-130 mm)
Location of the lesion	
RUL	44 (43%)
RML	0
RLL	10 (10%)
LUL	20 (19%)
LLL	19 (18%)
Central left	7 (7%)
Central right	4 (4%)
Final histological diagnosis	
Adenocarcinoma	30 (29%)
Squamous cell carcinoma	63 (61%)
Large Cell neuro-endocrine carcinoma	4 (4%)
NSCLC-NOS	7 (6%)
T stage after surgery	
pT4	36 (34%)
pT3	21 (21%)
pT2	33 (31%)
pT1	14 (13%)

Final Diagnosis

Of the 104 patients analysed, surgical-pathological staging showed tumor invasion (T4) in a total of 36 (34%) patients, based on vascular invasion (n=17), mediastinal invasion (n=15), both vascular and mediastinal invasion (n=3), or oesophageal invasion (n=1). The remaining 68 (66%) patients had no T4-status at surgical-pathological staging. An overview of accuracy estimates for EBUS, CT and combined CT/EBUS is provided in Table 6.2.

Table 6.2 *Accuracy estimates for diagnosing T4-status in patients with NSCLC*

	EBUS (n=104) (95%CI)*	**CT (n=72) (95%CI)****	**CT/EBUS combined (n=33) (95%CI)*****
Sensitivity	63.9% (46.2% to 79.2%)	61.5% (40.5% to 79.8%)	90.9% (58.7% to 99.8%)
Specificity	92.6% (83.7% to 97.6%)	37.0% (23.2% to 52.5%)	77.3% (54.6% to 92.2.%)
Positive predictive value	82.1% (65.6% to 91.7%))	35.6% (27.5% to 44.6%)	66.7% (47.5% to 81.6%)
Negative predictive value	82.9% (75.7% to 88.2%)	63.0% (47.9% to 75.9%)	94.4% (72.1% to 99.1%)
Accuracy	82.7% (74.0 to 89.4%)	45.8% (34.9% to 58.0%)	81.8% (64.5% to 93.0%)

**For EBUS, all 104 patients were included in the analysis.*
***For CT, 72 patients with a high-quality CT (with contrast and less than 3 mm slice thickness) available for re-evaluation were included in the analysis.*
****For EBUS/CT combined, 33 patients where CT and EBUS had non-conflicting results for T4 evaluation (i.e. both were positive or both were negative) were included in the analysis.*

Diagnostic accuracy of EBUS

At EBUS, 28 patients were judged to have stage T4 tumors, of which 23 were confirmed at subsequent surgical-pathological staging. Of these 23 true positive cases, T4-status was established based on mediastinal invasion (n=12) or vascular invasion (n=11: pulmonary artery (n=9), pulmonary vein (n=1) or azygos vein (n=1)). For the five false positive cases, the endoscopist reported invasion of the mediastinum (n=1), the pulmonary artery (n=3), or the pericardium (n=1), which was not confirmed at surgical-pathological staging.

The remaining 76 patients did not demonstrate signs of tumor invasion at EBUS. Surgical-pathological staging showed T4 disease in 13 of them. These false negative cases included patients with mediastinal invasion (n=3), vascular invasion (n=9), both mediastinal and vascular invasion (n=1). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of EBUS for diagnosing T4-status was 63.9% (95%CI 46.2-79.2%), 92.6% (83.7-97.6%), 82.1% (65.6-91.7%), 82.9% (75.7-88.2%), respectively.

Diagnostic accuracy of chest CT scan

For six included patients, chest CT scan was not available for re-review, and for 26 patients, CT was deemed of insufficient quality for re-review, leaving 72 (69%) patients suitable for CT reassessment.

Based on chest CT, 45 patients were judged to have T4 status, of which 16 were confirmed at subsequent surgical-pathological staging. Of these 16 true positive cases, T4-status was established based on mediastinal invasion (n=8) or vascular invasion (n=8: n=6 pulmonary artery and n=2 vena cava superior)). For the 29 false positive cases, the radiologist reported invasion of the mediastinum (n=22) or vasculature (n=7), which was not confirmed at surgical-pathological staging.

Out of 27 cases where tumor invasion was not detected through CT, surgical-pathological staging revealed T4 disease in 10 patients. These included patients with mediastinal invasion (n=6) and vascular invasion (n=4). The sensitivity, specificity, PPV, and NPV of chest CT for diagnosing T4-status was 61.5% (95%CI 40.6-79.8%), 37.0% (23.2-52.5%), 35.6% (27.5-44.6%), 63.0% (47.9-75.9%), respectively (Table 6.2).

Due to technical reasons only 48 of the 72 CT scans were re-reviewed by the second radiologist. The Kappa statistic for this subset was 0.558 (95%CI 0.331-0.785) which corresponds to moderate agreement.

Diagnostic accuracy of combined CT/EBUS

Overall, 33 of the 104 patients had concordant CT and EBUS outcomes regarding T4-status. Of these, 15 were judged to have T4 at both CT and EBUS, of which 10 were confirmed at subsequent surgical-pathological staging. Of the 18 patients without T4-status at combined CT/EBUS, only one patient (3%) turned out to have a T4 tumor at surgical-pathological staging. The sensitivity, specificity, PPV and NPV of combined CT/EBUS for diagnosing T4-status was 90.9% (95%CI 58.7-99.8%), 77.3% (54.6-92.20%), 66.7% (47.5-81.6%), and 94.4% (72.1-99.1%), respectively.

DISCUSSION

In this study, we retrospectively evaluated the diagnostic accuracy of EBUS for the assessment of T4-status in patients with NSCLC. We found that the overall sensitivity and specificity of EBUS is moderate and may be insufficient to rule-in or rule-out T4-status. Likewise, chest CT had limited sensitivity and specificity. However, a combination of a negative EBUS with a negative chest CT rules-out T4-status with a relatively high level of certainty, and these patients may be referred for thoracotomy.

The role of endosonography in the diagnosis and staging of lung cancer has been expanding rapidly over the past decades. EBUS and EUS(-B) can be used for assessment of mediastinal lymph node metastases, and for diagnosis of centrally located lung tumors.[12, 13, 19] Instead of linear EBUS, radial EBUS can also be used to assess a more peripheral location of the lung tumour, but it is inappropriate for T4 assessment.(20) In a recent retrospective study, we showed that among 74 subjects with lung cancer (26% of whom were diagnosed as mediastinal or vascular T4sensitivity and specificity of EUS for assessing T4-status were 42% (95%CI 20-67) and 95% (85-99), respectively, compared to 76% (50-93) and 61% (46-75) for chest CT, and 83% (36-100) and 100% (88-100) for EUS and chest CT combined (in case of concordant results between both tests).[21]

The confirmation of direct mediastinal and/or great vessel invasion by a lung tumor (T4, stage IIIB), has profound consequences for treatment and prognosis of patients with NSCLC. With the exception of some highly selected cases, who may benefit from a radical surgical approach,[22, 23] the majority of patients are best treated with combined chemoradiotherapy with/without immunotherapy.[24] Therefore, accurate T4 assessment is crucial. So far, there has been limited evidence about the potential role of EBUS in this process. Alici et al showed in a retrospective cohort of 55 patients that EBUS was able to discern vascular tumor invasion, although only nine cases had surgical-pathological confirmation of the tumour status[25].

We here report the largest study thus far on the potential role of EBUS in T4 assessment. Our findings show that EBUS alone may be insufficiently accurate for making a final diagnosis of T4-status. Sensitivity and specificity were 63.9% and 92.6%, which resulted in a PPV and NPV of 82.1% and 82.9%, respectively. This could imply an unacceptable number of false positives and negatives. However, when combined with other clinical information, EBUS may certainly have added value for T4 assessment. This is illustrated in the subgroup of patients with both a negative chest CT and a negative EBUS, in which sensitivity and NPV were 90.9% and 94.4%, respectively. As such, T4-status may be ruled-out with a high level of certainty in these patients. Combined chest CT and EBUS seems less accurate for ruling-in T4-status: specificity was 77.3% and PPV 66.7%.

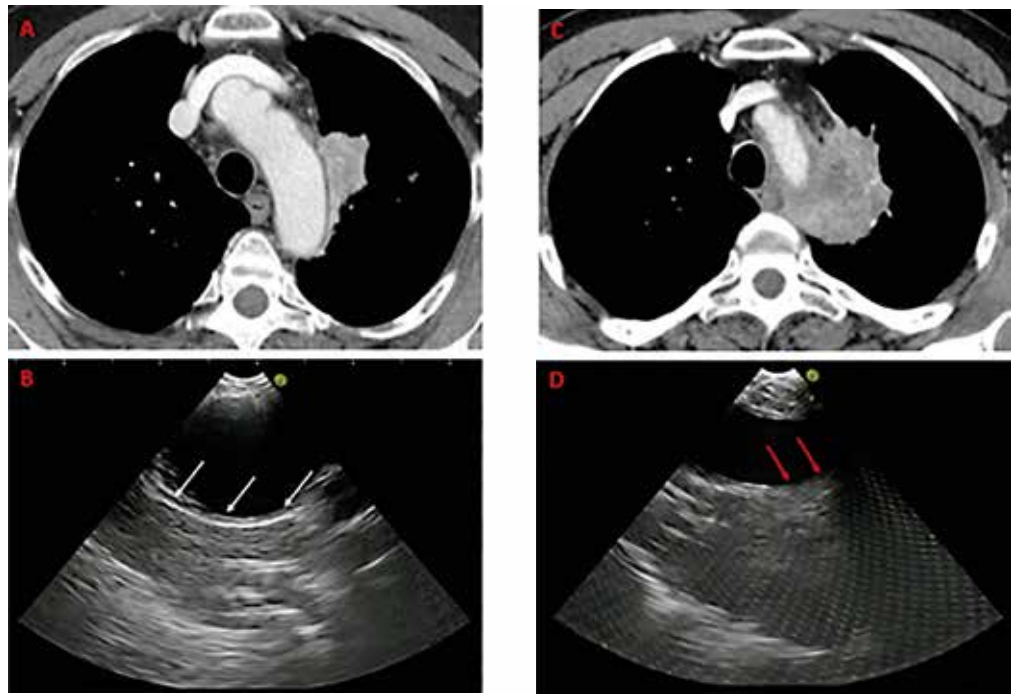
Endosonography (either EBUS or EUS(-B)) has as added benefit over chest CT due to its ability for a higher-resolution and real-time, dynamic assessment of the relationship between tumour and adjacent structures. For instance, sliding of the lung tumor adjacent to the aorta excludes tumor invasion at this specific site. The use of colour Doppler might

be helpful in selected cases to detect vascular structures and demonstrate or exclude tumor invasion at that location. However, assessment of intrapulmonary tumors through endosonography is limited to the immediate vicinity of the major airways as interposition of any air-containing/aerated tissue between tumor and probe precludes adequate visualisation. Especially left upper lobe tumors with possible aortic arch invasion lend themselves for a careful assessment *(figure 6.3)*.

Strong aspects of the current data are the large sample size, the international multicentre aspect and the excellent reference standard. However, some limitations apply to this study. The interpretation of the results is limited by inherent flaws in the study design associated with retrospective studies. A considerable proportion of potentially eligible patients needed to be excluded due to a missing follow-up, or because the EBUS report did not mention the T4-status. Endoscopists were not blinded to CT results and their T4-status interpretation may have been influenced by this, although this reflects clinical practice. Chest CT scans were not available/suitable for re-review in all patients. Data were analysed from four centres with highly experienced endoscopists; less experienced endoscopists may not achieve similar results.

In our opinion, the findings of this study show that there is a role for EBUS in T4 assessment of patients with potentially resectable lung tumors adjacent to the airways, however not as a standalone test. Future studies need to show if our findings can be confirmed in a prospective setting, and if there are subgroups of patients in whom EBUS as an add-on to CT may rule-in or rule-out T4-status with a higher level of certainty.

Figure 6.3 *EBUS assessment of aortic arch invasion from a left upper lobe central tumor*



Panel A : *Chest CT demonstrating a clear separation between the aorta and a left upper tumor (no T4).*
Panel B : *corresponding EBUS image showing the intimal layer of the aorta is constantly visible at EBUS (with arrows), indicating lack of vessel infiltration by the tumor (no T4), this was confirmed after thoracotomy.*
Panel C *demonstrates a Chest CT scan where there are clear sign of vascular invasion (T4).*
Panel D *shows the corresponding EBUS image showing a lack of visualization of the intimal layer of the aorta (red arrows) in a large part of the EBUS window, indicating vessel wall infiltration by the tumor. After tumor board meeting this patient was referred for chemo/radiation therapy.*

Endobronchial ultrasound for T4 staging in patients with resectable NSCLC Part 2. Chapter 6

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Esophageal ultrasound (EUS) assessment of T4 status in NSCLC patients.

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ABSTRACT

Background subkop

Mediastinal and central large vessels (T4) invasion by lung cancer is often difficult to assess preoperatively due to the limited accuracy of computed tomography (CT) scan of the chest. Esophageal ultrasound (EUS) can visualize the relationship of para-esophageally located lung tumors to surrounding mediastinal structures.

Aim

To assess the value of EUS for detecting mediastinal invasion (T4) of centrally located lung tumors.

