THE ROLE OF ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION IN THE DIAGNOSIS OF MEDIASTINAL LYMPHADENOPATHY

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DECLARATION

I, Neal Navani, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a novel procedure for the diagnosis of mediastinal lymphadenopathy. Its utility in clinical practice for the diagnosis of patients presenting with mediastinal lymphadenopathy is unknown. This thesis describes the learning curve associated with EBUS-TBNA using cumulative sum analysis and then the diagnostic yield of EBUS-TBNA in different clinical scenarios.

EBUS-TBNA was combined with standard bronchoscopy in patients with suspected sarcoidosis in a prospective trial. The role of EBUS-TBNA in patients with tuberculous lymphadenopathy and also patients with extra-thoracic malignancy was then clarified in multi-centre studies. A further prospective trial (REMEDY) aimed to ascertain whether mediastinoscopies could be prevented in patients presenting with isolated mediastinal lymphadenopathy. The utility of the specimens from EBUS-TBNA for sub-typing and genotyping of non-small cell lung cancer are also described in a multi-centre study. Finally, the results from a major multi-centre randomised controlled trial (Lung-BOOST) are presented, investigating whether EBUS-TBNA should be implemented as a first test in patients with suspected lung cancer.

The data included in this thesis demonstrate that EBUS-TBNA has high diagnostic yield in patients with sarcoidosis, tuberculosis and extra-thoracic malignancy. For the first time, the REMEDY trial demonstrates that EBUS-TBNA can prevent 87% of mediastinoscopies in patients with isolated mediastinal lymphadenopathy. In patients with lung cancer, specimens from EBUS-TBNA are suitable for sub-typing and genotyping of NSCLC and results from the randomised Lung-BOOST trial demonstrate that when EBUS-TBNA is used as an initial investigation in patients with suspected lung cancer the time to treatment decision is significantly reduced.

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DEDICATION

I am very fortunate to have my wonderful daughter Anya, my beautiful wife Roopa and my incredible parents. This thesis is dedicated to them for their love, sacrifice and support throughout.

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ABBREVIATIONS

ACCP	American College of Chest Physicians
APW	Aorto-pulmonary window
ASTER	Assessment of surgical staging versus endoscopic ultrasound in lung cancer: a randomised clinical trial
ATS	American Thoracic Society
BTS	British Thoracic Society
CDS	Conventional diagnosis and staging
CHART	Continuous hyperfractionated accelerated radiotherapy
CI	Confidence interval
CT	Computed tomography
CUSUM	Cumulative sum
CWU	Conventional work-up
EBB	Endobronchial biopsy
EBUS-TBNA	Endobronchial ultrasound-guided transbronchial needle aspiration
EGFR	Epidermal growth factor receptor
ERS	European Respiratory Society
EUS-FNA	Endoscopic ultrasound-guided fine needle aspiration
FN	False negative
FP	False positive
H&E	Hematoxylin and eosin

IML	Isolated mediastinal lymphadenopathy
MGG	May-Grunwald-Giemsa
MLN	Mediastinal lymphadenopathy
M.tb	*Mycobacterium tuberculosis*
NICE	National Institute for Health and Clinical Excellence
NPV	Negative predictive value
PET	Positron emission tomography
PET-CT	Integrated positron emission tomography – computed tomography
PFS	Progression-free survival
ROI	Region of interest
ROSE	Rapid on-site evaluation
SUV	Standardised uptake value
SUVmax	Maximum standardised uptake value
TBLB	Trans-bronchial lung biopsy
TBNA	Transbronchial needle aspiration
TN	True negative
TP	True positive
TTF-1	Thyroid Transcription Factor-1

CHAPTER 1: INTRODUCTION

1.1 MEDIASTINAL LYMPHADENOPATHY

Mediastinal lymphadenopathy refers to the enlargement of lymph nodes within the mediastinum and determining the diagnosis of mediastinal lymphadenopathy is a common problem faced by respiratory physicians. The differential diagnosis of enlarged mediastinal lymph nodes (MLN) includes neoplasm, granulomatous disease, infection and reactive hyperplasia. Neoplastic causes are most commonly metastatic lung cancer, lymphoma or metastatic disease from the oesophagus, breast, kidney or head and neck. Sarcoidosis and tuberculosis result in granulomatous lymphadenopathy. Fungal infections such as histoplasmosis and coccidiodomycosis may also cause enlarged MLNs. Rarer causes of mediastinal lymphadenopathy include Castleman's disease, angioimmunoblastic lymphadenopathy, chronic berylliosis, Wegener's granulomatosis and chronic mediastinitis.

In UK practice, the most common causes of mediastinal lymphadenopathy are sarcoidosis, metastatic lung cancer, tuberculosis and lymphoma. These four important conditions have vastly different treatments and prognoses. Moreover their symptoms are often non-specific. Fevers, night sweats and weight loss may be a common feature of each diagnosis and does not help with their differentiation. Therefore, a tissue diagnosis of mediastinal lymphadenopathy is critical to allow patient management.

1.2 MEDIASTINAL LYMPH NODE MAP

Assigning a location to mediastinal lymph nodes is important to allow accurate and reproducible lymph node sampling and to facilitate discussion between clinicians and researchers. There are 2 lymph node maps currently in use internationally. The Japanese Naruke lymph node map has largely been replaced by the American Thoracic Society mediastinal lymph node classification, first described by Mountain and Dresler (1997) and shown in Figure 1.1.

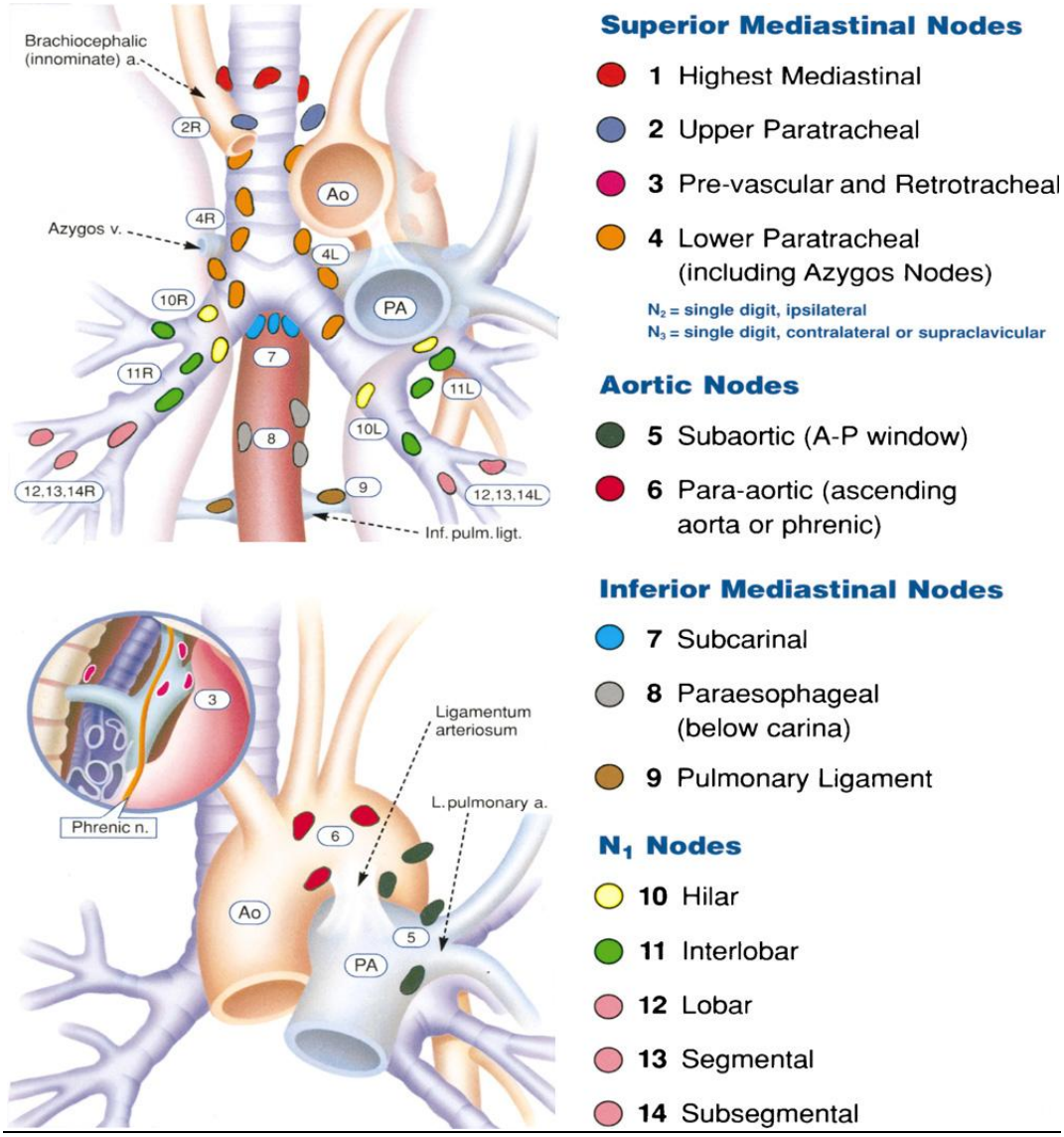


Figure 1: The mediastinal lymph node map. (Mountain and Dresler 1997).

1.3 CURRENT TECHNIQUES FOR THE DIAGNOSIS OF MEDIASTINAL LYMPHADENOPATHY

A combination of radiological, minimally invasive and invasive techniques is currently employed in the diagnosis of mediastinal lymphadenopathy. Computed tomography (CT) with intravenous contrast is a first line investigation in order to delineate the location of enlarged MLNs. Positron Emission Tomography (PET) and integrated PET-CT are more sensitive and specific tests than CT for mediastinal nodes and are currently recommended in the staging non-small cell lung cancer but have limited utility in other disease processes. Tissue sampling of mediastinal lymph nodes may currently be performed by bronchoscopy with conventional transbronchial needle aspiration. Surgical techniques and in particular mediastinoscopy are currently considered to be the gold standard for the diagnosis of MLNs. The newer techniques of endoscopic ultrasound guided fine needle aspiration (EUS-FNA) and endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) are emerging for the diagnosis of benign as well as malignant mediastinal lymphadenopathy. Each of these techniques is discussed below in the context of non-small cell lung cancer and isolated mediastinal lymphadenopathy.

1.4 MEDIASTINAL STAGING OF NON-SMALL CELL LUNG CANCER

1.4.1 Importance of mediastinal lymph node staging in NSCLC

The mediastinal staging of non-small cell lung cancer (NSCLC) is a critical process that determines treatment options and prognosis as well as allowing accurate

comparison in clinical trials. In patients with NSCLC who are fit for surgery and have no evidence of extra-thoracic disease, the status of the mediastinum differentiates operable from inoperable candidates (Rusch et al. 2007). Mediastinal staging may be best achieved with a multidisciplinary approach that involves pulmonary, surgical, oncology, and radiology input to establish whether curative surgical resection is possible. Those patients with no evidence of mediastinal metastases on staging investigations may be offered surgery. The remaining patients with mediastinal spread are offered chemotherapy and external beam radiotherapy or neo-adjuvant treatment in the context of a clinical trial (Spira & Ettinger 2004).

Currently, clinical stage IA disease has a 5-year survival rate after surgery of 50% (Goldstraw et al. 2007). Of the recurrences, most occur from metastatic involvement at presentation, which are missed by existing staging modalities. Deficiencies of staging techniques therefore result in 21-45% of thoracotomies being futile at 1 year (Fischer et al. 2009;Herder et al. 2002;van Tinteren et al. 2002) with the consequence of removing lung function without curing the disease. Patients with clinical stage II disease (T1N1M0 or T2N1M0) have a 5-year survival rate after surgery of 25% and benefit from adjuvant treatment. At clinical stage IIIA, the 5-year survival rate is 18%, and at stage IIIB it is only 8% (Goldstraw et al. 2007). Patients with clinical stage IIIA-N2 disease, however, represent a heterogeneous group with widely ranging survival rates. Those with bulky central mediastinal disease have a worse prognosis than those with single station ipsilateral disease. All patients with N2 mediastinal lymph node involvement remain poor candidates for initial surgical resection even if neoplastic invasion is limited to a single mediastinal station (Cerfolio & Bryant 2008;Ohta et al. 2006). The 5-year survival rate for

patients with stage IV disease is virtually nil, and this disease is treated either with chemotherapy and supportive care or with supportive care alone.

Therefore, it is of paramount importance to stage accurately as the treatment modalities and subsequent patient outcomes vary widely based on stage designation. By staging patients with NSCLC more accurately, patients are more likely to receive appropriate treatment, with a reduction in futile thoracotomies and improvement in morbidity and mortality.

1.4.2 Techniques for the mediastinal staging of non-small cell lung cancer

1.4.2.1 Computed tomography scan

Contrast enhanced computed tomography (CT) scanning is the first step in the assessment of mediastinal lymph node (MLN) staging for NSCLC. It is widely available and provides excellent anatomical detail, but relies on the size of MLN to differentiate potentially benign from malignant lymphadenopathy. A criterion of 1 cm in short-axis is generally employed to distinguish potentially malignant MLNs (≥ 1 cm in diameter) from benign MLNs (< 1 cm in diameter). Using this paradigm, a meta-analysis of 43 studies demonstrated that the sensitivity of CT in the diagnosis of MLN metastasis is low at 51% with a specificity of 86%, in a population with a median prevalence of mediastinal metastases of 28% (Silvestri et al. 2007). Therefore, MLNs less than a 1cm in short-axis may still harbor malignancy in up to 20% of cases. Even in cases of clinical stage 1A disease on the basis of CT, mediastinal lymph node dissection may demonstrate MLN metastases in 10% of cases (Cerfolio et al. 2005). Importantly, 40% of enlarged mediastinal nodes may be

benign (Kerr et al. 1992), particularly in the context of obstructive pneumonitis (McLoud et al. 1992). Therefore, in patients with discrete nodal enlargement, relying on CT alone for mediastinal staging would both over and under-stage patients with the potentially catastrophic consequences of a missed opportunity to operate or futile surgery. These limitations highlight the importance of pathological confirmation of lymph node status and MLN staging cannot be judged on CT appearances alone. However, CT does remain an important initial investigation and delineates the anatomy of the mediastinum allowing selection of the appropriate invasive staging tool.

1.4.2.2 Positron emission tomography

Functional imaging with positron emission tomography (PET) and fusion PET-CT is a valuable addition in the assessment of stage in NSCLC. The radio-labeled glucose analogue 18-fluoro-2-deoxy-D-glucose (FDG) undergoes the same cellular uptake as glucose and is phosphorylated, generating 18F-FDG-6-phosphate. The accumulation of 18F-FDG-6-phosphate in malignant cells can then be identified using a PET camera. By utilizing the abnormally high function of malignant cells within lymph nodes, PET can differentiate normal from malignant cells. Therefore, PET has superior sensitivity and specificity in staging the mediastinum as compared with CT and has an important clinical role in the diagnosis, staging, re-staging, therapy planning and monitoring of disease in non-small cell lung cancer.

The PLUS randomized controlled trial highlighted the importance of PET in the staging of NSCLC. 188 patients from nine hospitals were assigned to undergo either conventional workup (CWU) or CWU and PET. Thoracotomy was regarded as futile if the patient had benign disease, explorative thoracotomy, pathological stage IIIA-

N2/IIIB, or postoperative relapse or death within 12 months of randomization. The study included a high incidence of thoracotomy for benign lesions and in addition half of the comparator CT scans were performed without intravenous contrast, excluded the liver and were non-spiral. However, by detecting previously unsuspected metastatic disease, the addition of PET to conventional workup prevented unnecessary surgery in one out of five patients. Conversely, another randomized trial of pre-operative PET suggested that it did not alter the number of thoracotomies performed (Viney et al. 2004).

The standardized uptake value (SUV) is the measure of metabolic activity detected by PET and provides predictive information regarding treatment response and survival. The maximum SUV (SUVmax) in a region of interest (ROI) has been adopted as an approach to characterize metabolically active lesions. A cut-off of 2.5 is generally applied. However, non-neoplastic lesions, in particular granulomatous disorders and infections may also generate positive lesions on PET scanning. Therefore, an overlap exists between true and false positives in MLNs with an SUVmax ≥ 2.5. PET scanning falsely identifies malignancy in 25% of patients with nodes that are enlarged for other reasons (Silvestri et al. 2007). Labeling PET positive MLNs as malignant without pathological confirmation, may result in a missed opportunity to operate. Consequently, current guidelines advocate that PET positive mediastinal nodes should be invasively investigated, before surgery is precluded (Detterbeck et al. 2007). An SUV of 5.3 has been proposed in an attempt to improve specificity and accuracy without significant loss to sensitivity (Bryant et al. 2006). A retrospective study of 110 patients who underwent CT, PET scan and mediastinoscopy suggested that mediastinoscopy could be avoided in patients with MLNs having an SUVmax of less than 5.3 (Lee et al. 2008a). This however requires

further confirmation in prospective trials to determine its effect on patient outcomes, before this approach can be adopted.

Although PET positive mediastinal lesions require pathological confirmation of malignancy, it is generally considered that PET negative (SUV ≤ 2.5) lesions reliably exclude malignancy, particularly in lymph nodes < 1cm in short axis. However, recent evidence has tempered enthusiasm for PET since false negatives can and do occur and performance characteristics are dependent on nodal size. A meta-analysis by De Langen et al. (2006) of patients with lymph nodes measuring ≥ 16 mm on CT and a negative FDG-PET result demonstrated a post-test probability for N2 disease of 21%. The authors concluded that patients with MLN ≥ 16mm should be planned for invasive mediastinal staging prior to possible thoracotomy to prevent futile surgery in this subset of patients. Size of MLN is therefore a key factor in the accuracy of PET.

Previously unsuspected metastatic mediastinal disease is seen in 10% of patients with negative CT and PET studies of the mediastinum and it is generally accepted that these patients can be referred for radical treatment without further clinical staging tests (Vansteenkiste 2003). However, specificity and accuracy decrease significantly in larger mediastinal lymph nodes (Al-Sarraf et al. 2008). Therefore, MLN ≥ 1cm should be invasively sampled, even if they are judged to be metabolically inactive by PET scan (Detterbeck et al. 2007). PET remains justified however for patients with enlarged MLNs, because of its ability to direct invasive biopsy and also to detect previously unsuspected M1 disease (Silvestri et al. 2007).

Integrated PET-CT has superior sensitivity to either technique in isolation, with a faster learning curve for radiologists. In a prospective study of 50 patients with

known or suspected NSCLC, PET-CT had higher accuracy for MLN metastases than PET alone or visual correlation of PET and CT (Cerfolio et al. 2004). However, problems with false positives and negatives persist. Sensitivity and specificity of fusion PET-CT range from 64 – 86% and 81 – 94% respectively (Silvestri et al 2007). To date, pre-operative PET-CT has been examined in 2 randomised controlled trials. Fischer and colleagues (2009) compared PET-CT and conventional staging procedures followed by further invasive diagnostic procedures (such as mediastinoscopy) versus conventional staging and invasive diagnostic procedures. In both groups, mediastinoscopy was mandatory. The primary endpoint chosen was futile thoracotomies as per the PLUS trial. It was intended that 430 patients were to be recruited, but the trial was closed after enrolment of 189 participants due to slow accrual. 98 patients were allocated to the PET-CT arm. PET-CT significantly reduced the number of thoracotomies and the number of futile thoracotomies (52% to 35%) by detecting previously unrecognized metastases in 13 patients (9 distant and 4 mediastinal metastases). However, survival was similar in the two arms (Fischer et al. 2009). One limitation of the trial was that mediastinoscopy was routinely employed, which is not the current standard of care in most institutions and may have masked the benefit of PET-CT of the mediastinum. The second randomised trial of PET-CT in NSCLC demonstrated that PET-CT identifies more patients with mediastinal and extra-thoracic disease than conventional staging and therefore spared more patients from stage-inappropriate surgery (Maziak et al. 2009). However, PET-CT also incorrectly upstaged disease in more patients.

Despite the apparent advance in imaging modalities, pre-operative invasive mediastinal lymph node sampling cannot be prevented and the need for mediastinoscopy may have even increased by the detection of false positive nodes

(Tournoy et al. 2007). Current guidelines recommend that invasive mediastinal staging remains indicated for FDG-avid mediastinal disease, PET positive hilar N1 disease (Hishida et al. 2008), where there is low FDG uptake of the primary tumour or for any MLNs ≥ 1cm on CT scan (Detterbeck et al. 2007).

1.4.2.3 Surgical techniques for mediastinal lymph node staging

Mediastinoscopy is currently considered the gold standard technique for pre-operative MLN staging in NSCLC. Performed under general anesthesia, an incision is made above the suprasternal notch and the mediastinoscope is inserted along the trachea, allowing visualization and biopsy of MLNs. The procedure is usually performed as a day-case with low morbidity (2%) and mortality is rare (Porte et al. 1998). Mediastinoscopy affords access to paratracheal nodes (stations 2R, 2L, 4R, 4L), pre-tracheal nodes (station 3) and anterior subcarinal nodes (station 7). However, lymph nodes in the aorto-pulmonary window (station 5), sub-aortic fossa (station 6), posterior subcarinal nodes (station 7) and inferior mediastinal nodes (stations 8 and 9) are not accessible by this technique (see figure 1.1). Biopsy samples allow for micro-metastases in normal sized lymph nodes to be detected and the procedure enables the surgeon to determine extra-capsular spread, conferring inoperability. However, the sensitivity of detecting mediastinal metastases in patients with NSCLC is as low at 80% (range 67 – 92%) with a false negative rate of 10% (Detterbeck et al. 2007). This inaccuracy (for the gold standard technique) is in part explained by the limited access of mediastinoscopy to the mediastinum.

Mediastinoscopy is also considerably underutilized in routine clinical practice. Little et al. (2005) collated data from 11,668 patients undergoing thoracotomy for lung cancer in 729 US hospitals. Only 27% of patients had pre-operative mediastinoscopy and of those performed only 47% had lymphoid tissue samples obtained. Smulders and colleagues (2005) demonstrated that only 40% of mediastinoscopies were performed according to gold standard techniques in non-university teaching hospitals in the Netherlands. The authors also showed that systematic mediastinal lymph node sampling was performed in only 50% of cases and estimated that 18% of thoracotomies could have been avoided if gold standard techniques had been observed. Of 39 cases with unexpected N2 disease at thoracotomy, 16 were accessible by mediastinoscopy (Smulders et al. 2005). Therefore, mediastinoscopy tends to be underemployed in the mediastinal staging of lung cancer. Furthermore, when it is carried out, only half of the procedures obtain diagnostic tissue. Even in expert hands, the entire mediastinum is inaccessible. Despite these clear limitations and lack of standardization of the technique, mediastinoscopy currently remains the gold standard for the staging of mediastinal lymphadenopathy. At present, it is not routinely recommended for pre-operative mediastinal staging of lymph nodes that are less than 1cm in short axis and negative on FDG-PET scan, due to low yield and lack of cost-effectiveness (Meyers et al. 2006).

The advent of videomediastinoscopy has improved the standard procedure allowing better visualization and in addition to usual MLN stations, sampling of posterior station 7 lymph nodes. Video-assisted mediastinal lymphadectomy (VAMLA) may also be performed using this technique and allows complete lymph node dissection without the need for thoracotomy. VAMLA offers a standardized approach to surgical MLN sampling and may be superior to standard cervical mediastinoscopy

with an accuracy of 88% and negative predictive value of 83% in a recent cohort of 234 patients (Leschber et al. 2008). Surgical expertise in this technique however is far from universal.

Nodes in the aorto-pumonary window (APW), to which left upper lobe cancers commonly spread, can be accessed by anterior mediastinotomy, also known as the Chamberlain procedure. An incision is made under general anesthetic in the 2nd or 3rd intercostal space just to the left of the sternum and overnight stay is usually required. Few studies have addressed the accuracy of the procedure although it is employed as the definitive staging technique for nodes in the APW. Extended cervical mediastinoscopy is performed in some centres. In one series this has a sensitivity of 69% and false negative rate of 11% in 100 patients with a prevalence of mediastinal disease of 29% (Ginsberg et al. 1987). Video-assisted thorascopic surgery (VATS) can access one side of the mediastinum with the right side being technically easier. Few prospective data are available, although the procedure appears to have an acceptable safety and accuracy profile and may be regarded as an adjunct procedure to standard techniques. Its role may be best limited to the diagnosis of lesions inaccessible by other means, assessment of mediastinal invasion (T4 disease) and sampling of APW nodes.

1.4.2.4 Trans-bronchial needle aspiration

Blind transbronchial needle aspiration (TBNA) is a safe procedure for mediastinal lymph node staging and was first described in 1978 (Wang, Terry, & Marsh). It is planned with the aid of CT (and PET if available) to identify the lymph node to be sampled and its relationship to bronchial landmarks is noted. During standard bronchoscopy, a TBNA needle is introduced into the biopsy channel and punctures

the bronchial wall allowing the mediastinal lymph node to be aspirated. This is most safely done in the subcarinal lymph node station but lower paratracheal and hilar lymph nodes can also be sampled. The procedure may be carried out with a 18, 19 or 22 gauge needle.

A meta-analysis of patients undergoing TBNA showed a pooled sensitivity of 39% for the technique when the prevalence of mediastinal metastases was 34% (Holty, Kuschner, & Gould 2005). Four modifiable factors have been shown to optimize the diagnostic yield of blind TBNA. First, at least 5 and up to 7 passes in the same area may maximize diagnostic tissue (Chin, Jr. et al. 2002;Diacon et al. 2007). Second, the presence of a cytologist within the endoscopy suite to evaluate aspirates as they are produced, may significantly improve accuracy (Diacon et al. 2005). Rapid on-site evaluation (ROSE) allows an immediate diagnosis of malignancy or can confirm the adequacy of a specimen by identifying lymphocytes (Baram, Garcia, & Richman 2005). Third, the use of CT fluoroscopy allows imaging of MLN during TBNA and may improve yield (Garpestad et al. 2001). Finally, since blind TBNA is highly operator dependent, focused education and experience in the technique are invaluable in improving results (Haponik et al. 1995;Hsu, Liu, & Ko 2004). The highest yield is seen in lymph nodes >1cm in the right paratracheal and subcarinal locations and it is this patient group that may benefit most from TBNA (Harrow et al. 2000).

Although blind TBNA is an important technique in selected patients, it remains underemployed for MLN sampling in NSCLC. However, uptake in the US may be improving. In a survey by Haponik and Shure (1997) only 10% of pulmonary fellows in the US routinely practiced TBNA. More recently, 91% of fellows were trained in TBNA and 69% were competent according to fellowship directors (Pastis,

Nietert, & Silvestri 2005). Figures outside the US are likely to be considerably lower.

Assessment of the endobronchial tree is performed at the same sitting as TBNA. In addition, it is cheap and a well tolerated outpatient procedure that can be performed under conscious sedation. A positive result from TBNA may obviate the need for further invasive tests, particularly when combined with PET (Bernasconi et al. 2006). However, the generally low diagnostic yield and negative predictive value mean that further tests are necessary in the event of a non-diagnostic sample.

1.4.2.5 Endoscopic ultrasound guided fine needle aspiration

Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) of mediastinal lymphadenopathy has been available for over fifteen years (Silvestri et al. 1996). Under conscious sedation, an endoscope is placed in the oesophagus. Radial echoendoscopes provide cross-sectional imaging but do not allow tissue samples to be obtained and have been superseded by an integrated linear ultrasound probe that allows visualization of the mediastinum (Figure 1.2). Aspiration with a 22-guage adjustable-length Echotip needle is performed through the wall of the esophagus under direct vision. Due to the anatomical location of the esophagus, EUS-FNA is able to sample mediastinal lymph nodes in stations 2L, 4L, 5, 7, 8 and 9. Furthermore, aspiration of the celiac axis nodes, left lobe of the liver and left adrenal gland via EUS can provide additional important staging information (Singh et al. 2007).

Samples obtained by EUS-FNA with a 22-guage needle are suitable for cytopathological analysis. Consistency and reproducibility of reporting of cytology samples can be easily achieved, particularly by experienced pathologists and those

with less experience have a steep learning curve (Skov et al. 2007). Occasionally core samples are obtained by EUS-FNA and may be sent for histopathological investigation. Core tissue samples may be more reliably obtained using a 19-guage Trucut needle. This method requires that the mediastinal lymph node be at least 20mm in the direction of the biopsy. Adding Trucut biopsy to fine needle aspiration may improve the diagnostic accuracy and the adequacy of sampling (Wittmann et al. 2006). Another factor that may affect the adequacy of the sample is the availability of rapid on-site evaluation (ROSE). This involves a cytologist being present in the endoscopy suite and by using rapid staining techniques (e.g. modified May-Grunwald–Giemsa stain), the cytologist is able to make an immediate assessment of the sample with high accuracy, eliminating inconclusive or inadequate samples (Tournoy et al. 2005). This arrangement has also been shown to be cost-effective (Pellise et al. 2007). The issue of the number of passes into the lymph node by EUS-FNA for optimal diagnostic yield has also been addressed. Diagnostic yield can be maximized by performing 3 – 5 passes (Leblanc et al. 2004;Wallace et al. 2001).

Cohort studies have demonstrated that EUS-FNA is a safe and efficacious procedure for the mediastinal staging of NSCLC. A meta-analysis of 18 studies (Micames et al. 2007), totaling 1201 patients demonstrated a pooled sensitivity of 83% (range 45% – 100%) and specificity of 97% (range 88% – 100%), with a median prevalence of mediastinal disease of 65% . These studies only included patients with mediastinal lymph nodes accessible to EUS-FNA.

The position of EUS in the lung cancer staging algorithm is yet to be fully addressed in randomized trials. Tournoy et al recruited 40 patients requiring invasive mediastinal staging and randomly allocated them to undergo mediastinoscopy (21 patients) or EUS-FNA (19 patients). Negative EUS-FNA results were followed by

mediastinoscopy. A negative mediastinoscopy in each arm allowed thoracotomy and definitive MLN sampling. The authors showed that only 32% of patients allocated EUS required mediastinoscopy ($p<0.001$). EUS therefore significantly reduced the need for mediastinoscopy in patients with NSCLC requiring invasive MLN staging (Tournoy et al. 2008). Another randomized study of 104 patients compared conventional work-up (CWU) to a strategy where all patients were offered EUS-FNA in addition to CWU (Larsen et al. 2005). Fifty-one patients underwent CWU and 54 patients were allocated to routine EUS-FNA and CWU. The number of futile thoracotomies was again chosen as the primary endpoint and defined as an exploratory thoracotomy without tumour resection or death or evidence of tumour recurrence during follow-up. PET scanning was only available for 30-50% of patients. Preliminary results demonstrated that the number of futile thoracotomies was 5 (9%) in the routine EUS-FNA group and 13 (25%) in the CWU group ($P = 0.03$) after a median follow-up of 1.3 years.

Unfortunately, in these 2 randomized controlled trials, healthcare costs were not reported and patients undergoing mediastinoscopy typically required an overnight stay, which is not the current standard of care in the USA. The studies do however suggest that EUS-FNA may reduce the number of mediastinoscopies and futile thoracotomies in patients requiring invasive MLN staging and further randomized studies including healthcare cost analysis are awaited.

Although randomized controlled trials of unselected patients may provide the highest level of evidence for a new procedure (Van den Bruel et al. 2007), cohort studies are important to demonstrate efficacy in different situations (Bossuyt, Lijmer, & Mol 2000). EUS-FNA has been examined in patients as a first test after CT, in patients with enlarged or small and PET positive or PET negative mediastinal nodes. Singh

and colleagues employed EUS-FNA as a first test after CT scan in 93 patients (Singh et al. 2007). By sampling the mediastinum, coeliac axis nodes, left lobe of the liver and left adrenal they were able to provide a tissue diagnosis and stage in a single test in 70% of cases. They detected metastases to celiac axis nodes in 11% of cases, half of which had not been suspected on CT scan. The study also highlighted the improved accuracy of EUS-FNA over CT and PET scanning for the detection of metastases from lung cancer and the poor prognosis of coeliac axis nodal involvement.

EUS-FNA is able to provide minimally invasive sampling of the posterior areas of the mediastinum that cannot be reached by standard mediastinoscopy. Annema et al. showed that adding EUS-FNA to routine mediastinoscopy in 100 patients changed pre-operative staging in 16% of cases (Annema et al. 2005). In the study, all 80 patients with negative mediastinoscopy (regardless of EUS-FNA findings) underwent thoracotomy and lymph node dissection. Two (7%) false positive results from EUS-FNA of subcarinal nodes were identified, assuming these nodes were adequately sampled at lymph node dissection. If EUS is performed after CT, PET scan and negative mediastinoscopy, malignant N2/N3 disease may still be detected in 37% of patients (Eloubeidi et al. 2005b). EUS-FNA detected metastases in lymph nodes inaccessible to mediastinoscopy, re-enforcing the importance of test selection based on lymph node distribution seen on non-invasive imaging.