Methods

Patients who underwent EUS for the diagnosis and staging of lung cancer and in whom the primary tumor was detected by EUS and who subsequently underwent surgical- pathological staging (2000 -2016) were retrospectively selected from two university hospitals in The Netherlands. T status of the lung tumor was reviewed based on EUS, CT and thoracotomy findings. Surgical- pathological staging was the reference standard.

Results

In 426 patients, a lung malignancy was detected by EUS of which 74 subjects subsequently underwent surgical- pathological staging. 19 patients (26%) were diagnosed with stage T4 based on vascular (n= 8, 42 %) or mediastinal (n=8, 42%) invasion or both (n= 2, 11 %), one patient (5%) had vertebral involvement. Sensitivity, specificity, PPV and NPV for assessing T4 status were: for EUS (n=74); 42%, 95%, 73%, 83%, for chest CT (n=66); 76%, 61%, 41%, 88% and the combination of EUS and chest CT (both positive or negative for T4, (n=34); 83%, 100%, 100% 97%.

Conclusion

EUS has a high specificity and NPV for the T4 assessment of lung tumors located para-esophageally and offers further value to chest CT scan.

INTRODUCTION

Patients with non-small cell lung cancer (NSCLC) and tumor invasion of the mediastinum including the invasion of the large vessels and other mediastinal structures (T4, stage IIIB) have a 5 year survival of less than 28%[1]. Mediastinal tumor invasion (T4) is defined as invasion into the diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body and/or carina[2]. Accurate preoperative assessment of mediastinal tumor invasion is challenging as computed tomography (CT) scan of the chest is of limited value (sensitivity, specificity varies from 40-84% and 57-94%) [3'4] and FDG - positron emission tomography (PET) offers minimal additional information due to its poor anatomical/spatial resolution[5]. In addition, MRI of the chest has low specificity and accuracy (33% and 46%) [6]. Accurate staging is crucial to allow patients to receive the most appropriate therapy; subjects with vascular or mediastinal lung tumor infiltration (T4, stage IIIB) are most commonly treated with multimodality therapy including chemotherapy and/or radiotherapy[7]. Unfortunately, due to inappropriate or inaccurate pre-operative T staging, patients with suspected mediastinal tumor invasion still undergo explorative and futile thoracotomies[8'9'19].

Current lung cancer staging guidelines advocate the use of endosonography (endobronchial (EBUS) and/or esophageal (EUS) in patients with centrally located tumors[10'23]. EUS can be used for diagnosing lung cancer in those patients with a centrally located lung malignancy adjacent to the esophagus that is not accessible by standard flexible bronchoscopy[11'12'13]. In addition to tissue sampling for diagnostic purposes, EUS can assess the anatomical relationship of the lung tumor with centrally located vessels and the mediastinum[14'15]. To date, the value of EUS for T4 assessment in lung cancer remains unknown.

AIM

To assess the role of EUS for detecting mediastinal invasion (T4) of centrally located lung tumors.

METHODS

Study design and patients

This is a retrospective study undertaken in Leiden University Medical Center (LUMC) Leiden (2000-2011) and the Academic Medical Center (AMC) Amsterdam (2012-2016), The Netherlands.

Patients were eligible for enrolment into the study if the following criteria were present:

1) EUS (performed with the regular GI EUS scope) or EUS (B) (EUS performed with the EBUS scope) was used for the diagnosis and staging of lung cancer AND
2) The primary lung tumor was detected by EUS AND
3) Surgical- pathological staging including verification of tumor status was performed within 6 weeks following EUS

Patients were not eligible for analysis if neo-adjuvant therapy was administered prior to surgical exploration.

All cross sectional imaging of the chest, EUS reports, cyto-pathological, surgical and pathological reports were collected. The CT scan of the chest was reviewed for study purposes by an expert thoracic radiologist (IB) who assessed for the presence or absence of mediastinal/vascular tumor invasion.
The primary endpoint of this study is the sensitivity and specificity of EUS for the assessment of mediastinal or vascular invasion by the lung malignancy using surgical- pathological staging as the reference standard. Secondary endpoints are the sensitivity and specificity for T4 status for chest CT scan alone and for the combined CT/EUS approach.

PROCEDURES

Endosonography

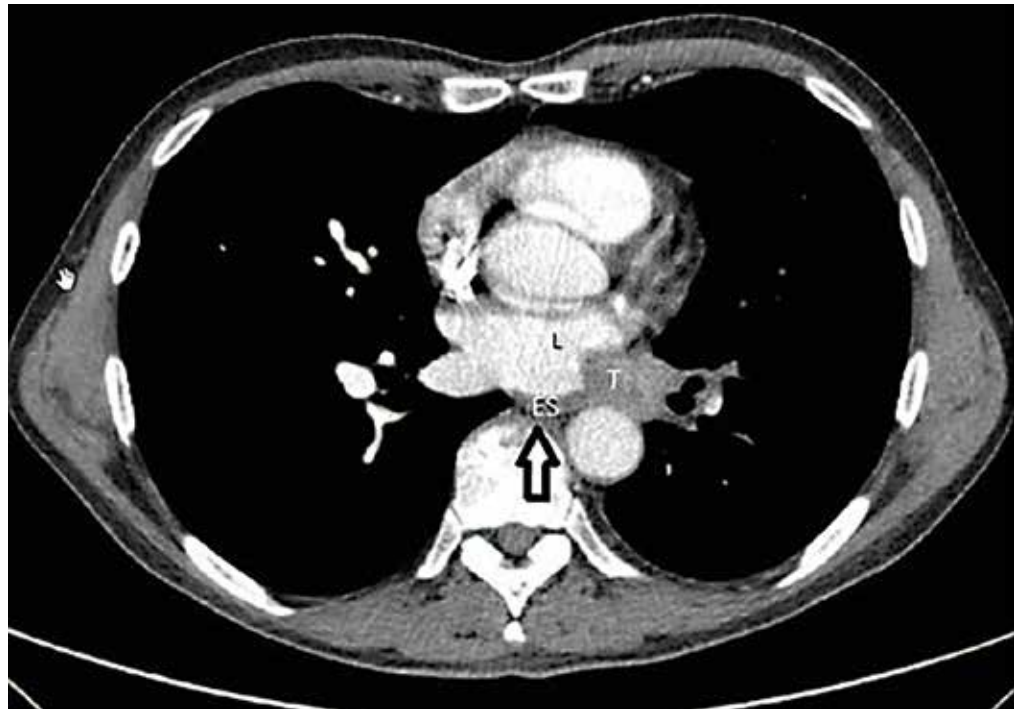
All EUS procedures were performed in an ambulatory setting using midazolam/fentanyl or propofol sedation by two experienced pulmonologists (JTA/KFR). Esophageal investigations were performed with either a (linear) Pentax FG 34 UX or EG 3270 UK (EUS) (Tokyo, Japan) or Pentax EB-1970UK (EBUS) scope (Pentax Medical, Hamburg, Germany) in combination with a Hitachi ultrasound scanner (EUB 6500 or Hivision Preirus, model EZU-MT28-S1). Ultrasound frequencies between 5 and 7.5 MHz were used with imaging depth up to 10 cm.

Assessment of T4 staging

All EUS procedures undertaken for the diagnosis and staging of lung cancer were performed in a systematic manner. Firstly, if indicated, the left adrenal gland was assessed. This was followed by the systematic assessment of all mediastinal nodal stations visible from the esophagus and, if deemed indicated, these stations were sampled[16]. Thirdly, the intrapulmonary tumor was visualised from the esophagus by rotating the ultrasound transducer in a 360 degree fashion from the distal esophagus at the level of the liver to the aortic arch. In cases where the intrapulmonary tumor was detected by EUS, the surrounding environment (vessels/ left atrium/ mediastinum) was scanned for possible tumor invasion.
Vascular invasion (T4) as assessed by EUS was defined as an interruption of the intimal layer of a great vessel or evidence of tumor encroachment into the vasculature (see figure 7.1A) or left atrium. Possible vascular invasion was further evaluated using colour Doppler ultrasonography.
Mediastinal invasion (T4) was documented if there was continuous opposition of the tumor with the mediastinal space without a separation between the two structures, e.g. invasion of the tumor, in the parietal pleura and centrally located vascular structures. An assessment regarding tumor invasion was further supported by dynamic manoeuvres of the endoscope. In selected cases, where the procedure was performed under conscious sedation, the patient was asked to take a deep breath and an assessment was made whether there was evidence of sliding between the lung tumor and the mediastinum.

In the EUS report the following was systematically noted: detection of the primary lung tumor (yes/no) and if so the presence of endosonographic signs of vascular or mediastinal invasion (yes/no).

Figure 7.1A *Patient with a centrally located squamous cell carcinoma of the left lower lobe*

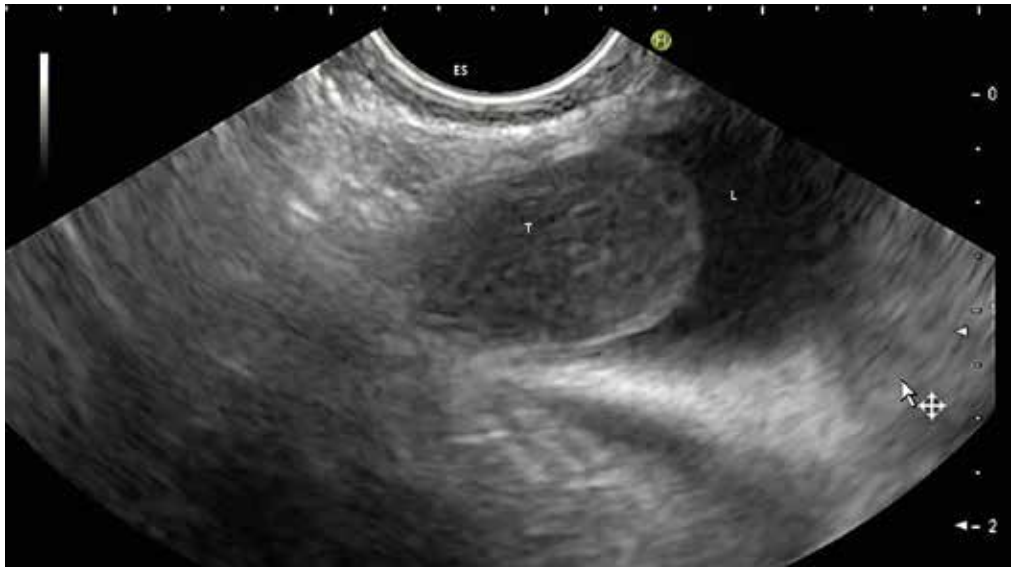


CT scan of the chest, demonstrating a left lower lung tumor (T) with suspected invasion left atrial lung tumor (T) invading the left atrium (L).
ES= lumen of the esophagus

CT scan of the chest

CT scans of the chest were collected for study purposes and were reviewed by an independent thoracic radiologist (IB) who was unaware of the initial CT report, EUS, intraoperative or pathology findings. For study purposes, the supplemental study report included details on the size and location of the tumor, T4 status (yes/no) and the nature of the T4 status.
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, obvious invasion of vessels, tumor contact with more than one fourth circumference of the vessel, obliteration of fat planes that are normally seen adjacent to vessels.[17] *(see figure 7.1B)*

Figure 7.1B



EUS image (GI scope) demonstrating clear invasion of the tumor (T) in the left atrium (L).
ES= lumen of the esophagus

Surgical pathological staging

In all cases a lobectomy/pneumonectomy was performed according to current standards and guidelines[18,24].

Surgical pathological T4 was defined as tumor invasion of any degree into the diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, carina, esophagus or vertebral body[2].

FINAL DIAGNOSIS AND ANALYSIS

The final tumor status was determined by a multidisciplinary tumor board including surgeons, pathologists, respiratory physicians and radiologists.

The final analysis was performed on cases where the T status of the intrapulmonary tumor was assessed by EUS and verified by surgical- pathological staging. The tumor status as assessed by EUS and CT was compared to the final surgical- pathological staging (reference standard). Sensitivity, specificity, positive predictive value and negative predictive value of EUS, CT scan of the chest and the combination of EUS/CT thorax for T4 status were calculated.

The EUS findings were classified into four separate categories.

1: True negative: the absence of tumor invasion at EUS and confirmed at surgical-pathological staging.
2: True positive: tumor invasion as judged by EUS was confirmed by surgical-pathological staging.
3: False negative: the tumor as seen at EUS and judged not to be T4 whereas the final surgical-pathological staging demonstrated definite tumor invasion
4: False positive: the tumor defined as T4 by EUS was down staged to T1-T3 at final surgical-pathological assessment.

Interpretation errors were defined as follows: tumor detected by EUS and a decision regarding the T4 status was made but proved to be wrong at surgical pathological staging. A diagnostic error was defined when the subsequent thoracotomy confirmed the presence of tumor invasion in an area of the mediastinum or great vessels which had not been assessed by EUS.

The combination of EUS and CT findings were also correlated to the final surgical- pathological staging. Patients were included in this analysis if both EUS and CT findings were concordant regarding the T4 stage.

Statistical analysis was performed using SPSS version 21.0 (SPSS Inc, Chicago, and III).

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.

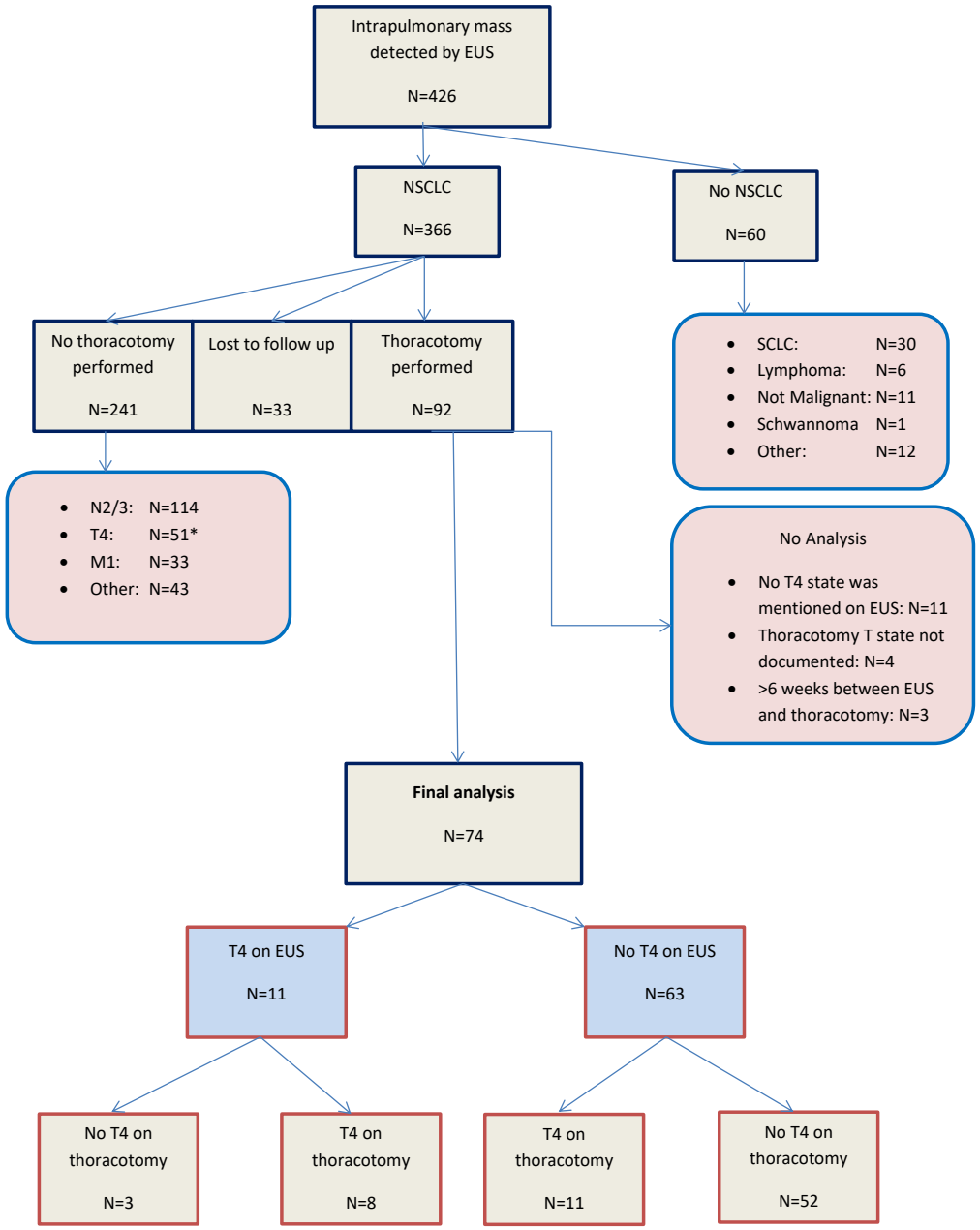
RESULTS

Patients

In total, 426 consecutive patients with known (or suspected) lung cancer were identified between May 2000 and January 2016 in whom the primary lung tumor was assessed by EUS n=399 (94%) or EUS (B) n=27 (6%). 366 (86%) patients were diagnosed with NSCLC and 60 patients (14%) had an alternative final diagnosis. 241/ 366 (66%) patients did not undergo a thoracotomy, primarily because of N2/N3 disease. 33/366 (9%) patients were lost to follow up. In total, 92/366 (25%) patients subsequently underwent a thoracotomy. Of these cases, 11 patients were excluded from analysis as the presence or absence of mediastinal tumor invasion was not documented at EUS. In 4 cases the surgical- pathological report did not document T4 status and in 3 cases the time interval between the EUS staging procedure and the final thoracotomy exceeded 6 weeks.

Therefore, a total of 74 cases were included in the final analysis (Figure 7.2, Flowchart). Baseline patient characteristics are presented in Table 7.1. In summary, most tumors were left sided, the median age of patients was 61 years and 64% were male. The chest CT scan was available for review in 66 out of 74 (89%) cases.

Figure 7.2 *Flow chart of patients with a centrally located lungtumor which was detected by EUS and who underwent subsequently surgical-pathological staging to confirm T status*



Final Diagnosis

Of the 74 patients included in the final analysis, surgical-pathological staging demonstrated mediastinal tumor invasion (T4) in 19 (26%) patients based on vascular (n=8, 42%) or mediastinal (n=8, 42%) invasion or both (n=2, 11%); a single patient (5%) had vertebral involvement. The remaining 55 (74%) patients had no surgical-pathological evidence of T4. Finally, 80% of all resections were R0 resections.

EUS

At EUS, 11 patients were judged to have stage T4 tumors, of which 8 were confirmed at subsequent surgery. The 8 true positive cases included invasion in of the mediastinum (n=5), the pulmonary artery (n=1) and pulmonary vein (n=2). The 3 false positive cases all involved patients with suspected vascular invasion into the pulmonary vein.

Number of patients analyzed:	74
Age (yrs, median (range)	61 (39-79)
Male	48 (64%)
Mean tumor size	53mm (20-130)
Location of primary tumor:	
LUL	31 (41%)
LLL	15 (20%)
RUL	14 (20%)
RLL	14 (19%)
EUS	71 (96%)
EUS-B	3 (4%)
Final histological diagnosis:	
Squamous cell carcinoma	34 (45%)
Adenocarcinoma	26 (36%)
NSCLC undifferentiated	14 (19%)

Table 7.1 *Baseline characteristics of patients of whom the lungtumor was detected by EUS and who subsequent underwent a thoracotomy*

The remaining 63 patients did not demonstrate signs of tumor invasion at endosonography. At thoracotomy, 11 of patients were diagnosed with T4 disease. These false negative cases included patients with mediastinal invasion (n=5) and vascular invasion (n=5). Finally, one patient had thoracic spine involvement. (Interpretation error (n =5), diagnostic error (n=3) both (n=3). Sensitivity, specificity, positive predictive value, negative predictive value of endosonography for diagnosing T4 was 42%, 95%, 735, 83% respectively *(Table 7.2).*

Table 7.2 *Diagnostic parameters for mediastinal tumor invasion (T4) for EUS, CT and both techniques; surgical-pathological staging was the reference standard*

	EUS (n=74)(CI)	**CT (n=66) CI)**	**EUS/CT combined #(n=34)** **(CI)**
Sensitivity	42% (20-67%)	76% (50-93%)	83% (36-100%)
Specificity	95% (85-99%)	61% (46-75%)	100% (88-100%)
PPV	73% (44-90%)	41% (31-52%)	100%
NPV	83% (76-87%)	88% (76-95%)	97% (82-99%)
Accuracy	81%	65%	97%

 # calculated in those patients in whom EUS and CT findings were the same regarding the presence or absence of tumor invasion.

CT scan of the chest
In 66 of 74 (89%) patients, a CT scan of the chest was retrospectively evaluated for study purposes. In a total of 8 (11%) patients the CT scans were not available for review. Intravenous contrast was used in all scans. Mean CT slice thickness was 3.5 mm (range 1- 10 mm). T4 stage was documented in 32/66 patients (48%) of which 13 cases (41%) were confirmed at thoracotomy. 19 cases (59%) were falsely staged as T4. Of the remaining 34/66 cases (52%), 4 cases (12%) were false negative. The sensitivity, specificity, PPV, NPV of CT of the chest was 76%, 61%, 41%, 88%. *(Table 7.2)*

In the 66 patients in whom both EUS and CT data were available for analysis the sensitivity for mediastinal tumor invasion was 41% for EUS and 77% for CT (p =0.109) with a specificity of 96% for EUS and 61% for CT (p <0.0001).

Combined CT/EUS analysis
Overall, 34 of the 74 patients had concordant EUS and CT outcomes regarding the T4 status. Of these cases, 5 (15%) were judged to have T4 at the combined CT and EUS: these findings were all confirmed at subsequent thoracotomy. Of the 29/34 patients (85%) without tumor invasion at combined CT and EUS assessment, a single patient (3%) turned out to have a T4 tumor at surgical pathological staging. The sensitivity, specificity, PPV and NPV of combined EUS-CT was 83%, 100%, 100%, 97% respectively. *(Table 7.2)*

DISCUSSION

In this study the role of esophageal ultrasound (EUS) for the assessment of mediastinal/vascular tumor invasion (T4) in patients with non-small cell lung cancer (NSCLC) was evaluated. We found that the sensitivity, specificity, positive predictive value and negative predictive value of EUS for diagnosing mediastinal/great vessel invasion was 42%, 95%, 73%, 83% respectively. The specificity and PPV of EUS were superior to chest CT findings (95% vs 61% and 73% vs 41%). Importantly, the combination of EUS and CT had an excellent specificity, positive and negative predictive values. The outcomes of this study are important because the addition of EUS assessment of para esophageal located lung tumors improves loco regional staging and can avoid exploratory thoracotomies.

Data regarding mediastinal tumor invasion (T4) in lung cancer is scarce. Varadarajulu et al diagnosed T4 tumors by EUS criteria in 10 out of 175 lung cancer patients[14]. However, in their study, only 2 patients had EUS documented T4 status confirmed at thoracotomy. In the remaining 165 patients without EUS evidence of T4, there was one false negative case where aortic invasion was not detected by either EUS or CT. This study was limited by the use of different EUS scopes including radial probes.
Schröder et al investigated the role of EUS for aortic wall involvement in lung cancer patients with a left sided tumor abutting the aorta. In 97 patients, the results of EUS and CT/MRI were compared with surgical/pathological results. EUS had significantly higher sensitivity (83%) compared to CT interpretation (17%) for the evaluation of T4 status[15].

EUS has excellent access to nodes in the lower mediastinum and the left paratracheal region and complements the role of EBUS by assessing a different range of nodal stations[10]. In addition to nodal staging, a recent review described the feasibility and safety of diagnosing para-esophageally located lung tumors by EUS[13].The yield of EUS for diagnosing the intrapulmonary lung tumor is very high in selected cases. An advantage of high resolution EUS imaging over CT scan is the detailed assessment of tumour to nearby structures - mediastinum interface, the real time character and the ability to assess dynamic features.
For instance, sliding of the lung tumor adjacent to the aorta excludes tumor invasion at this specific site. The use of colour Doppler might be helpful in selected cases to detect vascular structures and demonstrate or exclude tumor invasion at that location.

The confirmation of direct mediastinal and/or great vessel invasion by a lung tumor (T4, stage IIIB), has a profound effect on treatment decisions and subsequent prognosis

of patients with NSCLC. With the exception of some highly selected cases, who may benefit from a radical surgical approach[19,20], the majority of patients are best treated with combined chemo-radiotherapy[21].

Some limitations apply to this study. This is a retrospective study and the interpretation of the results is limited by inherent flaws in the study design associated with retrospective studies. In addition, this study analysed data from 2 centres with expert EUS operators. It remains unclear if less experienced endoscopists can achieve similar results. Although it seems that EUS(B) using the EBUS scope has similar operating characteristics when compared to a regular GI EUS scope for pulmonary indications[10,22], it has to be stated that the vast majority of cases in the present study were evaluated using a GI EUS scope. The regular GI EUS scope contains more ultrasound crystals resulting in superior imaging and greater depth assessment. Whether the findings will be reproduced when performed with an EBUS scope has to be confirmed in future studies. Also visualization of intrapulmonary tumors from the esophagus is only possible if the tumor is located very near or immediately adjacent to the oesophagus. As the esophagus is located in the left posterior chest, this most often applies to central located left sided tumors. It is important to realize that the presence of air between the esophagus and the intrapulmonary tumor inhibits visualisation. A number of potential limitations with respect to cross sectional imaging must also be highlighted including the variety of CT protocols undertaken, slice thickness, and in addition the study spanned a 15 year period. These factors may in part offer an explanation for the overall moderate accuracy of the CT Thorax assessment alone. Important aspects of this study include the large sample size and excellent reference standard.

We believe the findings of this study provide important lessons to physicians who assess patients with (suspected) lung cancer. In those subjects with lung tumors abutting the esophagus who are possible candidates for surgical resection, staging by EUS provides important additional T information which is complementary to findings at chest CT. Endosonography is indicated for mediastinal nodal staging and those with centrally located lung tumors who are candidates for surgical resection. Based on the present data, T4 assessment by EUS should be considered following a mediastinal nodal examination to improve T staging and therefore reduce the number of exploratory and futile thoracotomies.