The size of mediastinal nodes and their avidity for ^{18}FDG, as well as their location are important determinants of diagnostic yield by EUS-FNA. In patients with enlarged lymph nodes seen on CT scan, pooled sensitivity from meta-analysis was 90% and specificity was 97% (Micames et al. 2007). Several studies have evaluated the sensitivity of EUS in patients with no CT evidence of metastatic disease.

However, when lymph nodes <1cm are considered, pooled sensitivity drops to 58%, with a specificity of 98%. Nonetheless, the detection of mediastinal metastases in posterior lymph nodes less than 1cm in short axis on CT scan is an important finding. Since posterior lymph nodes are inaccessible to standard mediastinoscopy, these patients would have previously undergone futile thoracotomy.

Several studies have demonstrated the value of EUS-FNA in diagnosing metastases in FDG-avid lymphadenopathy. Annema and colleagues (2004) recruited 36 patients with FDG avid mediastinal lymph nodes, each of whom underwent EUS-FNA. N2/N3 positive disease was diagnosed by EUS-FNA in 25 out of the 36 patients. Mediastinal metastases were missed in 1 PET positive and 1 PET negative lymph node. The sensitivity, negative predictive value and accuracy of EUS-FNA in this small study was 93%, 80% and 94% respectively. Another study based in the Netherlands evaluated PET positive lesions in 81 patients with proven or suspected lung cancer (Kramer et al. 2004). 50 patients in this trial had a positive diagnosis of metastatic disease by EUS-FNA, conferring inoperability. Metastases in 19 patients were missed by EUS-FNA and confirmed by surgery or clinical follow-up. However, the study showed that EUS-FNA targeting of PET nodes was a cost-effective strategy, reducing surgical staging procedures by more than 50% and saving 40% of staging costs. The unreliability of negative EUS-FNA samples, however, means that surgical staging techniques still have an important role.

Other studies have compared the performance of CT, PET and EUS-FNA and concluded that EUS-FNA has a higher positive predictive value and overall accuracy than the other staging modalities (Eloubeidi et al. 2005a;Fritscher-Ravens et al. 2003). Several studies have also specifically evaluated the ability of EUS-FNA to detect metastases in mediastinal lymph nodes < 1cm in short axis (Leblanc et al.

2005;Wallace et al. 2004). Their results suggested that metastatic disease could be detected in 25% of patients, resulting in a change of management. However, these studies did not employ routine PET scanning as part of their protocol and currently the routine deployment of EUS-FNA (or mediastinoscopy) in PET and CT negative nodes cannot currently be justified, due to the high negative predictive value of PET and CT combined in stage 1 disease. A US study of 153 patients, included 136 patients thought to have clinical N0 disease and with no evidence of extra-thoracic disease (Cerfolio, Bryant, & Eloubeidi 2006). These patients underwent routine EUS-FNA and mediastinoscopy, followed by thoracotomy if both were negative. The authors found that EUS-FNA and mediastinoscopy detected metastatic mediastinal disease in only 3.7% and 2.9% of cases respectively. Unsuspected N2 disease was found in a further 4.4% of patients at thoracotomy. Therefore, although EUS-FNA can detect metastases in MLNs <1cm , it is not required if PET scan demonstrates an SUVmax of <2.5. The yield of detection of pre-operative N2 disease is much higher in patients with clinical hilar (N1) disease at 41% and therefore invasive mediastinal staging should currently be advocated for this group of patients (Hishida et al. 2008).

Primary NSCLCs of the left upper lobe have a predilection for metastasis to mediastinal lymph nodes in the aorto-pulmonary window (station 5) and para-aortic lymph nodes (station 6), often skipping left hilar (N1) nodes (Cerfolio & Bryant 2006). Although EUS-FNA is often able to sample station 5 nodes, the para-aortic area is generally inaccessible to minimally invasive techniques (Figure 1.1). Few studies have evaluated the best approach for the mediastinal staging of patients with left upper lobe tumours. A retrospective study of 112 patients with suspected metastases in lymph node stations 5 or 6 suggested that EUS-FNA had an accuracy

of 66%, whereas the preferred staging procedure was left VATS, with an accuracy of 100% in this group of patients (Cerfolio, Bryant, & Eloubeidi 2007).

EUS-FNA has been utilized as a tool to determine mediastinal invasion of the primary tumour (T4 disease). In one small retrospective analysis, 3 out of 10 patients thought to have mediastinal invasion by EUS, were found to have T2 disease at surgery and underwent successful resection (Varadarajulu et al. 2004). Accurately determining mediastinal invasion is of paramount importance as patients with T4 (stage IIIB) disease have a five-year survival of less than 5% and are generally not offered surgery. Although further data from other centres is required, assessing mediastinal invasion by EUS alone cannot currently be advocated due to its high false positive rate.

Employing EUS-FNA in the staging algorithm for NSCLC may represent significant healthcare cost-savings by minimizing the number of surgical procedures. Various staging strategies have been compared for a hypothetical patient with NSCLC and 1cm subcarinal lymphadenopathy (Harewood et al. 2002). If the sensitivity of EUS-FNA exceeded 76% or the probability of the node being malignant was greater than 24%, EUS-FNA as a first test was the most cost efficient model. Mediastinoscopy, PET, CT–guided biopsy, blind TBNA but not endobronchial ultrasound was included in the evaluation. Another study demonstrated that EUS in an ambulatory setting before mediastinoscopy as an inpatient is a cost effective strategy, even with a negative predictive value of EUS-FNA as low as 22% (Aabakken et al. 1999).

EUS-FNA represents an important advance for the accurate mediastinal staging of NSCLC. It is cost efficient and may be a particularly useful method for sampling posterior FDG-avid MLN. However, issues around utilization, availability, training

and expertise in the procedure remain. A recent survey in the United States suggested that over 60% of oncologists felt that EUS would not impact on staging NSCLC (Reddy et al. 2008). Furthermore, in centres where EUS was available, less than 20% of oncologists would employ the service for lung cancer staging. The recent incorporation of EUS-FNA into lung cancer staging guidelines and the increased multidisciplinary approach to lung cancer should encourage its uptake.

1.5 ISOLATED MEDIASTINAL LYMPHADENOPATHY

1.5.1 Differential diagnosis and importance of pathological diagnosis

Isolated mediastinal lymphadenopathy refers to enlarged MLNs (≥1cm in short axis) in the absence of a lung parenchymal lesion ≥1cm in short axis. In UK clinical practice the most common causes are reactive (or anthracotic) lymph nodes, sarcoidosis, lung cancer, tuberculosis and lymphoma (Table 1.1).

Table 1.1: Causes of isolated mediastinal lymphadenopathy according to disease frequency

The differential diagnosis of isolated mediastinal lymphadenopathy	
Common	Reactive hyperplasia
	Sarcoidosis
	Thoracic malignancy
	Tuberculosis
	Lymphoma
Uncommon	Metastases from extra-thoracic malignancy
	Histoplasmosis
	Coccidiodomycosis
	Castleman's disease
	Angioimmunoblastic lymphadenopathy
	Chronic berylliosis
	Wegener's granulomatosis

Since each of these conditions have different prognoses and treatments it is important to differentiate them. Patients with enlarged lymph nodes due to sarcoidosis (stages I and II) often have a benign course and occasionally may require oral corticosteroid treatment. Patients with tuberculous lymphadenopathy require anti-tuberculous chemotherapy and have an excellent outlook. However, those patients with enlarged MLNs due to malignancy have a poor prognosis. Metastatic spread with incurable disease is implied and treatment is often chemotherapy with palliative intent. The exception is lymphoma which may respond satisfactorily to chemotherapy and/or radiotherapy. Conversely, reactive lymphadenopathy requires no further intervention. The diverse treatments for isolated MLNs highlight the importance of establishing an accurate diagnosis. The current available methods for the diagnosis of isolated MLNs are discussed below.

1.5.2 Techniques for the diagnosis of isolated mediastinal lymphadenopathy

1.5.2.1 Chest radiograph and Computed tomography scan

On the chest radiograph, the ease with which MLN enlargement can be recognized depends on the particular location. Enlargement of the right upper paratracheal nodes causes uniform or lobular widening of the right paratracheal stripe, and an increase in density of the superior vena cava of which the border may become convex to the lung. Enlarged right lower paratracheal nodes push the azygos vein laterally increasing the diameter of the combined opacities of both node and azygos arch. Aorto-pulmonary nodes may cause a bulge in the angle between the aortic arch and the main pulmonary artery. If they are substantially enlarged, the left upper paratracheal nodes induce mediastinal widening. The radiographic features of

subcarinal node enlargement include the displacement of the azygo-oesophageal line that becomes convex to the lung, an increased opacity of the subcarinal space on the posteroanterior film and a lack of visibility of the external surface of the medial wall of the intermediate bronchus. Enlargement of the anterior mediastinal nodes may be substantial to be visible on the chest films. In such case, mediastinal widening is frequently bilateral and lobulated in outline. The radiographic signs of enlargement of hilar lymph nodes are hilar enlargement, or a rounded mass in a portion of the hilum.

On CT scan, lymph node enlargement is defined on the basis of a short-axis node diameter exceeding 1 cm. A coalescence of enlarged nodes suggests infection, granulomatous disease or malignancy. Diffuse mediastinal involvement is more typical of lymphoma, large cell undifferentiated carcinoma and acute or chronic mediastinitis. Computed Tomography (CT) can also be used to define the density of lymph nodes. Enlarged nodes may be calcified, or low in density and necrotic in appearance or can enhance following intravenous injection of contrast media. Low attenuation lymph nodes after administration of contrast media, with or without rim enhancement typically reflect the presence of necrosis. This finding is commonly seen in patients with tuberculosis, metastatic carcinoma and lymphoma. Post-contrast enhancement of enlarged hilar and MLNs may suggest Castleman's disease, angioimmunoblastic lymphadenopathy or vascular metastases in particular from renal cell carcinoma. This feature of enhancement may however also be found in sarcoidosis and tuberculous lymphadenopathy. Therefore, CT appearances are insufficiently specific to allow a definitive diagnosis and pathological diagnosis remains necessary. CT does however provide accurate anatomical information and acts as a road-map for further investigations. In addition, CT also provides images of

the lung parenchyma which may aid in the diagnosis of isolated MLNs. In particular, patients with sarcoidosis may have characteristic lung appearances, most commonly with small nodules (<1cm) in a perilymphatic distribution and along the fissures.

1.5.2.2 Positron emission tomography

PET enables detection of MLNs with abnormally high functional activity (e.g. tumour metastases), a feature that CT lacks. Because of this advantage and because of the limitations of using size criteria with CT to diagnose malignant MLNs, PET has superior sensitivity, specificity and accuracy in diagnosing mediastinal metastases as compared with CT. However, reactive and inflammatory mediastinal lymph nodes especially due to tuberculosis or sarcoid may also be positive on PET scanning.

Scientific data on the role of PET or integrated PET-CT in sarcoidosis is limited. One study suggested that the sensitivity of PET in detecting sarcoid was high for radiographic stages I and II (where enlarged MLNs are a feature) and may predict disease activity and reponse to treatment (Teirstein et al. 2007). However, specificity remains low. SUVmax values between 2 and 15 have been reported in MLNs due to sarcoid and therefore FDG avid mediastinal lymph nodes are non-specific and require pathological diagnosis.

Functional imaging with 18-FDG PET and integrated PET-CT increase the sensitivity and specificity of lymphoma assessment and may also predict outcome and direct future therapies (Zinzani et al. 2009). Once again, however there are no specific appearances on PET images that will preclude the need for pathological diagnosis. Active tuberculosis (TB) infection including asymptomatic and extra-pulmonary disease may be detected with PET-CT (Hofmeyr, Lau, & Slavin 2007). It

may also be a useful tool in the assessment of latent TB, to exclude active disease prior to treatment. PET/CT has the potential for monitoring response to anti-tuberculosis treatment. Metabolic response may also indicate clinical response and guide duration of anti-mycobacterial therapy.

Despite advances in imaging techniques, pathological confirmation of mediastinal lymphadenopathy remains necessary.

1.5.2.1 Mediastinoscopy

Cervical mediastinoscopy is currently considered the best investigation for the diagnosis of mediastinal lymphadenopathy. The procedure is performed under general anaesthesia and provides access to the upper and lower paratracheal lymph nodes and occasionally the anterior subcarinal station. Although rare, complications do occur. One percent of patients experience complications including haemorrhage, vocal cord dysfunction, tracheal injury and pneumothorax. Mortality rate is considered to be 0.1%, usually from damage to major vessels intra-operatively.

The largest published series of mediastinoscopy examined 2145 procedures over a nine year period in a single centre (Lemaire et al. 2006). In patients with lung cancer, their false negative rate was 5.5% when the disease prevalence was 23.5%.

A recent study of 47 patients with isolated MLN examined the diagnostic yield of mediastinoscopy and compared it to the clinical diagnosis (McManus et al. 2008). The sensitivity and specificity of the pre-operative clinical diagnosis was 87% and 78% respectively. 1 patient with suspected tuberculosis was revealed to have lymphoma on biopsy. Five out of the 12 patients with a pre-operative diagnosis of malignancy had a final diagnosis of sarcoidosis. Nine cases of isolated MLN were

identified incidentally. Of these, 7 had tuberculosis, 1 sarcoid and 1 non-small cell lung cancer. All but one patient had a definitive diagnosis reached at mediastinoscopy.

Another large study of mediastinoscopy for the diagnosis of MLN, prospectively evaluated 271 patients with isolated MLN and 127 patients with a pulmonary or hilar lesion of unknown aetiology (Porte et al. 1998). Overall there were 17 false negative results (4.3%). The sensitivity of mediastinoscopy in patients with isolated MLN was 96% and in patients with a pulmonary or hilar lesion the sensitivity was 92%. Interestingly, 76% of the samples were performed in the right paratracheal lymph node station (4R), with 12.5% from the subcarinal lymph node station (7) and 7.8% in the left paratracheal lymph node station (4L). There were no deaths and morbidity was low (2.25%). Importantly, mediastinoscopy altered the pre-operative suspected diagnosis in 74 patients (18.5%).

Mediastinoscopy therefore offers a sensitive and safe technique for the diagnosis of mediastinal lymphadenopathy and is currently considered the gold standard investigation. However, several limitations of the procedure must be recognised. First, standard cervical mediastinoscopy does not allow complete access to the mediastinum. In particular, posterior subcarinal nodes (station 7), the aorto-pulmonary window (stations 5 and 6) and inferior lymph node stations (stations 8 and 9) are usually inaccessible to the standard technique. Also, general anaesthesia is required and overnight inpatient stay is still necessary in the UK for the majority of patients. These latter considerations in addition to surgical time are responsible for high healthcare costs associated with the procedure.

1.5.2.4 Bronchoscopy and transbronchial needle aspiration

Standard bronchoscopy is commonly performed for the diagnosis of isolated MLNs. During standard bronchoscopy, a dedicated transbronchial aspiration needle is introduced into the biopsy channel and blindly punctures the bronchial wall allowing the mediastinal lymph node to be aspirated. As discussed above, in patients with lung cancer sensitivity is low at 39%, with a false negative rate of 28%, when the prevalence of mediastinal metastases was 34% (Holty, Kuschner, & Gould 2005).

One study has examined the utility of conventional TBNA for the diagnosis of isolated MLN (Cetinkaya et al. 2004). TBNA procedures were performed using a flexible bronchoscope and a 22-gauge Wang needle in 60 consecutive patients with isolated MLN. A diagnosis was reached in 45 of 60 patients (75%). Diagnoses included tuberculosis (n=21), sarcoidosis (n=21), carcinoma (n=15), and lymphoma (n=3). TBNA had high sensitivity for TB, but diagnosed 1 case (out of 3) of lymphoma.

Several other studies have examined the role of conventional TBNA in patients with MLN due to suspected sarcoid and tuberculosis. They have found similar sensitivities of the procedure of 75 – 79% (Bilaceroglu et al. 2004;Trisolini et al. 2008). However, overall, the relatively low diagnostic yield and high negative predictive value mean that TBNA is poorly utilized (Haponik & Shure 1997) and further tests are commonly necessary in the event of a non-diagnostic or negative sample.

1.5.2.5 Endoscopic ultrasound-guided fine needle aspiration

As discussed above, studies have clearly demonstrated the utility of EUS-FNA in the mediastinal staging of NSCLC and data is now emerging on the utility of EUS for the diagnosis of sarcoid (Annema, Veselic, & Rabe 2005). In one study, EUS-FNA

demonstrated non-caseating granulomas without necrosis in 41 of 50 patients (82%) with a final diagnosis of sarcoidosis. Similar high yields were obtained in patients with intra-thoracic lymph node tuberculosis (Puri et al. 2010;Song et al. 2010). Currently, there are no reports on its value in patients with MLNs due to lymphoma.

Although EUS-FNA is a promising tool for the diagnosis of isolated MLNs, there are several restrictions. EUS cannot sample right-sided or hilar lymph nodes stations and these areas are commonly involved in patients with sarcoidosis and tuberculosis (particularly 4R). In addition, EUS does not allow visualization of the endobronchial tree which may provide additional diagnostic information in patients with granulomatous diseases. The equipment and skilled personnel are also not widely available and this has meant that EUS (like conventional TBNA) is underutilized for the diagnosis of isolated MLN.

1.6. ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION

1.6.1 The equipment and procedure

EBUS-TBNA, using a linear echoendoscope, was first described in 2003 (Krasnik et al. 2003). The procedure allows TBNA with a 22 or 21-gauge needle under real-time ultrasound guidance. This progress in technology allows the respiratory physician and thoracic surgeon for the first time to sample the majority of the mediastinum in a minimally invasive manner with high sensitivity. Lymph nodes stations 1, 2, 4, 7, 10 and 11 are readily accessible, representing an increased range compared to standard mediastinoscopy. EBUS-TBNA routinely provides samples from the posterior sub-

carinal space and hilar areas that are out of reach of cervical mediastinoscopy. Nodes in stations 5 and 6 as well as the lower paraoesophageal stations (8 and 9) are not accessible by EBUS-TBNA.

Prior to 2003, the procedure was initially performed by placing a catheter with a radial ultrasound mini-probe at the tip, in the working channel of the bronchoscope. When the lymph node to be sampled had been located, the catheter was withdrawn and replaced with a TBNA needle. The lymph node was then sampled blindly with TBNA. More recently, an integrated curvilinear ultrasound bronchoscope has been developed and allows TBNA under real-time ultrasound guidance (Figure 1.2b). This progress in technology allows sampling of the mediastinum in a minimally invasive manner with high sensitivity.

The integrated bronchoscope [Olympus BF-UC160F-OL8, Olympus Medical Systems, Tokyo, Japan] has a convex ultrasound transducer at the distal end with a 7.5Mhz frequency and allows visualization of para-bronchial structures up to a depth of 5cm. The outer diameter of the insertion tube is 6.2mm and that of the tip is 6.9mm. The distal end of the scope can be adjusted 160° upwards and 90° downward and the endoscope has a biopsy channel of 2 mm. The fibre-optic lens is oblique forward-viewing at 30° and the ultrasound image is in parallel to the scope, with a scanning angle of 50°. After intubation with the EBUS scope, a saline-filled balloon is inflated to maintain contact with the airway wall. Vascular structures are located using the power Doppler. Once the target lymph node is identified on the ultrasonography monitor, a dedicated 22 or 21 gauge needle (XNA-202C; Olympus Ltd) is inserted into the working channel. The needle can then be observed to pierce and enter the lymph node under direct ultrasound vision. Suction is applied and the needle is moved to and fro within the lesion. Using this technique, mediastinal

lymph nodes as small as 4mm may be sampled. The procedure is carried out in the outpatient setting, under conscious sedation. Lymph nodes that may represent N3 (contralateral mediastinal or hilar) metastases are sampled first, followed by N2 nodes and finally ipsilateral hilar nodes, so that any contamination will not result in false over-staging. A diagnostic plateau is reached after 3 passes per lymph node (Lee et al. 2008b).

Lymph nodes stations 1, 2, 3, 4, 7, 10 and 11 are readily accessible, representing an increased range compared to standard mediastinoscopy. EBUS-TBNA routinely provides samples from the posterior subcarinal space and hilar areas that are out of reach of cervical mediastinoscopy. Previous randomized studies have compared the use of the mini-probe ultrasound guided TBNA to the blind technique with conflicting results (Herth, Becker, & Ernst 2004;Shannon et al. 1996). Data now exists that confirms the theoretical benefit of real-time linear EBUS-TBNA over the blind method. A single centre study from the US prospectively examined 138 consecutive patients with suspected or proven lung cancer (Wallace et al. 2008). Each patient sequentially underwent blind TBNA, EBUS-TBNA (and EUS-FNA) and 30% of patients in the cohort had mediastinal metastases. The study demonstrated that linear real-time EBUS-TBNA had a significantly superior sensitivity for detecting mediastinal disease than standard TBNA (69% vs 36%).

1.6.2 Current evidence in the mediastinal staging of non-small cell lung cancer

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) may represent a significant advance in the mediastinal staging of NSCLC. To date, however there are no completed randomized trials investigating the effects of EBUS-

TBNA on patient outcome and healthcare costs. An early study of EBUS-TBNA from Japan in 2004 demonstrated a sensitivity of 95.7% for MLN involvement in 70 patients with a prevalence of disease of 67% (Yasufuku et al. 2004). In a large cohort of 502 patients from Germany, the US and Denmark, a sensitivity of 93.5% in 502 patients was observed (Herth et al. 2006a). Meta-analysis of EBUS-TBNA in 1299 patients from expert centres has demonstrated a pooled sensitivity of 93% (Gu et al. 2009). Reported complications of linear EBUS-TBNA are rare but include pneumothorax requiring intercostal drainage (Bauwens et al. 2008) and an infected pericardial space (Haas 2009).

Given the high diagnostic accuracy, it is a natural progression to compare EBUS-TBNA to the gold standard of mediastinoscopy. One study has suggested that EBUS-TBNA is superior to mediastinoscopy for MLN staging in patients with enlarged mediastinal nodes (Ernst et al. 2008). Consecutive patients with suspected lung cancer on the basis of clinical and CT findings were included. All patients had enlarged mediastinal adenopathy (≥ 10mm in short axis) confined to lymph node stations 2, 4 or 7 and data from PET was not available. Cervical mediastinoscopy was performed on all patients. EBUS-TBNA was also performed on all patients, either as a separate procedure 1 week before or at the same time as mediastinoscopy. Surgical lymph node dissection was the diagnostic standard. One hundred and twenty mediastinal lymph nodes (in 66 patients) were sampled by EBUS-FNA and mediastinoscopy. 7 patients had benign disease and of the remaining 59 patients, 57 had NSCLC, 1 had small cell lung cancer and there was 1 case of lymphoma. The diagnostic yield (combining true positives and true negatives) of EBUS-FNA was 109/120 (91%). This was statistically superior to the yield from mediastinoscopy 94/120 (78%) with a P value of 0.007. The observed difference was due to the

supremacy of EBUS-FNA over mediastinoscopy for subcarinal nodes and may be explained by the fact that posterior subcarinal nodes are beyond the reach of mediastinoscopy. Another important finding of this trial was that all positive results from EBUS-TBNA were verified by surgical lymph node dissection, confirming a specificity of 100% for the technique.

The false negative (FN) rate from invasive mediastinal tests (EBUS-TBNA, EUS-FNA, mediastinoscopy) may result from either limitation in mediastinal access or sampling error within a lymph node. The FN rate for mediastinoscopy may be explained by the fact that only certain MLN stations can be accessed. The technique does however provide histological samples, allowing detection of micro-metastases. EBUS-TBNA has greater mediastinal (and hilar) access than mediastinoscopy but relies on needle aspiration. Sampling error within a MLN may therefore be responsible for the FN rate seen with needle aspiration techniques. Up to 25% of malignant MLNs may contain metastatic disease in the marginal area of the lymph node only, corresponding closely to the FN rate observed in clinical studies. Due to this FN rate, negative EBUS-TBNA results (including adequate samples) should be investigated further with surgical staging investigations.

Figure 1.2: Endoscopic (a) and endobronchial (b) ultrasound echoendoscopes

As with EUS-FNA, EBUS-FNA has been evaluated in specific patient groups. A Belgian study looked at 102 patients with NSCLC and FDG-avid MLN (Bauwens et al. 2008). They found a prevalence of mediastinal disease of 58% and EBUS-TBNA had a sensitivity of 95% with a NPV of 91%. Mediastinoscopy was prevented in 59 cases and therefore EBUS-TBNA was a highly effective initial alternative to mediastinoscopy in patients with PET positive MLNs.

Yasufuku and colleagues performed CT, PET and EBUS-TBNA on 102 radiologically operable patients on the basis of CT scan (Yasufuku et al. 2006). They found that the sensitivity of CT, PET and EBUS-TBNA for detecting mediastinal spread of NSCLC in this context was 76.9%, 80.0%, and 92.3% respectively. This illustrates that CT and PET miss mediastinal metastases in patients with clinical stage 1 disease and that this disease may be detected by EBUS-TBNA.

Herth et al. (2006b) examined 100 patients with a radiographically normal mediastinum on staging CT scan. Current guidelines do not advocate the use of ultrasound guided mediastinal aspiration in patients with MLNs less than 1cm in short axis. However, in this study mediastinal metastasis was detected in 1 in 6

patients, with a sensitivity of 92% and NPV of 96%. Furthermore, EBUS-TBNA can detect malignancy in 9% of MLNs that are less than 1cm in short axis and negative on PET scan with a sensitivity of 89% (Herth et al. 2008). A further 60 patients in the study by Wallace et al. (2008) also were negative on CT and PET. 12 patients were found subsequently to have MLN metastases, half of which were detected by EBUS-TBNA. Currently, patients with no enlarged MLNs which are also negative on PET scan are offered curative surgery or radical radiotherapy, in the absence of proven extra-thoracic disease. These studies therefore argue that EBUS-TBNA can detect MLN metastases in small nodes and may have an important role in the pre-operative assessment of patients with NSCLC and may prevent futile thoracotomies.

In the absence of available cost-effectiveness data and also the variable yield associated with blind TBNA, EBUS-TBNA has been slow to be incorporated into international diagnostic and staging algorithms for NSCLC, despite advantages over other staging modalities (see Table 2). Currently in the US, issues surrounding re-imbursement and in the UK, a lack of a specific NHS tariff may hinder the uptake of EBUS-TBNA. The advent of EBUS-TBNA and EUS-FNA and emerging data regarding their use for the MLN staging in NSCLC do however represent a significant advance for patients with NSCLC and the multi-disciplinary team charged with their care. However, the position of EBUS-TBNA in the diagnostic algorithm for NSCLC requires clarification.

Table 1.2: Comparison of different techniques for mediastinal lymph node staging in non-small cell lung cancer. Sensitivities from American College of Chest Physician Clinical Practice Guidelines (Silvestri et al. 2007; Detterbeck et al. 2007).

Investigation	Sensitivity	Specificity	Advantages	Disadvantages
Computed tomography	51%	86%	Delineates anatomy.	Uses 1cm short-axis cut-off for malignancy. 40% of enlarged nodes are benign. 20% of normal sized nodes contain malignancy.
Positron emission tomography	74%	85%	High negative predictive value for stage 1 disease. Accurate systemic staging.	25% false positive rate. Inaccurate in lymph nodes >1 cm .
Transbronchial needle aspiration	78%	99%	Cost effective. Allows simultaneous airway inspection.	Variability in results and utilization. Usually limited to enlarged nodes in stations 4 and 7.
Mediastinoscopy	78%	100%	Considered gold standard. Allows detection of micro-metastases and extra-capsular extension.	Risks of general anaesthesia and surgery. Lymph nodes stations 5, 6, 8, 9 and 11 not accessible to standard technique.
Endoscopic ultrasound	84%	99.5%	High sensitivity in para-esophageal lymph node stations. Access to celiac axis nodes, liver, left adrenal gland. Can detect malignancy in normal sized nodes. Minimally invasive and complimentary to EBUS-TBNA.	Requires specialized training and equipment. Lymph node stations 2R, 4R, 5, 10 and 11 and endobronchial tree cannot be assessed.
Endobronchial ultrasound	90%	100%	High sensitivity for majority of mediastinum. Can detect malignancy in normal sized nodes and may be easily repeated. Minimally invasive and complimentary to EUS-FNA.	Requires specialized training and equipment. Lymph node stations 5, 6, 8 and 9 cannot be assessed.

1.6.3 Current evidence in the diagnosis of isolated mediastinal lymphadenopathy

Prior to this thesis, the published utility of EBUS-TBNA in the diagnosis of sarcoidosis is limited to under 200 patients, which suggest a sensitivity of 85 – 90% (Garwood et al. 2007;Oki et al. 2007;Wong et al. 2007). One randomised trial has confirmed that EBUS-TBNA is superior to conventional TBNA in patients with sarcoidosis (Tremblay et al. 2009). However, there is only a case report of a diagnosis of tuberculosis with EBUS-TBNA in an HIV positive individual (Steinfort et al. 2009) and a just single cohort study of 11 patients with lymphoma out of whom a diagnosis was reached in 10 with EBUS-TBNA (Kennedy et al. 2008). To date, the role and performance characteristics of EBUS-TBNA for the prospective diagnosis of isolated MLNs have not been investigated.

1.7 COMBINING ENDOBRONCHIAL AND ENDOSCOPIC ULTRASOUND

EUS and EBUS provide complimentary access to the entire mediastinum with the exception of the station 6 MLN. This combined approach is able to access mediastinal stations beyond the scope of mediastinoscopy. Several centres have employed combined EUS-FNA and EBUS-TBNA under conscious sedation at the same sitting for minimally invasive mediastinal staging in NSCLC. Initial results are encouraging. In one study of 33 patients, 31 were able to undergo both procedures sequentially under the same sedation (Vilmann et al. 2005). EBUS-TBNA provided additional information to EUS-FNA and vice versa. Using the combined procedure, sensitivity for detecting mediastinal metastases was 100%. Importantly, the combined procedure was well tolerated. In addition, EUS-FNA is able to detect extra-thoracic disease and EBUS allows visualization of the endobronchial tree. Applying both techniques in combination is therefore a very attractive prospect. The European randomized ASTER trial comparing EBUS-TBNA and EUS-FNA in a combined procedure (followed by

mediastinoscopy if negative) versus mediastinoscopy alone has recently been reported (Annema et al. 2010). One hundred and twenty three patients were randomised to the endosonography arm and 118 were allocated to the surgical staging arm. The sensitivity of endosonography followed by surgical staging (in 65 patients) was 94%, compared to 79% by surgical staging alone. The sensitivity of endosonography alone was 85% and was not statistically superior to surgical staging alone. There were significantly fewer unnecessary thoracotomies in the endosonography group (7% vs. 18%).

The new standard of re-operative mediastinal staging of NSCLC may therefore be regarded as combined endoscopic and endobronchial ultrasound followed by mediastinoscopy if endosonography is negative. However, questions linger over the widespread applicability of this approach. Currently, resources and expertise in EUS-FNA and EBUS-TBNA are limited, particularly outside the US. A further consideration is the healthcare costs of this combined methodology, particularly when compared to a radiological or PET targeted approach to the mediastinum. Finally, a limitation of the ASTER trial was that data on individual EBUS-TBNA and EUS-FNA procedures alone was not collected and so it is unknown if similar results could have been achieved with EBUS-TBNA or EUS-FNA alone.

CHAPTER 2:

THE LEARNING CURVE FOR ENDOBRONCHIAL ULTRASOUND

2.1 INTRODUCTION

It has been previously recognised that outcomes of complex procedures improve with operator experience. This association has been demonstrated in studies involving endoscopic ultrasound (EUS). It has been reported that the sensitivity of EUS-guided aspiration of pancreatic masses improves after the first 30 cases (Mertz & Gautam 2004), while assessment of the T stage of oesophageal cancer with EUS may require 100 procedures before optimal results are achieved (Fockens et al. 1996). As the use of EBUS-TBNA extends to more hospitals, the learning curve of the procedure becomes increasingly important for training, validation and patient safety. This study reports our learning curve for the application of EBUS-TBNA in consecutive unselected patients.

2.2 METHODS

In our institution, a London teaching hospital, EBUS-TBNA has been carried out by two respiratory physicians (Neal Navani, Sam M Janes) since February 2008. Both operators were proficient in standard bronchoscopy and attended a two day course dedicated to EBUS-TBNA, but had limited prior experience in conventional TBNA (less than 20 procedures each). All patients had CT scan (or integrated PET-CT) and enlarged (≥1cm in short-axis) mediastinal lymph nodes or masses in areas accessible to EBUS-TBNA. Pathological evaluation of mediastinal lymph nodes was clinically indicated in all cases, as determined by

the referring physician, surgeon or multi-disciplinary team. Lymph node location was described according to the Mountain and Dresler (1997) classification.

EBUS-TBNA was performed in all cases under conscious sedation with an integrated linear ultrasound fibre-optic bronchoscope. In patients requiring mediastinal staging of malignancy, N3 nodes were sampled before N2 and then N1 nodes in order to prevent over-staging. In cases where endobronchial disease was visible, EBUS-TBNA of enlarged mediastinal / hilar lesions was performed with minimal suction before being replaced with a standard bronchoscope for sampling of the endobronchial lesion. The colour Doppler function was used to confirm the location of vascular structures to be avoided. Once identified, the para-bronchial lymph node or mass was aspirated with a dedicated 22G fine needle. Each lymph node was aspirated 1 to 4 times, depending on the macroscopic appearance of the material obtained or the on-site cytopathologist's evaluation of adequacy, or both. Cytological samples were smeared directly onto slides. Any histological specimens obtained were fixed in formalin. When available, on-site microscopic evaluation of the cell content of the samples was performed using the modified rapid Giemsa stain.

Ethical approval for this retrospective study was not required due to the observational nature of the study. Samples from EBUS-TBNA were judged to be negative when they contained lymphoid cells only with no specific pathology, and were referred for surgical sampling where possible. The reference standard for negative EBUS-TBNA samples was considered to be surgical pathological sampling by mediastinoscopy, VATS, mediastinal lymph node dissection at thoracotomy or clinical and radiological follow-up of at least 6 months duration. The results of EBUS-TBNA were therefore classified as true positive (TP), true negative (TN) or false negative (FN) per patient. Standard definitions of sensitivity (TP / [TP + FN]), specificity (TN / [TN + false positives]), positive predictive value (TP / (TP + false

positives]), negative predictive value (TN / [TN + FN]) and accuracy ([TP + TN] / [TP + TN + FP + FN]) were used.

The results were analysed using cumulative sum analysis (CUSUM) to assess the learning curve associated with the procedure (Bolsin & Colson 2000;Wohl 1977). A CUSUM chart was created, with an acceptable diagnostic inaccuracy rate of 5% and an unacceptable rate of 15%. Alert and alarm lines were plotted at 80% and 95% confidence respectively to show whether acceptable accuracy rates had been obtained and maintained. So as not to overestimate the level of accuracy, patients found to be without disease were excluded from this analysis.

In the CUSUM analysis, a positive result from EBUS-TBNA is s and that of a false negative procedure is (1-s). Using the methods described by Bolsin and Colson (2000), the value of s was determined by the acceptable and unacceptable failure rates (5% and 20% respectively) to be 0.15. The CUSUM graph climbs by s when the success rate is below that expected. When the success rate of EBUS-TBNA is below expected, the CUSUM graph climbs by 1-s. This means that more than 1 successful procedure is needed to redress the balance following a false negative EBUS-TBNA.

Univariate logistic regression was used to investigate the relationship between lymph node size and disease prevalence. A two-sided significance level of 0.05 was used. Analyses were carried out using STATA version 10 (Stata Corp., College Station, TX).

2.3 RESULTS

Between February 2008 and November 2008, 120 patients underwent EBUS-TBNA of 136 nodes. 81 (68%) patients were male and the median age was 64 (range 24 - 88) years. The

indications for EBUS-TBNA and their frequency are listed in Table 2.1, with isolated mediastinal lymphadenopathy (in the absence of a known malignancy) being the most common indication. No complications of EBUS-TBNA were observed. The diagnoses obtained are summarised in Table 2.2.

Assuming that patients would have been referred for mediastinoscopy in the absence of EBUS-TBNA, mediastinoscopies were prevented in 55 (46%) patients. The sensitivity of EBUS-TBNA for the overall cohort was 90% with a diagnostic accuracy of 93% and negative predictive value of 83% when the disease prevalence was 68%. No false positives were observed and therefore the specificity and positive predictive values were 100%.

In order to assess our learning curve, we separated the cohort into 6 groups of 20 consecutive patients each. The sensitivity, accuracy and negative predictive value per group, along with mean lymph node size, location and utilisation of on-site cytopathology are shown in Table 2.3. There was no difference between the groups for lymph node size, location, number of passes and number of patients with on-site cytopathological evaluation. There was no significant difference in sensitivity or accuracy between any of the groups. A CUSUM analysis (Figure 2.1) indicated no significant learning curve, with a high level of accuracy obtained from the initial patients. The chart shows a very slight learning curve over the first twenty patients – although levels of accuracy never approach unacceptable levels – but after this, accuracy of at least 90% is observed over the whole series, and no periods of poor performance were identified. The right paratracheal (station 4R) and subcarinal (station 7) lymph node areas were the most frequently sampled. However, sensitivity and accuracy did not depend upon lymph node location. The size of mediastinal lymph nodes was noted to be significantly associated with disease prevalence (Table 2.4), with lymph node size being highly significant (p-value <0.001) in the logistic regression.

Table 2.1 Indications for EBUS-TBNA

Indication	Number of procedures (%)
Mediastinal staging of known or suspected lung cancer	42 (35)
Para-bronchial mass	13 (11)
Isolated mediastinal lymphadenopathy	53 (44)
Re-staging following chemotherapy	3 (3)
Suspected metastases of extra-pulmonary malignancy	9 (8)

Table 2.2: Diagnoses by EBUS-TBNA

Diagnosis	Number (%)
Reactive, anthracotic or normal lymph node	38 (32)
Non-small cell lung cancer	37 (31)
Sarcoidosis	17 (14)
Tuberculosis	11 (9)
Small cell lung cancer	9 (8)
Breast cancer	3 (3)
Colorectal carcinoma	2 (2)
Lymphoma	1 (1)
Oesophageal carcinoma	1 (1)
Endometrial carcinoma	1 (1)

Table 2.3: Sensitivity, negative predictive value and diagnostic accuracy of EBUS-TBNA according to operator experience

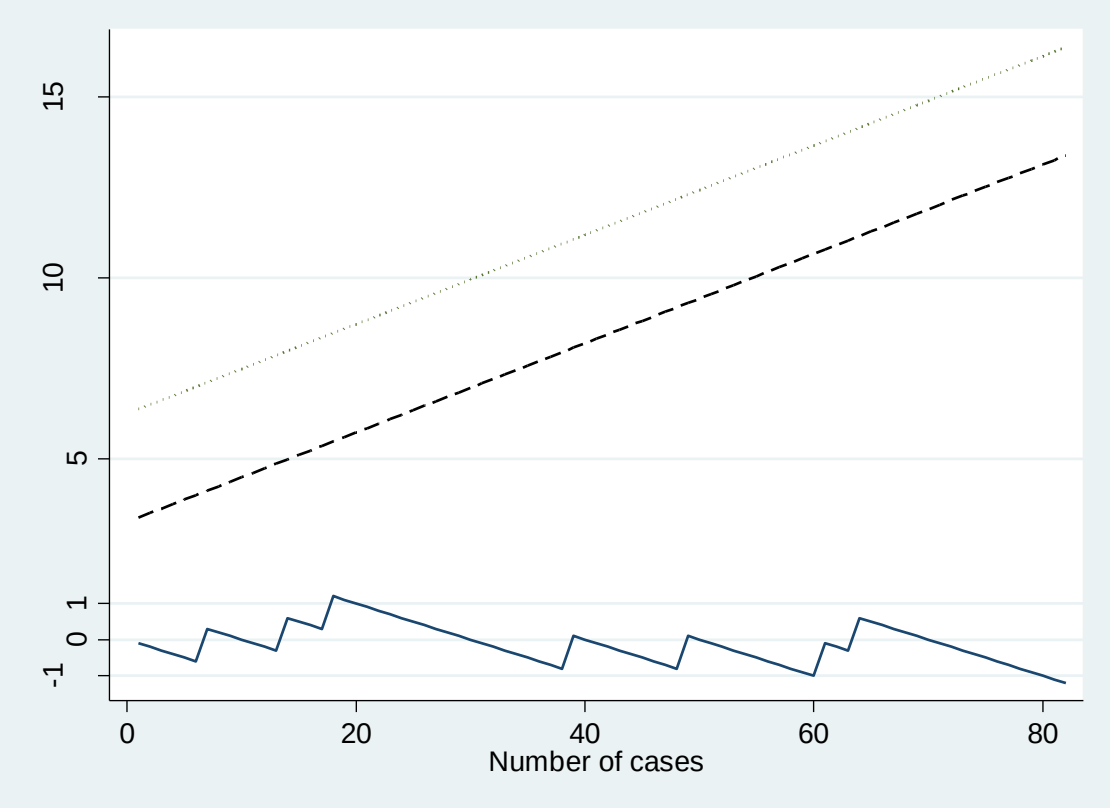
Group	Patient number	Sensitivity (%)	NPV (%)*	Accuracy (%)	Disease prevalence (%)	Mean lymph node size (mm)	Number of patients with on-site evaluation of samples
1	1 – 20	90	91	95	50	18	5
2	21 – 40	85	78	90	65	19	2
3	41 – 60	94	75	95	85	21	8
4	61 – 80	86	75	90	70	22	0
5	81 – 100	86	75	90	70	22	3
6	101 – 120	100	100	100	70	18	5
Overall		90	83	93	68	20	23

NPV - Negative Predictive Value

Table 2.4: Disease prevalence and diagnostic accuracy varies according to lymph node size

Lymph node size	No of patients	Disease prevalence (%)	Sensitivity (%)	Negative predictive value (%)
≤15mm	50	45	79	85
16 - 20mm	18	56	100	100
21 – 25mm	19	94	100	100
>25mm	33	93	93	50

Figure 2.1: CUSUM chart of the learning curve for EBUS-TBNA.



CUSUM chart of the learning curve of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) at University College London Hospital using an acceptable false-negative rate of 5% and an unacceptable rate of 20%. Internationally recognised standards were achieved after 20 cases.

2.4 DISCUSSION

The results of this study suggest that there is no significant learning curve for performing EBUS-TBNA and high standards of sensitivity and diagnostic accuracy can be reached after only 20 patients. These results should provide an impetus for centres that currently do not perform TBNA to consider EBUS-TBNA given its clear advantages over other modalities for the diagnosis of mediastinal lymphadenopathy (MLN).

First, EBUS-TBNA provides a greater range of access to the mediastinum than other invasive modalities. Lesions adjacent to the main airways and MLN in the upper and lower paratracheal regions, subcarinal area and hilar lymph node stations are sampled with EBUS-TBNA. Endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) provides samples from left-sided and posterior MLNs due to the anatomical location of the oesophagus, while cervical mediastinoscopy allows direct vision and biopsy of anterior and superior lymph nodes. Notably, standard mediastinoscopy cannot visualise the posterior subcarinal space nor the hilar lymph nodes.

A second advantage of EBUS-TBNA over mediastinoscopy is that general anaesthesia is not required. In this study, the procedure was carried out under conscious sedation in all patients, using titration of intravenous midazolam and fentanyl. No complications related to sedation or the procedure were observed in this cohort. Reports of a pneumothorax requiring intercostal drainage in one patient

(Bauwens et al. 2008) and post-procedure infectious complications in two patients (Haas et al. 2009) have been documented following linear EBUS-TBNA. However, mediastinoscopy is associated with an important but small morbidity and mortality (<1%) (Lemaire et al. 2006).

A further advantage of EBUS-TBNA is that it may be easily combined with other procedures to maximize diagnostic information at the same sitting. In cases of lung cancer, following mediastinal lymph node sampling with EBUS-TBNA, the scope may be replaced with a standard videobronchoscope for careful inspection of the airways and sampling of the primary tumour. In cases of suspected sarcoid, transbronchial biopsy may also be performed. By combining EBUS-TBNA with EUS-FNA it is possible to sample the entire mediastinum with the exception of the para-aortic lymph node station. Previous studies have shown this to be a feasible prospect (Vilmann et al. 2005; Wallace et al. 2008). More recently, it has been suggested that the EBUS scope may be placed in the oesophagus (after the pulmonary route has been utilised in the interests of hygiene) to allow more convenient access to left paratracheal lymph nodes, the posterior subcarinal area and lower paraoesophageal mediastinal nodes (Hwangbo et al. 2009).

American college of chest physician guidelines (referring to the radial miniprobe technique of EBUS) suggest that practioners should perform at least 50 EBUS-TBNA procedures in a supervised setting to establish competency (Ernst, Silvestri, & Johnstone 2003). The joint ERS/ATS statement also suggests a long learning curve for EBUS (Bolliger et al. 2002). However, in addition to the current study, a previous report from a thoracic surgical unit suggested a considerably shorter

learning period for linear real-time EBUS-TBNA (Groth et al. 2008). In that study however, all procedures were performed via an endo-tracheal tube under general anaesthesia, on-site evaluation of samples was utilised for every case and lymph node size was not reported. The current data are also consistent with the opinion of Sheski and Mathur (2008) who suggested approximately 20 procedures are required to achieve competence. Analyses of a learning curve for conventional TBNA have provided conflicting evidence. Traditionally, TBNA was thought to have a protracted training period (Hsu et al. 2004) that in part was responsible for poor uptake of the procedure. However, a more recent report has suggested that the learning curve for conventional TBNA has been exaggerated (Hermens et al. 2008). The EBUS-TBNA operators in this study (NN, SMJ) had limited experience in conventional TBNA and therefore proficiency in conventional TBNA does not appear to be a pre-requisite for EBUS-TBNA.

Several factors may comprise the learning process in EBUS-TBNA. Initially, manipulation of the EBUS scope requires practice as the viewing angle is 30 degrees oblique to the direction of the scope. Interpretation of the ultrasound image, 3-dimensional knowledge of the mediastinal anatomy in different planes to CT scans and operation of the dedicated TBNA needle are also skills that need to be acquired. However, attendance at an EBUS course followed by cases performed in an auto-educational setting allow the experienced bronchoscopist to be proficient in EBUS-TBNA after a relatively few cases. The preparation and interpretation of cytology specimens are similar to other samples already obtained in secondary care (e.g. bronchial brushings smeared onto slides) and therefore their analysis is not associated with a learning curve.

Correct interpretation of the results from EBUS-TBNA is important. This study highlights that larger mediastinal nodes have higher disease prevalence and therefore a lower negative predictive value is expected when sampling enlarged lymph nodes. In a meta-analysis of 918 patients who underwent EBUS-TBNA, the pooled negative predictive value for the cohort was 80%, when the disease prevalence was 68% (Detterbeck et al. 2007). It is therefore recommended that negative (as well as inadequate) samples from EBUS-TBNA of MLNs should be invasively sampled again by EUS-FNA or mediastinoscopy. The interpretation of positive results is subject to review at the multi-disciplinary meeting. No false positive results have been observed in ours or other studies (Ernst et al. 2008; Herth et al. 2008).

Several limitations of the data must be recognised. Lymph nodes less than 1cm in short-axis on CT scan were excluded from the learning curve period of the study. Since smaller nodes have lower disease prevalence, it may be expected that sensitivity of the procedure may fall. This may be avoided in cases of NSCLC by employing FDG PET-CT scanning prior to EBUS-TBNA and aspirating those <1cm nodes that are FDG avid, in addition to all other nodes that are ≥1cm in short axis, regardless of FDG-avidity. Although the study is retrospective, a dedicated database was set-up prior to the first case and data was entered prospectively. The study also represents the experience of a single centre and 2 operators. However, patients were consecutive and unselected and therefore not subject to selection bias and represent real clinical practice.

The indications for EBUS-TBNA are increasing and the evidence to support its use has is gathering. As well as the mediastinal staging of NSCLC, EBUS-TBNA is likely to gain prominent roles in the diagnosis of para-bronchial lesions, isolated mediastinal lymphadenopathy due to sarcoid and tuberculosis and also restaging of the mediastinum following treatment of NSCLC. As further supporting data emerges, it is likely that the use of EBUS-TBNA will increase. A growing number of physicians will consider using EBUS and this study demonstrates that the use of EBUS-TBNA by experienced bronchoscopists is associated with a short learning curve of less than 20 patients.

CHAPTER 3:

COMBINATION OF ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION WITH STANDARD BRONCHOSCOPIC TECHNIQUES IN PATIENTS WITH STAGE I AND II SARCOIDOSIS

3.1 INTRODUCTION

A pathological diagnosis of sarcoidosis is required in patients to exclude other differential diagnoses and to justify the use of immunosuppressive treatment. It may only be avoided in patients with clear evidence of bilateral hilar lymphadenopathy on chest radiograph, arthritis and erythema nodosum - Loefgren's syndrome (Iannuzzi, Rybicki, & Teirstein 2007). In patients with enlarged intra-thoracic lymph nodes due to suspected sarcoidosis, other diagnoses such as tuberculosis and malignant disorders must be excluded.

Pathological confirmation of pulmonary sarcoidosis is most commonly accomplished with flexible bronchoscopy which has a yield of approximately 70%, with higher yields obtained in patients with more advanced radiographic stages (Bilaceroglu et al. 1999). Flexible bronchoscopy under conscious sedation permits transbronchial needle aspiration (TBNA) and transbronchial lung biopsy (TBLB). Additional endobronchial biopsy (EBB) is also routinely recommended in addition, and may demonstrate non-caseating granulomas even when no endobronchial

disease is evident (Shorr, Torrington, & Hnatiuk 2001). Despite combining TBLB and EBB approximately one-third of bronchoscopies do not provide a diagnosis of sarcoidosis.

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) is gaining momentum as an important new technique for the diagnosis of enlarged lymph nodes due to sarcoidosis. Recent randomised data have demonstrated its superiority to conventional TBNA with a 19 gauge needle in the diagnosis of pulmonary sarcoidosis (Tremblay et al. 2009). Cohort studies of highly selected patients with radiographic stage I and II sarcoidosis and high disease prevalence (>90%) suggest sensitivities of between 85 and 93% (Wong et al. 2007; Oki et al. 2007; Garwood et al. 2007; (Nakajima et al. 2009).

No data is currently available on the safety and efficacy of combining the standard bronchoscopic techniques of TBLB and EBB with EBUS-TBNA for the diagnosis of sarcoidosis. We hypothesised that the diagnostic yield from the combination of EBUS-TBNA and standard bronchoscopy carried out under the same conscious sedation would be higher than with standard bronchoscopy alone. A prospective study was therefore conducted to evaluate the safety and diagnostic yield from EBUS-TBNA, TBLB, EBB and their combination in consecutive patients with enlarged intra-thoracic lymph nodes due to suspected sarcoidosis.

3.2 METHODS

3.2.1 Patients

Consecutive patients with enlarged intra-thoracic lymph nodes (≥1cm in short-axis) and suspected sarcoidosis were recruited between August 2008 and July 2009 at University College London Hospital, a tertiary London teaching hospital. Informed written consent was obtained from all patients and the institutional review board approved this prospective study. In all patients, pathological confirmation was clinically required to exclude other diseases or to support systemic treatment of sarcoidosis. All patients underwent chest X-ray, computed tomography (CT) or positron emission tomography (PET) scanning and on the basis of the clinical scenario and radiology were suspected to have stage I or II sarcoidosis (Figure 3.1a). Lymph node location was described according to the American Thoracic Society lymph node map proposed by Mountain and Dresler (1997). Patients underwent sequential EBUS-TBNA followed by TBLB and EBB under conscious sedation with midazolam and fentanyl and topical anaesthesia with 2 and 4% lidocaine. All procedures were conducted in the ambulatory care setting without the presence of an anaesthetist. In all cases, EBUS-TBNA was performed prior to standard bronchoscopy in order to avoid airway contamination following TBLB and EBB.

3.2.2 EBUS-TBNA procedure

An integrated linear ultrasound fibre-optic bronchoscope was used (BF-UC160F-OL8, Olympus, Tokyo), which scans in a direction parallel to the insertion of the bronchoscope. The scope offers endobronchial views (at a 35 degree forward oblique angle) and when in contact with airway wall, the 7.5MHz convex ultrasound transducer provides imaging of parabronchial structures (figure 3.1b). A balloon may be inflated around the tip of the scope in order to maintain contact with the airway wall. Once the target lymph node has been located (and vascular structures excluded

with the Doppler function) a compatible 22 or 21 gauge needle is placed in the working channel of the EBUS scope. The tip of the sheath of the needle is seen on the endobronchial view, and then the needle is allowed to pierce the airway wall and enter the lymph node using the jabbing technique under direct ultrasound guidance (figure 3.1b). Suction is applied and the needle is moved to and fro within the lymph node. A minimum of 4 passes per node were performed, in accordance with previous data (Garwood et al. 2007). Samples were smeared directly onto slides and air-dried before being transferred to the laboratory for cytological analysis. If histological cores were obtained these were placed in formalin. On site evaluation of samples was not employed.

3.2.3 Standard bronchoscopic procedure

After the EBUS scope was withdrawn, it was immediately replaced with a standard flexible videobronchoscope. Further topical lidocaine was applied when required. TBLB was performed from the lobe that was demonstrated to be abnormal on imaging. In patients with normal lung parenchyma (stage 1 sarcoidosis) TBLB was performed from the most convenient location at the operator's discretion. At least 4 TBLBs per patient were performed in order to maximize diagnostic tissue as recommended by current guidelines (Bradley et al. 2008). Fluoroscopy was not utilised and all TBLBs were carried out by experienced bronchoscopists (NN and SMJ) who each perform more than 100 bronchoscopies per year. After the completion of TBLBs, EBBs were performed. Areas of endobronchial cobblestoning were sampled preferentially. Where no endobronchial macroscopic abnormalities were evident an area chosen to be suitable by the operator was biopsied. At least 4

EBBs were obtained to maximize diagnostic yield. Broncho-alveolar lavage was also performed in selected cases depending upon the clinical scenario. All patients underwent routine post-procedure chest radiograph in order to detect pneumothorax.

3.2.4 Diagnostic criteria for sarcoidosis

Non-caseating granulomas on cytology (Figure 3.1c and 3.1d) or histology with negative mycobacterial and fungal cultures in the absence of malignancy were deemed to be consistent with sarcoidosis. All patients were followed up clinically and radiologically for at least 6 months. The reference standard for negative EBUS-TBNA samples was considered to be surgical pathological sampling by mediastinoscopy, VATS, mediastinal lymph node dissection at thoracotomy or clinical and radiological follow-up of at least 6 months duration. The results of EBUS-TBNA, TBLB and EBB were each classified as true positive (TP), true negative (TN) or false negative (FN) per patient.

3.2.5 Statistical analysis

We used standard definitions of sensitivity (TP / [TP + FN]), specificity (TN / [TN + FP]), positive predictive value (TP / (TP + FP]), negative predictive value (TN / [TN + FN]) and accuracy ([TP + TN] / [TP + TN + FP + FN]). The unit of analysis was the patient. Comparison of yield from diagnostic modalities was carried out using the chi-squared test or Fischer's exact test. A p value of <0.05 was taken to denote statistical significance.

3.3 RESULTS

Forty consecutive patients with suspected sarcoidosis were scheduled to undergo EBUS-TBNA, TBLB and EBB. 22 patients were male and mean age was 46 years (range 19 - 68). On radiological grounds, 27 patients were considered to have stage I sarcoidosis, while 13 patients were considered to have stage II sarcoidosis. Thirty-four patients had symptoms of cough, fevers or weight loss. Six patients were asymptomatic but required a tissue diagnosis due to immunosuppression for another disorder, infection with the human immunodeficiency virus or prior malignancy. Patient characteristics are summarised in Table 3.1.

Thirty-nine patients had sequential EBUS-TBNA, TBLB and EBB under conscious sedation. One patient was unable to undergo standard bronchoscopy following EBUS-TBNA due to intolerance of sedation. Total procedure time ranged from 40-55 minutes. Overall, 27 patients were diagnosed with sarcoidosis, 8 had TB, 2 had reactive lymphadenopathy, 2 had lymphoma (diagnosed on EBUS-TBNA and confirmed on bone marrow biopsy) and 1 had metastatic adenocarcinoma (Figure 2). All patients were followed up for at least 6 months duration and reviewed in a multi-disciplinary setting. No false positive results were obtained. 71 nodes in 40 patients were sampled with EBUS-TBNA with a median of 4 passes per lymph node (range 3-5). All patients had enlarged lymph nodes sampled in stations 4, 7 or 10. The mean size of the lymph nodes sampled was 24 mm (range 10 - 45mm). The sensitivity of EBUS-TBNA for obtaining non-caseating granulomas in patients with sarcoidosis was 85% (23/27). The sensitivity of standard bronchoscopic techniques alone was significantly lower at 35% (9/26) ($p<0.001$). Yield per procedure according to stage of sarcoidosis is summarised in Table 3. There was no significant difference in

diagnostic yield with EBUS-TBNA between stage I and II sarcoidosis. However, the sensitivity of standard bronchoscopic techniques was significantly higher in stage II (78%) versus stage I (12%) disease ($p=0.001$).

In patients with negative EBUS-TBNA, non-caseating granulomas were obtained by TBLB of radiologically normal lung parenchyma in one patient and EBB of normal appearing endobronchial mucosa in one patient. The sensitivity of combined EBUS-TBNA and standard bronchoscopic techniques for the diagnosis of sarcoidosis was 93% (25/27) and was significantly higher than standard bronchoscope techniques alone ($p<0.0001$). Overall diagnostic accuracy for EBUS-TBNA in the cohort was 88% (35/40) and the combination of EBUS-TBNA with standard bronchoscopic techniques had a diagnostic accuracy of 93% (37/40). One patient experienced a pneumothorax, requiring overnight admission but not intercostal drainage.

Table 3.1 – Characteristics of patients with suspected sarcoidosis undergoing EBUS-TBNA and bronchoscopy

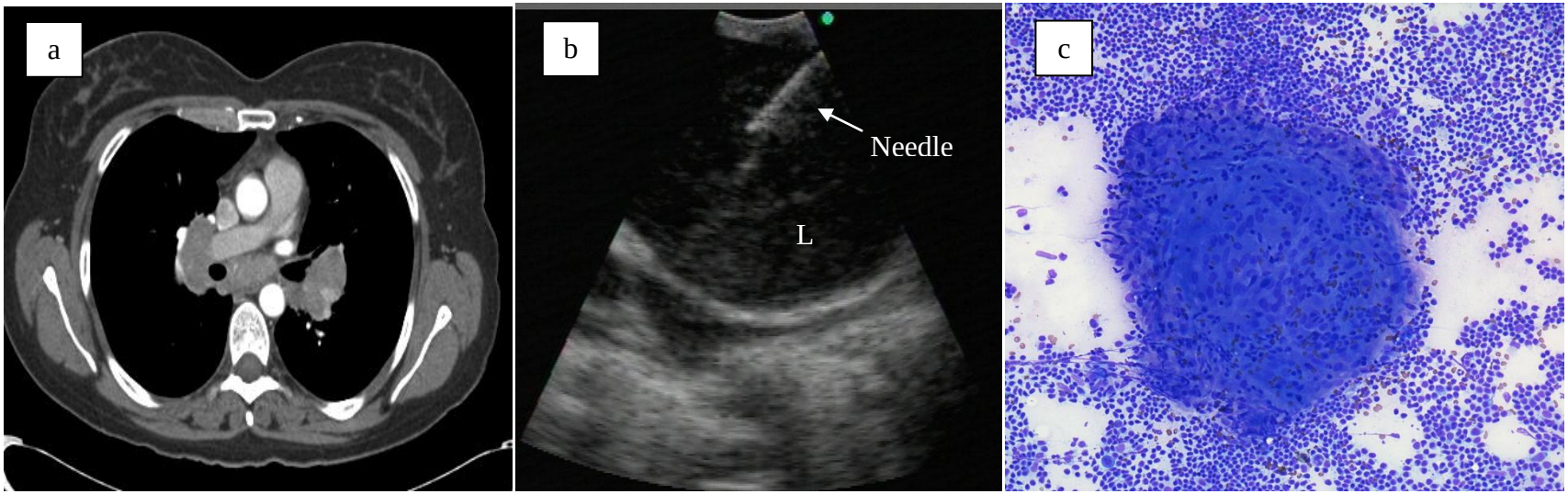
Age range		19 – 68
Gender	Male	22
	Female	18
Ethnicity	African or Caribbean	11
	Asian	2
	Caucasian	27
Symptoms	Cough	29
	Fevers	5
	Weight loss	7
	Asymptomatic	6
Lymph node stations sampled with EBUS-TBNA	4R	21
	4L	3
	7	35
	10R	10
	10L	2

Table 3.2: Diagnostic yield of endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA), standard bronchoscopy and their combination according to stage of sarcoidosis

	Number of patients with positive diagnostic yield (%)				
	EBUS-TBNA	Transbronchial lung biopsy (TBLB)	Endobronchial biopsy (EBB)	Standard bronchoscopy – TBLB and EBB	Combined EBUS-TBNA + standard bronchoscopy
Stage 1 sarcoidosis (n=18)	16 (89%)	2 (12%)*	0 (0%)*	2 (12%)*	17 (94%)*
Stage 2 sarcoidosis (n=9)	7 (78%)	6 (67%)	3 (33%)	7 (78%)	8 (89%)
Overall (n=27)	23 (85%) †	8 (31%)	3 (11%)	9 (35%)*	25 (93%) ††

*One patient with stage I sarcoidosis did not undergo standard bronchoscopy after EBUS-TBNA.† $p<0.001$ for the comparison of yields from EBUS-TBNA versus standard bronchoscopy. †† $p<0.0001$ for the comparison of yields from combined EBUS-TBNA and standard bronchoscopy versus standard bronchoscopy alone.

Figure 3.1



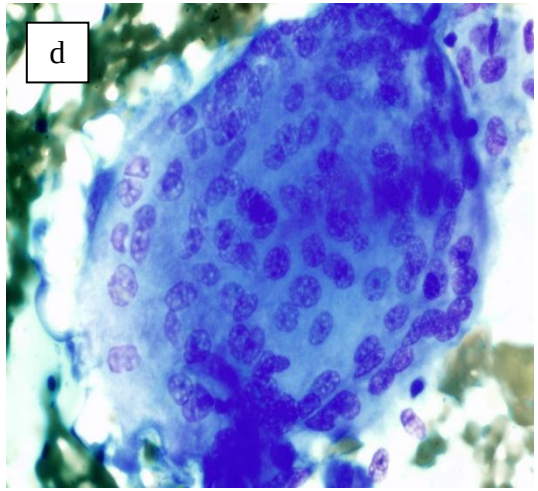


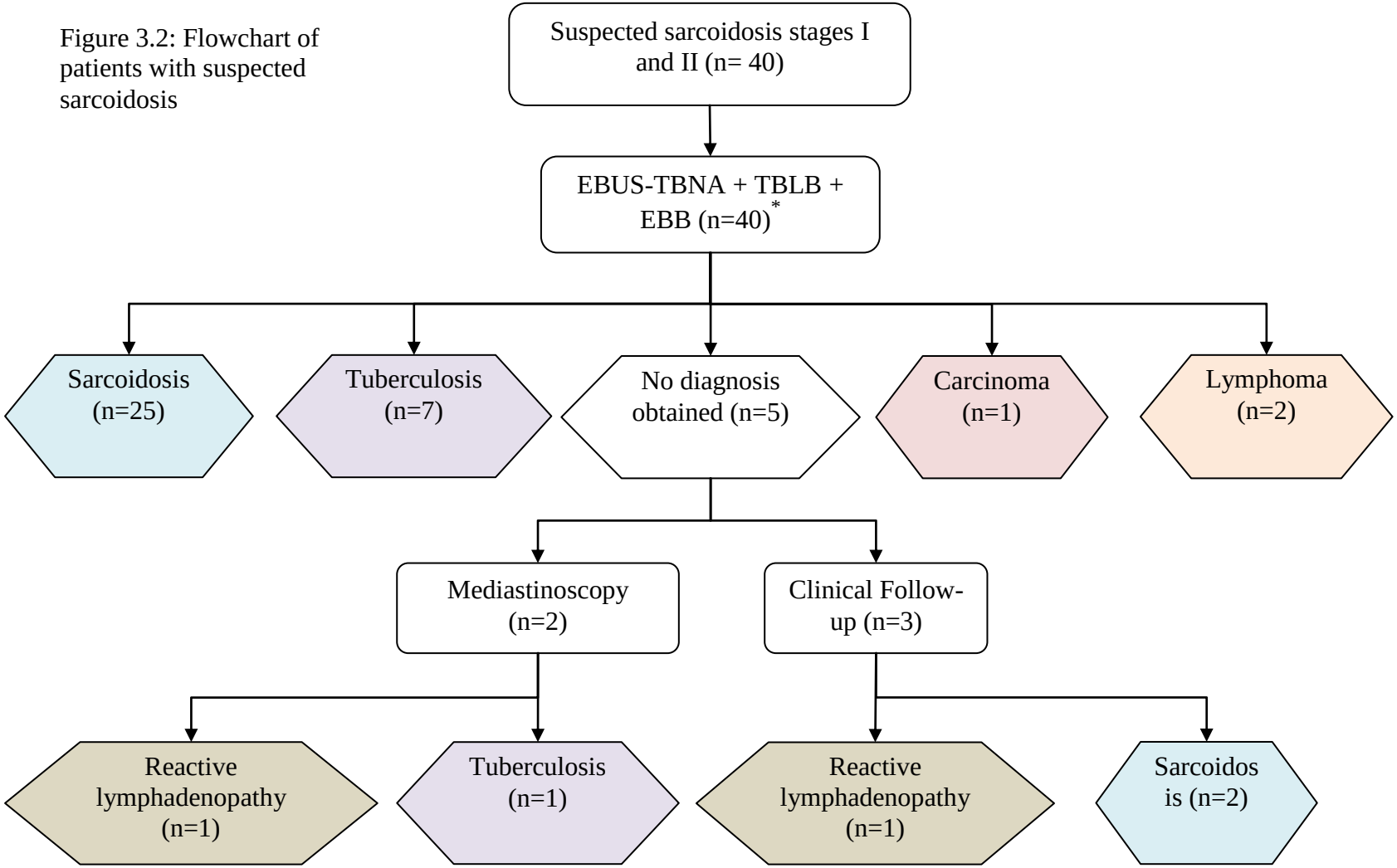
Figure 3.1a: Computed tomography with contrast image of enlarged bilateral hilar and subcarinal lymphadenopathy due to sarcoidosis.