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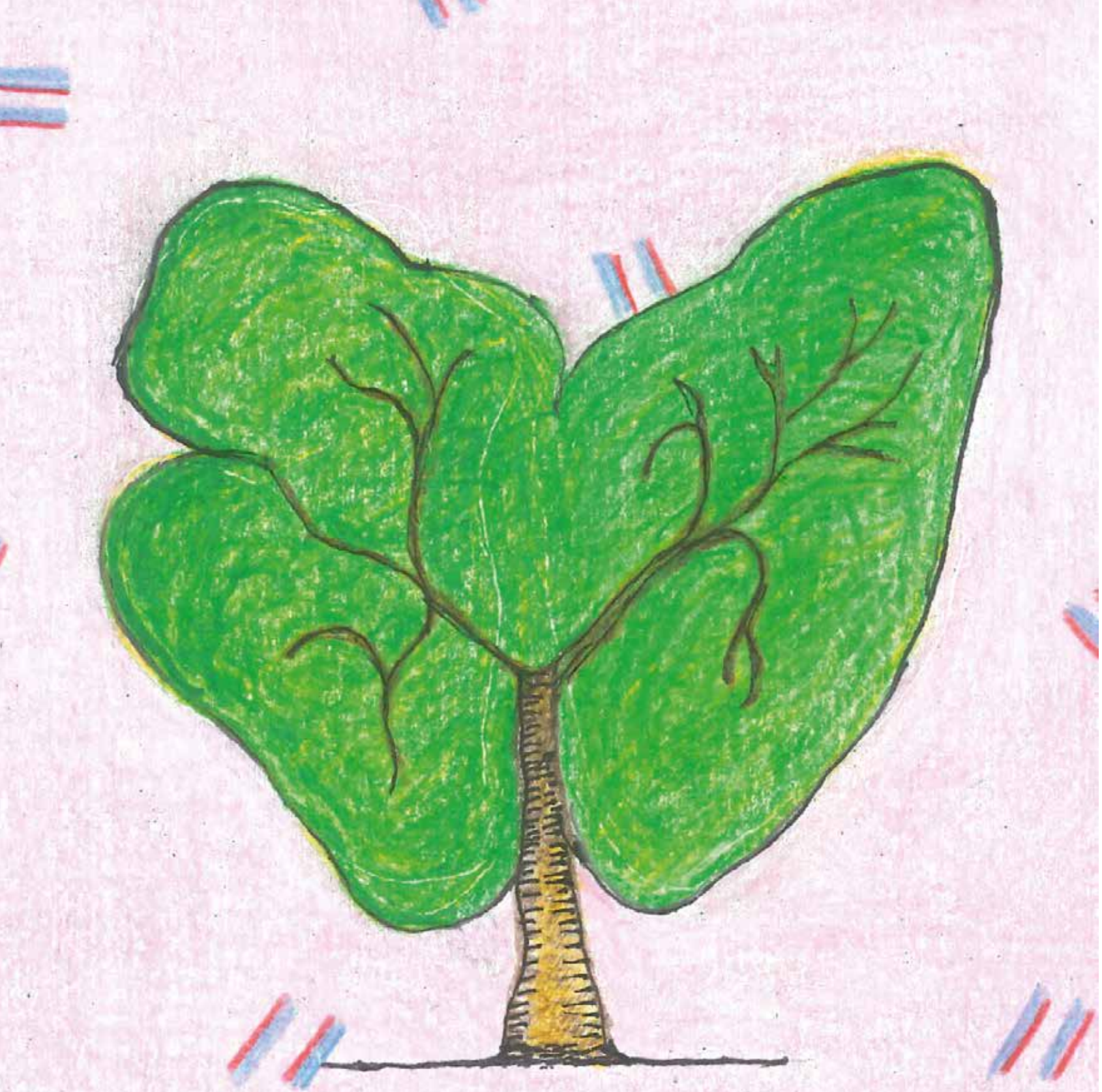

Part 3

Novel lung cancer staging strategies and its impact on survival

PART THREE CHAPTER 8

5 year survival after endosonography vs mediastinoscopy for mediastinal nodal staging of lungcancer.

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INTRODUCTION

Lung cancer accounts for the highest cancer-related mortality rate worldwide.[2] Accurate mediastinal nodal staging is crucial in the management of non-small cell lung cancer (NSCLC) as it directs therapy and has prognostic value.[1,3]

ASTER (Assessment of Surgical Staging vs Endosonographic Ultrasound in Lung Cancer: a Randomized Clinical Trial) compared a surgical (mediastinoscopy) with an endosonographic staging strategy (combined use of endobronchial and transesophageal ultrasound, followed by mediastinoscopy if negative).[4] The endosonographic strategy was significantly more sensitive for diagnosing mediastinal nodal metastases than surgical staging (94% vs 79%).

If mediastinal staging is improved, more patients should receive optimal treatment and might survive longer. The current post-hoc analysis evaluated survival in ASTER.

METHODS

Of 241 patients with potentially resectable NSCLC, 123 were randomized to the endosonographic and 118 to the surgical staging strategy in 4 tertiary referral centers in Leiden (the Netherlands), Ghent and Leuven (Belgium) and Cambridge (United Kingdom) between February 2007 and April 2009.[4] Surgical-pathological staging was the reference standard for mediastinal nodal assessment. The current analysis was either approved or waived by the involved ethical committees.

Between 30 June and 15 October 2015, survival data were obtained through patient records, death registers or contact with general practitioners.

The proportion of survivors at 5 years for both staging strategies and odds ratios with 95% confidence intervals were calculated. Kaplan-Meier analysis was performed to compare median survival across the strategies. Patients with no date of death were censored on the date they were last known to be alive. Subgroup analysis was performed for patients with nodal stages N2/N3 and N0/N1. Data were analyzed using SPSS v.22. (SPSS Inc, Chicago, Illinois).

RESULTS

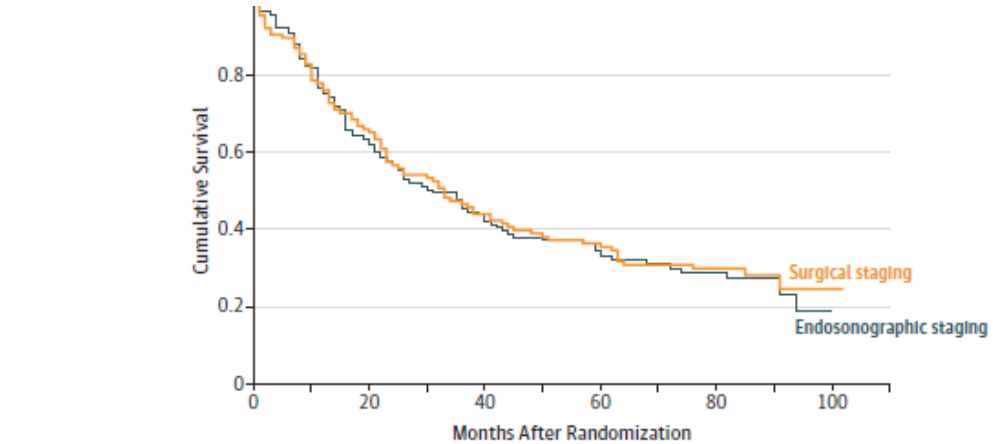
Survival data were obtained for 237/241 patients (98%) - 182 males (77%) - with a mean age at randomization of 65 years (SD 9).

Survival at 5 years was 35% (42/121) for the endosonographic versus 35% (41/116) for the surgical strategy (odds ratio 0.97 (95% CI 0.57-1.66)) (Table 8.1). The estimated median survival was 31 months (95% CI 21-41) versus 33 months (95% CI 23-43), respectively (hazard ratio 1.04 (95% CI 0.77-1.40) (Figure 8.1).

Table 8.1 *Survival of the endosonographic versus the surgical staging strategy*

	Survival at 5 years n/N (%)	Odds ratio (95% CI)	Estimated median survival in months (95%CI)	Hazard ratio (95%CI)
Overall	**83/237 (35)**		**33 (26-40)**	
Endosonographic staging strategy	42/121 (35)	0.97 (0.57-1.66)	31 (21-41)	1.04 (0.77-1.40)
Surgical staging strategy	41/116 (35)		33 (23-43)	
N2/N3	**21/116 (18)**		**21 (17-25)**	
Endosonographic staging strategy	11/64 (17)	0.87 (0.34-2.25)	21 (15-27)	1.04 (0.70-1.55)
Surgical staging strategy	10/52 (19)		22 (15-27)	
N0/N1	**62/121 (51)**		**62 (39-85)**	
Endosonographic staging strategy	31/57 (54)	1.27 (0.62-2.60)	72 (38-106)	0.91 (0.57-1.44)
Surgical staging strategy	31/64 (48)		57 (30-84)	

Figure 8.1 *Survival among patients with lungcancer in the endosonographic vs surgical staging strategies*



Adjusted for mediastinal nodal metastases status (N0/N1 vs N2/N3) (adjusted hazard ratio, 0.98 [95%CI, 0.73-1.32]). The median duration of follow-up was 33 months (interquartile range [IQR], 13-76) for surgical staging and 31 months (IQR, 13-75) for endosonographic staging.

In the subgroup of patients with N2/N3 metastases, survival was 17% (11/64) in the endosonographic versus 19% (10/52) in the surgical group (odds ratio 0.87 (95%CI 0.34-2.25)). In the subgroup of patients with N0/N1 metastases, survival was 54% (31/57) versus 48% (31/64), respectively (odds ratio 1.27 (95% CI 0.62-2.60)).

DISCUSSION

No survival difference was found 5 years following randomization to an endosonographic or surgical staging strategy of patients with NSCLC. Since the original results of ASTER were published, clinical guidelines on lung cancer management underwent major revisions and now advocate endosonography instead of mediastinoscopy- as the initial step for mediastinal nodal staging.[1,3] The endosonographic strategy is more accurate, less invasive, reduces unnecessary thoracotomies [4] and is cost-effective.[5]

Data from a recent randomized trial shows prolonged survival in patients who underwent endosonography compared to conventional staging.[6] However, most patients in the latter group underwent bronchoscopy instead of mediastinoscopy. To our knowledge, ASTER is the first randomized trial to evaluate survival outcomes between endosonographic and surgical staging strategies.

Why did improved mediastinal staging not lead to improved survival? Missing data occurred in less than 2% and therefore are an unlikely source of bias. However, by chance, the prevalence of mediastinal nodal metastases in the surgical group was lower compared to the endosonography group (44% versus 54%). This might have negatively affected survival in the latter group. Also, ASTER was powered to detect a difference in diagnostic sensitivity, not survival. This is the main limitation of the current analysis and reflected by the wide confidence intervals. If a survival difference between the strategies exists, it is likely to be small and a larger sample size may be needed to detect it. However, randomized trials to detect a survival difference upon staging strategy are not likely to be conducted as the endosonographic strategy is now advised in clinical guidelines.[1,3]

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PART THREE

CHAPTER 9

General discussion

The aim of this thesis was to further explore the role of endosonography in the diagnosis and staging of lung cancer with a focus on lung tumor diagnosis and assessment of tumor invasion. This thesis consists of three parts.

In part 1 titled "Endobronchial and esophageal ultrasound for diagnosing lung tumors" (chapters 1-5), we evaluated the diagnostic accuracy and safety of endosonography (EBUS, EUS-B and EUS) in obtaining a tissue diagnosis in lung tumors located adjacent to the major airways and the esophagus. First, a systematic review and meta-analysis regarding EBUS for diagnosing central lung tumors was performed. Second, the role of EBUS, EUS-B and EUS for diagnosing centrally located lung tumors located near or adjacent to the major airways, respectively the esophagus was evaluated. Third, two cases of atrial tumors are presented diagnosed by endosonography. In the final chapter of this part, a protocol of an ongoing randomized controlled trial comparing two different EBUS needles was presented.

In part 2 titled "Endobronchial and esophageal ultrasound for T staging of lung tumors" (chapters 6 and 7) the role of EBUS and EUS for assessing the T4 status (e.g. lung tumor invasion in the mediastinum or central vessels) in patients with lung cancer was evaluated, as well as its added value to CT-scan of the chest.

Part 3 is titled "Novel lung cancer staging strategies and its impact on survival" (chapter 8). The 5 year survival rates of lung cancer patients are discussed of patients that were initially staged by endosonography vs those that underwent immediate surgical staging. The final chapters of this thesis contain the summary, key findings and general discussion including future perspectives.

Background

In patients with suspected lung cancer, it is important that diagnosis and staging is performed quick, safe and accurate in order to initiate appropriate therapy promptly. Multiple diagnostic tests can be used for this purpose. Imaging tests, like CT scan of the chest and FDG –PET scanning provide information about tumor size, its invasion in surrounding structures and the presence of metastases inside and outside the thorax.[1-4] Subsequently, (minimally) invasive techniques are needed to obtain a tissue diagnosis regarding the type of lung cancer including molecular analysis and its immunologic profile.[5, 6] International guidelines advise tissue verification of mediastinal and hilar nodes in case CT or PET-CT shows nodal enlargement.[7] EBUS is recommended, if possible, combined with EUS or EUS-B to optimize regional nodal staging .[7, 8] For patients presenting with a centrally located lung tumor or a tumor >3 cm, staging of the mediastinum and hilar region with endosonography is also recommended, even in the absence of nodal enlargement.[7]

Although abundant literature exists regarding the role of endosonography in regional nodal staging, its role in primary lung tumor diagnosis is limited and data regarding its value in assessing tumor invasion are scarce.