Figure 3.1b: Endobronchial ultrasound image demonstrating transbronchial needle aspiration of lymph node with a 22 gauge needle. LN – lymph node.

Figure 3.1c: Non-caseating granuloma (low power, May-Grunwald-Giemsa stain) consistent with sarcoidosis obtained via endobronchial ultrasound guided transbronchial needle aspiration.

Figure 3.1d: Multi-nucleate giant cell (high power, May-Grunwald-Giemsa stain) obtained via endobronchial ultrasound guided transbronchial needle aspiration

Figure 3.2: Flowchart of patients with suspected sarcoidosis



*1 patient was unable to undergo standard bronchoscopy after EBUS-TBNA

3.4 DISCUSSION

In this prospective cohort study of patients with suspected sarcoidosis, combining EBUS-TBNA with standard bronchoscopic techniques optimised diagnostic yield and resulted in a higher diagnostic accuracy than bronchoscopy alone. Thirty-nine out of 40 patients were able to undergo the combined procedure under conscious sedation. The current British Thoracic Society guidelines (Bradley et al. 2008) do not mention the utility of EBUS-TBNA in the diagnosis of sarcoidosis. However this study provides further evidence that EBUS-TBNA is an important minimally invasive approach that may be combined with standard bronchoscopy and considered a first line investigation in patients with suspected sarcoidosis.

Forty patients with suspected sarcoidosis were enrolled into this study. Of these, only 27 were finally diagnosed with sarcoidosis while 8 patients were identified to have tuberculosis (Figure 3.2). This discrepancy illustrates the inaccuracy of clinical diagnosis and the benefit of obtaining a tissue diagnosis for these patients, particularly in tuberculosis endemic areas. This data is in contrast to previous cohort reports of patients with suspected sarcoidosis where the disease prevalence was 93% – 98% in whom the necessity for pathological diagnosis is questioned. Prior analysis of asymptomatic patients with presumed stage I sarcoidosis suggested invasive sampling was not required due to the low probability of detecting alternative diagnoses (Reich et al. 1998). This paradigm may however not be justified in tuberculosis and HIV prevalent regions.

A further area of controversy is the use of cytology from lymph node aspirates to determine a reliable diagnosis of sarcoidosis. Non-caseating granulomas have been observed in mediastinal lymph nodes as a reaction to malignancy or anthracotic pigment. However the presence of giant cells is thought to be specific for a true granulomatous disorder. Given that non-caseating granulomas may be observed in both tuberculosis and sarcoidosis, and Langhans type giant cells, although characteristic of tuberculosis, are not always easy to identify, a diagnosis of sarcoidosis is often made by exclusion. Additional histological material demonstrating non-caseating granulomas from TBLB or EBB add considerable weight to a diagnosis of sarcoidosis, whereas microbiological investigations of lymph node aspirate or broncho-alveolar lavage may confirm tuberculosis. In all cases, the pathological findings should be interpreted within the clinical context.

In this study, the sensitivity of standard bronchoscopic techniques of TBLB and EBB for the diagnosis of sarcoidosis was 35%. This is considerably lower than 70% previously reported in retrospective series. The operators in the current study were highly experienced in TBLB and EBB and an appropriate number of specimens were obtained in each case as recommended by current guidelines (Bradley et al. 2008). An explanation for the apparent discrepancy is that 67% patients in this cohort had radiographic stage I sarcoidosis with enlarged intra-thoracic lymphadenopathy only. The prevalence of parenchymal and endobronchial disease in this group of patients is lower than in higher radiographic stages resulting in a lower diagnostic yield for standard bronchoscopic procedures. A high diagnostic rate from standard bronchoscopic techniques was obtained in the 9 patients with stage II disease (78%).

In this subgroup there was no statistically significant benefit from the addition of EBUS-TBNA although this analysis is underpowered to draw further conclusions.

The sensitivity of EBUS-TBNA in this study (85%) is consistent with previous data. To date, the largest published study of patients with suspected sarcoid was completed in expert EBUS centres in Japan, Hong Kong and Germany (Wong et al. 2007). EBUS-TBNA was performed on 65 patients, 61 of whom had sarcoidosis. The sensitivity for the procedure was 92%. A recent retrospective study has compared EBUS-TBNA, TBLB and BAL in thirty-eight patients (Nakajima et al. 2009). Of these, 35 patients were diagnosed with sarcoidosis (31 stage I and 4 stage II). As observed in the current study, the sensitivity was higher for EBUS-TBNA (90.3%) than for TBLB (40%). The authors of the retrospective study did not however include EBB and therefore may have underestimated the diagnostic yield from bronchoscopy.

Conventional TBNA without EBUS guidance was not employed in this study. However, yield from conventional TBNA in practice has been variable and in a recent randomised trial the sensitivity of conventional TBNA (using a 19G needle) in patients with suspected sarcoidosis and enlarged intra-thoracic lymph nodes was 53.8% (Tremblay et al. 2009). This was significantly inferior to the yield from EBUS-TBNA (83.3%). Therefore the addition of conventional TBNA is unlikely to have improved the overall sensitivity.

In this study, despite the small sample size, a statistically reliable effect from the addition of EBUS-TBNA to standard bronchoscopic techniques was observed. The results reflect a single centre experience with a relatively high number of EBUS-TBNA procedures (>200) performed each year after a learning curve for EBUS-TBNA was completed. The patients were unselected and consecutive and reflect clinical practice. Of note, the additional role of EBUS-TBNA in patients with suspected sarcoidosis stages III and IV cannot be extrapolated from these results and requires further clarification. Since these radiographic stages are not associated with enlarged lymph nodes, it is likely that the benefit of additional EBUS-TBNA samples in these patients will be lower.

3.5 CONCLUSION

Combining EBUS-TBNA with standard bronchoscopic techniques is a safe and feasible procedure and optimizes the diagnostic yield in patients with pulmonary sarcoidosis and enlarged intra-thoracic lymph nodes. EBUS-TBNA in combination with standard bronchoscopy may be considered a new first-line investigation in patients with suspected sarcoidosis and enlarged intra-thoracic lymphadenopathy.

CHAPTER 4:

THE UTILITY OF ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION IN PATIENTS WITH TUBERCULOUS INTRA-THORACIC LYMPHADENOPATHY

4.1 INTRODUCTION

The global threat of tuberculosis (TB) remains undiminished with the World Health Organization (WHO) estimating there were 9.4 million incident cases worldwide in 2009 (World Health Organisation 2010a). The incidence in the UK has risen year on year over the last 2 decades, and this trend continued in 2009 with a 4.2% rise. Successful chemotherapy requires a combination of drugs for at least six months, but this may need to be substantially increased if resistance to the first line agents is present. Indeed, the emergence of drug resistant, multi-drug resistant and extremely-drug resistant (XDR) TB over the last 20 years has emphasised the importance of establishing the correct diagnosis and drug susceptibilities of the mycobacterium before starting anti-tuberculous therapy(World Health Organisation 2010b).

While the number of pulmonary tuberculosis cases has fallen in many developed countries over recent years the notification of extra pulmonary disease has increased. In both the United States and United Kingdom tuberculosis lymphadenitis (TBLA) is the commonest extra-pulmonary manifestation amongst all ethnic groups (Fiske et

al. 2010). Mediastinal TBLA represented 9% of cases reported in the UK in 2009(Health Protection Agency 2010) and presents significant diagnostic challenges. Clarifying the aetiology of isolated mediastinal lymphadenopathy is essential to exclude alternative diagnoses such as lymphoma, carcinoma and sarcoidosis. However the lack of specific clinical and radiological features necessitates pathological or microbiological diagnosis whenever possible. Mediastinal lymph node sampling is commonly performed by conventional transbronchial needle aspiration (TBNA), endoscopic ultrasound guided fine needle aspiration or mediastinoscopy.

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) has now emerged as an important tool for the diagnosis of mediastinal and hilar lymphadenopathy. In addition to the nodal stations accessible by conventional TBNA, EBUS guidance also allows safe aspiration of hilar nodes and nodes less than 10mm. In patients with lung cancer and sarcoidosis, EBUS-TBNA has been shown to increase the yield and sensitivity when compared to standard bronchoscopic techniques including conventional TBNA (Wallace et al. 2008; Tremblay et al. 2009). However, the role of EBUS-TBNA in the diagnosis of tuberculous intra-thoracic lymphadenopathy has not been established. This multi-centre study, for the first time, describes the diagnostic utility of EBUS-TBNA for the diagnosis of intra-thoracic tuberculous lymphadenopathy.

4.2 METHODS

4.2.1 Patients

Consecutive patients with intra-thoracic lymph node tuberculosis who were referred for EBUS-TBNA were included in this study. A final diagnosis of intra-thoracic tuberculosis lymphadenitis was confirmed by positive pathology, microbiology or an unequivocal clinical and radiological response to anti-tuberculous therapy at least 6 months after presentation by the referring physician. Patients were excluded if sputum or bronchial washings were positive for acid fast bacilli on either smear or culture prior to EBUS-TBNA, or if the diagnosis was available from sampling of extra-thoracic disease. Demographic data and HIV status were recorded. All patients were followed up for at least 6 months. The participating centres were University College Hospital (London), St Mary's Hospital (London), Guy's and St Thomas' Hospital (London) and Papworth Hospital (Cambridgeshire). Informed consent was obtained from each patient prior to undergoing EBUS-TBNA. The observational nature of the study meant that ethical approval was not required.

4.2.2 Intervention

Following contrast-enhanced thoracic computed tomography to assess the size and location of the lymphadenopathy, EBUS-TBNA was carried out as an outpatient under local anaesthesia and moderate sedation using midazolam and fentanyl. The procedure was performed with an echo-bronchoscope (BF-UC160F-OL8 Olympus, Tokyo) which allows endoscopic views and simultaneous linear ultrasound of mediastinal and hilar structures. The location, number, and size of the intra-thoracic lymph nodes were recorded. Vascular structures were confirmed using the colour Doppler function. A dedicated aspiration needle (22 or 21 gauge) was then placed in the working channel and advanced into the lymph node under ultrasound guidance. Once the tip of the needle was visualised in the lymph node, the stylet was

withdrawn and suction applied to the needle and the needle was then moved to and fro within the lymph node. Two centres employed on-site evaluation of the cell content of samples which determined the number of passes. Where on-site evaluation was not available, at least 3 passes per lymph node were obtained. Smears were prepared directly onto slides for cytological analysis. Samples were also expelled directly into formalin for cell block analysis. Needle contents from at least one dedicated pass were submitted in saline for microbiological analysis. The microbiological specimens were analyzed by fluorescence microscopy using direct auramine stains and Middlebrook 7H9 medium (an element of the *BACTEC^{TM} MGIT 960^{TM}* System) was used to culture *Mycobacterium spp.*

4.2.3 Assessment of samples

Pathological findings were classified into five grades as documented previously (Bezabih, Mariam, & Selassie 2002): Grade I—epithelioid granulomatous reaction with caseation; Grade II— epithelioid granulomatous reaction without caseation; Grade III—non-granulomatous reaction with necrosis; Grade IV—non-specific; Grade V—inadequate sample. Grades I – III were considered to be consistent with a diagnosis of tuberculous intra-thoracic lymphadenitis in the context of clinical features, supportive tuberculin skin test (TST) or interferon gamma release assay (IGRA) and a clinical response to treatment. Microbiological investigations were considered positive for tuberculosis if the smear was positive for acid fast bacilli or culture isolated *Mycobacterium tuberculosis*.

4.2.4 Statistical analysis

The standard definition for diagnostic sensitivity was employed. Since the prevalence of tuberculosis in the cohort was 100%, predictive values were not

calculated. Categorical variables were compared using the Chi-squared test. Predictors of a positive culture for tuberculosis were modelled using logistic regression. Continuous variables were not categorised in the regression analyses. Significant variables in univariate analysis (at the 20% level) or those deemed clinically important were included in the multivariate model. This study and its report conforms to the standards for the reporting of diagnostic accuracy studies (STARD) statement (Bossuyt et al. 2003).

4.3 RESULTS

Between 1st January 2008 and 1st February 2010, 156 consecutive patients who subsequently received a final diagnosis of intra-thoracic lymph node tuberculosis underwent EBUS-TBNA at 4 centres. The median age at the time of the procedure was 39 years (range 18 –86 years). There were 80 males (51%). The most common clinical symptom was cough in 94 (60%) of the patients. Other presenting symptoms included weight loss, cough, haemoptysis and night sweats and are summarised in table 4.1.

At EBUS, mediastinal and hilar lymph nodes ranging in size from 5 to 60 mm (median 22 mm) were detected. The sub-carinal lymph node station (station 7) was the most common location for EBUS-guided sampling (44% of nodes sampled), followed by the right paratracheal lymph node station (4R) (29% of nodes sampled) (table 2). 61 patients (39%) had 2 or more nodal stations sampled.

EBUS-TBNA was diagnostic of tuberculosis in 146 patients (94%, 95% confidence interval 88 – 97%). Pathological findings were consistent with tuberculosis in 134

(86%) patients. 68 (44%) had granulomas with necrosis; 58 (37%) had granulomas without necrosis. 8 (5%) had necrosis alone. 19 (12%) patients had lymphocytes alone from EBUS-TBNA and in 3 (2%) patients the sample was inadequate for pathological assessment.

Microbiological investigations of EBUS-TBNA yielded a diagnosis of tuberculosis in 82 (53%) patients. In 27 (17%) patients smear of the EBUS-guided aspirate was positive for acid fast bacilli. Seventy-four (47%) patients had a positive culture for *Mycobacterium tuberculosis* with a median time to positive culture of 16 days (range 3 – 84 days). In our cohort, 8 (5%) patients were proven to have isoniazid-resistant tuberculosis. In 15 (10%) patients, pathology was negative but a firm diagnosis of tuberculosis was obtained on Auramine/ Ziehl-Neelsen stain or culture.

The logistic regression model included age, ethnicity, lymph node size, lymph node location, retroviral infection status, abnormal lung parenchyma, number of lymph nodes sampled, number of needle passes and lymph node pathology showing necrosis. Univariate analysis found that lymph node size > 20mm (P=0.022) was associated with an outcome of positive culture from EBUS-TBNA aspirate (Figure 4.1). In the multivariate analysis (Figure 4.1), the presence of necrosis on EBUS-TBNA pathology and sampling more than one lymph node may be associated with a positive culture in the multivariate regression model (table 4.3). A significant interaction between lymph node size and necrosis on pathology was observed with a positive culture less likely to occur in larger nodes with necrosis. In 8 (5%) cases the EBUS aspirate stained positive for acid fast bacilli, however culture was negative.

Ten (6%) patients did not have a specific diagnosis following EBUS-TBNA. Of these, 4 underwent mediastinoscopy which confirmed the diagnosis of tuberculosis in all cases, while 6 patients received empirical anti-tuberculous therapy (figure 4.2).

One patient undergoing EBUS-TBNA experienced a serious complication necessitating inpatient admission. The patient was a 32 year old man of south Asian origin in whom a 35mm right paratracheal lymph node was aspirated under EBUS guidance. Four uncomplicated passes into the lymph node were made and the procedure yielded necrotising granulomas which was also positive for acid fast bacilli on Ziehl-Neelsen staining. Anti-tuberculous therapy was initiated. However, two days after the procedure, the patient presented with sepsis and blood cultures were positive for a beta-haemolytic group G streptococcus. The patient improved with appropriate antibiotics and was discharged on the 8th day post-procedure without further complications. The episode was ascribed to insertion of the bronchoscope itself rather than performance of TBNA, as previously described (Steinfort, Johnson, & Irving 2010).

Table 4.1: Baseline characteristics of patients with tuberculous intra-thoracic lymphadenopathy

Total number of patients	156
Male	80 (51%)
Median age (range)	39 (18 – 86)
Ethnicity:	
African	47 (30%)
Caucasian	25 (16%)
Caribbean	6 (4%)
South Asian	56 (36%)
East Asian	12 (8%)
Other	10 (6%)
Presenting symptoms:	
Fever / night sweats	76 (49%)
Weight loss	72 (46%)
Cough	94 (60%)
Haemoptysis	12 (77%)
No symptoms	34 (22%)
Abnormal lung parenchyma on CT	54 (35%)
HIV positive	17 (11%)

Table 4.2: Results for endobronchial ultrasound guided transbronchial needle aspiration of 220 lymph nodes in 156 patients with intra-thoracic tuberculous lymphadenopathy according to lymph node location

Lymph node station	Number of nodes sampled at EBUS-TBNA	Number of nodes from which pathological grades I-III were obtained	Number of nodes from which positive culture for tuberculosis was obtained
2R	3	3	0
2L	1	1	0
4R	63	54	27
4L	13	11	7
7	96	87	53
10R	28	26	10
10L	13	10	5
11R	1	1	1
11L	2	2	1

* According to Mountain-Dresler Lymph node map

Table 4.3: Univariate and multivariate analyses of factors to predict positive culture of *Mycobacterium tuberculosis* in patients undergoing EBUS-TBNA.

Covariate	**Unadjusted Odds ratio of positive culture**	**Univariate P value**	**Adjusted Odds ratio of positive culture (95% CI)**	**Multi-variate P value**
Age	0.993	0.487		
African or Asian	1.382	0.410		
LN >20mm	0.457	0.019	0.905 (0.361 – 2.264)	0.832
Station 7 lymph node	1.051	0.879		
HIV positive	1.281	0.631		
Abnormal lung parenchyma	1.170	0.641		
Multiple lymph nodes sampled	1.731	0.097	1.921 (0.965 – 3.823)	0.063
Number of needle passes	1.276	0.079		
Pathology showing necrosis (grades I or III*)	1.138	0.689	2.254 (0.870 – 5.839)	0.094
LN > 20mm * Necrosis - interaction			0.292 (0.075 – 1.128)	0.074

*Grade I - epithelioid granulomatous reaction with caseation; Grade III - - granulomatous reaction with necrosis

Figure 4.1: STATA output of univariate and multivariate logistic regression analysis. Outcome refers to a positive culture of *Mycobacterium tuberculosis* from EBUS-TBNA aspirate.

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. ** UNIVARIATE LOGISTIC REGRESSIONS. **
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. logistic outcome

Logistic regression                             Number of obs   =        155
                                                LR chi2(0)      =      -0.00
                                                Prob > chi2     =          .
Log likelihood = -107.27969                     Pseudo R2       =    -0.0000

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       _cons |   .9135802    .146911    -0.56   0.574     .6666022    1.252064
------------------------------------------------------------------------------

. logistic outcome age

Logistic regression                             Number of obs   =        155
                                                LR chi2(1)      =       0.49
                                                Prob > chi2     =     0.4858
Log likelihood = -107.03674                     Pseudo R2       =     0.0023

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         age |   .9927759    .010353    -0.70   0.487     .9726904    1.013276
       _cons |   1.234122   .5689504     0.46   0.648     .4999656    3.046323
------------------------------------------------------------------------------

. logistic outcome afr_asi

Logistic regression                             Number of obs   =        155
                                                LR chi2(1)      =       0.76
                                                Prob > chi2     =     0.3845
Log likelihood = -106.90149                     Pseudo R2       =     0.0035

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     afr_asi |   1.405152   .5522958     0.87   0.387     .6503666    3.035908
       _cons |         .7   .2439262    -1.02   0.306     .3535764    1.385839
------------------------------------------------------------------------------

. logistic outcome ln_20

Logistic regression                             Number of obs   =        150
                                                LR chi2(1)      =       5.32
                                                Prob > chi2     =     0.0211
Log likelihood = -101.10097                     Pseudo R2       =     0.0256

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ln_20 |   .4671385   .1556436    -2.28   0.022     .2431297    .8975389
       _cons |    1.30303   .3015575     1.14   0.253     .8278712    2.050908
------------------------------------------------------------------------------

. logistic outcome lns_7
```

```
                                                ebus_tb
Logistic regression                             Number of obs   =        155
                                                LR chi2(1)      =       0.05
                                                Prob > chi2     =     0.8313
Log likelihood =   -107.257                     Pseudo R2       =     0.0002

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       lns_7 |   1.072886   .3543963     0.21   0.831     .5615448    2.049854
       _cons |       .875   .2264278    -0.52   0.606     .5269128    1.453039
------------------------------------------------------------------------------

. logistic outcome hiv

Logistic regression                             Number of obs   =        155
                                                LR chi2(1)      =       0.21
                                                Prob > chi2     =     0.6494
Log likelihood = -107.17637                     Pseudo R2       =     0.0010

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         hiv |   1.263462   .6506451     0.45   0.650     .4604883    3.466614
       _cons |    .890411    .151849    -0.68   0.496     .6374237    1.243806
------------------------------------------------------------------------------

. logistic outcome ctapp

Logistic regression                             Number of obs   =        155
                                                LR chi2(1)      =       0.33
                                                Prob > chi2     =     0.5652
Log likelihood = -107.11433                     Pseudo R2       =     0.0015

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ctapp |   1.215221   .4120244     0.57   0.565     .6252486     2.36188
       _cons |   .7032017   .3396779    -0.73   0.466     .2728422    1.812376
------------------------------------------------------------------------------

. logistic outcome mult_nodes

Logistic regression                             Number of obs   =        155
                                                LR chi2(1)      =       2.58
                                                Prob > chi2     =     0.1081
Log likelihood = -105.98885                     Pseudo R2       =     0.0120

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
  mult_nodes |         1.7   .5637425     1.60   0.110     .8875222    3.256256
       _cons |   .7407407   .1545266    -1.44   0.150     .4921486    1.114901
------------------------------------------------------------------------------

. logistic outcome passes

Logistic regression                             Number of obs   =        155
                                                LR chi2(1)      =       3.25
                                                Prob > chi2     =     0.0716
Log likelihood = -105.65702                     Pseudo R2       =     0.0151

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      passes |   1.276214   .1770854     1.76   0.079     .9723268    1.675078
       _cons |   .3439023   .1987684    -1.85   0.065     .1107792    1.067609
------------------------------------------------------------------------------

. logistic outcome necros

Logistic regression                             Number of obs   =        152
```

```
                                                ebus_tb
                                                      LR chi2(1)      =       0.23
                                                      Prob > chi2     =     0.6320
Log likelihood = -105.03308                           Pseudo R2       =     0.0011

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      necros |   1.168421   .3799143     0.48   0.632     .6177739    2.209883
       _cons |   .8333333    .190724    -0.80   0.426     .5321147    1.305065
------------------------------------------------------------------------------
```

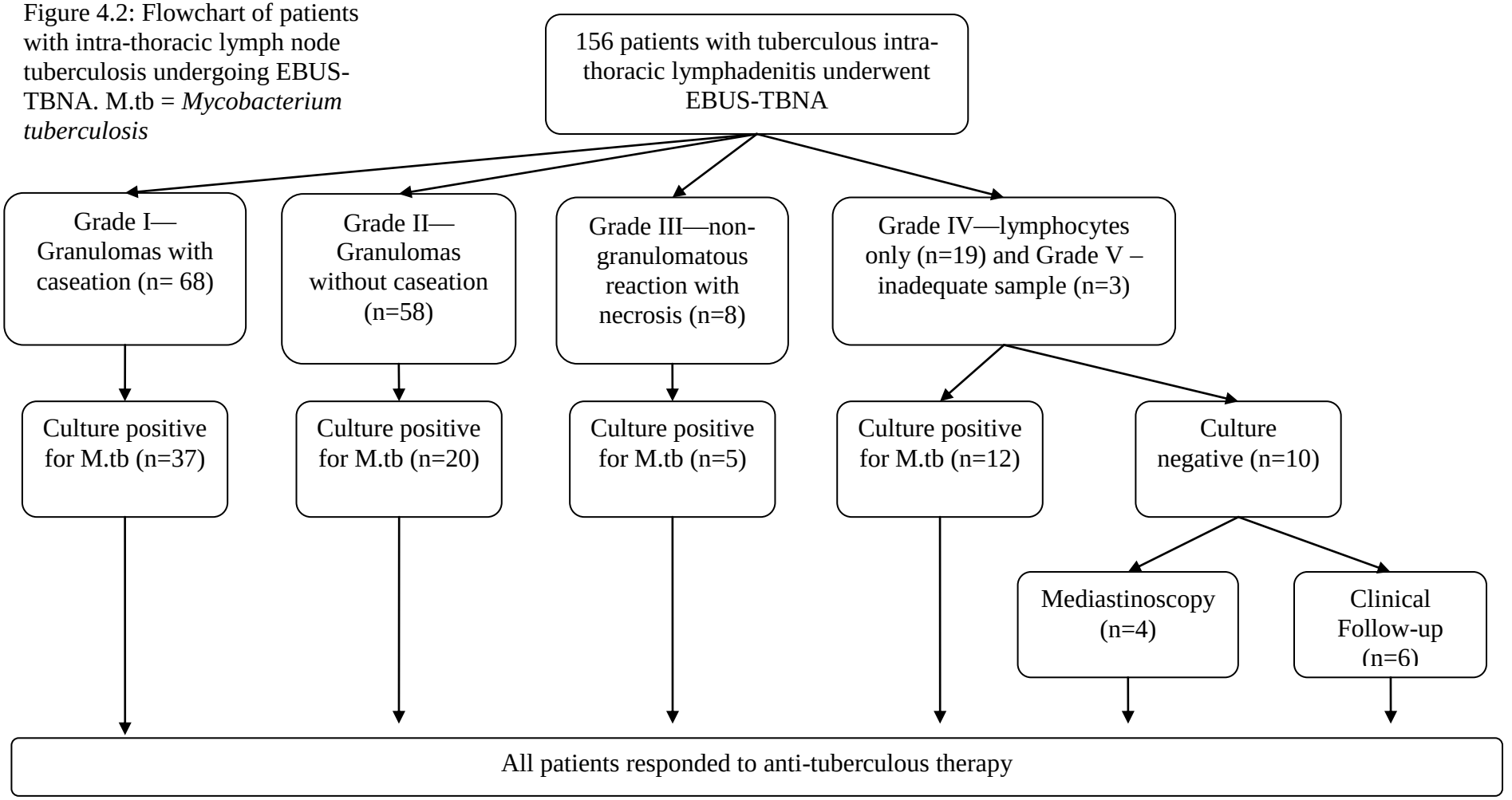
Figure 4.1 cont. Mulitvariate regression analysis with interaction term between presence of necrosis and lymph node size

```
. logistic outcome ln_20 necros inter1

Logistic regression                                   Number of obs   =        147
                                                      LR chi2(3)      =       7.95
                                                      Prob > chi2     =     0.0470
Log likelihood = -97.641259                           Pseudo R2       =     0.0391

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       ln_20 |   .8888889    .410522    -0.26   0.799     .3595265    2.197678
      necros |   2.236111   1.072369     1.68   0.093     .8735418    5.724045
      inter1 |   .2934783   .2001481    -1.80   0.072     .0771025    1.117078
       _cons |   .8571429   .2753212    -0.48   0.631     .4567099    1.608666
------------------------------------------------------------------------------
```

Figure 4.2: Flowchart of patients with intra-thoracic lymph node tuberculosis undergoing EBUS-TBNA. M.tb = *Mycobacterium tuberculosis*



4.4 DISCUSSION

This is the first study to assess the utility of EBUS-TBNA in the diagnosis of tuberculous intra-thoracic lymphadenopathy and demonstrates a sensitivity of 94% for the technique in 156 patients, with one complication observed. In 74 (47%) patients a positive culture of *Mycobacterium tuberculosis* was obtained.

In patients with isolated mediastinal lymphadenopathy due to tuberculosis, traditional techniques of bronchoscopy and sputum culture have a low yield for positive culture(Codecasa et al. 1998). Mediastinoscopy may be employed but requires general anaesthesia, carries a morbidity of 1-2% and also has the disadvantage that posterior subcarinal and hilar nodes are inaccessible. Recently, endoscopic ultrasound guided fine needle aspiration (EUS-FNA) of tuberculous mediastinal lymphadenopathy has been described (Puri et a. 2010; Song et al. 2010). In these studies, diagnostic yield was 90% - 93%. However, EUS-FNA does not allow access to the right paratracheal and hilar lymph node stations which are commonly involved in tuberculosis (Codecasa, Besozzi, De, Miradoli, Sabolla, & Tagliaferri 1998) and accounted for 47% of the nodal stations sampled in this study.

EBUS-TBNA now provides an important alternative in patients with tuberculous intra-thoracic lymphadenopathy. The procedure is well tolerated in the outpatient setting, provides access to the mediastinal and hilar lymph node locations commonly involved in tuberculosis and also allows bronchial washings to be performed at the same procedure. Successful isolation of an organism allows susceptibility testing, which is an increasingly necessary clinical need in the UK, as elsewhere, given the prevalence of isoniazid-resistant and multidrug resistant disease (Health Protection Agency 2010). Prior to the advent of EBUS-TBNA many of these patients would

have received empirical anti-tuberculous therapy. However, demonstration of a resistant organism (over 10% of the culture-positive patients in this cohort) significantly alters the anti-tuberculous regimen and duration of treatment and sub-optimal treatment may induce selection of further drug resistant strains. In addition it is well recognised that intra-thoracic TBLA may not alter radiologically on successful treatment and in fact a significant proportion may paradoxically increase during treatment. In this setting, a firm microbiological diagnosis avoids the risk of inappropriate antimicrobial escalation, but instead allows consideration of the use of anti-inflammatory treatments.

The culture rate of 47% observed in this study is similar to culture rates from lymph node sampling observed with other modalities. In a study of 29 patients with intra-thoracic TBLA who underwent mediastinoscopy, 14 (48%) patients had a positive culture for *Mycobacterium tuberculosis* (Farrow et al. 1985). Bilaceroglu et al(2004) report a culture rate of 26% (17/63) performing TBNA without EBUS, while in a study of EUS, culture rate was 21% (Song et al. 2010). Gupta and colleagues (1993) obtained a positive culture for tuberculosis in 49% of lymph node fine needle aspirates. Gulati et al (2000) demonstrated that tuberculosis was cultured in 7 out of 26 patients undergoing USS guided percutaneous mediastinal lymph node biopsies. These low culture rates are likely to represent the heterogeneity in bacillary load of intra-thoracic tuberculous lymph nodes and the yield obtained with EBUS is comparable with these other modalities. The mean time to culture was 16 days (range 3 to 84). One centre extended liquid mycobacterial culture beyond 6 weeks' incubation, and identified 3 additional isolates. Further investigation into the value of extended culture and of the potential application of rapid molecular techniques, such

as the GeneXpert MTB platform (Boehme et al. 2010), is warranted to try to further increase sensitivity.

The logistic regression model demonstrated that those EBUS procedures which obtained necrotic granulomas or necrosis alone were more likely to have a positive culture for tuberculosis. It may be postulated that the bacillary load in these lymph nodes is higher in order to cause necrosis and therefore the organism in these patients is more likely to be cultured. As has been previously demonstrated in patients with sarcoidosis undergoing conventional TBNA (Trisolini et al. 2008), sampling more than one lymph node station increases the diagnostic yield. Although many patients were observed to have matted and hypoechoic lymph nodes on the endobronchial ultrasound views, the significance of these findings was not assessed.

Seventeen patients included in the study were infected with the human immunodeficiency virus (HIV) and culture was positive in 6 of these patients. A previous report has demonstrated that EBUS-TBNA may diagnose non-tuberculous mycobacterial disease in a patient with acquired immune deficiency syndrome (Steinfort et al. 2009). Further data is required on the utility of EBUS-TBNA in HIV infected individuals.

Data from systematic review of patients with non-small cell lung cancer undergoing EBUS-TBNA indicate that EBUS-TBNA is a safe procedure with minimal complications (Gu et al. 2009). In this report we describe one complication of symptomatic bacteraemia following EBUS-TBNA of necrotic mediastinal lymphadenopathy. Von Bartheld and colleagues (2010) have described the formation of a mediastinal – oesophageal fistula in a patient following EUS-FNA with a 22 gauge needle of a heterogeneous subcarinal gland. In addition, there has

also been a report of mediastinitis following EUS guided aspiration of necrotic subcarinal lymph node in a patient with non-small cell lung cancer (Aerts et al. 2008). It may be postulated that the risk of infectious complications may be increased in patients undergoing aspiration of large necrotic lymph nodes and further safety data is required in this patient group.

Limitations of the current study are recognised. Tuberculosis has a high endemic rate in our predominantly London based population with an incidence of 44.3 per 100,000 per year(Health Protection Agency 2010). In addition, the centres included in the study have considerable experience with EBUS-TBNA and so the results may not be applicable to other areas. The retrospective nature of this study prevented the inclusion of patients with isolated mediastinal lymphadenopathy and therefore it is not possible to determine the disease prevalence of tuberculosis in our population undergoing EBUS-TBNA. In this study, the finding of granulomas without caseation along with supporting clinical evidence and response to therapy was considered to be consistent with a final diagnosis of tuberculosis. Although a positive tuberculin skin test or interferon gamma release assay adds weight to the diagnosis of tuberculosis, it is possible that these investigations may still be positive in patients with sarcoidosis from populations such as ours with a moderately high incidence of tuberculosis. However even allowing for this, it should be noted that in the group with non-caseating granulomas, 20 of the 58 cases in this cytological criteria were culture positive (in addition to clinical criteria of improvement on treatment) - hence indicating that this pathological finding is in itself still compatible with active tuberculosis.

In conclusion, EBUS-TBNA is a safe and effective first line investigation in patients with tuberculous intra-thoracic lymphadenopathy.

CHAPTER 5:

ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION FOR THE DIAGNOSIS OF INTRA-THORACIC LYMPHADENOPATHY IN PATIENTS WITH EXTRA-THORACIC MALIGNANCY

5.1 INTRODUCTION

Mediastinal lymphadenopathy is a common finding in patients with extra-thoracic malignancies and is a frequent diagnostic dilemma for respiratory physicians and oncologists. Enlarged mediastinal nodes are often discovered at the time of initial staging, when the demonstration of mediastinal metastases may significantly alter treatment and prognosis. Alternatively mediastinal lymphadenopathy may be discovered after treatment and require pathological evaluation in order to exclude or confirm disease recurrence.

Prior to the advent of endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA), sampling of intra-thoracic lymphadenopathy was most commonly performed by mediastinoscopy or endoscopic ultrasound guided fine needle aspiration (EUS-FNA). However, mediastinoscopy is associated with a 1% complication rate (Lemaire et al. 2006) and requirement for general anaesthesia, while EUS-FNA does not allow access to the right paratracheal and hilar lymph nodes. EBUS-TBNA allows sampling of paratracheal, subcarinal and hilar

lymphadenopathy under sedation in the outpatient setting. The technique has a role in the diagnosis and staging of lung cancer with a sensitivity of over 90%, even early in the learning process. Prospective data is now available on the utility of EBUS-TBNA in the diagnosis of sarcoidosis (Tremblay et al. 2009) and the previous chapter has also demonstrated a high diagnostic yield in patients with tuberculous lymphadenopathy. Limited data however exists on the role of EBUS-TBNA in the diagnosis of extra-thoracic malignancies (Tournoy et al. 2011). This large multi-centre study, describes the diagnostic utility of EBUS-TBNA for the clarification of intra-thoracic lymphadenopathy in patients with extra-thoracic malignancy.

5.2 METHODS

5.2.1 Patients

Consecutive patients with an active or previous diagnosis of extra-thoracic malignancy and enlarged intra-thoracic lymphadenopathy who underwent EBUS-TBNA between 1st January 2007 and 1st December 2010 were included. Patients were suspected to have intra-thoracic lymph node metastases on the basis of CT or PET-CT findings. The participating centres were University College London Hospital, Papworth Hospital Cambridge, University Hospitals Birmingham, University Hospital of North Tees, and Lancashire Teaching Hospitals, Preston. The retrospective observational design of the study meant that ethical approval was not required.

5.2.2 Intervention

EBUS-TBNA was performed with a dedicated linear echo-endoscope (Olympus BF-UC160F-OL8) under moderate sedation with intravenous midazolam and fentanyl or midazolam alone. Systematic assessment of all EBUS accessible lymph nodes was made. Vascular structures were avoided using the Doppler function. Under direct ultrasound guidance the lymph node was then aspirated using either a 21 gauge or 22 gauge needle. Samples were expelled both, onto glass slides where air dried smears were made for cytology and also into liquid fixative suitable for cell block preparations. In cases where core biopsies were obtained for histology, these were placed directly into formalin. The appropriate immunohistochemical staining with antibodies to the cytokeratins, thyroid transcription factor-1, prostate-specific antigen, oestrogen and progesterone receptors and neuroendocrine markers, was utilised when required.

5.2.3 Analysis

Standard definitions of sensitivity, negative predictive value and diagnostic accuracy were employed. Positive malignant findings on EBUS-TBNA were not confirmed and specificity was assumed to be 100%. Non-malignant findings at EBUS-TBNA were subject to surgical confirmation or at least 6 months radiological and clinical follow-up. Predictors of malignant lymphadenopathy were modelled using logistic regression. Continuous variables were not categorised in the regression analyses. Significant variables in univariate analysis (at the 10% level) or those deemed clinically important were included in the multivariate model. Analysis was carried out with STATA version 10 (Stata corporation). This study conforms to the

standards for the reporting of diagnostic accuracy studies (STARD) initiative (Bossuyt et al. 2003).

5.3 RESULTS

All 161 patients successfully underwent EBUS-TBNA and no complications were observed. The median age of the patients was 64 (range 19 – 86). The most common extra-thoracic malignancies observed were breast, colorectal and oesophageal carcinomas. The patient characteristics are summarised in Table 5.1.

The median size of lymph nodes seen at EBUS-TBNA was 25 (range 6 – 48) mm and each node underwent a median of 4 passes (range 2 – 6). One hundred and ninety-six nodes were sampled in 160 patients, with no samples taken in one patient. The subcarinal or right paratracheal lymph node stations were the site of aspiration in 100 (62%) of patients. Twenty-eight (17%) patients had hilar lymph nodes sampled only. The sensitivity, negative predictive value (NPV) for malignancy and overall accuracy for EBUS-TBNA was 87%, 73% and 88% respectively. The final diagnosis was unknown in 6 patients. If we assume that the intra-thoracic lymph nodes in these patients harboured extra-thoracic malignancy (undiagnosed by EBUS-TBNA) the sensitivity, NPV and accuracy are 78%, 61% and 84%. Overall, 110 (68%) of the patients in the study were known to have had malignant intra-thoracic lymphadenopathy. EBUS-TBNA did not obtain a diagnosis in 13 patients with metastases to intra-thoracic nodes from an extra-thoracic malignancy. Four patients had breast cancer, 1 bladder cancer, 1 renal cell carcinoma, 1 seminoma, 1

leiomyosarcoma, 3 melanoma, 1 head and neck carcinoma and 1 patient had lymphoma.

In 14 (9%) patients EBUS-TBNA demonstrated granulomas alone and the final diagnosis in each of these patients was sarcoidosis. Of the 51 cases, in which EBUS-TBNA did not provide a malignant or alternative diagnosis, surgery was performed in 9 (18%) and a median of 15 months clinical and radiological follow-up was employed in the remainder (Figure 5.1).

Univariate analysis of lymph node size, number of passes per node and number of lymph nodes sampled revealed there was a statistically significant association between lymph node size and presence of metastatic lymphadenopathy (P=0.03). In the logistic regression multivariate model, lymph node size remained significantly associated with malignant lymphadenopathy (OR 1.04 (95% confidence interval 1.00 – 1.08). This implies that for every increase in lymph node size of 1mm the probability of the lymph node being malignant increases by 4%. Univariate analysis demonstrated there was no association between lymph node size and yield from EBUS-TBNA (P=0.279) (Figure 5.2).

Of the 71 patients with extra-thoracic malignancy diagnosed by EBUS-TBNA, morphological appearances alone were sufficient in 17 (24%). Immunohistochemistry was successfully performed in 54 (76%) patients whose EBUS-TBNA had diagnosed malignancy, elucidating the primary origin of the tumour (Figure 5.3).

Table 5.1: Characteristics of patients with extra-thoracic malignancy

Total number of patients	**161**
Male	73 (45%)
Median age (range)	64 (19 – 86)
Median lymph node size (mm, range)	25 (6 – 48)
Extra-thoracic malignancy primary site	
Breast	40
Colorectal	25
Oesophagus	13
Melanoma	12
Head and Neck	11
Renal cell	10
Prostate	9
Bladder	8
Lymphoma	7
Ovarian	5
Sarcoma	4
Testis	3
Cervix	3
Stomach	3
Endometrial	2
Penis	2
Teratoma, Anus, Vulva, Schwannoma	1 each

Figure 5.1: Flowchart of patients with extra-thoracic malignancy

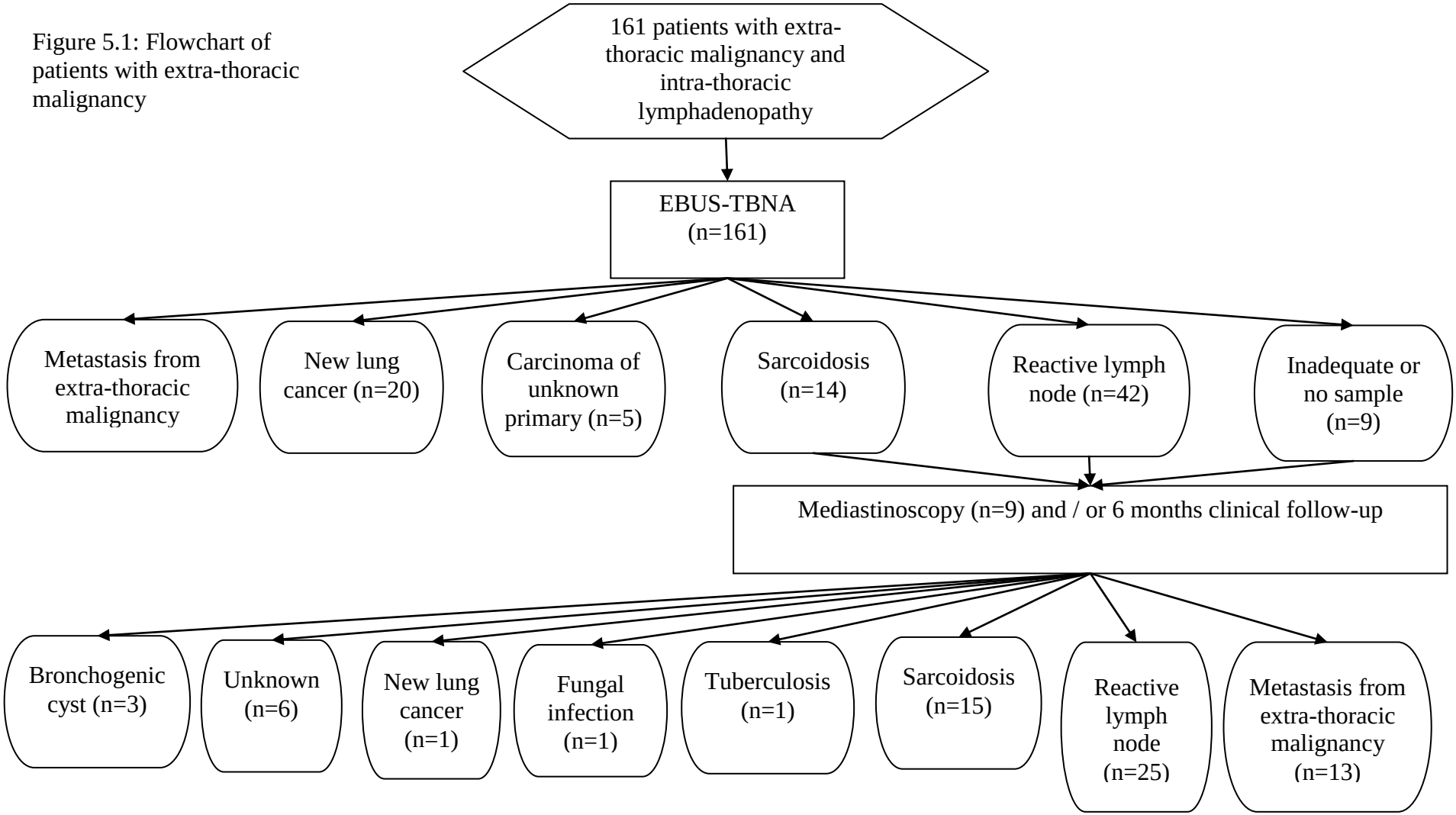


Figure 5.2: STATA output of univariate and multivariate regression analyses in patients undergoing EBUS-TBNA with extra-thoracic malignancy

Univariate analyses:

```
. logistic  finaldiagnosis  lymphnodesize

Logistic regression                               Number of obs   =        161
                                                  LR chi2(1)      =       4.81
                                                  Prob > chi2     =     0.0284
Log likelihood = -100.97493                       Pseudo R2       =     0.0232

------------------------------------------------------------------------------
finaldiagn~s | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
lymphnodes~e |   1.041615   .0199353     2.13   0.033     1.003266    1.081429
------------------------------------------------------------------------------


. logistic  ebuspathology  lymphnodesize

Logistic regression                               Number of obs   =        161
                                                  LR chi2(1)      =       1.19
                                                  Prob > chi2     =     0.2753
Log likelihood = -107.59558                       Pseudo R2       =     0.0055

------------------------------------------------------------------------------
ebuspathol~y | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
lymphnodes~e |   1.019304   .0180036     1.08   0.279     .9846218    1.055209
------------------------------------------------------------------------------


. logistic  ebuspathology  numberoflnstations

Logistic regression                               Number of obs   =        161
                                                  LR chi2(1)      =       0.28
                                                  Prob > chi2     =     0.5980
Log likelihood = -108.05158                       Pseudo R2       =     0.0013

------------------------------------------------------------------------------
ebuspathol~y | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
numberofln~s |   1.178568   .3710299     0.52   0.602     .6358937    2.184364
------------------------------------------------------------------------------
```

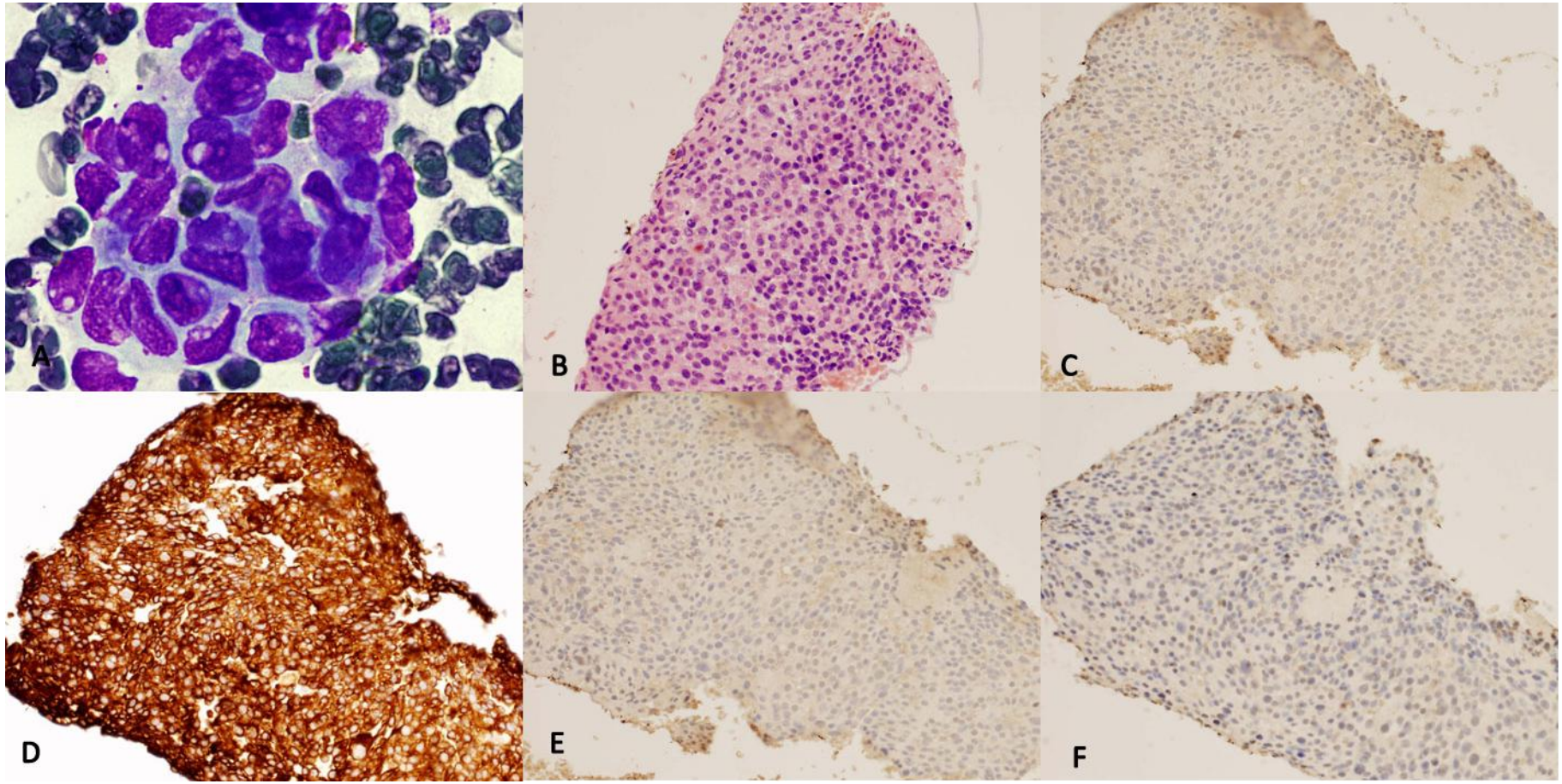
Multivariate analysis:

```
. logistic  finaldiagnosis  lymphnodesize  numberoflnstations

Logistic regression                               Number of obs   =        161
                                                  LR chi2(2)      =       4.87
                                                  Prob > chi2     =     0.0875
Log likelihood =  -100.9425                       Pseudo R2       =     0.0236