PART 1 ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR DIAGNOSING LUNG TUMORS

In **chapter 1**, we performed a systematic review and meta-analysis to evaluate the diagnostic yield and sensitivity of EBUS-TBNA for diagnosing centrally located lung tumors. We identified 14 studies, published between 2008 and 2018, overall including 1175 patients. On average, we found that EBUS-TBNA had a diagnostic yield of 89% (95%CI 80-92) in diagnosing the lung tumor and a sensitivity of 91% (95%CI 88-94) to diagnose malignancy. Complications were reported in 5% of patients and were mostly minor. All studies had a high risk of bias or applicability concerns when assessed by QUADAS-2,[9] which may have resulted in overestimations of accuracy. The main bias was the retrospective design and patient selection. Also, definitions of a centrally located lung tumor differed across studies, ranging from the inner one third (in line with the definition of the American College of Chest Physicians)[6] to the inner two thirds of the hemi-thorax (in line with the European Society of Thoracic Surgery guidelines and National Comprehensive Cancer Network).[10, 11]

In **chapter 2**, we report our own findings in a retrospective analysis of the diagnostic yield and safety of EBUS-TBNA in patients with a centrally located lung tumor without endobronchial abnormalities at conventional bronchoscopy. In this study, we defined a centrally located lung tumor, as a central lesion in which the medial margin stays within the inner third of the chest.[6] We added to this definition that the lesion should be adjacent to the larger airways and therefore in potential reach of EBUS. Of the 163 patients in whom the tumor could be visualized by EBUS, the tumor was sampled in 89% of cases, resulting in diagnostic yield of 83%. The sensitivity for diagnosis malignancy was 96%, which is similar to the meta-analysis presented in chapter 1. Diagnostic yield was independent of tumor location (i.e., paratracheal, mainstem, lobar, or segmental bronchus). No major complications occurred.
The findings of *chapter 1 and 2* imply that a lung tumor diagnosis can be safely performed by either EBUS-TBNA or EUS-FNA in the vast majority of selected patients with a lung tumor situated adjacent to the major airways or esophagus.

In **chapter 3**, we performed a similar retrospective analysis for EUS-B-FNA, by focusing on patients with a centrally located lung tumor adjacent to the esophagus. We included 58 patients and found a diagnostic yield of 90% in diagnosing the lung tumor, and a sensitivity of 90% for diagnosing malignancy. Most patients (91%) also underwent conventional bronchoscopy and EBUS, prior to performing EUS-B in the same session. In this study, diagnostic yield of bronchoscopy alone was 26%, which increased to 51% when adding EBUS, and to 91% when adding EUS-B. No complications occurred during the EUS-B procedures. These findings are in line with those of a previously published systematic review from our research group which evaluated the performance and safety of the conventional EUS scope in diagnosing centrally located lung tumors, diagnostic yield of 90% (95%CI 82-95), and a sensitivity of 92%(83-96).[12] These findings imply that both EUS and EUS-B can be used safely to diagnose centrally located lung tumors. An advantage of using EUS-B-over a conventional EUS-scope is that complete lung tumor diagnosis and

systematic mediastinal and hilar staging can be performed with just an EBUS scope and a single operator.[8, 13]

In **chapter 4**, we present two cases in whom a solitary left atrial tumor could be safely diagnosed by endosonography (one with the regular EUS scope, and one with the EBUS scope) under real-time ultrasound guidance. In both cases, cytology and cell block analysis provided a clear diagnosis, including the necessary immunohistochemistry. Similar cases have rarely been described in the literature.[14-17] Altough only very selected cases will be eligible for endosonography-based diagnosis of cardiac tumors, it is important to be aware of this minimally invasive alternative to diagnostic heart surgery. Operators should obviously be experienced and alternative diagnostic approaches should be carefully reviewed and discussed.

In **chapter 5**, we report on a protocol for a randomized clinical trial in which we will compare the recently developed Acquire™ versus the conventional 22G TBNA needle in EBUS/EUS-B guided sampling of mediastinal/hilar lymph nodes and primary lung tumors. This new three-plane symmetric Acquire needle with Franseen geometry may allow for a higher suitability rate for the assessment of PD-L1 expression on the cell block. Besides assessing a cancer diagnosis, assessment of PDL1 and molecular profiling is of increasing importance, therefore needles that enable to obtain more tumor cells from the lesion are needed.

To date, there are no data about the Franseen biopsy needle for diagnostic and staging purposes during EBUS/EUS-B procedures in patients with (suspected) lung cancer.

The main outcomes of this part show that in patients with lung tumor located adjacent to the larger airways or esophagus, a tissue diagnosis can be safely obtained by endosonography. These findings are important for clinical practice as high risk CT guided FNA and diagnostic surgical procedures can be avoided.

PART 2 ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR T STAGING OF LUNG TUMORS

In **chapter 6**, we retrospectively evaluated the diagnostic accuracy of EBUS for the assessment of mediastinal or vascular tumor invasion (T4-status) in 104 patients with NSCLC who subsequently underwent lung tumor resection as the reference standard. Sensitivity, specificity, PPV and NPV for diagnosing T4-status were 64% (95%CI 46-79%), 94% (84-98%), 82% (66-92%) and 83% (76-88%), respectively. We concluded that the overall accuracy of EBUS for tumor staging is moderate and, in most cases, insufficient to rule-in or rule-out T4-status as a stand-alone test. However, the combination of a negative EBUS and a negative chest CT (absence of tumor invasion) resulted in an NPV of 94% (95%CI 72-99), which ruled out T4 disease with a high level of certainty. These patients in particular may benefit from surgical lung tumor resection.

In **chapter 7**, we retrospectively evaluated EUS for the assessment of T4 status. In 74 patients with NSCLC, staged by EUS who subsequently underwent surgical tumor resection, we found that the sensitivity, specificity, PPV and NPV were 42% (95%CI 20-67%),95% (85-99%), 73% (44-90%) and 83% (76-87%), respectively. These findings show that also EUS is moderately accurate in diagnosing T4-status as a stand-alone test. However, a combination of a negative EUS with a concordant negative chest CT (absence of tumor invasion) resulted in a specificity of 100% (95%CI 88-100%) and a NPV of 97% (95%CI 82-99), rule out tumor invasion with a high degree of certainty.

The outcomes of the studies reported in chapter 6 and 7 are important because the assessment of tumor invasion, by carefully assessing the relation of the tumor with its surroundings, improves tumor staging. Improved tumor staging potentially reduces exploratory thoracotomies and allocates more patients to the appropriate treatment.

PART 3: NOVEL LUNG CANCER STAGING STRATEGIES AND ITS IMPACT ON SURVIVAL

In **chapter 8** we reported on the 5 year follow-up analysis of the ASTER randomized trial,[18] which compared an immediate surgical staging strategy (mediastinoscopy) with an endosonographic centered staging strategy (combined use of endobronchial ultrasound (EBUS-TBNA) and transesophageal ultrasound (EUS-FNA), and if negative followed by mediastinoscopy). In that trial, 241 patients with resectable NSCLC were randomized, showing a significant difference in sensitivity for diagnosing mediastinal nodal metastases, favoring the endosonographic strategy (79% versus 94%).[18] In the present follow-up analysis, we evaluated five-year survival across both strategies and found it to be similar (35% in both arms). These findings illustrate that improved nodal staging does not always result in improved patient important outcomes such as mortality. Among the many advantages of the newly adopted endosonographic staging approach are, its improved nodal staging accuracy, its minimally invasive approach, the associated reduction of unnecessary thoracotomies and its cost effectiveness.[19]

Key findings and considerations

We found that in patients with lung tumors located adjacent or close to the airways or the esophagus, endosonography is able to obtain a tumor tissue diagnosis in the vast majority of cases. These findings are important, because besides nodal staging, a tumor diagnosis can be established during the same procedure. As a consequence, high risk CT guided biopsies and exploratory thoracotomies can be avoided.

Additionally, we demonstrated that endosonography can assess tumor invasion in the surrounding structures (T4). Although moderate accurate as a stand- alone test, a negative EBUS/EUS with a concordant negative chest CT (absence of tumor invasion) can rule out tumor invasion in the surrounding structures. This implicates that, besides nodal staging and tumor diagnosis, staging of the T descriptor can also be performed with endosonography in selected patients. Improved tumor staging potentially reduces exploratory thoracotomies and the allocation of patients to the optimal treatment.

Most of the presented studies in this thesis regarding endosonographic tumor diagnosis and staging are retrospective and therefore the interpretation of the results are limited by inherent flaws in the study design and patient selection. However, the data are convincing, and therefore endosonography qualifies as a safe and minimally invasive diagnostic tool for central lung tumors presenting without endobronchial abnormalities. It will be important to spread the current knowledge with chest radiologists, pathologists and pulmonologists and train EBUS users not only in nodal assessment but also in tumor diagnosis and staging.

Data from the ASTER randomized[18] study showed that endosonographic staging does not result in improved 5-year survival when compared to immediate surgical staging. However, the ASTER trial was not powered to detect a difference in survival. If a survival difference between the strategies exists, it is likely to be small and a larger sample size may be needed to detect it. Data from another randomized staging trial showed prolonged survival in patients who underwent endosonography compared with conventional staging[20]. However, most patients in the latter study underwent bronchoscopy instead of mediastinoscopy. Randomized trials to detect survival difference based on staging strategy are not likely to be conducted as the endosonographic strategy is nowadays advised in clinical guidelines.[7]

Although survival benefit has not yet been demonstrated, endosonography staging has many advantages over initial surgical staging, including improved nodal staging accuracy, its minimally invasive approach, the associated reduction of unnecessary thoracotomies, reduce of use of operating theaters and cost effectiveness.[19] The use of confirmatory mediastinoscopy after tumor negative (absence of N2/3 disease) endosonography is under debate. In the MEDIAST trial (NTR 6528), NSCLC patients staged negative by endosonography were randomised between immediate surgical lung tumor resection and mediastinoscopy, if negative followed by tumor resection. Inclusion is completed and the data will shed light to what extent confirmatory surgical staging detects unforeseen nodal metastases and at what price. (Number of patients needed to undergo mediastinoscopy to detect a single patient with missed N2 disease, patient delay, complications, hospital costs etc). The MEDIAST trial will also assess 2- and 5-year survival data.[21]

Endosonography for lung cancer staging

How can optimal bronchoscopic and endosonographic diagnosing and staging of lung cancer be performed with the current knowledge? The combination of bronchoscopy with EBUS and EUS-B in a single session under deep sedation (e.g. propofol) by a single operation is optimal.[8, 13] With conventional bronchoscopy, a complete inspection of the airways is possible and in case of endobronchial abnormalities, biopsies can be taken. EBUS followed by EUS-B allows complete and systematic evaluation of the mediastinal and hilar nodes and assessment of the left adrenal gland.[8, 13, 22, 23] *(figure 9.1)*. Data from this thesis show the value of endosonography for the T descriptor, by obtaining a tissue diagnosis and improving T4 staging.

Figure 9.1A EBUS landmarks for a complete assessment of the hilar and mediastinal lymph nodes

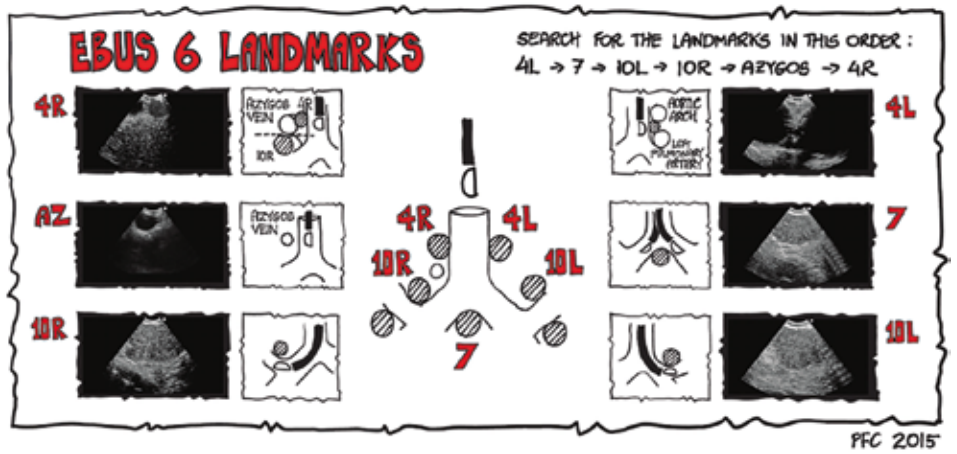
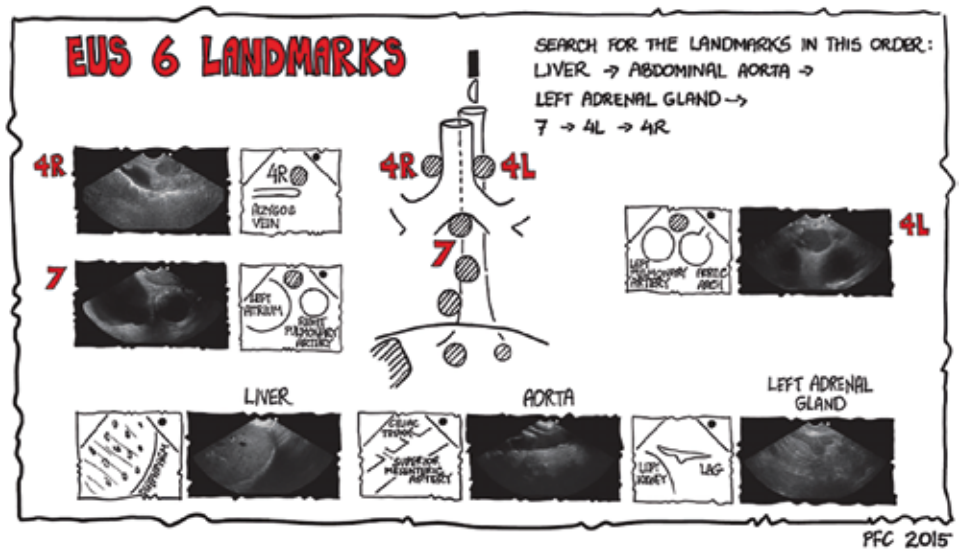