------------------------------------------------------------------------------
finaldiagn~s | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
lymphnodes~e |   1.041662   .0199049     2.14   0.033     1.003371    1.081414
numberofln~s |   1.085326   .3508359     0.25   0.800      .575978    2.045099
------------------------------------------------------------------------------
```

Figure 5.3: EBUS-TBNA samples demonstrating metastatic breast cancer. A) FNA smear shows malignant cells (MGG) B) cell block preparation contains numerous malignant cells (H&E) C) TTF-1 negative staining D) CK7 positive staining E) CK20 negative staining F) ER staining negative. EBUS-TBNA: endobronchial ultrasound guided transbronchial needle aspiration; MGG: May Grunwald Giemsa stain; H&E: hematoxylin and eosin; TTF-1: thyroid transcription factor-1; CK: cytokeratin; ER: estrogen receptor.



5.4 DISCUSSION

This multi-centre study of 161 patients is the largest to date to demonstrate the role of EBUS-TBNA in the diagnosis of intra-thoracic lymphadenopathy in patients with extra-thoracic malignancy. EBUS-TBNA demonstrated a sensitivity of 87% with an overall diagnostic accuracy of 88% and therefore is an important alternative to other techniques for the diagnosis of intra-thoracic lymphadenopathy in patients with extra-thoracic malignancy.

Clarification of mediastinal lymphadenopathy in the context of a known or suspected extra-thoracic malignancy is a common scenario faced by physicians and may have profound effects on the patient's treatment and prognosis. Although mediastinoscopy is considered the gold standard investigation in this clinical scenario, mediastinoscopy is associated with risks due to general anaesthesia, a serious complication rate of 1% and increased healthcare costs compared to minimally invasive techniques. Standard cervical mediastinoscopy also only provides access to the paratracheal and anterior subcarinal lymph nodes. Evidence of the utility of alternatives to mediastinoscopy in this patient group is beginning to emerge. A recent report of conventional TBNA in 5 patients with extra-thoracic malignancy (Bruno et al. 2010) showed that the procedure is able to provide suitable material for the diagnosis of extra-thoracic malignancy. A large study of 75 patients undergoing endoscopic ultrasound guided fine needle aspiration (EUS-FNA) demonstrated a sensitivity of 86% (Peric et al. 2010), while Tournoy and colleagues (2011) also showed a sensitivity of 85% for EBUS-TBNA in 61 patients with malignant intra-thoracic lymphadenopathy(Tournoy, Govaerts, Malfait, & Dooms 2011).

In this study of 161 patients with suspected metastases from extra-thoracic malignancy, only 84 (52%) had a final diagnosis of intra-thoracic lymph node metastases, highlighting the importance of pathological confirmation in this clinical scenario. The negative predictive value obtained in this cohort was low at 73%, emphasising the need for further investigation if EBUS-TBNA did not yield a malignant or other specific diagnosis.

Of note in this study, EBUS-TBNA demonstrated non-caseating granulomas in 14 cases, in all of whom a sarcoid-like reaction was the final diagnosis. Previous studies of granulomas in mediastinal lymph nodes in patients with early stage non-small cell lung cancer have suggested that this finding reliably excludes malignancy and suggested that clinical follow-up rather than further invasive sampling may be justified in this context (Steinfort & Irving 2009). The statement that the presence of granulomas may reliably exclude malignancy is questionable. The coexistence of granulomas and malignant cells in metastatic lymph nodes has been described (Trisolini, Cancellieri, & Patelli 2009) and, although we did not encounter this phenomenon in any of our patients initially diagnosed with sarcoid on EBUS TBNA, we would recommend further investigation when granulomas only are observed in EBUS-TBNA and the suspicion of malignancy remains.

Limitations of the current study are recognised. The retrospective nature means that only patients who were clinically selected for EBUS-TBNA were included. Although consecutive patients were included to minimise this bias, the characteristics of patients who were directly referred for mediastinoscopy outside of the study are unknown. The multi-centre collaboration strengthens conclusions about the generalisability of the data, however, due to differing pathology practices, standardised immunohistochemistry protocols were not followed.

In conclusion, pathological evaluation is important for diagnosis and staging of patients with extra-thoracic malignancy and suspected mediastinal or hilar lymph node metastases. EBUS-TBNA is a safe and sensitive technique and may be considered a first line investigation in these patients.

CHAPTER 6:

ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION PREVENTS MEDIASTINOSCOPIES IN PATIENTS WITH ISOLATED MEDIASTINAL LYMPHADENOPATHY

6.1 INTRODUCTION

Isolated mediastinal lymphadenopathy is a common presentation to respiratory physicians. Final diagnoses often include sarcoidosis, tuberculosis, lymphoma and metastatic carcinoma. However, symptoms are often non-specific and fevers, night sweats and weight loss may be a common feature of each diagnosis. A pathological and microbiological diagnosis is therefore commonly obtained to differentiate these conditions and guide further management.

Mediastinoscopy has traditionally been considered the gold-standard for lymph node sampling in these patients. Previous retrospective studies have demonstrated a high diagnostic yield for the procedure in this patient group with few complications Lemaire et al. 2006; Porte et al 1998). However, mediastinoscopy requires general anaesthesia, only allows access to the paratracheal and anterior subcarinal lymph nodes and in many cases requires an inpatient stay. Patients are left with a visible scar above the suprasternal notch which may be a cosmetic issue in young people.

Although complications from mediastinoscopy are rare they may be catastrophic and include vocal cord palsy, innominate vein damage and even death (Porte et al. 1998).

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was developed for the mediastinal staging of lung cancer and studies have demonstrated that it has a similar sensitivity to mediastinoscopy for detecting mediastinal metastases from non-small cell lung cancer. In the only prospective direct comparison of EBUS-TBNA and mediastinoscopy to date in patients with suspected lung cancer (Ernst et al. 2008), EBUS-TBNA demonstrated a significantly superior sensitivity (91% vs 78%, P=0.007). Data have now emerged on the utility of EBUS-TBNA in the diagnosis of benign mediastinal lymph nodes (Tremblay et al. 2009), lymphoma (Marshall et al. 2011) and extra-thoracic malignancy (Tournoy et al. 2011). However, given concerns about smaller biopsy sizes with EBUS-TBNA and inherent selection bias of retrospective studies, it is unknown whether EBUS-TBNA can replace mediastinoscopy as a first investigation in patients with isoIated mediastinal lymphadenopathy (IML). In this prospective multi-centre clinical trial, the aim was to determine whether EBUS-TBNA could be utilised as an alternative initial procedure in consecutive patients presenting with IML requiring pathological evaluation and also to describe the economic consequences of this strategy.

6.2 METHODS

6.2.1 Trial design

This was a multi-centre single arm prospective clinical trial of EBUS-TBNA in patients with isolated mediastinal lymphadenopathy. If EBUS-TBNA did not give a

definitive diagnosis, patients underwent mediastinoscopy. In order to clarify whether patients included in this study reflected patients previously referred for mediastinoscopy, data was also collected on 68 patients who underwent mediastinoscopy between 2007 and 2008, prior to the introduction of the EBUS-TBNA service. Although a randomised trial of EBUS-TBNA versus mediastinoscopy was originally planned, we anticipated difficulty with recruitment if all patients were not allowed to undergo EBUS-TBNA. The trial protocol was approved by the Moorfields and Whittington Research Ethics Committee (reference 09/H0721/23). The trial was registered as REMEDY (clinical trial of EBUS-TBNA in patients with isolated mediastinal lymphadenopathy) on www.clinicaltrials.gov as NCT00932854.

6.2.2 Participants

Consecutive patients with undiagnosed isolated mediastinal lymphadenopathy on CT or PET-CT who were referred for mediastinoscopy were approached for trial entry between July 2009 and April 2011. The participating centres were University College London Hospital, Whittington Hospital, North Middlesex University Hospital, Barnet General Hospital and Princess Alexandra Hospital. Patients with anterior mediastinal lymphadenopathy only, with a known lung cancer, without informed consent or absolute contra-indications to EBUS-TBNA or mediastinoscopy were all excluded from the trial.

6.2.3 Intervention

All patients were scheduled to undergo EBUS-TBNA as an initial procedure. The technique for EBUS-TBNA has been previously described (Chapter 3). Briefly, the procedure is conducted in the outpatient setting and patients receive intravenous sedation with midazolam and fentanyl in addition to topical lidocaine. A dedicated linear echoendoscope (Olympus BF-UC160F-OL8) was used in all cases and a systematic assessment of hilar and mediastinal lymph nodes was made. Under ultrasound guidance mediastinal and/or hilar lymph nodes were punctured with a dedicated 21-gauge or 22-gauge needle and suction was applied. Samples were transferred onto glass slides and also directly into liquid fixative for cell block processing. Any cores of tissue were placed into formalin. The site and number of lymph nodes punctured as well as the number of passes were at the operator's discretion. On-site evaluation of samples was not employed. Immunohistochemistry was performed as required, however flow cytometry was not used. The pathologists were blinded to the fact that the patient was in a clinical trial and were provided with clinical information, reflecting routine clinical practice.

Pathological and microbiological results were reviewed in a multi-disciplinary team (MDT) setting including radiologists, respiratory physicians, thoracic surgeons and pathologists. If a diagnosis agreed by the MDT was not obtained from EBUS-TBNA, then the patient underwent mediastinoscopy. Cervical mediastinoscopy was performed under general anesthesia via an incision above the suprasternal notch and lymph node stations 2, 4 and 7 were sampled. Any overnight inpatient stay was determined by the responsible surgeon.

In cases where EBUS-TBNA and mediastinoscopy failed to show a definitive diagnosis, the participant underwent serial imaging and clinical follow-up for at least 6 months duration.

6.2.4 Endpoints

The co-primary outcomes were the proportion of mediastinoscopies saved and healthcare costs compared to a strategy where all patients undergo mediastinoscopy. Other endpoints were the sensitivity, negative predictive value (NPV) and diagnostic accuracy of EBUS-TBNA in patients with isolated mediastinal lymphadenopathy. Complications of EBUS-TBNA were recorded if bleeding exceeded 10mls or peripheral oxygen saturations were recorded below 90%. Length of inpatient stay was also prospectively documented.

6.2.5 Economic analysis

The incremental cost of the EBUS-TBNA strategy (where negative EBUS-TBNA is followed by mediastinoscopy) versus mediastinoscopy alone in patients with IML was calculated from the perspective of the NHS. The analysis was based on a decision tree model (Figure 6.1). Patients with IML who received EBUS-TBNA either received a diagnosis or did not. In the case of the latter, they underwent mediastinoscopy, and if that failed to produce a diagnosis they received clinical follow-up until a diagnosis was available. They then received treatment depending on their diagnosis. Patients in the mediastinoscopy alone strategy either received a diagnosis from this procedure or not, and in the latter case received clinical follow-up until a diagnosis was available. We assume that treatment and treatment outcomes following diagnosis were the same irrespective of the method of diagnosis, and therefore treatment costs are omitted from the incremental analysis. Since treatment outcomes were the same, the two strategies were equally effective, and therefore the

economic evaluation is a cost-minimisation analysis; hence the EBUS-TBNA strategy represents good value for money to the NHS if it is less costly than the mediastinoscopy strategy.

In the model, the proportion of patients receiving EBUS-TBNA, mediastinoscopy and clinical follow-up for each strategy was obtained from the prospective trial. Unit costs were taken from manufacturers' prices and local hospital costs (see Table 6.1 for details of the cost-breakdown of the EBUS-TBNA procedure). Because all costs occurred within one year discounting was unnecessary. We investigated the sensitivity of the results to the cost of the EBUS-TBNA procedure, varying it between £503 (the 2010-11 NHS tariff for a flexible bronchoscopy) and £5259 (the 2010-11 NHS tariff for mediastinoscopy with complications) for the analysis.

6.2.6 Sample Size

It was assumed that an incremental cost of £500 per patient would be acceptable if EBUS-TBNA could reduce the number of mediastinoscopies by 60%. This supposes that 40% of patients would undergo both EBUS-TBNA and mediastinoscopy. A total of 75 patients were required to detect a mean difference of £500 in cost assuming an 80% power and 2.5% significance level (since the Bonferroni correction is applied to adjust for multiple significance testing). The sample size is also sufficient to give the trial adequate power to assess whether the proportion of patients undergoing mediastinoscopy is reduced by 60%, assuming the same power and significance level.

Figure 6.1: Decision tree model of patients with isolated mediastinal lymphadenopathy. Data is from the REMEDY trial.

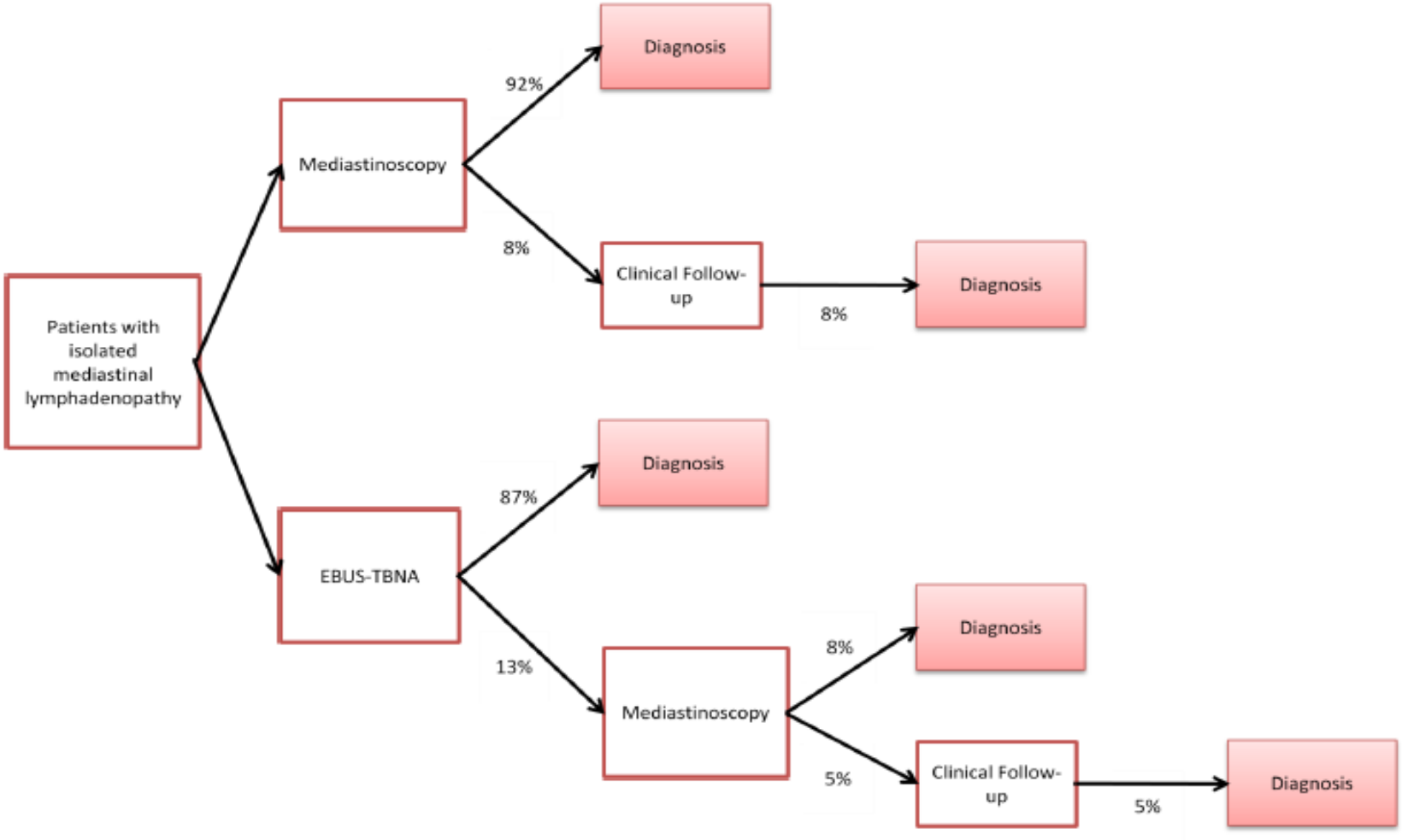


Table 6.1: Estimated cost of the EBUS-TBNA procedure to the NHS, assuming 250 cases per year and 3 cases per session

Resource	**Cost per year (£)**	**Cost per procedure (£)**
Capital costs of 2 EBUS echoendoscopes (£140,000 spread over 5 years)	28,000	112
EBUS-TBNA needle	43,750	175
Maintenance contract	9000	36
2 Consultants for 2.5 sessions per week	50,000	200
2 Nurses, 1 health care assistant, 1 recovery nurse per session	68,750	275
Sterilisation	13,750	55
Pathology	36,250	145
Administration	10,000	40
Overheads (endoscopy suite, portering, facilities, drug costs) and Indirect costs	86,000	344
Total cost of EBUS-TBNA	345,500	1382

6.2.7 Statistical methods

Demographic and clinical characteristics of the study population were summarised using mean, standard deviation, median, or counts and percentages, depending on their type and distribution. A one sample z test was used to determine if there was a significant reduction in mediastinoscopies due to EBUS-TBNA. The one sample t-test was used to investigate whether the strategy of EBUS-TBNA initially (followed by mediastinoscopy if negative) differs significantly in cost from that associated with mediastinoscopy alone. Test accuracy of EBUS-TBNA and mediastinoscopy was calculated using the sensitivity and NPV with 95% binomial confidence intervals. Specificity of EBUS-TBNA and mediastinoscopy samples was assumed to be 100%. Statistical calculations have been performed using STATA version 10 (Statcorp., USA). The design, conduct, analysis and report of this study conform to the Standard of Reporting Diagnostic Accuracy Guidelines (Bossuyt et al. 2003).

6.3 RESULTS

Seventy-seven patients were recruited during the study period with a median age of 42 years. The total number recruited exceeded the required sample size as several patients had consented to enter the trial at different sites on the final day of rectuitment. The most common diagnosis was sarcoidosis and their characteristics are summarized in Table 6.2. The table also includes the characteristics and final diagnoses of patients undergoing mediastinoscopy only between 2007 and 2008. Patients in the prospective trial and historical controls had similar age and symptom distributions.

EBUS-TBNA prevented 87% of mediastinoscopies (97.5% CI 78 – 96%) but failed to provide a diagnosis in 10 patients (figure 6.2, page 132). All 10 patients proceeded to mediastinoscopy. Mediastinoscopy provided a specific diagnosis in 7 cases while the remaining 3 patients had further clinical and radiological follow-up of at least 6 months duration. There were no losses to follow-up and all patients were included in the analysis. The final diagnosis was correctly determined by EBUS-TBNA in 67 cases giving an overall diagnostic sensitivity of 92% (95% confidence interval 83 – 95%). NPV was 40% (95% CI 12 – 74%) and diagnostic accuracy of 92% (95% CI 84 – 97%). EBUS-TBNA successfully diagnosed sarcoidosis in 32 (94%) out of 34 patients with the condition (Table 6.3a, page 129). Twenty-eight patients in the trial had a final diagnosis of tuberculosis and EBUS-TBNA provided pathological evidence of tuberculosis in 26 (93%) and cultured Mycobacterium tuberculosis in 11 (40%) cases. Two patients were diagnosed with Hodgkin's lymphoma following EBUS-TBNA. A further patient with lymphoma was not definitively diagnosed by EBUS-TBNA and required mediastinoscopy to confirm the diagnosis.

No major complications from EBUS-TBNA were observed. Four patients experienced transient hypoxia and 1 patient had self-limiting bleeding. These complications did not result in early termination of the procedure in any case and all procedures were day cases. The median number of passes per lymph node, the frequency of lymph node stations sampled and diagnostic yield of EBUS-TBNA is shown in Table 6.3b (page 130). The ten patients undergoing mediastinoscopy (after negative EBUS-TBNA) accumulated a total of 15 inpatient nights and no serious complications were observed.

In the retrospective study of mediastinoscopy in patients with isolated mediastinal lymphadenopathy, mediastinoscopy provided a specific diagnosis in 53 patients out

of 68 patients. In the 15 patients with no specific diagnosis, the final diagnosis was sarcoidosis in 4, adenocarcinoma in 1 and reactive lymphadenopathy in the remainder. The sensitivity of mediastinoscopy in this retrospective cohort was 92% (95 CI 81% - 97%).

The mean cost of EBUS-TBNA procedure per patient was £1382 (Table 6.1). The standard price for mediastinoscopy is £3228 according to the 2010-11 NHS payment by results tariff. In the base case analysis, the mean cost per patient of the EBUS-TBNA strategy was £1822; for the mediastinoscopy only strategy the cost was £3268 (Table 6.4). Hence the incremental cost per patient of the EBUS-TBNA strategy versus the mediastinoscopy strategy was –£1446. Therefore, the EBUS-TBNA strategy was significantly cheaper than mediastinoscopy strategy. A univariate threshold sensitivity analysis which varied the potential cost of EBUS-TBNA demonstrated that under the conditions of the trial, the EBUS-TBNA strategy was less costly than the mediastinoscopy strategy if the cost per EBUS-TBNA procedure was less than £2828 (Figure 6.3, page 133).

Table 6.2: Clinical characteristics of patients with isolated mediastinal lymphadenopathy

	EBUS-TBNA (n=77)	Mediastinoscopy only (n=68)
Age		
< 30	15 (19%)	3 (4%)
30 – 49	34 (44%)	25 (37%)
50 – 69	16 (21%))	29 (43%)
>69	12 (16%)	11 (16%)
Median (range)	42 (17 – 79)	53 (25 – 85)
Gender		
Male	45 (58%)	38 (56%)
Female	32 (42%)	30 (44%)
Ethnicity		
Caucasian	25 (32%)	36 (53%)
Asian	29 (38%)	22 (32%)
African	15 (19%)	9 (13%)
Caribbean	6 (8%)	0 (0%)
Other	2 (3%)	1 (1%)
Symptoms		
Cough	27 (35%)	31 (46%)
Dyspnoea	11 (14%)	8 (12%)
Weight loss	13 (17%)	8 (12%)
Fevers / night sweats	13 (17%)	5 (7%)
Chest pain	3 (4%)	10 (15%)
Other	2 (3%)	2 (3%)
None	8 (10%)	4 (6%)

Table 6.2 continued: Clinical characteristics of patients with isolated mediastinal lymphadenopathy

	EBUS-TBNA (n=77)	Mediastinoscopy only (n=68)
Final Diagnosis		
Sarcoidosis Stage 1	31 (40%)	31 (46%)
Sarcoidosis Stage 2	3 (4%)	2 (3%)
Tuberculosis	28 (36%)	17 (25%)
Lymphoma	3 (4%)	5 (7%)
Extra-thoracic malignancy	4 (5%)	2 (3%)
Lung cancer	4 (5%)	1 (1%)
Reactive lymphadenopathy	4 (5%)	10 (15%)

Table 6.3a: Diagnoses obtained by EBUS-TBNA in the REMEDY trial

	Number of diagnoses obtained by EBUS-TBNA	Sensitivity
Sarcoidosis Stage 1	29	94%
Sarcoidosis Stage 2	3	100%
Tuberculosis	26	93%
Lymphoma	2	66%
Extra-thoracic malignancy	4	100%
Non-small cell lung cancer	3	75%
Overall	**67**	**92%**

Table 6.3b: Results of EBUS-TBNA in 77 patients with isolated mediastinal lymphadenopathy

Lymph node station	Number of nodes sampled (%)	Mean lymph node size (mm)	Median number of passes	Sensitivity
4R	21	25	5	90%
4L	1	35	5	100%
7	66	26	5	92%
10R	1	15	3	100%
Total	**99**	**23**	**4**	**92%**

Table 6.4: Costs of different strategies for the investigation of patients with isolated mediastinal lymphadenopathy

	Parameter value			Costs		
	EBUS strategy	Mediastinoscopy strategy	Unit cost	EBUS strategy	Mediastinoscopy strategy	Difference
EBUS-TBNA	1.00	0	£1382	£1382	0	
Mediastinoscopy	0.13	1.00	£3228	£420	£3228	
Clinical Follow-up	0.04	0.08	£500	20	40	
Total cost				£1822	£3268	£1446

Figure 6.2: Flowchart of patients in the REMEDY trial

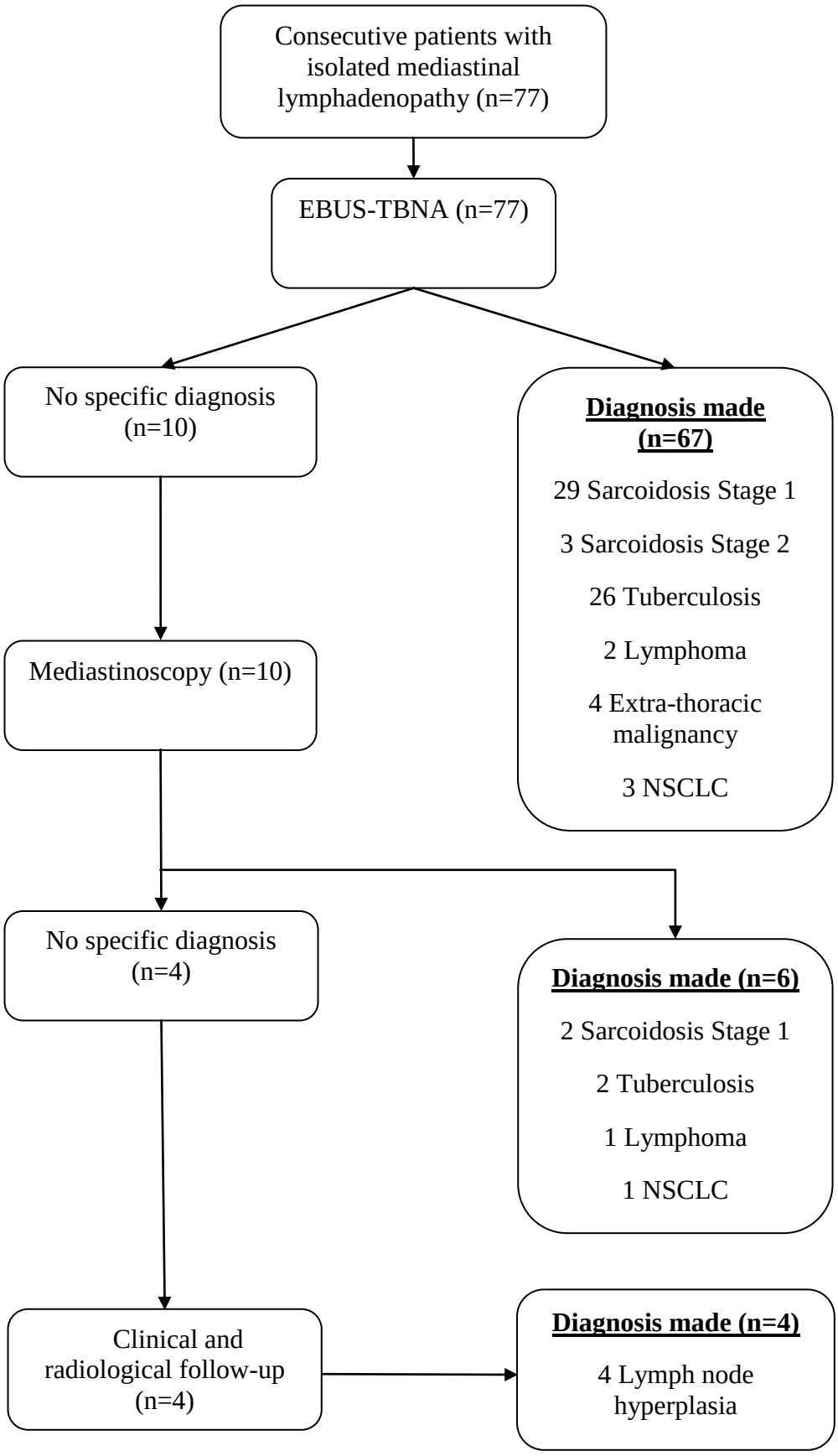
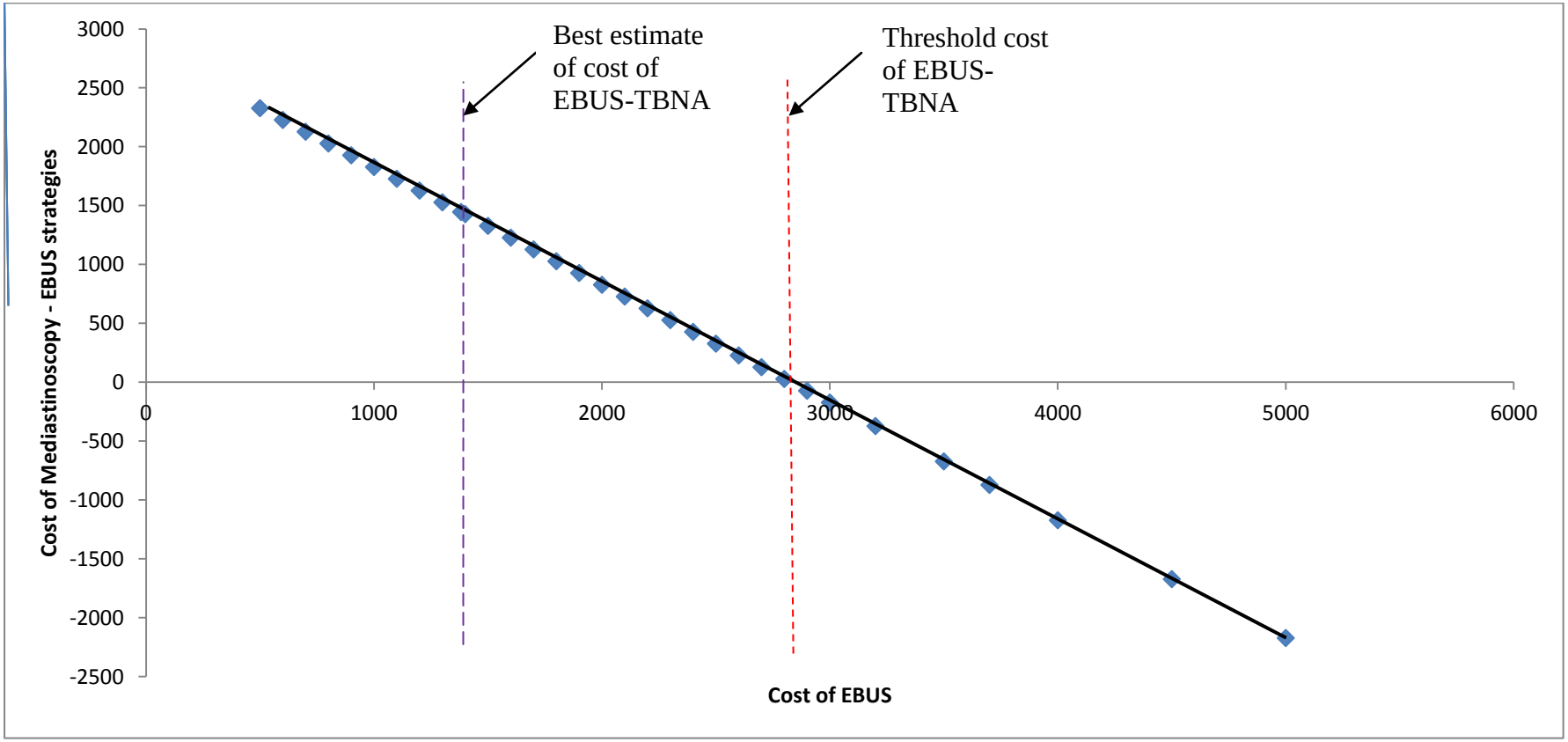


Figure 6.3: Univariate threshold sensitivity analysis. Threshold sensitivity analysis demonstrating that the cost of mediastinoscopy alone is more expensive than a strategy of EBUS-TBNA (followed by mediastinoscopy if EBUS-TBNA is negative) as long as EBUS-TBNA costs less than £2828 (red dashed line). Above this threshold cost for EBUS-TBNA, mediastinoscopy alone is the less costly strategy. The best estimate cost of EBUS is £1382 (purple dashed line).



6.4 DISCUSSION

This is the first prospective clinical trial to demonstrate the utility and cost-savings of using EBUS-TBNA as an initial investigation for patients with IML requiring pathological diagnosis. The study demonstrates that EBUS-TBNA is a highly effective diagnostic modality and can prevent 87% of mediastinoscopies in this scenario. The negative predictive value however is low at 40% and therefore mediastinoscopy is recommended after negative EBUS-TBNA, which is an important consideration when obtaining consent from patients for the procedure.

Considerable evidence is now available on the efficacy of EBUS-TBNA in patients with lung cancer (McComb et al. 2011). Data is also expanding on the effectiveness of EBUS-TBNA in patients with mediastinal sarcoidosis (Navani et al. 2011a), tuberculosis (Navani et al. 2011b), lymphoma (Marshall et al. 2011) and extra-thoracic malignancy (Tournoy et al. 2011). However, much of the data is from retrospective cohort studies and therefore subject to selection bias. In particular, in many of the studies, patients referred for mediastinoscopy were excluded and therefore their characteristics are unknown. In addition, in prior studies, many patients undergoing EBUS-TBNA would not have been otherwise referred for mediastinoscopy. Therefore, previous inferences on mediastinoscopies prevented are prone to bias. For example in Yasufuku and colleagues' large retrospective report of patients with undiagnosed mediastinal masses (Yasufuku et al. 2011), the authors concluded that EBUS-TBNA can spare more invasive procedures but it is not clear that the patients would have been subjected to the more invasive procedures in the absence of EBUS-TBNA. In the current prospective trial, only patients who were

referred for mediastinoscopy were included and were similar to historical controls undergoing mediastinoscopy, minimizing this bias as much as possible.

The diagnosis of lymphoma by EBUS-TBNA is an area of controversy since the management of lymphoma often depends on pathological subtype and grade and EBUS-TBNA obtains smaller specimens than mediastinoscopy. EBUS-TBNA may be particularly useful for the mediastinal staging of lymphoma and in the diagnosis of lymphoma recurrence, however its role for primary diagnosis is currently under debate. In one study of EBUS-TBNA in patients with lymphoma, EBUS had a sensitivity of 76% and 19% of the diagnosed patients still required a further invasive procedure (Steinfort et al. 2010a). In this trial of 77 consecutive patients, only 3 patients had a lymphoma. EBUS-TBNA provided a conclusive diagnosis in 1 patient and prevented mediastinoscopy in another (who went on to have the diagnosis confirmed by bone marrow biopsy). The low prevalence of lymphoma in this typical cohort of patients further highlights that EBUS-TBNA is a good initial test for patients with IML.

EBUS-TBNA was diagnostic of tuberculosis in 26 out of 28 cases in this study. Of these, 11 (40%) were culture positive and 1 isolate of Mycobacterium tuberculosis were found to be resistant to isoniazid. This is consistent with the larger multi-centre cohort of patient with mediastinal lymph node tuberculosis (Chapter 4) which demonstrated an overall diagnostic sensitivity of 94% in 156 patients and culture rate of 47%. The emergence of drug resistant tuberculosis emphasises the importance of sampling MLNs in this scenario and also the utility of EBUS-TBNA in this group of patients. In some cases however, diagnostic difficulty remains in distinguishing sarcoid from tuberculosis as non-caseating granulomas obtained from EBUS-TBNA may also be consistent with tuberculosis. The merit of PCR based tests on samples

obtained from EBUS-TBNA is currently under evaluation. The advent of miniforceps and transbronchial needle forceps may help to improve diagnostic yield further in patients with suspected mediastinal lymphoma or tuberculosis (Herth et al. 2011).

This study is the first health economic analysis of EBUS-TBNA in patients with IML. The cost of EBUS-TBNA to the NHS in this trial was estimated at £1382 per procedure. This is slightly higher than the costs of EBUS-TBNA estimated in Singapore (Ang et al. 2010) of SGD 2623 (£1337) and the US (Harewood et al. 2010) of $1711 (£1051). A decision tree analysis in patients with lung cancer from an Australian perspective (Steinfort et al. 2010b) employed a mean cost of EBUS-TBNA of $1361(£905). The current NICE guidance on lung cancer utilises a cost of £1252 per EBUS-TBNA (Medford et al. 2009). If we used these lower costs per procedure then the cost savings achieved by the EBUS-TBNA strategy would increase by a small amount. The estimate of the cost to the NHS of EBUS-TBNA of £1382 per procedure (detailed in Table 6.1) is based on a model of 2 lists per week and 3 cases per list resulting in approximately 250 cases per year. This necessitates 2 dedicated endobronchial ultrasound scopes and a single processor which have been factored into the costings as capital costs.

In the current analysis a cost-minimisation approach was considered the most appropriate as the same final diagnosis, treatment and treatment outcomes would have been reached regardless of whether EBUS-TBNA or mediastinoscopy were employed as the initial procedure. In addition, complications from EBUS-TBNA and mediastinoscopy were not observed or included in this study; this is possibly a conservative assumption. A systematic review of studies of EBUS-TBNA in patients with lung cancer up to 2008 (Gu et al. 2009) has demonstrated that the procedure is

very safe with only 2 complications recorded in 1299 procedures (1 patient with a pneumothorax and 1 patient with hypoxia). In a large retrospective single-centre study of 2145 patients undergoing mediastinoscopy the complication rate was 1% which comprised of 1 death, 7 haemorrhages, 2 tracheal injuries, 2 pneumothoraces and 12 patients with vocal cord dysfunction (Lemaire et al. 2006). The low rate of complications was not included in the decision tree model but in view of reported complication rates in the literature which are higher for mediastinoscopy, their inclusion would have further favoured the EBUS-TBNA strategy.

Limitations of the study are acknowledged. EBUS-TBNA was performed in a tertiary centre with a high volume of procedures carried out by physicians with expertise in the procedure. The reporting pathologists also have considerable experience in the interpretation of EBUS-TBNA specimens. The sensitivity obtained and proportion of mediastinoscopies prevented in this study therefore may not be immediately reproducible in other centres. The trial excluded patients with anterior mediastinal lymphadenopathy (inaccessible by EBUS-TBNA) and therefore the results cannot be applied to these patients. Although the cost of EBUS-TBNA to the NHS has been approximated, sensitivity analysis has been carried out and the effect has been reported for a wide range of potential EBUS-TBNA costs.

In conclusion, EBUS-TBNA is a safe, highly sensitive and cost-saving initial investigation in patients with IML being referred for mediastinoscopy. The low negative predictive value of EBUS-TBNA in this setting indicates that mediastinoscopy should be performed in cases of negative EBUS-TBNA.

CHAPTER 7:

SUITABILITY OF ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION SPECIMENS FOR THE SUBTYPING AND GENOTYPING OF NON-SMALL CELL LUNG CANCER

7.1 INTRODUCTION

Traditionally, the pathology of lung cancer has been divided into non-small cell lung cancer (NSCLC) and small cell lung cancer, reflecting the different tumour biology and treatments. In recent years, it has become necessary to further subdivide NSCLC and subtyping and genotyping of NSCLC is now central to treatment decisions for patients with advanced NSCLC. Late phase clinical trials have provided 3 major observations that certain treatment agents only have efficacy or safety in particular subtypes or genotypes of NSCLC. First, a large randomised non-inferiority trial of Pemetrexed and Cisplatin in 1725 patients with NSCLC (Scagliotti et al. 2008) demonstrated that Pemetrexed is only of benefit in patients with non-squamous histology, while in patients with squamous subtype Pemetrexed was inferior to the standard treatment of Cisplatin and Gemcitabine. This has been reflected in guidance from the National Institute of Health and Clinical Excellence (NICE) which has recommended pemetrexed as a first line treatment for patients with

adenocarcinoma or large cell carcinoma in September 2009 (National Institute of Health and Clinical Excellence 2009).

Second, a randomised phase II trial of bevacizumab plus carboplatin and paclitaxel versus carboplatin and paclitaxel alone revealed that fatal pulmonary haemorrhage was significantly higher in patients with squamous subtype of NSCLC (Johnson et al. 2004). Consequently, bevacizumab is contra-indicated in patients with squamous cell lung cancer (SQCC). Third, phase III randomised trials in East Asia have demonstrated that the tyrosine kinase inhibitors only have improved progression-free survival (PFS) in patients with NSCLC harbouring an activating EGFR mutation. In patients without an EGFR mutation, standard chemotherapy may offer superior PFS (Mok et al. 2009;Zhou et al. 2011) . Further targeted agents in patients with specific cancer genotypes are set to emerge (Kwak et al. 2010).

Coupled with the emergence of personalised therapies for advanced NSCLC has been the rapid expansion of endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) which allows sampling of mediastinal and hilar lymphadenopathy under direct vision. The technique employs a 21 or 22 gauge needle and therefore obtains smaller samples than biopsy via mediastinoscopy. Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) was initially developed for the nodal staging of lung cancer. However, it is now commonly used as an initial investigation in patients with suspected NSCLC after computed tomography scan as it may provide a tissue diagnosis and accurate nodal staging in a single investigation (Navani, Spiro, & Janes 2009). However, given the concern regarding the smaller samples obtained it is unknown whether aspirates

from EBUS-TBNA in routine practice provide sufficient material to allow subtyping and genotyping of NSCLC in order to guide treatment. We therefore conducted a large pragmatic multi-centre study to clarify whether samples from EBUS-TBNA were suitable for subtyping of NSCLC and EGFR mutation testing.

7.2 METHODS

7.2.1 Patients and EBUS-TBNA samples

Patients included in this retrospective study were known or suspected to have NSCLC and underwent EBUS-TBNA between January 2009 and March 2011 across 5 centres in the UK. The participating centres were University College London Hospital, University Hospital Birmingham, University Hospital of North Tees, Lancashire Teaching Hospital and Papworth Hospital, Cambridge. Patient demographic data was collected and included age, gender and ethnicity. Information on the EBUS-TBNA procedure regarding the lymph stations and the size of lymph nodes sampled was also documented. Following systematic assessment of the mediastinal and hilar lymph node stations, the target lymph node was aspirated under direct ultrasound vision using a dedicated EBUS-TBNA needle (22 or 21 gauge). Three to 5 passes per lymph node were made. Rapid on-site evaluation of samples was not employed. The samples obtained were expelled from the needle using the stylet and placed into liquid fixative for cell-block processing. Needle contents were also flushed with saline into the liquid fixative. The specimen was centrifuged to form a pellet, suspended in agar, fixed in neutral buffered formalin and processed as a cell block from which a single hematotoxylin and eosin (H&E) stained section was cut. Further sections were cut and used for immunohistochemical staining as

required (Wallace et al. 2007). When cores of tissue were obtained at EBUS-TBNA these were placed directly into formalin.

7.2.2 Pathological and molecular techniques

Interpretation of the EBUS-TBNA specimens was carried out by the locally reporting pathologist and there was no centralised reporting. Classification of NSCLC was based upon morphological appearances (H&E stain) according to the criteria summarised in Table 7.1. Immunostaining was performed if the sample was sufficient and clinically indicated (Figure 71a-d). Antibodies to cytokeratins 5/6 (CK5/6) and p63 were deemed to be consistent with squamous cell carcinoma (Kaufmann et al. 2001;Khayyata et al. 2009). Antibodies to Thyroid transcription factor 1 (TTF-1) were also employed and TTF-1 is known to be expressed in approximately 75% of lung adenocarcinomas (Stenhouse et al. 2004;Yatabe, Mitsudomi, & Takahashi 2002).

The decision to submit the sample for EGFR mutation testing was made following discussion by the multi-disciplinary team. EGFR mutations were detected using DNA sequencing techniques and patients were considered to be positive for EGFR mutation if 1 of 29 EGFR mutations was detected by polymerase chain reaction based assays (Figure 7.1e). Four centres employed the commercially available amplification refractory mutation system (ARMS) kit (Qiagen) which is able to detect an EGFR mutation in samples which contain 1% tumour. The remaining centre employed a matrix-assisted laser desorption/ionization mass spectroscopy system for detecting EGFR mutations (Sequenom MassARRAY).

7.2.3 Endpoints and statistical analysis

The primary endpoint of the study was the proportion of patients with NSCLC undergoing EBUS-TBNA in whom it was not possible to subtype the lung cancer and therefore were classified as NSCLC not otherwise specified (NSCLC-NOS). The co-primary endpoint was the proportion of samples that were not suitable for EGFR testing as determined by the local testing centre. The rate of NSCLC-NOS were determined according to age, lymph node location (hilar versus mediastinal) and size (greater or less than 1cm in short-axis), pathological differentiation and whether immunohistochemistry was carried out in univariate and multivariate analyses. The unit of analysis was the patient.

Each patient was followed up for at least 6 months duration and each EBUS-TBNA procedure was therefore classified as a true positive, true negative or false negative result. False positive results from EBUS-TBNA were assumed not to occur. Standard definitions for the calculation of the sensitivity and negative predictive value of EBUS-TBNA (secondary endpoints) in patients with NSCLC were applied. Proportions were compared using the Chi-squared test. Predictors of NSCLC-NOS were modelled using logistic regression. Covariates demonstrated to be significant at the 20% level on Univariate analysis were entered into the multivariate model. All statistical calculations were carried out using STATA version 10 (Statacorp., USA). Ethical approval was not required given the observational nature of the study. All results were fully disclosed to the patients and also discussed in multi-disciplinary team meetings in order to determine the treatment strategies.

Table 7.1: Morphological criteria used on EBUS-TBNA samples for differentiating between adenocarcinoma and squamous cell lung carcinoma. Data from Sturgis et al. (2000).

	Adenocarcinoma	Squamous Cell Carcinoma
Background	Cell debris, foamy macrophages	Necrosis
Cell distribution	Small aggregates	Individually dispersed
Architecture	Glandular, acinar, papillary	Solid, trabecular
Cell group	Morulae	Pearl formations
Cell membrane	Poorly defined	Well defined
Cytoplasm	Scanty, vacuolated	Large, dense, keratinized
Nuclei	Round-oval, lightly stained	Irregular, hyperchromatic
Nucleoli	Prominent (well differentiated)	Inconspicuous (keratinized) Prominent (non-keratinized)
Nuclear pseudoinclusions	Present	Absent

Figure 7.1a: EBUS-TBNA smear demonstrating adenocarcinoma (May–Grunwald-Giemsa stain).

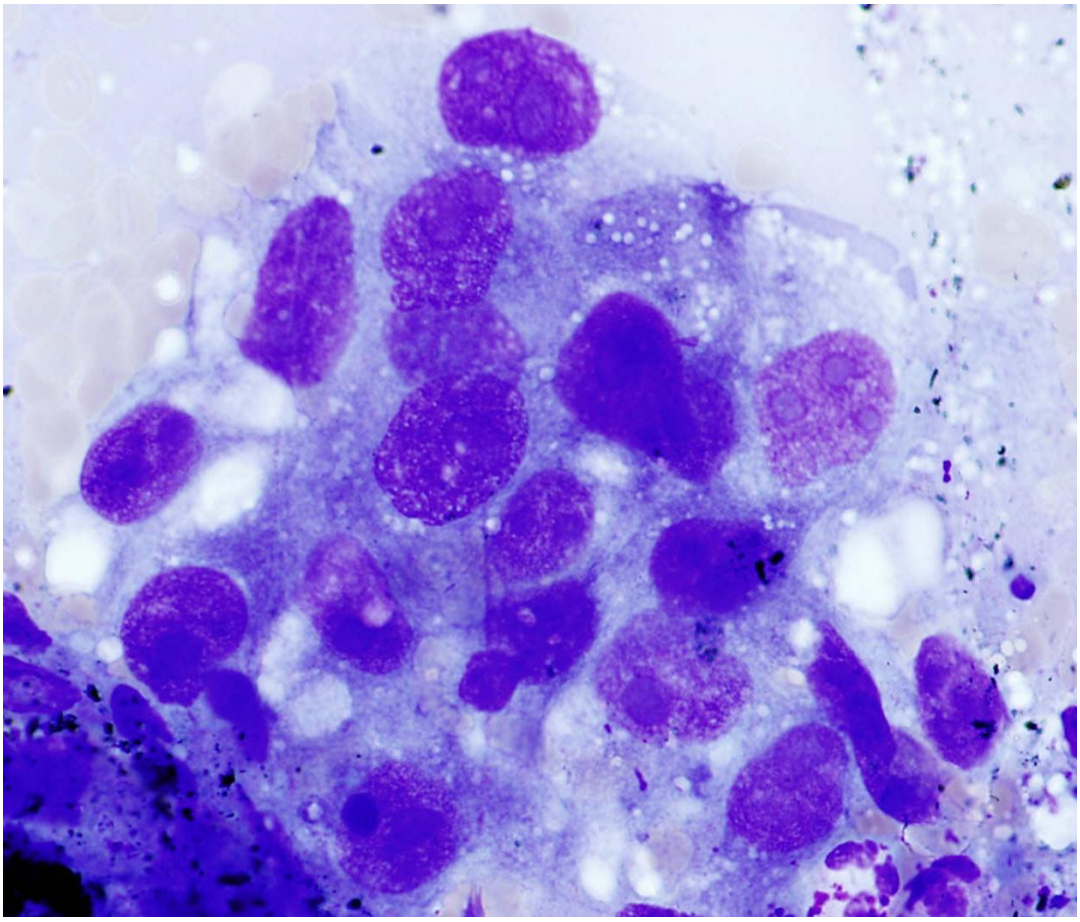


Figure 7.1b: Cell block obtained from EBUS-TBNA demonstrating adenocarcinoma (H&E stain)

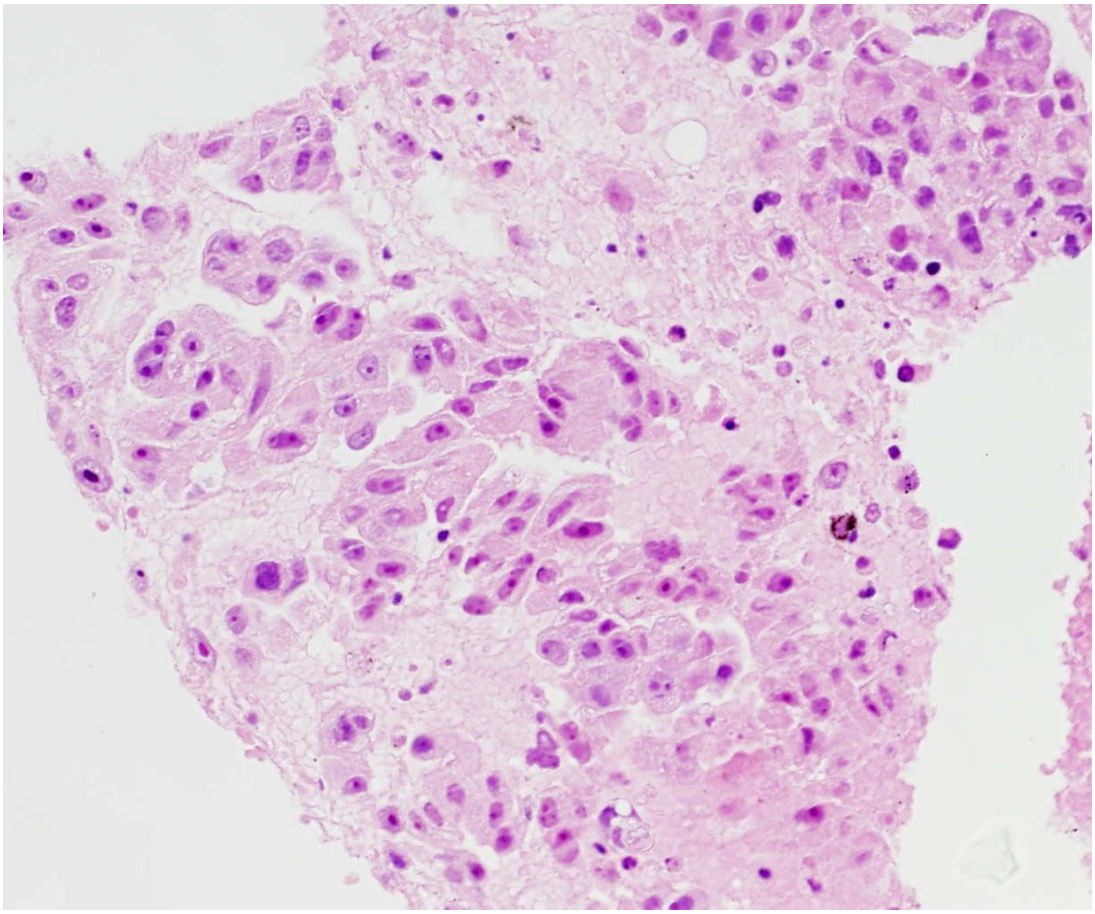


Figure 7.1c: Adenocarcinoma from EBUS-TBNA cell block positive for TTF-1, confirming lung origin

TTF-1; thyroid transcription factor -1

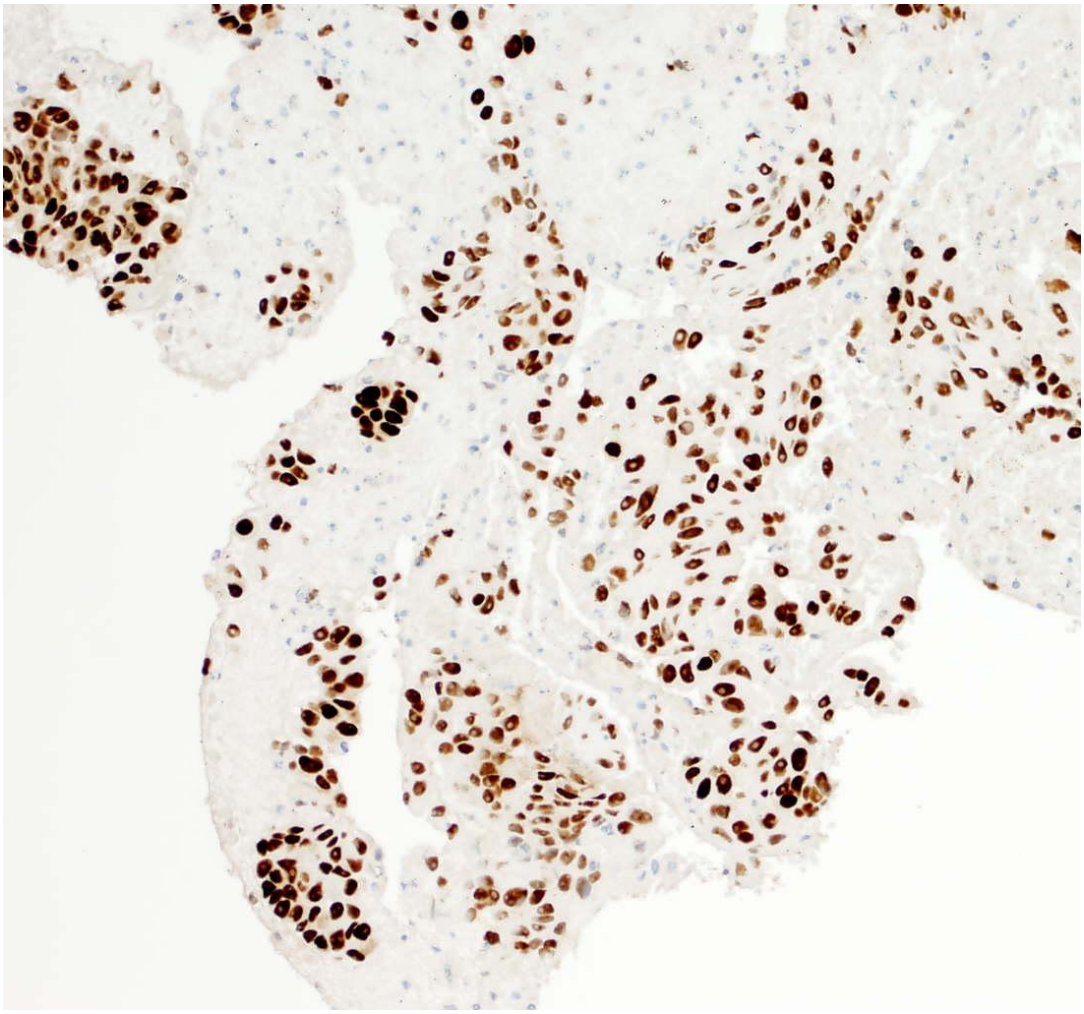


Figure 7.1d: EBUS-TBNA cell block demonstrating adenocarcinoma to be ERCC1 positive, suggesting resistance to platinum-based chemotherapies

ERCC1: excision repair cross-complementing group 1

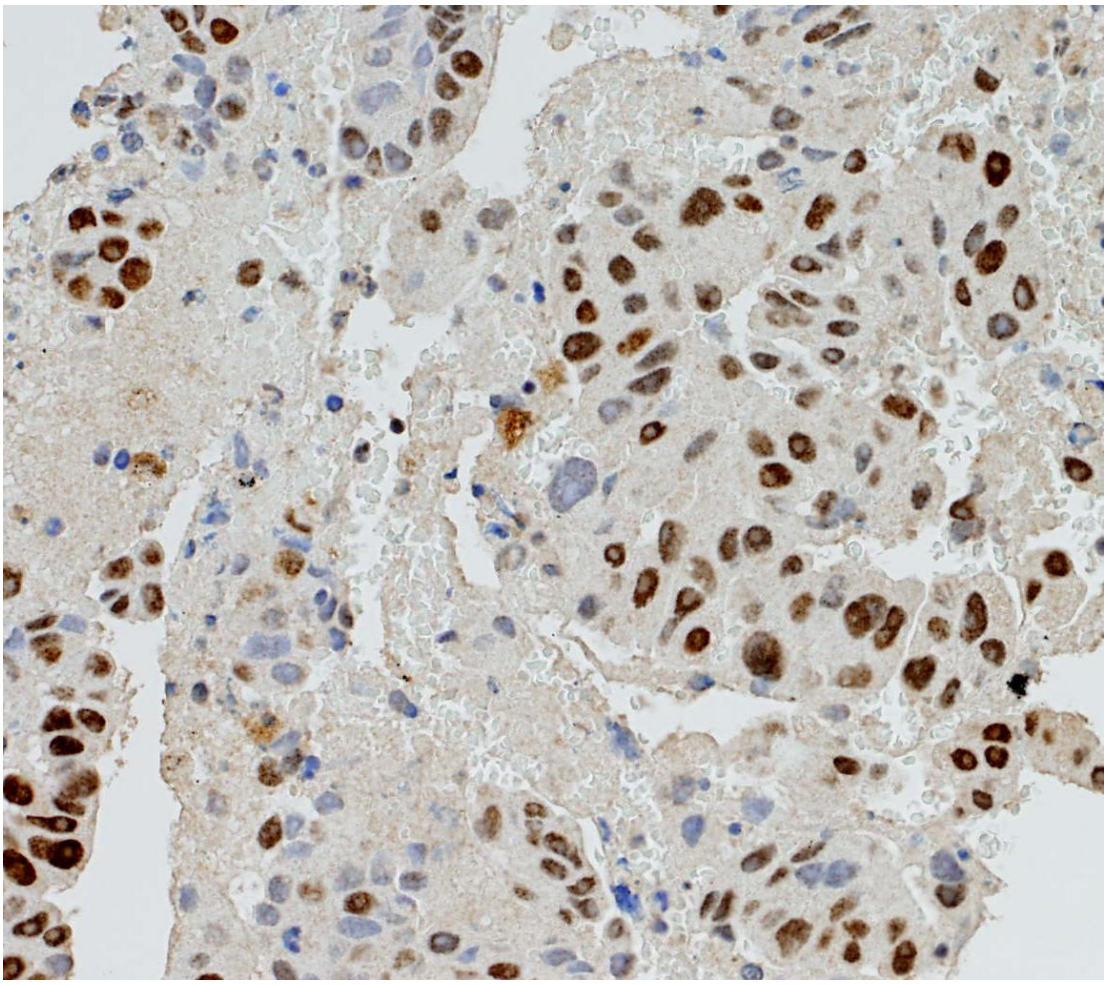
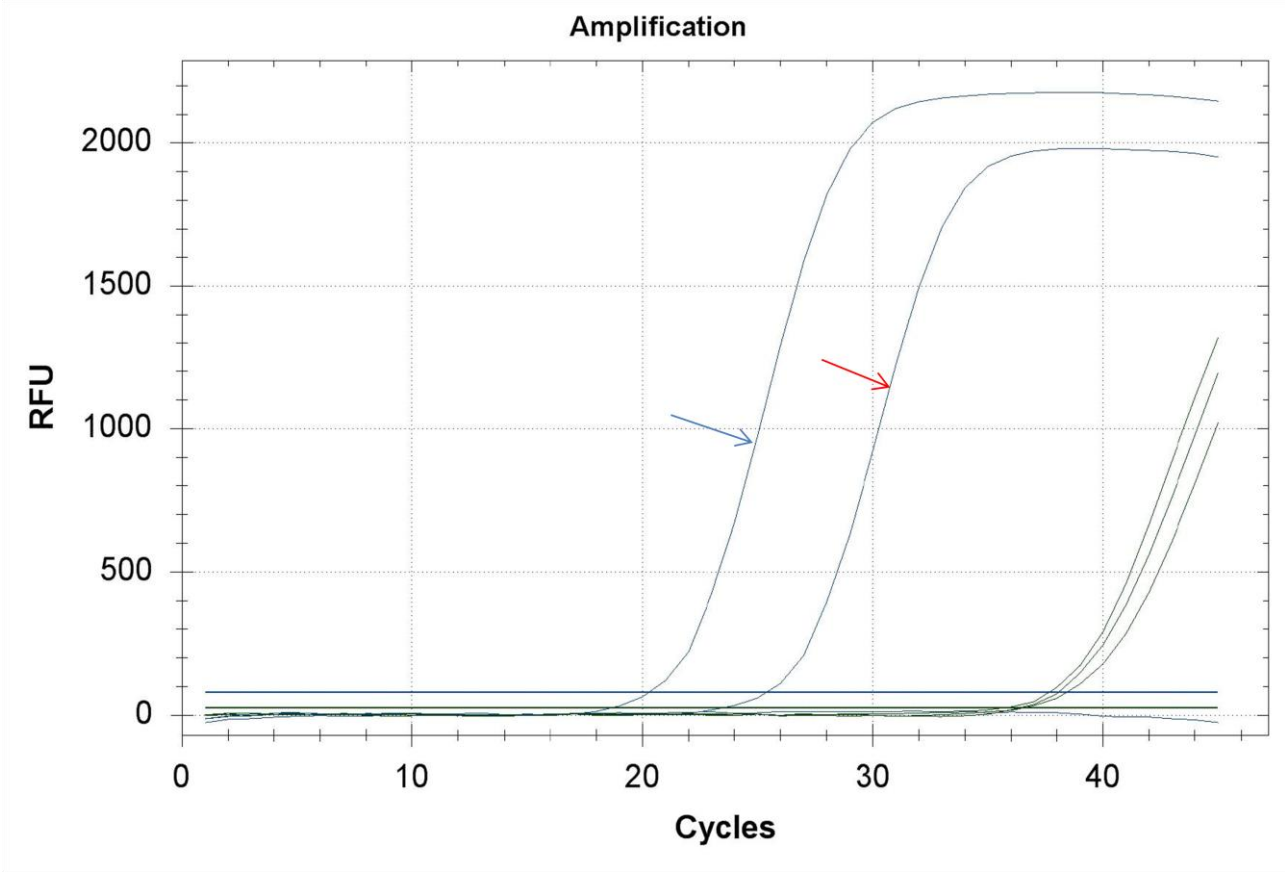


Figure 7.1e: Real-time polymerase chain reaction plot (RT-PCR) showing exon 19 deletion in the Epidermal Growth Factor Receptor gene. DNA is extracted from EBUS-TBNA specimens and the mutations are detected by real-time PCR amplification and hybridisation using fluorescently labelled probes. There is RT-PCR amplification of EGFR control (blue arrow) and exon 19 deletion (red arrow). The exogenous control is the green line.



7.3 RESULTS

Between 2009 and 2011, 615 patients with known or suspected NSCLC underwent EBUS-TBNA at 5 UK centres. Three-hundred and fifty-five (58%) were male and the median age of patients with NSCLC was 69 (range 35 – 88) years. Baseline characteristics are summarised in Table 7.2 (page 156). Two-hundred and fifty-eight patients had more than 1 lymph node sampled and in total 893 lymph nodes were aspirated. The size and location of lymph nodes sampled and the diagnostic yield are shown in Table 7.3 (page 157).

The pathological subtypes of NSCLC diagnosed by EBUS-TBNA are shown in Figure 7.2 (page 152). In total, 478 had a final diagnosis of NSCLC in intra-thoracic lymph nodes. The number of patients with a final diagnosis by EBUS-TBNA of NSCLC – NOS (the primary endpoint) was 86 (21%, 95% CI 18% - 26%). 250 (62%) patients had their EBUS-TBNA specimens submitted for immunostaining and this was possible in 233 (93%, 95% CI 89% - 96%). In univariate analysis, there was no association between NSCLC-NOS and age, lymph node size, lymph node location, number of lymph nodes aspirated and pathological differentiation. However, a highly significant relationship was seen on univariate and multivariate analysis (Figure 7.3i-viii, page 153-5) between immunohistochemistry not performed and the final diagnosis of NSCLC-NOS (Table 7.4, page 158). The multivariate model also included pathological differentiation. When immunostaining was possible, the risk of the NSCLC tumour being unclassified was halved in the multivariate analysis (OR 0.50, 95% CI 0.28 – 0.88, P=0.016).

Three hundred and eighty-one patients had lymph nodes aspirated that were greater than 1cm in short axis. Of these, 281 had NSCLC diagnosed by EBUS-TBNA and

the number of patients diagnosed with NSCLC –NOS was 46 (16% 95% CI 11% – 19%) in this subgroup. In the 54 patients with recorded lymph node size less than or equal to 1cm in short axis, the prevalence of malignancy was 61% (33 patients) and the number of patients diagnosed with NSCLC-NOS was 3 (9%, 95% CI 2 – 24%). In 180 patients the lymph node size was not recorded. There was no statistically significant difference (P=0.60) in the NOS-NSCLC rate in nodes greater or less than 1cm in short axis. 458 patients had sampling with a 22 gauge needle while the larger 21 gauge needle was used in the remainder and was associated with a NSCLC-NOS rate of 22% and 10% respectively (P=0.40).

Ninety-three (22%) patients who underwent EBUS-TBNA had EGFR mutation analysis requested on the sample. Of these, 47 were adenocarcinoma, 18 had squamous cell carcinoma, 9 had large cell carcinoma and 19 had NSCLC-NOS. EGFR mutation analysis was possible (the co-primary endpoint) in 84 (90%, 95% CI 82% – 95%) cases and 5 (5%) patients with EGFR mutations were identified. Of the 5 patients who had an EGFR mutation, all were Caucasian and had adenocarcinoma. The median age of these patients was 58 years (range 53 – 68) and 4 (80%) were female. Two out of the 5 EBUS-TBNA samples which had an EGFR mutation EBUS-TBNA were also noted to stain for TTF-1.

Overall, EBUS-TBNA had a sensitivity of 88% (95% CI 85% - 91%), negative predictive value of 71% (95% CI 64% - 77%) and diagnostic accuracy of 91% (95% CI 88% - 93%). Four hundred and twenty-two patients had NSCLC diagnosed by EBUS-TBNA. Fifty-six patients had a false negative EBUS procedure. In each of these cases lymphoid cells only were aspirated and subsequent surgery, mediastinoscopy or clinical follow-up confirmed malignancy (Figure 7.2, page 152).

None of the 32 specimens in whom granulomas were aspirated were proven to be false negative results.

The sensitivity from aspiration of hilar lymph nodes (stations 10 and 11) was 78% (95% CI 67% - 87%) and was significantly inferior to the sensitivity from mediastinal lymph nodes (88%, 95% CI 84% - 91%, P=0.049). The median size of hilar lymph nodes was 15mm (range 7 – 40).

Sensitivity in patients with lymph nodes ≤1cm in short-axis was 61% (95% CI 44% - 75%), and significantly lower than the sensitivity of 90% (95% CI 87% - 93%, P<0.001) in nodes >1cm. There was no interaction between lymph node location and size.

One patient's EBUS procedure resulted in a death. The patient was an 81 year old female who presented with stage IV adenocarcinoma of the lung. The EBUS-TBNA procedure was uncomplicated and the patient was discharged home after the procedure with normal vital observations. Twenty-four hours later the patient was admitted to hospital with clinical features of severe pneumonia and sepsis. Group A Streptococcus was isolated from blood cultures and also from a throat swab. The patient deteriorated from sepsis and respiratory failure and died within 48 hours of admission. The scenario was attributed to the carriage of organisms by the EBUS scope from the pharynx into the lungs. No other complications were reported.

Figure 7.2: Flowchart of patients

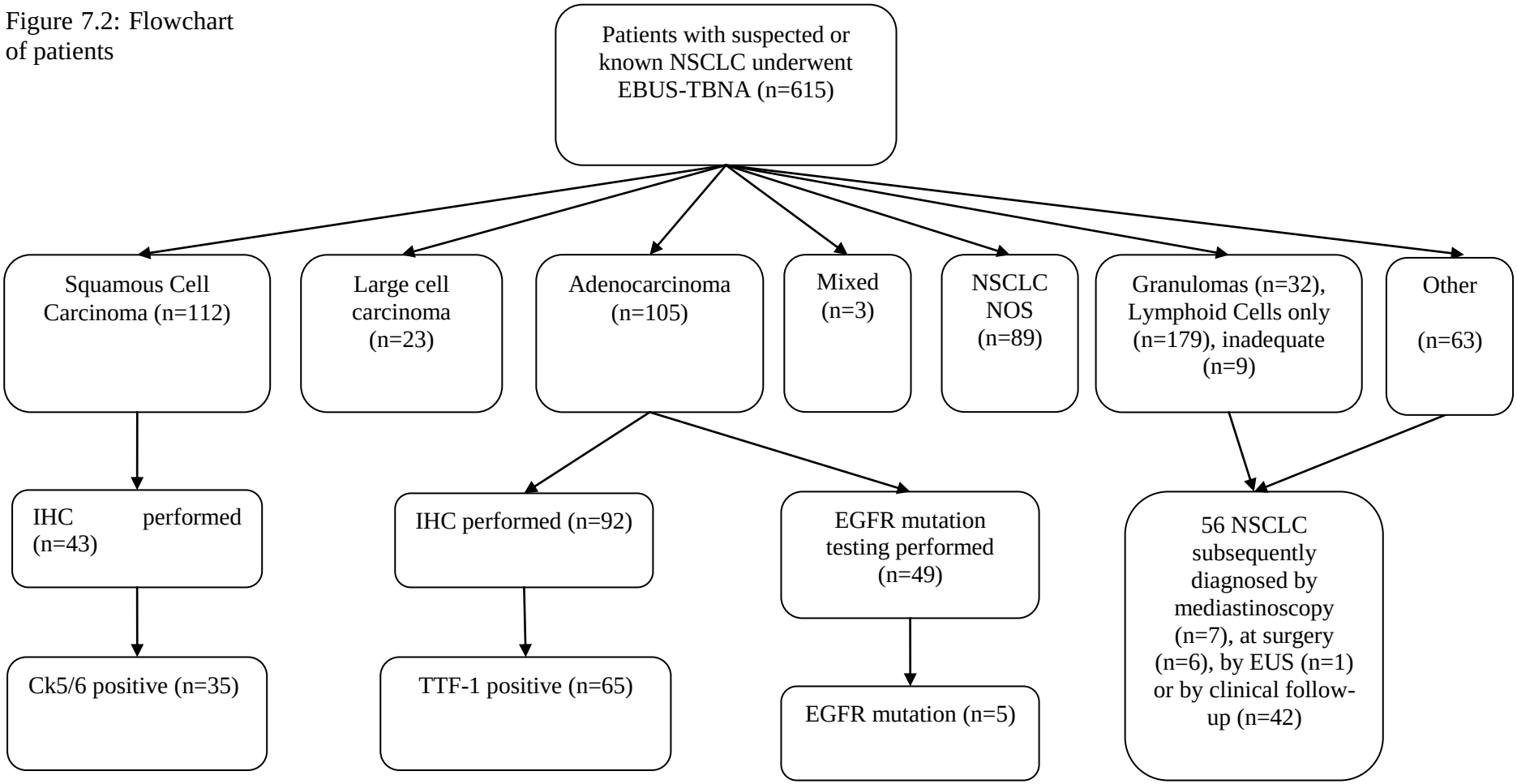


Figure 7.3: STATA output of univariate and multivariate analyses to predict occurrence of NSCLC-NOS from EBUS-TBNA specimens

(i) No relationship between NOS rate and age (P=0.93)