Figure 9.1B EUS landmarks for the assessment of the mediastinal lymph nodes and the left adrenal gland

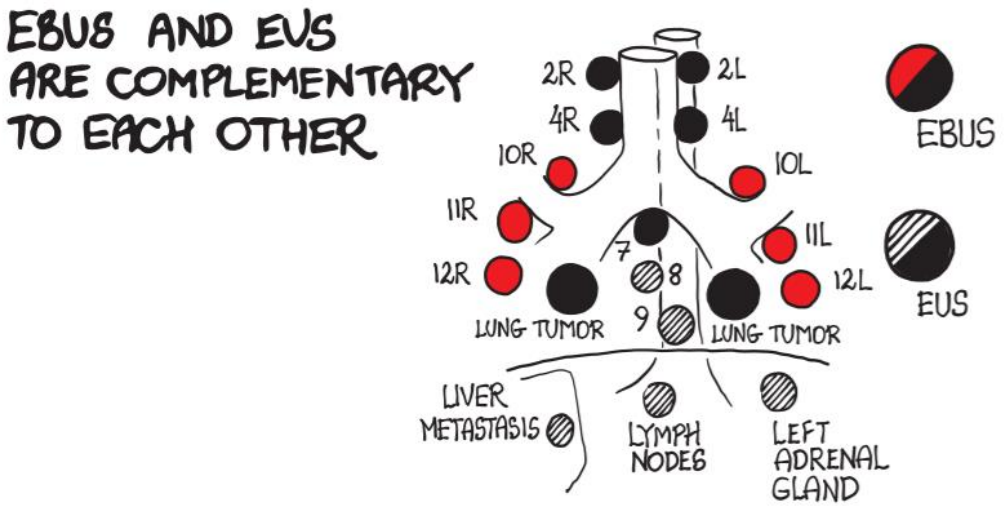


drawings by Paul Frost Clementsen

For an optimal and complete assessment of mediastinal and hilar nodal stations, evaluation and if possible tissue sampling of at least three different mediastinal nodal stations (4R, 4L, 7) is advised.[7] However, common practice for mediastinal nodal staging with endosonography mostly involves the so-called *"hit-and-run"* strategy, by which only the (single) node suspected on PET-CT is sampled.[24, 25] Crombag et al showed that after a systematic EBUS followed by EUS-B, the sensitivity of mediastinal nodal staging increases with 9% compared to the targeted approach.[8] The combined approach did not result in

more complications and the procedure takes just 10 minutes longer when EUS-B is performed after EBUS.[8] A recent systematic review showed that the addition of EUS(-B) to EBUS leads to a 12% gain in the detection of mediastinal nodal metastases[13]. Besides the complete mediastinal nodal staging with EBUS and EUS-B, the left adrenal gland can also be assessed in the same session. The left adrenal gland is important to assess, because the adrenal glands are predilection sites for distant metastases.[2] For left adrenal gland tissue sampling, the EBUS scope can be used (placed in the esophagus: (EUS-B) as well as the regular GI EUS scope[22, 23, 26] *(figure 9.2)*.

Figure 9.2 Diagnostic reach of EBUS and EUS (B) showing their complementary diagnostic reach



Drawing by Paul Frost Clementsen[30].

EBUS/ EUS training

For the spread and implementation of EBUS/ EUS-B, training is of key importance. The quality and safety of endosonography is highly dependent on the skill and experience of the operator. Diagnostic yield has been shown to increase, and the number of complications to decrease with operator experience.[27-29] Learning curves studies have shown that performance of 50 procedures does not ensure basic competency, although this varies considerably across endoscopists. Specific tools for assessment of performance in endosonography can be used for monitoring trainees' progression, and all programmes should continuously monitor their outcomes.[30, 31] The EBUS assessment tool (EBUS AT) evaluates both anatomical knowledge and EBUS skills to safely aspirate lymph nodes.[30-32] This tool can be used both to evaluate trainees on the simulator as on patients in clinical practice.[32]

Simulator training has shown to be more effective than traditional apprenticeship training.[32] A dedicated EBUS training program has been developed by the European Respiratory Society (ERS). This three-part training program teaches participants to perform endobronchial ultrasound (EBUS) independently and competently. Part one of the program covers the theoretical knowledge that is required for EBUS, part two focuses on clinical and simulation training, and part three includes self-practice and assessment.[33]

FUTURE PERSPECTIVES

To date, it is clear that endosonography has a central position in the diagnosis and staging of lung cancer. What can be expected in the future and what are the current challenges? In our opinion, it is important that EBUS users will learn, besides a 'targeted nodal sampling", the following: 1) how to perform a complete systematic EBUS nodal evaluation; 2) how to perform EUS-B; 3) how to diagnose and stage lung tumors and 4) how to find and sample the left adrenal gland. Furthermore, needles might be improved and new technologies developments are needed to asses lung lesions that are out of diagnostic reach with current available equipment.

Novel tools for obtaining a tissue diagnosis in suspected lung cancer

Obtaining a tissue diagnosis including immunologic and molecular profiling is crucial in the management of a patient with (suspected) lung cancer. For endosonography, several needle types and sizes (25-, 22-, 21-, and 19G) are commercially available.[34] There are limited randomized data and there is no expert consensus regarding the optimal needle size, type and needle handling performance (e.g. stylet and suction use). A recent meta-analysis about EBUS-TBNA needle size and tissue acquisition found no difference in terms of diagnostic yield for a specific needle gauge nor suction use.[35] Most commonly, the 22G needle is used. A recent studie failed to demonstrate superior outcomes using a larger 19G needle over 22G.[36] With the upcoming of immunotherapy and targeted therapies, assessment of PD-L1 expression and molecular profiling of lung malignancies has become crucial. In daily clinical practice, EBUS-TBNA and EUS- FNA samples have a variable success rate for assessment of PD-L1 expression and molecular profiling. Optimally, large histological tissue samples are required for such profiling.[37-39]

The development of new sampling tools such as a transbronchial needle forceps (TBNF) or transbronchial needle biopsy (TBNB) needles might improve the diagnostic yield of EBUS TBNA in this respect.[40] The EchoTip ProCore needle, is designed to provide core biopsies, and has a fissure close to the tip for histological sampling.[41] A retrospective analysis showed a statistically significant difference in the diagnostic sensitivity of sampling mediastinal lymphadenopathy using a ProCore needle compared with standard fine needle aspiration.[42] but more data are needed to make a more defenite assessment. Recently, a three-plane symmetric needle with Franseen geometry (Acquire™ 22G transbronchial needle biopsy (TBNB)) was developed in pancreatic cysts. The Acquire™ 22G needle allowed improved, true histological core tissue acquisition in pancreatic cysts.[43, 44] To date, a randomized controlled clinical trial is ongoing (NTR nr NL7701) evaluating the efficacy and outcomes of these needle in comparison to conventional 22G needles.

In addition to the development of safe techniques for obtaining tissues, sensitive laboratory diagnostic methods that can obtain accurate results with a small amount of tissue specimens should be developed. There are increasing arguments that lung tumors have a heterogeneous distribution of tumor cells and types. Often, a single biopsy will not provide the complete information about the molecular characteristics of the lung tumor.[45] Single-cell genome profiling technology provides the ability to assess intra-tumor heterogenicity [46,47]

EBUS technology

The diagnostic yield of conventional bronchoscopy for lung cancer – in the absence of endobronchial abnormalities, is low. The size and location, and visibility of lesion are important factors that influence the diagnostic yield.[48] If the centrally located tumor is located adjacent to the major airways or the esophagus EBUS and EUS(B) can be used to obtain a tissue diagnosis.[7] Even if the lung tumor is not directly located against the esophagus but in close proximity, it is possible during the EUS(B) procedure to obtain a tissue diagnosis.[49]

In most cases the lung tumor is not centrally located, but more peripheral located. The definition of peripheral pulmonary nodules/tumors, includes a nodule that is not visible endobronchially and is completely surrounded by lung parenchyma without associated atelectasis, effusion, or enlarged lymph nodes.[50] Regarding future EBUS equipment, miniaturization of the probes enabling real-time sampling of more peripherally located tumors will extent the use of EBUS.

Also, for peripheral located tumors/nodules a tissue diagnosis is crucial but given the risks of transthoracic approaches and invasiveness of surgical biopsy, recent advancements have been developed regarding image guidance to extend the bronchoscope's reach.

New imaging and diagnostic techniques for diagnosis lung tumor

New techniques have been developed to examine the lung in more detail and to go beyond the large airways. One of these is navigational bronchoscopy. A virtual image is made, and the needle is helped to reach places that cannot be seen or reached with a conventional bronchoscope.[51] Electromagnetic Navigation Bronchoscopy (ENB) is based on the high resolution CT and an electromagnetic (EM) field generated around the patient. A virtual image is reconstructed from the chest CT. The special probe is then inserted through the bronchoscope in the smaller airways to make a virtual map of the selected point [51, 52]
A meta-analysis of 16 studies demonstrated a sensitivity of 71% to detect malignancy in patients with pulmonary masses, with pneumothoraxes only occurring in 3%.[53] Another recent development is the robotic bronchoscopy. This technique uses a similar virtual map generated from reconstructed high-resolution CT and EMfield mapping but has redesigned the bronchoscope and utilizes robotic arms to maneuverer and drive it forward. Existing technologies, including radial EBUS, fluoroscopy and navigational bronchoscopy are integrated into these robot techniques.[54] Needle-based CLE (nCLE) is a promising imaging modality that allows real-time cancer detection. CLE is a laser-based technique that is executed with a fluorescent dye and enables real-time visualization of cell shapes, thereby acting as a real-time microscope.[55, 56] Interpretation of nCLE lung tumor imaging is based on three main characteristics. The presence of dark enlarged pleomorphic cells and dark clumps most accurately indicate the presence of a pulmonary malignancy.[57, 58]
In a recent study 15 patients with suspected peripheral lung cancer based on (PET-)CT scan underwent radial EBUS and fluoroscopy guided flexible bronchoscopy and in 87% a high quality video was obtained, without complications.[59] Combining robot technology (maneuvering to the target lesion) with real-time nCLE guided bronchoscopic tumor sampling is currently under evaluation (Clinical Trials.cov NCT04441749).

In conclusion, in this thesis the diagnostic and staging value of endosonography for lung tumors was evaluated. We showed that in patients with a centrally located lung tumor located adjacent or close to the airways or the esophagus, endosonography is able to obtain a tissue diagnosis of the tumor in the vast majority of cases. Additionally, endosonography can be used to assess tumor invasion in the surrounding structures (T4) and combined with CT improves tumor staging. The findings of this thesis are immediately applicable in daily clinical practice and many patients are expected to benefit from the suggested recommendations.

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PART THREE

CHAPTER

10

English summary

Introduction
Lung cancer is one of the most common cancers in the world. Obtaining a tissue diagnosis is very important because it directs both treatment and prognosis. Several diagnostic tests can be performed to obtain a tissue diagnosis. If there is a centrally located lung tumor, without a visible abnormality in the larger airways, it is often difficult to obtain a tissue diagnosis of the tumor. The invasion of the tumor in the surrounding structures such as the mediastinum and the great vessels (T4) is also important because this determines the treatment options.

The aim of this thesis was to further investigate the role of endosonography (EBUS, EUS-B and EUS), specifically in the diagnosis of the primary lung tumor itself and demonstrating or excluding local tumor invasion.

PART 1: ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR DIAGNOSING LUNG TUMORS

Chapter 1 provides the results of a review and meta-analysis where we evaluate the performance of linear endobronchial ultrasound guided – transbronchial-needle aspiration (EBUS-TBNA) for the diagnosis of centrally located lung tumors. 5,657 manuscripts were identified; of these 14 were included, including 1175 patients who underwent EBUS-TBNA for diagnosing an intrapulmonary tumor. Average yield of EBUS-TBNA for diagnosing centrally located lung tumors was 89% (95%CI 84-92) and average sensitivity for diagnosing malignant tumors was 91% (95%CI 88-94). Among studies reporting this information, EBUS related complications occurred in 5% of patients (42/721). This analysis implies that EBUS-TBNA is a safe procedure with a high yield and sensitivity for diagnosing centrally located lung tumors. However, caution should be taken to extrapolate these results into routine real life practice due to the lack of high-quality studies included, as its limitations in patient selection.

Chapter 2 shows the results of an international multicenter retrospective analysis (2013-2018) of linear EBUS databases in Bologna, Italy and Amsterdam, The Netherlands. Where patients with a centrally located lung tumor without endobronchial abnormalities who underwent lung tumor search with linear EBUS were included. Diagnostic yield to sample centrally located intrapulmonary tumor was 83% (136/163) and it was independent of tumor location. There were no major complications found. This implies that 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.

In chapter 3 we assessed the feasibility and diagnostic yield of EUS-B-FNA in para-esophageal located lung tumors and its added value to bronchoscopy and EBUS. We have performed a retrospective, multi-center international study (from 01-2015 until 01-01-2018) of patients with suspected lung cancer, undergoing a bronchoscopy, EBUS and EUS-B in one session by a single operator (pulmonologist), in which the primary lung tumor was detected and aspirated by EUS-B. We investigated 58 patients and in 26 (45%) the primary tumor was only detected by EUS-B. Adding EUS-B to conventional bronchoscopy and EBUS increased the diagnostic yield for diagnosing lung cancer in paraesophageally located lung tumors from 51% to 91%. These findings argue in favor of performing EUS-B in the same endoscopy session after non-diagnostic bronchoscopy and EBUS.

In chapter 4 we present two exceptional cases of patients with an intracardiac tumor in the left atrium who were successfully, minimally invasive and safely assessed by EUS and EUS-B and invasive open-heart surgery was prevented.

Chapter 5 provides the protocol of our randomized controlled trial in which we will compare the new Acquire™ 22 G needle vs the standard Expect 22 G needle in patients who undergo EBUS/EUS-B sampling for mediastinal/hilar lymph node or primary tumor evaluation. With the currently available 22G needles, it is regularly not possible to determine the PDL 1 status, which leads to additional diagnostics and delay in the initiation of treatment. This new symmetrical three-plane needle with French geometry can provide improved diagnostics of the sample. To date, there is no literature on the French biopsy needle for diagnostic and staging purposes during EBUS / EUS-B procedures in patients with (suspected) lung cancer.

PART 2 ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR T STAGING OF LUNG TUMORS

Chapter 6 reports the value of EBUS for detecting mediastinal or vascular (T4) invasion of centrally located lungtumors provided adjacent to the major airways. We performed a retrospective analysis in which 104 patients were included with NSCLC who subsequently underwent thoracotomy as the reference standard. Sensitivity and specificity for diagnosing T4-status were 64% (95%CI 46-79%) and 94% (84-98%), and PPV and NPV were 82% (66-92%), 83% (76-88%), respectively. Based on this, it seems that the overall accuracy of EBUS is moderate and may not sufficient enough to rule-in or rule-out T4-status but combining this with the results of the CT report, T4 can be ruled out if both studies are negative for T4. This patients can be referred for thoracotomy.

In chapter 7 we assess the value of EUS for detecting mediastinal invasion (T4) of centrally located lung tumors. We have performed a retrospective analysis of 426 patients who underwent EUS for the diagnosis and staging of lung cancer and the primary tumor was detected by EUS and 74 patients who subsequently underwent surgical- pathological staging (2000 -2016). Of these 19 patients (26%) were diagnosed with stage T4 based on vascular (n= 8, 42 %) or mediastinal (n=8, 42%) invasion or both (n= 2, 11 %), one patient (5%) had vertebral involvement. Sensitivity, specificity, PPV and NPV for assessing T4 status were: for EUS (n=74); 42%, 95%, 73%, 83%, for chest CT (n=66); 76%, 61%, 41%, 88% and the combination of EUS and chest CT (both positive or negative for T4, (n=34); 83%, 100%, 100% 97%. This implicates that EUS has a high specificity and NPV for the T4 assessment of lung tumors located para-esophageally and offers further value to chest CT scan.

PART 3: NOVEL LUNG CANCER STAGING STRATEGIES AND ITS IMPACT ON SURVIVAL

Chapter 8 shows the post-hoc analysis of the ASTER trial and evaluated long term survival of patients included in this analysis. Survival data at 5 years were obtained for 237 of 241 patients (98%); Survival was 35% in both arms [95% CI, 0.57-1.66]. The estimated median survival was 31 months (95% CI, 21-41) for the endosonographic strategy vs 33 months (95% CI, 23-43) for the surgical strategy (adjusted hazard ratio, 0.98 [95% CI, 0.73-1.32]). Since the original results of ASTER were published, clinical guidelines on lung cancer management underwent major revisions and now advocate endosonography instead of mediastinoscopy as the initial step for mediastinal nodal staging. The endosonographic strategy is more accurate, less invasive, and reduces unnecessary thoracotomies and is cost-effective.

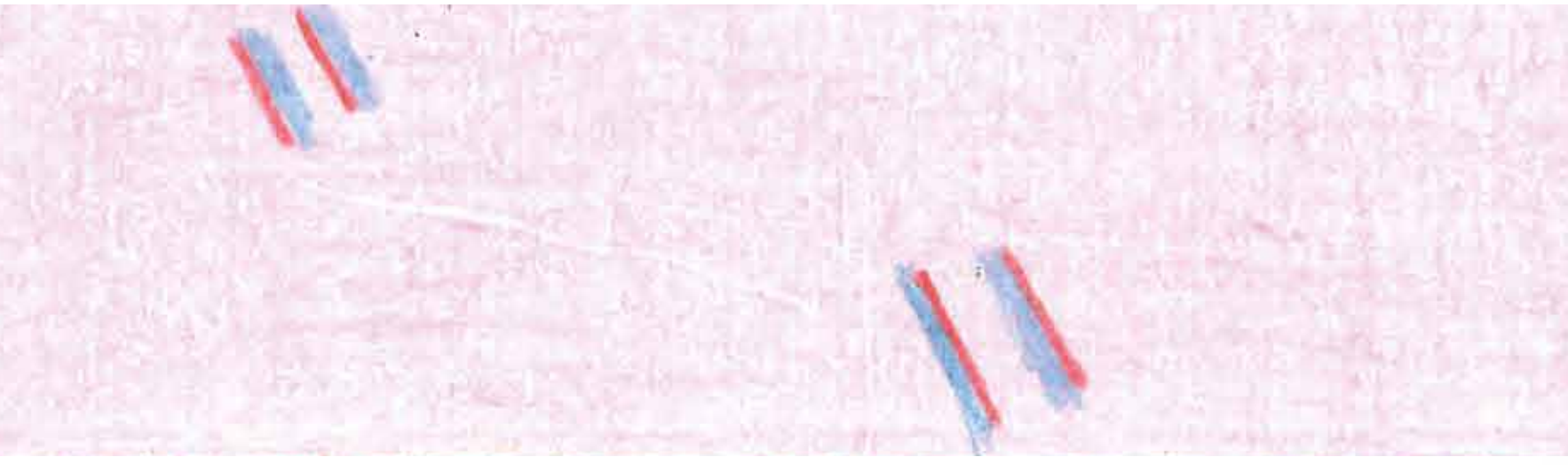
Discussion

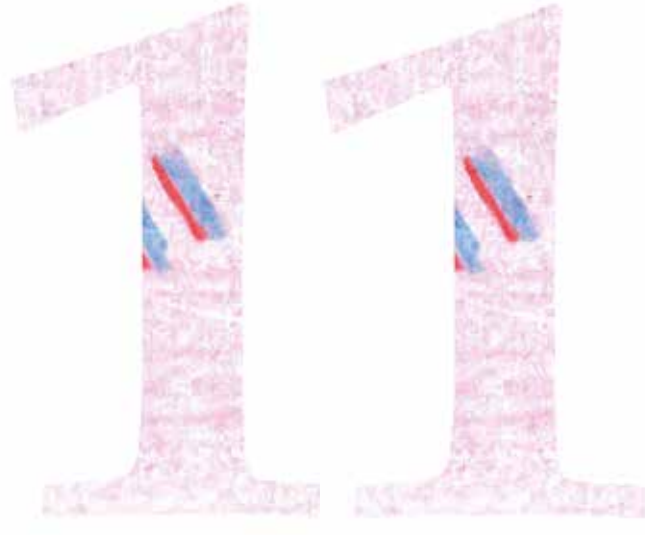
This part contains the general discussion, key findings, considerations, future perspectives and summary **(chapter 9)** of this thesis. The last chapter of this thesis **(chapter 10)** contains the Dutch summary.

SCAN ME

PART THREE

CHAPTER





Nederlandse samenvatting

Introductie
Longkanker is een van de meest voorkomende vorm van kanker ter wereld. Het verkrijgen van een weefseldiagnose is erg belangrijk omdat hiervan zowel de behandeling als ook de prognose afhangt. Om een weefseldiagnose te kunnen verkrijgen kunnen meerdere diagnostische tests verricht worden. Als er sprake is van een centraal gelegen longtumor, zonder dat er sprake is van een zichtbare afwijking in de grote luchtwegen, is het vaak ingewikkeld om een weefseldiagnose van de tumor te verkrijgen. Ook doorgroei van de tumor in de omgevingsstructuren als het mediastinum en de grote vaten (T4) is belangrijk, omdat dit de behandeling bepaalt.

Het doel van dit proefschrift was om de rol van endo-echografie (EBUS, EUS-B en EUS) verder te onderzoeken, specifiek met de diagnosestelling van de primaire longtumor zelf en het aantonen of uitsluiten van lokale tumor ingroei.

DEEL 1: ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR DIAGNOSING LUNG TUMOR

Hoofdstuk 1 geeft de resultaten van een review en meta-analyse waarin we de prestaties evalueren van lineaire endobronchiale echogeleide transbronchiale naaldaspiratie (EBUS-TBNA) voor de diagnose van centraal gelegen longtumoren. Er werden 5.657 manuscripten geïdentificeerd; van deze werden er 14 geïncludeerd in de studie, met daarin 1175 patiënten die EBUS-TBNA ondergingen voor de diagnose van een centraal gelegen longtumor. De gemiddelde opbrengst van EBUS-TBNA voor het diagnosticeren van centraal gelegen longtumoren was 89% (95% BI 84-92) en de gemiddelde sensitiviteit voor het diagnosticeren van maligne tumoren was 91% (95% BI 88-94). Van de onderzoeken die deze informatie rapporteerden, traden EBUS-gerelateerde complicaties op bij 5% van de patiënten (42/721). Deze analyse impliceert dat EBUS-TBNA een veilige procedure is met een hoge opbrengst en sensitiviteit voor het diagnosticeren van centraal gelegen longtumoren. De data moet echter wel voorzichtig worden geïnterpreteerd bij het extrapoleren van deze resultaten naar de dagelijkse praktijk vanwege beperkingen in de kwaliteit van de studie in o.a. patiënt selectie.

Hoofdstuk 2 toont de resultaten van een internationale multicenter retrospectieve analyse (2013-2018) van lineaire EBUS-databases in Bologna, Italië en Amsterdam, Nederland. Waarbij patiënten met een centrale longtumor zonder endobronchiale afwijkingen die longtumor diagnostiek ondergingen met lineaire EBUS werden geïncludeerd. De diagnostische opbrengst van de puncties van centraal gelegen longtumoren was 83% (136/163) en was onafhankelijk van de tumorlocatie. Er werden geen grote complicaties gevonden. Dit houdt in dat longtumoren die zich presenteren zonder endobronchiale afwijkingen en zich naast de belangrijkste luchtwegen bevinden, veilig kunnen worden gepuncteerd middels EBUS-TBNA, wat resulteert in een hoge diagnostische opbrengst.

In **Hoofdstuk 3** wilden we de haalbaarheid en diagnostische opbrengst van EUS-B-FNA in, naast de oesophagus gelegen, longtumoren beoordelen en de toegevoegde waarde ervan voor bronchoscopie en EBUS onderzoeken. We hebben een retrospectieve, multi-

center internationale studie uitgevoerd (van 01-01-2015 tot 01-01-2018) bij patiënten met verdenking op longkanker, die in één sessie, een bronchoscopie, EBUS en EUS-B ondergingen door een enkele endoscopist (longarts), waarbij de primaire longtumor werd gedetecteerd en gepuncteerd middels EUS-B. We onderzochten 58 patiënten en bij 26 (45%) werd de primaire tumor alleen gedetecteerd door EUS-B. Het toevoegen van EUS-B aan conventionele bronchoscopie en EBUS verhoogde de diagnostische waarde voor het diagnosticeren van longkanker in para-oesofageale longtumoren van 51% naar 91%. Deze bevindingen pleiten voor het verrichten van EUS-B in dezelfde endoscopie sessie na een niet-diagnostische bronchoscopie en EBUS.

In **hoofdstuk 4** presenteren we twee exceptionele casus van patiënten met een tumor gelegen in het linker atrium, waarbij op een veilige en minimaal invasieve wijze een weefsel diagnose verkregen werd door EUS en EUS-B. Invasieve openhartchirurgie werd hierbij voorkomen.