```
. logistic  cytology  age

Logistic regression                                     Number of obs   =        332
                                                        LR chi2(1)      =       0.01
                                                        Prob > chi2     =     0.9335
Log likelihood = -192.99876                             Pseudo R2       =     0.0000

------------------------------------------------------------------------------
    cytology | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         age |   .9989435   .0126495    -0.08   0.933      .974456    1.024046
------------------------------------------------------------------------------
```

(ii) No relationship between NOS rate and lymph node location (mediastinal vs hilar lymph nodes)

```
. logistic cytology  lymphnodelocation1

Logistic regression                                     Number of obs   =        332
                                                        LR chi2(1)      =       1.57
                                                        Prob > chi2     =     0.2106
Log likelihood = -192.21845                             Pseudo R2       =     0.0041

------------------------------------------------------------------------------
    cytology | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
lymphnodel~1 |   1.556467   .5404004     1.27   0.203     .7881439    3.073792
------------------------------------------------------------------------------
```

(iii) No relationship between number of lymph nodes sampled and NOS rate

```
. logistic cytology  numberoflymphnodessampled

Logistic regression                                     Number of obs   =        332
                                                        LR chi2(1)      =       0.18
                                                        Prob > chi2     =     0.6742
Log likelihood =  -192.9139                             Pseudo R2       =     0.0005

------------------------------------------------------------------------------
    cytology | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
numberofly~d |   1.095411   .2368968     0.42   0.673     .7169584    1.673634
------------------------------------------------------------------------------
```

(iv) No relationship between NOS rate and size of largest lymph node sampled

```
. logistic  cytology sizeoflargestnodesampledmm

Logistic regression                               Number of obs   =        213
                                                  LR chi2(1)      =       0.01
                                                  Prob > chi2     =     0.9231
Log likelihood = -114.87328                       Pseudo R2       =     0.0000

------------------------------------------------------------------------------
    cytology | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
sizeoflarg~m |   1.002251     .02334     0.10   0.923     .9575334    1.049056
------------------------------------------------------------------------------
```

(v). Non-significant relationship between NOS rate and pathological differentiation (P=0.089)

Patients with a poorly or undifferentiated tumour have 1.7 times the odds of their NSCLC being undifferentiated

```
. logistic  cytology pathologicaldifferentiation

Logistic regression                               Number of obs   =        332
                                                  LR chi2(1)      =       2.80
                                                  Prob > chi2     =     0.0943
Log likelihood = -191.60293                       Pseudo R2       =     0.0073

------------------------------------------------------------------------------
    cytology | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
pathologic~n |   1.666418   .5007746     1.70   0.089     .9246762    3.003158
------------------------------------------------------------------------------
```

(vi) Highly significant relationship between NOS rate and lack of immunohistochemistry

```
. logistic  cytology immunohistochemistry

Logistic regression                               Number of obs   =        332
                                                  LR chi2(1)      =       7.59
                                                  Prob > chi2     =     0.0059
Log likelihood = -189.20649                       Pseudo R2       =     0.0197

------------------------------------------------------------------------------
    cytology | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
immunohist~y |   .4675082   .1338022    -2.66   0.008     .2667921    .8192294
------------------------------------------------------------------------------
```

(vii) Raw data showing relationship between NOS rate (cytology=1) and presence of immunochemistry

```
. tab  cytology immunohistochemistry
```

Cytology	Immunohistochemistry 0	1	Total
0	150	93	243
1	69	20	89
Total	219	113	332

(viii) In a multivariate model that also included pathological differentiation, immunohistochemistry retained its significant association with NOS rate.