In **hoofdstuk 5** wordt het protocol beschreven voor een gerandomiseerde klinische studie waarin we EBUS/ EUS-B diagnostiek van mediastinale/ hilaire lymfeklieren of primaire longtumoren zullen vergelijken met de recent ontwikkelde Acquire™ 22G TBNB-naald versus een conventionele naald.
Met de huidige beschikbare 22G naalden is het regelmatig niet mogelijk de PDL 1 status te bepalen wat leidt dat extra diagnostiek en vertraging tot inzet van de behandeling. Deze nieuwe symmetrische naald met drie vlakken met Franseen-geometrie kan een verbeterde diagnostiek van het sample geven. Tot op heden is er geen literatuur over de Franseen biopsienaald voor diagnostiek en stadiëringsdoeleinden tijdens EBUS/ EUS-B procedures bij patiënten met (verdenking op) longkanker.

DEEL 2 ENDOBRONCHIAL AND ESOPHAGEAL ULTRASOUND FOR T STAGING OF LUNG TUMORS

In **hoofdstuk 6** hebben we retrospectief de diagnostische waarde van EBUS bekeken, voor de beoordeling van mediastinale of vasculaire tumorinvasie (T4-status) bij 104 patiënten met NSCLC die vervolgens een thoracotomie hadden ondergaan als referentiestandaard. Sensitiviteit en specificiteit voor het diagnosticeren van de T4-status waren 64% (95% BI 46-79%) en 94% (84-98%), en PPV en NPV waren 82% (66-92%), 83% (76-88%) %).
Op basis hiervan lijkt het erop dat de algehele nauwkeurigheid van EBUS matig is en mogelijk onvoldoende is om de T4-status aan te tonen of uit te sluiten. Een combinatie van een negatieve EBUS met een negatieve CT-thorax resulteerde in een NPV van 94% (95% BI 72-99), waardoor T4-ziekte met een hoge mate van zekerheid werd uitgesloten, deze patiënten kunnen worden verwezen voor thoracotomie.

In **hoofdstuk 7** beoordelen we de waarde van EUS voor het detecteren van mediastinale en vasculaire invasie (T4) van centraal gelegen longtumoren. We voerden een retrospectieve analyse uit van 426 patiënten die EUS ondergingen voor de diagnose en stadiëring van longkanker en de primaire tumor werd gedetecteerd door EUS en 74 patiënten die vervolgens chirurgisch-pathologische stadiëring ondergingen (2000-2016). Van deze

werden 19 patiënten (26%) gediagnosticeerd met stadium T4 op basis van vasculaire (n = 8, 42%) of mediastinale (n = 8, 42%) invasie of beide (n = 2, 11%), één patiënt (5 %) had vertebrale betrokkenheid. Sensitiviteit, specificiteit, PPV en NPV voor het beoordelen van de T4-status waren: voor EUS (n = 74); 42%, 95%, 73%, 83%, voor CT-scan (n = 66); 76%, 61%, 41%, 88% en de combinatie van EUS en CT-thorax (beide positief of negatief voor T4, (n = 34); 83%, 100%, 100% 97%. Dit impliceert dat EUS een hoge specificiteit en NPV voor de T4-beoordeling van longtumoren die para-oesofageaal gelegen zijn.

DEEL 3: NOVEL LUNG CANCER STAGING STRATEGIES AND ITS IMPACT ON SURVIVAL

In **hoofdstuk 8** beschrijven we een vervolganalyse van de ASTER-trial, waarin een chirurgische stadiëringsstrategie (mediastinoscopie) werd vergeleken met een endosonografische stadiëringsstrategie (gecombineerd gebruik van endobronchiale echografie (EBUS-TBNA) en transoesofageale echografie (EUS -FNA), en indien negatief gevolgd door mediastinoscopie). In dat onderzoek werden 241 patiënten met operatief te verwijderen NSCLC gerandomiseerd en werd er een significant verschil in gevoeligheid voor het diagnosticeren van mediastinale nodale metastasen vastgesteld, in het voordeel van de endo-echografische strategie (79% versus 94%). In de huidige follow-up analyse hebben we de overleving na vijf jaar geëvalueerd voor beide strategieën, maar we hebben vastgesteld dat dit vergelijkbaar was (35% in beide armen). Deze bevindingen illustreren dat een verbeterde diagnose niet altijd leidt tot verbeterde belangrijke uitkomsten voor de patiënt, zoals mortaliteit. Endo-echografie is tegenwoordig de eerste keus in de internationale richtlijnen voor het stadiëren en diagnosticeren van het mediastinum. Niet alleen is de endo-echografische strategie nauwkeuriger, het is vooral minder invasief en vermindert onnodige thoracotomieën en is tevens kosten effectiever.

Hoofdstuk 9 bevat de algemene discussie, belangrijkste bevindingen, overwegingen en toekomstperspectieven van dit proefschrift.

Appendices

CURRICULUM VITAE

Jolanda Corina Kuijvenhoven werd op 11 juni 1983 geboren te Amsterdam en is de oudste in het gezin van 2 kinderen. Zij behaalde haar middelbare schooldiploma in 2001 aan het Gymnasium van het Tabor college locatie Werenfridus te Hoorn. Datzelfde jaar ging zij geneeskunde studeren aan de Rijks Universiteit Groningen. Na het behalen van het arts-examen in 2008 ging zij werken als ANIOS longziekten op de longafdeling van het Medisch Spectrum Twente te Enschede, waarna zij aldaar in 2008 ook startte met de opleiding tot longarts. In 2015 werd de opleiding tot longarts afgerond na een afsluitende stage in het Academische Medisch Centrum Amsterdam. Hierna startte haar werkzame leven als longarts in het Spaarne Gasthuis als Chef de Clinique en tegelijkertijd startte zij haar promotie traject bij Prof. Dr. J.T. Annema in het Academisch Medisch Centrum Amsterdam met als resultaat dit proefschrift. Sinds 2017 is Jolanda als longarts gevestigd in de Maatschap Friese Longartsen en werkzaam in het Medisch Centrum Leeuwaren.

Jolanda is getrouwd met Matthijs Varkevisser en samen hebben zij twee zonen (Jip 2013 en Robbert 2017)

PHD PORFOLIO

Name PhD student: Jolanda Kuijvenhoven

PhD period: 9/2016- 1/ 2020

Name PhD supervisor: Prof.dr. Jouke Annema

Name PhD Co-supervisor: Dr. Daniel Korevaar

1. PhD training

	Year	Workload (Hours/ECTS)
General courses		
- Update GCP	2021	0.5
- GCP exam	2017	1.0
- Practical biostatistics	2019	1.1
- CE 1: Randomized Controlled Trials	2019	1.5
- Pubmed course	2019	0.2
- Castor online modules	2019	0.5
- Endnote	2021	0.2
Seminars, workshops and master classes		
- Skills workshop EUS and EUS-B, international ERS part 2 EBUS course	2019	0.2
- Skills workshop EUS and EUS-B, international ERS part 2 EBUS course	2018	0.2
- Docent en workshopleider, ERS EUS/EBUS course, AMC	2016, 2017, 2018, 2019, 2021	0.4
- Docent echo thorax cursus AMC	2015,2016, 2017	0.3

Presentations		
- Endosonography for tumor diagnosis and T staging, lecture at the international ERS EBUS course, AMC, The Netherlands	2019,2021	1.5
- Endosonography for tumor diagnosis and T staging, lecture at international ERS course, AMC, The Netherlands	2018	1.5
- EBUS/EUS tumor diagnostics and T4 staging, ERS EBUS/EUS cursus AMC, Nederland	2017	1.5
(Inter)national conferences		
- Endobronchial Ultrasound (EBUS) for T4 staging in patients with resectable NSCLC, poster presentation virtual ERS	2020	1.0
- The expanding role of endobronchial ultrasound in patients with centrally located intrapulmonary tumors., poster presentation ERS Madrid	2019	1.0
- EUS-B for diagnosing tumors adjacent to the esophagus, Poster discussion at ERS Paris.	2018	1.5
- EBUS for diagnosing lungtumors a systematic review and meta-analysis, poster discussion at ERS Paris	2018	1.5
- EUS for mediastinal tumor invasion, poster discussion ERS congress London	2016	1.5
- Systematic vs targeted endosonographic staging of lungcancer improves loco- reginal nodal staging, oral presentation 19e WCIP/WCBE world congress, Florence	2015	2.0
- EUS assessment of T4 status in patients with NSCLC, oral presentation 19e WCIP/WCBE world congress, Florence	2015	2.0
- Mediastinoscopy vs endosonography for mediastinal nodal staging: does it affect 5 year survival? Oral presentation 19e WCIP/WCBE world congress, Florence	2015	2.0
Other		
- Faculty Virtual ERS live endoscopy	2020, 2021	1.0
- Faculty live endoscopy ERS	2015, 2016, 2017, 2018, 2019	2.0 0.5
- Chair thematic poster ERS 2018	2018 2019	0.5
- Skills workshop ERS congress		2.5

2. Teaching		
	Year	**Workload (Hours/ECTS)**
Supervising		
- Chef de Clinique Respiratory Medicine, Spaarne Gasthuis	2015-2017	
- Consultant Respiratory Medicine, Medisch Centrum Leeuwarden.	2017-present	
- Training residents, physicians and medical students	2017-present	
- Supervising scientific program MD fellows (Luca Morandi, Fausto Leoncini, Haizea Alvarez-Martinez	2017-2018, 2018-2019, 2019-2020	

3. other	
- Member of ERS (member of assembly 1 (respiratory clinical care) and assembly 14 (clinical techniques, imaging, endoscopy)) - Member of NVALT, sectie Pulmonale Interventies, slaapgrelateerde ademhalings aandoeningen en COPD - President commissie Bronckhorst Colloquium. (Twee daagse nascholing voor longartsen in Nederland en België met een jaarlijks wisselend onderwerp).	
no parameters of esteem	

LIST OF PUBLICATIONS

1 Alvarez Martinez H.A.M*, **Kuijvenhoven J.C***, Annema J.T;
Intracardiac EUS guided FNA for diagnosing cardiac tumors.
Respiration 2021, in press

2 **Kuijvenhoven J.C**, Leoncini F, Crombag L.C, Spijker R, Bonta P.I, Korevaar D.A, Annema J.T.
Endobronchial Ultrasound for the Diagnosis of Centrally Located Lung Tumors: A Systematic Review and Meta-Analysis.
Respiration. 2019 Nov 15:1-10. doi: 10.1159/000500363

3 **Kuijvenhoven J.C**, Livi V, Morandi L, Cancellieri A, Annema J.T, Trisolini R.
The expanding role of endobronchial ultrasound in patients with centrally located intrapulmonary tumors.
Lung Cancer. 2019 Aug;134:194-201. doi: 10.1016/j.lungcan.2019.06.006. Epub 2019 Jun 24.

4 Skovgaard Christiansen I*, **Kuijvenhoven J.C***, Bodtger U, Naur T.M.H, Ahmad K, Singh Sidhu J, Nessar R, Salih G.N, Høegholm A, Annema J.T, Clementsen P.F.
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5 **Kuijvenhoven J.C**, Crombag L, Breen D.P, van den Berk I, Versteegh M.I.M, Braun J, Winkelman T.A, van Boven W, Bonta P.I, Rabe K.F, Annema J.T.
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6 **Kuijvenhoven J.C**, Korevaar D.A, Tournoy K.G, Malfait T.L, Dooms C, Rintoul R.C, Annema J.T.
Five-Year Survival After Endosonography vs Mediastinoscopy for Mediastinal Nodal Staging of Lung Cancer.
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Safety and Tolerability of Nebulized Amoxicillin-Clavulanic Acid in Patients with COPD (STONAC 1 and STONAC 2).
COPD. 2016 Aug;13(4):448-54. doi: 10.3109/15412555.2015.1107893. Epub 2016 Jan 8.

* Shared authorship

LIST OF ABBREVIATIONS

CLE	Confocal laser endomicroscopy (CLE)
CP-EBUS	Convex probe endobronchial ultrasound
CT	Computed tomography
CT guided TTNA	CT guided transthoracic needle aspiration
EBUS	Endobronchial ultrasound
EBUS-TBNA	Endobronchial ultrasound-guided transbronchial-needle aspiration
EBUS AT	EBUS Skills and Tasks Assessment Tool
EUS-B	Endoscopic (esophageal) ultrasound with an EBUS scope
EUS-B-FNA	Esophageal ultrasound fine needle aspiration (using an EBUS scope)
EUS-FNA	Endoscopic ultrasound- guided fine needle aspiration
GI	Gastro-intestinal
IASLC	International Association for the Study of Lung Cancer
MR:	Magnetic Resonance Imaging
nCLE	Needle-based confocal laser endomicroscopy
NSCLC	Non-small cell lung cancer
NSCLC NOS	Non-Small Cell Lung Cancer not otherwise specified
NPV	Negative Predictive Value
OCT	Optical coherence tomography (OCT) and
PET-CT	Positron Emission Tomography and Computed Tomography
PPV	Positive Predictive Value
R-EBUS	Radial EBUS
ROSE	Rapid on-site cytological evaluation
SCLC	Small Cell Lung cancer
TBNB	Transbronchial needle biopsies
TBNF	Transbronchial needle forceps
TTNA	Trans thoracic needle aspiration
TTB	Trans thoracic biopsy

Appendices

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Graag wil ik allen die direct of indirect hebben bijgedragen aan het tot stand komen van dit proefschrift heel erg hartelijk bedanken.

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