```
. logistic  cytology  pathologicaldifferentiation  immunohistochemistry

Logistic regression                               Number of obs   =        332
                                                  LR chi2(2)      =       8.95
                                                  Prob > chi2     =     0.0114
Log likelihood = -188.52805                       Pseudo R2       =     0.0232
```

cytology	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
pathologic~n	1.435791	.441307	1.18	0.239	.7860761	2.622515
immunohist~y	.4963152	.144514	-2.41	0.016	.2804852	.8782239

Table 7.2: Baseline characteristics of 615 patients with suspected lung cancer who underwent EBUS-TBNA

	Number (%)
Gender	
Male	355 (58%)
Female	260 (42%)
Age	
<50	34 (6%)
50 – 75	437 (71%)
>75	144 (23%)
Smoking	
Current	161 (26%)
Former	406 (66%)
Never or <10 pack years	48 (8%)
Ethnicity	
Caucasian	546 (89%)
South Asian	9 (1%)
East Asian	1 (0%)
African	0 (0%)
Caribbean	0 (0%)
Other	0 (0%)
Unknown	59 (10%)
Total	615

Table 7.3: Lymph node stations sampled in 615 patients undergoing EBUS-TBNA

Lymph node station	Number of nodes sampled	Mean size of lymph node (mm)	Prevalence of NSCLC	Sensitivity	Negative Predictive Value	Diagnostic accuracy
2R	12	18	56%	80%	80%	89%
2L	1	20	100%	100%	N/A	100%
3P	2	18	0%	N/A	100%	100%
4R	225	21	86%	90%	62%	91%
4L	72	18	72%	75%	61%	82%
7	361	22	74%	89%	77%	92%
10R	87	17	71%	100%	100%	100%
10L	44	18	78%	94%	83%	96%
11R	16	15	93%	85%	33%	86%
11L	4	13	100%	50%	0%	50%
Overall	824	21	78%	88%	72%	91%

Table 7.4: Univariate and multivariate analyses of factors to predict NSCLC-NOS in patients undergoing EBUS-TBNA. On the basis of univariate results, only pathological differentiation and performance of immunohistochemstry

Covariate	Unadjusted OR of NSCLC-NOS	Univariate P value	Adjusted OR of NSCLC-NOS	Multivariate P value
Age	0.99	0.93		
Lymph node location	1.55	0.20		
Lymph node size	1.0	0.92		
Pathological differentiation	1.66	0.09	1.44	0.24
Immunohistochemistry performed	0.47	0.008	0.50	0.016

7.4 DISCUSSION

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

EBUS-TBNA is an important investigation for the diagnosis of mediastinal and hilar lymphadenopathy in patients with lung cancer. It has been recommended as an initial investigation by NICE (2011) in patients with enlarged mediastinal lymph nodes as it may provide an inoperable disease stage and a pathological diagnosis in a single investigation. This large multi-centre pragmatic implementation study demonstrates that routine samples from EBUS-TBNA are able to provide sufficient information to allow subtyping in 79% and genotyping in 90% of patients with NSCLC.

The proportion of patients with NSCLC in whom EBUS-TBNA diagnosed NSCLC-NOS in this multi-centre study was 21%. This is consistent with data from alternative biopsy techniques. An analysis of the California Cancer Registry of 175,298 patients diagnosed with lung cancer between 1989 and 2006 demonstrated a NSCLC-NOS rate of 22.1% (Ou & Zell 2009). The rate of NSCLC-NOS was higher in the patients who had a cytological diagnosis alone (37%). The National Lung Cancer Audit (NLCA) recently published data on 37,637 patients diagnosed with lung cancer in Great Britain and Northern Ireland in 2009 (NHS Information Centre 2011). These patients underwent diagnosis and staging of lung cancer in a real world

setting in England and Wales and the audit demonstrated an overall NSCLC-NOS rate of 30.5%. The improved rate of NSCLC-NOS in EBUS-TBNA samples does not entirely reflect a change in pathological assessment with a drive to lower NOS rate since part of the EBUS cohort was also carried out in 2009. It does highlight that EBUS-TBNA samples at the very least are as good as other sample acquisition techniques for subtypying, such as bronchoscopy and CT guided biopsy. EBUS-TBNA is also able to sample central parenchymal lung lesions that would otherwise not be accessible without a considerably more invasive approach (Tournoy et al. 2009). Therefore increased application of EBUS-TBNA may improve the rates of histological confirmation in patients with NSCLC, which currently stand at a mean of 75.6% in the NLCA (NHS Information Centre 2011).

Previous studies have shown that samples from cytology are valid when compared to subsequent larger samples. Indeed, the morphologic features that distinguish squamous cell carcinoma (predominantly keratinized cytoplasm and intercellular bridges) from adenocarcinoma (mucin vacuoles and gland formation) span less than the 250-μm inner diameter of a 25-gauge fine needle (Fischer et al. 2011). In a recent retrospective study of 48 patients (Wallace & Rassl 2011), cell block samples from EBUS-TBNA were compared to histological specimens obtained by alternative procedures such as bronchoscopy and CT guided biopsy. All subtypes diagnosed by EBUS-TBNA were validated by histological samples. When immunohistochemistry was performed on cell blocks, there were six cases diagnosed with NSCLC-NOS on EBUS-TBNA samples which were diagnosed with a specific cell type on alternative histological samples (3 adenocarcinomas, 2 squamous cell carcinomas and 1 large cell undifferentiated carcinoma). A further study of 101 individuals demonstrated a 93% concordance between small biopsy and cytology specimens (Sigel et al. 2011).

As in this study, lack of supporting immunohistochemistry contributed to unclassified cytology cases. In another report, 158 (85%) cases of NSCLC were typed by cytology and 28 (15%) were classified as NSCLC-NOS (Nizzoli et al. 2011). Utilising histological specimens from the same patients, 183 (98%) of cases were subtyped by histology and only 3 (2%) cases were classified as NSCLC-NOS. There was 88% concordance between cytological and histological typing. The available data therefore confirm that cytological specimens are reliable for subtyping with no false positive results from cytological subtyping observed and that use of immunohistochemistry can reduce the NSCLC-NOS rate.

Immunohistochemistry profiles do not feature in the diagnostic criteria for squamous cell or adenocarcinoma in the current WHO classification of NSCLCs which is based on resected surgical specimens (Travis WD 2004). However when morphological criteria are unable to distinguish subtypes in smaller samples, a panel of antibodies including TTF-1, p63 and CK5/6 as well as a mucin stain has been recommended in order to minimise the rate of NSCLC tumour that remain unclassified and to make the key distinction between squamous and non-squamous subtypes (Nicholson et al. 2010). The current large pragmatic study shows that samples obtained by EBUS-TBNA are suitable for this approach from any accessible lymph node station and even when sampling lymph nodes less than 1cm in size.

The EGFR-tyrosine kinase inhibitors erlotinib and gefitinib have become established as first-line treatments for patients with advanced lung cancer that harbour an EGFR mutation. Current European Society of Medical Oncology guidelines recommend that all never or former light smokers (<15 packs per year) or patients with non-squamous histology should be tested for EGFR mutation status regardless of performance status (Felip et al. 2011). Cytological samples in alcohol based fixatives

may preserve nucleic acids better than formalin (Vincek et al. 2003) and molecular profiling of cytology samples has been shown to be reliable when compared with histological samples from the same patient (van Eijk et al. 2011). In this study, EGFR mutation testing was requested in 93 patients and the test was possible and deemed reliable in 84 (90%) cases. In the remaining cases, there was insufficient tumour sample to perform the investigation. Five patients with an EGFR mutation were observed out of 49 with adenocarcinoma. Previous studies have assessed the utility of EBUS-TBNA samples for EGFR testing with variable results. In one study EGFR mutation testing was possible in 27 out of 35 patients (77%) undergoing EUS-FNA or EBUS-TBNA (Schuurbiers et al. 2010). Another study of 36 patients in Spain undergoing EBUS-TBNA suggested EGFR mutation analysis was feasible in 26 cases (Garcia-Olive et al. 2010). Billah demonstrated that 96% of specimens from EBUS-TBNA in a US cancer centre were able to undergo EGFR mutation testing (Billah et al. 2011). Similarly high rates of reliable EGFR mutation testing of EBUS-TBNA samples have been observed by Nakajima and colleagues (2007;Nakajima et al. 2011). A recent study, in which cell blocks were prepared from 128 lung cancer cytology specimens, demonstrated that molecular analysis was possible in 98% of specimens (Rekhtman et al. 2011). The low prevalence of EGFR mutation in patients with adenocarcinoma (10%) in our study reflects the predominantly Caucasian smoking population.

It is widely accepted that NSCLC may contain areas of mixed adenocarcinoma, large and squamous cell carcinoma. Up to 25% of small cell carcinomas are thought to contain areas of NSCLC differentiation (Anraku & Waddell 2006). This pathological heterogeneity implies that smaller cytological samples may not be representative of the entire lesion. Another area of controversy that is currently developing in NSCLC

is that of genetic tumour heterogeneity. Conflicting evidence exists. Three studies comparing EGFR mutation status in primary tumour and local lymph node metastases demonstrated significant discrepancies between the sites (Park et al. 2009;Schmid et al. 2009;Sun et al. 2011). However a recent study showed that when highly sensitive techniques for mutation detection are employed, no discordant mutation patterns were detected among 77 paired primary and metastatic tumours (Yatabe, Matsuo, & Mitsudomi 2011). These authors suggested that weak EGFR mutation signals in an area without EGFR amplification may not reach the threshold of detection because of the mixture with normal cells resulting in pseudoheterogeneity. The authors concluded that true genetic heterogeneity is rare (Yatabe, Matsuo, & Mitsudomi 2011). This latter view would support EGFR mutation status being assessed in the most accessible tissue only, rather than multiple sites being sampled.

This study confirms the high yield from EBUS-TBNA of detecting malignancy in intra-thoracic lymph nodes in a real world setting. A sensitivity of 88% in 615 patients was observed (disease prevalence of 78%) which is similar to a sensitivity of 93% observed in a meta-analysis of 1299 patients (Gu et al. 2009). This study contains the first reported death attributed to EBUS-TBNA. The complication may be attributed to the process of introducing pharyngeal micro-organisms into the lower respiratory tract. The patient was elderly and immunosuppressed due to widely metastatic malignancy and succumbed to sepsis within 72 hours of the procedure.

The large number of patients included in this study renders subgroup analyses powerful. Hilar lymph nodes and lymph nodes less than 1cm were noted to have lower sensitivities than mediastinal nodes and nodes greater than 1cm respectively. This may be due to the increased technical difficulty of sampling hilar and smaller

lymph nodes. However, when hilar or small lymph nodes were sampled successfully, the samples were still suitable for NSCLC sub-typing and EGFR mutation analysis.

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

Recent guidance has suggested a novel algorithm for the diagnosis of adenocarcinoma in small biopsies and cytological samples (Travis et al. 2011). In patients with positive cytology and classic morphology for adenocarcinoma or squamous cell carcinoma no further markers are required and those with adenocarcinoma can be submitted directly for EGFR mutation testing. Samples which are classified as NSCLC-NOS on morphology are recommended to undergo a panel of immunohistochemistry that includes one squamous cell carcinoma marker and one adenocarcinoma marker +/- mucin staining. If the NSCLC tumour still remains unclassified then molecular analysis is still recommended. This multi-centre study clearly demonstrates that samples from EBUS-TBNA obtained in routine practice are suitable for entry into this new diagnostic algorithm and provides further impetus for the use of EBUS-TBNA as an initial diagnostic procedure in patients with suspected lung cancer.

CHAPTER 8:

THE LUNG-BOOST TRIAL – A PRAGMATIC RANDOMISED CONTROLLED TRIAL OF ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION AS AN INITIAL INVESTIGATION IN PATIENTS WITH SUSPECTED LUNG CANCER

8.1 INTRODUCTION

Lung cancer remains one of the most challenging of all the malignant diseases. There are 1.35 million new cases diagnosed each year and lung cancer is the biggest killer of men and women who die of any cancer across the world (Parkin et al. 2005). Lung cancer in never smokers has risen to be included in the top ten causes of death from cancer in the Western World; yet smoking, the primary cause for 85% of sufferers, is being met with variably successful smoking cessation programmes across the globe. Smoking, especially amongst young women continues to rise.

The clinical staging of non-small cell lung cancer (NSCLC) is a critical process that determines treatment options and guides prognosis. This is currently best achieved via a multi-disciplinary approach involving surgical, respiratory, oncology and radiology input. In patients with NSCLC who are fit for surgery and have no

evidence of extra-thoracic spread, the disease status of the mediastinal lymph nodes (MLN) is used to differentiate operable from inoperable candidates. Patients with no clinical evidence of MLN metastases are eligible for surgery while those with clinically detected N2 or N3 disease are referred for multi-modality treatment.

Several invasive and non-invasive techniques are available to diagnose and stage lung cancer. Patients commonly undergo a computed tomography (CT) scan of the thorax and upper abdomen. Approximately 50% of patients present with metastatic disease evident outside the thorax and in these patients a biopsy from the most convenient location by the least invasive modality allows management of the patient. However, in patients with intra-thoracic disease (stages I – IIIA) only on initial presenting CT scan, the diagnostic and staging algorithm is more complex. A biopsy of the primary lesion is commonly undertaken by bronchoscopy or CT guided biopsy before attention turns to nodal staging. CT and PET-CT scanning of mediastinal nodes are associated with significant problems of sensitivity in nodes <1cm in short axis and specificity in lymph nodes >1cm in short axis. Therefore, unless the patient has all mediastinal lymph nodes <1cm in short axis which are negative on PET-CT scan, current guidelines recommend invasive mediastinal sampling.

The complete diagnosis and staging of patients with intra-thoracic disease therefore usually requires several procedures, often taking several weeks, which is a time of anxiety for patient. A further consideration is that the current approach to mediastinal staging of NSCLC (CT, PET-CT, mediastinoscopy) can result in inaccurate nodal staging in 25% of operable patients (Navani et al. 2010). This is unsurprising when we consider that the results of meta-analyses have calculated the sensitivity for the detection of mediastinal metastases by CT scan as 51%, by PET-CT as 74% and mediastinoscopy as 78% (Silvestri et al. 2007).

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a newer technique that allows minimally invasive sampling of all intra-thoracic lymph nodes adjacent to the bronchial tree. A pooled analysis of 1299 patients with known or suspected NSCLC undergoing EBUS-TBNA demonstrated that the procedure had a sensitivity of 90% for the detection of nodal metastases (Gu et al. 2009). At the time of trial inception, guidelines recommended EBUS-TBNA as an alternative to mediastinoscopy in patients who required invasive mediastinal sampling after PET-CT scan.

We aimed to investigate whether EBUS-TBNA could be utilised as an initial investigation in patients with suspected lung cancer. Since the procedure was able to provide a tissue diagnosis and an inoperable disease stage in a single investigation, we hypothesised that EBUS-TBNA as a first test would reduce the time to treatment decision by reducing the number of investigations and outpatient appointments required in patients with suspected lung cancer. It was recognised that patients with N2 disease evident from EBUS-TBNA may remain candidates for radical treatment with chemo-radiotherapy, continuous hyperfractionated accelerated radiotherapy (CHART) or surgery and so would still require PET-CT scanning for definitive systemic staging. Therefore we conducted a pragmatic, multi-centre randomised controlled trial to test the hypothesis that EBUS-TBNA as an initial investigation after staging CT scan would reduce the time to treatment decision for patients with suspected lung cancer.

8.2 METHODS

8.2.1 Patients

Patients with suspected stage I – IIIA lung cancer on the basis of CT scan of the neck, thorax and upper abdomen were eligible for trial entry. Patients were at least 18 years of age and fit enough to undergo thoracotomy and lung resection. Exclusion criteria were significant concurrent malignancy and contra-indication to EBUS-TBNA or mediastinoscopy. Patients with evidence of extra-thoracic malignancy, supraclavicular lymphadenopathy or pleural effusion were also excluded.

This investigator initiated trial was approved by the ethics committees of the 6 participating centres (University College London Hospital, Whittington Hospital, North Middlesex University Hospital, Princess Alexandra Hospital, Barnet General Hospital and Nottingham Hospital). The Lung-BOOST trial was registered on clinicaltrials.gov as NCT00652769.

8.2.2 Study design

Participants were randomly assigned (1:1) to either conventional diagnosis and staging (CDS) or EBUS-TBNA as an initial investigation followed by conventional diagnosis and staging techniques as required (EBUS-CDS). Telephone randomisation using permuted blocks of four generated by computer was employed. Randomisation was stratified according to the presence of mediastinal lymph nodes >1cm in short axis and recruiting centre. Following the informed consent process by the investigator, randomisation was carried out by research assistants telephoning the

randomisation line at the North London Cancer Research Network office. The random allocation sequence was concealed from participants and investigators until the interventions were assigned. Due to the nature of the intervention and the pragmatic nature of the trial, blinding of participants and investigators was not possible. Data was collected on paper case record forms (Appendix 1) and entered (using double data-entry) by an independent data clerk onto a secured trial database on a dedicated trial computer.

8.2.3 Conventional diagnosis and staging

Participants allocated to conventional diagnosis and staging (CDS) underwent investigations as determined by the multidisciplinary team. A suggested algorithm for CDS was provided in the trial protocol based on best available evidence and published guidelines (Figure 8.1). Patients were recommended to undergo CT guided biopsy or bronchoscopy depending upon whether the primary lesion was peripheral or central. Conventional transbronchial needle aspiration was utilised at the operator's discretion. If the patient was a candidate for radical treatment, a PET-CT scan was recommended. Mediastinoscopy was advised if the presence of FDG avid lymph nodes precluded a radical treatment option. Invasive mediastinal sampling was also recommended in the trial protocol if any mediastinal lymph node was > 1cm in short axis and its result would alter management. However, the protocol did not mandate any specific investigations (other than the exclusion of EBUS-TBNA) and all investigations and their order, including the need for PET-CT scan and mediastinoscopy, were determined by the multi-disciplinary team.

8.2.4 Endobronchial Ultrasound

Patients randomised to the EBUS-TBNA arm of the trial underwent EBUS-TBNA as an initial procedure after staging CT scan. The procedure was performed in the outpatient setting under sedation with midazolam and fentanyl. Topical lidocaine was applied for local anaesthesia. EBUS-TBNA was performed with a dedicated bronchoscope with linear ultrasound integrated into the distal end (BF-UC160F-OL; Olympus, Tokyo). A systematic examination of all mediastinal and hilar lymph node stations was made. Nodes that were highlighted to be suspicious of metastasis on CT scan due to size or location were sampled and labelled according to the Mountain – Dressler (1997) lymph node map. If no abnormal nodes were identified, aspirates were taken using a 22 or 21-gauge needle from a lymph node station that is most likely to drain the primary lesion. Standard videobronchoscopy was permitted as an additional investigation at the same sitting at the operator's discretion. Endosopic ultrasound guided fine needle aspiration (EUS-FNA) was permitted as an alternative to EBUS-TBNA if a target lesion was not amenable to EBUS-TBNA. Three to 5 passes per lymph node were made. Rapid on-site evaluation of samples was not performed. Specimens obtained were smeared onto slides and also spun down for cell block analysis. Any cores obtained were transferred directly into formalin and subsequent histolopathological examination. Samples from EBUS-TBNA underwent routine laboratory processing. Results from EBUS-TBNA were discussed in multi-disciplinary team meetings in the referring hospitals and further investigations were requested as required. A suggested investigative pathway was provided in the trial protocol but not mandated (Figure 8.2).

8.2.5 Treatment after diagnosis and staging

In the event of mediastinal metastases being identified by clinical staging procedures, patients were referred for multi-modality therapy. This included palliative chemotherapy, palliative radiotherapy, radical radiotherapy, CHART or combined (concurrent or sequential) chemo-radiotherapy. When mediastinal metastases were not identified, patients were referred for surgery or radical radiotherapy. A PET-CT was recommended in all cases prior to a decision to treat with radical intent.

8.2.6 Endpoints

The primary endpoint was the time from first outpatient appointment to treatment decision, after completion of diagnosis and staging procedures. The sensitivity, negative predictive value and diagnostic accuracy of EBUS-TBNA were also calculated. Pre-specified secondary endpoints were (i) the health care costs of diagnosing and staging lung cancer (ii) the number of investigations and outpatient visits a patient required to be diagnosed and staged with lung cancer (iii) the proportion of lung cancer patients that are diagnosed and staged with a single test after CT scan (iv) the number of PET-CT scans and mediastinoscopies, (v) the number of futile thoracotomies. A futile or unnecessary thoracotomy was defined as either an exploratory (open and shut) thoracotomy, unexpected pT4 disease, unexpected mediastinal nodal metastases (pN2 or pN3), death within 1 year after surgery or evidence of disease recurrence within 1 year of surgery. The rate of complications due to diagnostic and staging techniques were also documented.

8.2.7 Economic Analysis

The incremental cost of the strategy of EBUS-TBNA as an initial investigation versus conventional diagnosis and staging in patients with suspected lung cancer was calculated from the perspective of the NHS. The analysis was based on data from the trial only up until the point of treatment decision. Unit costs were taken from NHS tariffs or local hospital costs. Estimation of the cost of EBUS-TBNA has been previously described (Table 6.1). Because all costs per patient occurred within one year, discounting was unnecessary. The sensitivity of the results to the cost of the EBUS-TBNA procedure was calculated, varying it between £503 (the 2010-11 NHS tariff for a flexible bronchoscopy) and £5259 (the 2010-11 NHS tariff for mediastinoscopy with complications).

8.2.8 Statistical Analysis

For the analysis of the primary endpoint (time to treatment decision), the Kaplan Meier method was utilised on a complete-case intention-to-treat basis. Standard definitions of sensitivity for the detection of nodal metastases were employed. The final diagnosis of nodal staging was determined by clinical follow-up and positive pathology from EBUS-TBNA, conventional TBNA, EUS-FNA, mediastinoscopy or mediastinal lymph node dissection. It was agreed with the ethics committee that malignant diagnoses from techniques that generate pathological specimens did not require further verification.

A sample size of at least 168 was initially planned to give 99% power, on the basis that patients in the CDS arm of the trial would require 3 investigations and a median time to treatment of 30 days, while patients in the EBUS-TBNA arm would require a single investigation and a median of 14 days. The trial was closed on the 1st July 2011 due to funding expiry at which point 133 patients had been recruited, giving the trial 95% power to detect a difference if one existed (Table 8.1).

The Fisher exact test was used for the analysis of categorical data, while unpaired t tests were used to compare groups of continuous normally distributed variables. All tests performed were 2-sided and 5% was taken as the cut-off for statistical significance. Statistical analyses were performed using STATA version 10 (Statacorp., USA). This trial report conforms to CONSORT guidance and is registered on www.clinicatrials.gov as NCT00652769.

Figure 8.1: Conventional diagnostic and staging pathway for lung cancer. Adapted from NICE and ACCP guidelines.

Blue boxes represent procedures, tests and outpatient appointments necessary for diagnosis and staging. Red boxes represent outcome.

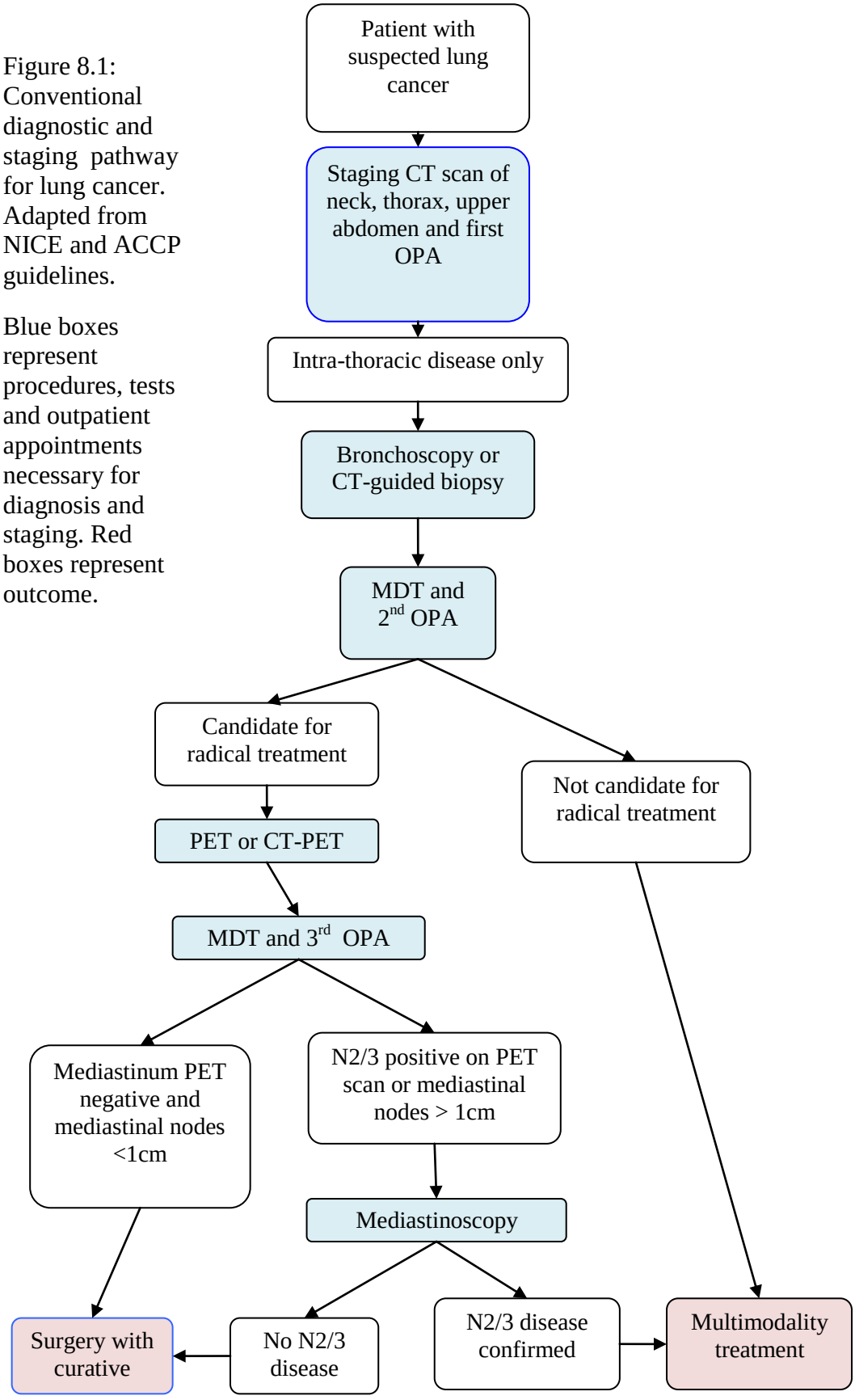


Figure 8.2: Novel pathway for lung cancer diagnosis and staging with EBUS-TBNA as an initial test after CT scan

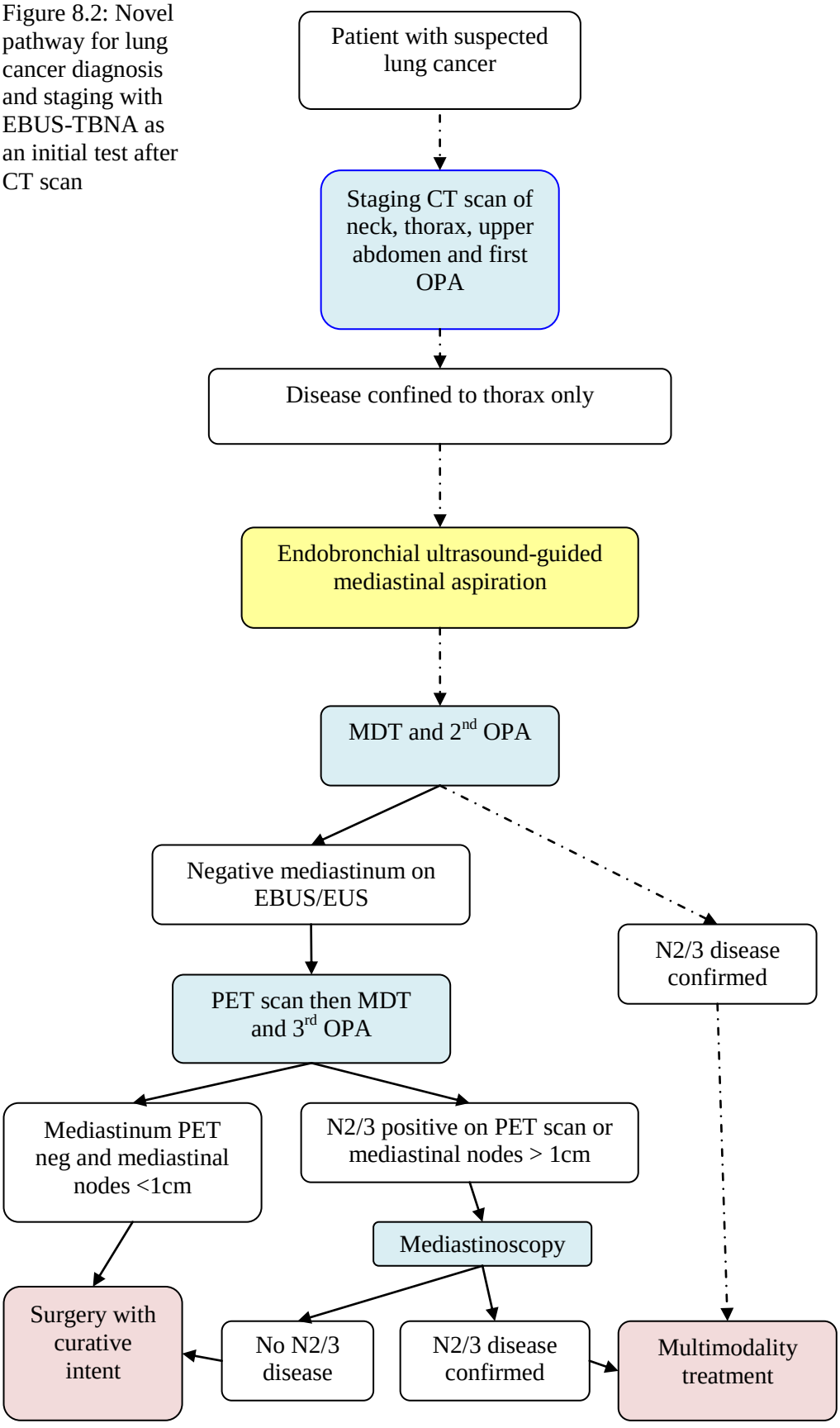


Table 8.1: Sample size estimation with varying power but static type 1 error and clinical assumptions

Time to treatment decision				
Conventional diagnosis and staging	EBUS-TBNA	Power	Type 1 error	Sample size
66% of patients within 30 days and 33% within 14 days	66% of patients within 14 days and 33% within 30 days	80%	5%	82
66% of patients within 30 days and 33% within 14 days	66% of patients within 30 days and 33% within 14 days	90%	5%	104
66% of patients within 30 days and 33% within 14 days	66% of patients within 30 days and 33% within 14 days	95%	5%	126
66% of patients within 30 days and 33% within 14 days	66% of patients within 30 days and 33% within 14 days	99%	5%	170

8.3 RESULTS

Between June 2008 and July 2011, 133 patients with suspected lung cancer were randomised, 67 to conventional diagnosis and staging and 66 to initial EBUS-TBNA. One patient (previously randomised to the control arm) withdrew consent before any further investigations were carried out. Both groups were well balanced for all major clinical characteristics (Table 8.2, page 183).

8.3.1 Conventional Diagnosis and Staging arm

In the 66 patients who underwent CDS, 49 patients underwent bronchoscopy, 5 of whom had a conventional TBNA; 29 had CT guided biopsy, 50 had a PET-CT scan, 2 had a bone scan and 8 patients a mediastinoscopy (Figure 8.3a, page 193). Four patients crossed over and underwent EBUS-TBNA at the request of the multi-disciplinary team meeting. One patient in the CDS arm of the trial had both EUS-FNA and EBUS-TBNA. Other investigations included MRI of the neck, ultrasound of the liver, CT of the brain, video-assisted thoracoscopic surgical lung biopsy and repeat bronchoscopy. The final diagnosis (Table 8.3, page 184) was NSCLC in 50 patients (21 adenocarcinoma, 21 squamous cell carcinoma, 3 large cell carcinoma, 2 adenosquamous and 3 not otherwise specified). Seven patients had a small cell lung cancer. Further final diagnoses were metastatic melanoma in 2, metastatic breast cancer in 1, folded lung in 1, tuberculosis in 1 and bacterial infection in 4 patients. The total number of investigations in the 66 patients in the conventional diagnosis and staging arm was 158 (Table 8.4). Mediastinal metastases (representing N2 or N3 disease) were found by clinical staging techniques in 37 (56%) patients. Seventeen patients (26%) underwent thoracotomy with mediastinal lymph node dissection.

Four of these patients did not have NSCLC. Clinically unsuspected nodal disease was not found on pathological staging in any patients and 3 patients had an open and shut thoracotomy due to unsuspected T4 disease (Table 8.5, page 186).

8.3.2 EBUS-TBNA initial staging arm

EBUS-TBNA was performed in 64 patients and detected mediastinal nodal metastases in 37 (59%). Two patients had EUS-FNA as their initial investigation and 1 patient with N2 disease was identified using this technique. Five patients required CT guided biopsy and 33 patients had a PET-CT and 7 underwent mediastinoscopy. One patient had a video-assisted thoracoscopic surgical lung biopsy. Two patients had mediastinal metastases detected by mediastinoscopy so that overall mediastinal metastases were diagnosed by clinical staging techniques in 40 (61%) patients. The total number of investigations in 66 patients who underwent EBUS-TBNA as an initial test was 112. The final diagnosis was NSCLC in 46 patients (26 adenocarcinoma, 17 squamous cell carcinoma, 1 large cell carcinoma, 1 adenosquamous and 1 not otherwise specified). Four patients had small cell lung cancer. The final diagnosis was metastatic prostate cancer in 1, metastatic breast cancer in 1, folded lung in 2, tuberculosis in 2, lung abscess in 1 and bacterial infection in 9 patients. Seventeen patients (26%) without evidence of mediastinal nodal metastases underwent thoracotomy and mediastinal lymph node dissection. Three patients were found not to have NSCLC and surprise mediastinal metastases were found in 1 patient at pathological staging. No patients had an open and shut thoracotomy.

8.3.3 Primary Endpoint

Using a complete-case intention-to-treat analysis (Figure 8.3b), the median time to treatment decision in the conventional diagnosis and staging arm of the trial was 29 days, compared to a median of 14 days in the EBUS arm. Using Kaplan Meier analysis (Figure 8.4), the hazard ratio was 2.02 (95% CI 1.419 – 2.884, P<0.0001). Therefore patients in the EBUS-CDS arm of the trial were likely to reach a treatment decision twice as fast as patients in the conventional diagnosis and staging arm.

8.3.4 Secondary Endpoints

The number of PET scans in the EBUS-TBNA arm was significantly reduced compared to the number in the conventional diagnosis and staging arm. There was no difference in the number of mediastinoscopies in each arm. The mean number of investigations per patient in the conventional diagnosis and staging arm and EBUS-TBNA arm were 2.39 and 1.70 respectively (P<0.0001). Twelve percent of patients were diagnosed and staged with a single investigation using the conventional strategy while 45% (P<0.0001) required an EBUS-TBNA or EUS-FNA as their sole investigation in the EBUS-CDS arm of the trial.

Analysis of unnecessary thoractomoies is preliminary since not all patients have undergone 1 year of follow-up. Using our a priori definition, unnecessary thoracotomies occurred in 8 (62%) patients out of 13 undergoing surgery in the CDS arm and in 6 out of 14 (43%, P=0.269) patients in the EBUS+CDS arm (Table 5). In an exploratory analysis, if we exclude patients who only had disease recurrence within 12 months of surgery, the unnecessary thoracotomy rate was higher in the

CDS arm (46%) compared to the EBUS-CDS arm (14%, P=0.10). The primary and secondary endpoints are summarised in Table (Table 8.6).

8.3.5 Performance characteristics of endoscopic investigations

Results of the 64 patients who underwent EBUS-TBNA are shown in Table 8.7. The median size of lymph nodes sampled was 12 mm and lymph node stations 4R (right paratracheal) and 7 (subcarinal) were the most commonly sampled. The sensitivity of EBUS-TBNA was 92% (95% CI 78% – 98%). The negative predictive value of EBUS-TBNA was 90% (95% CI 72% – 97%) and diagnostic accuracy was 95% (95% CI 86% - 99%).

Two patients underwent EUS-FNA, both of station 5 lymph nodes in the EBUS-CDS arm of the trial. The procedure yielded a malignant diagnosis in one case. In the CDS arm of the study, 5 patients underwent conventional TBNA. In two patients there was a benign final diagnosis and in 1 patient conventional TBNA provided a diagnosis of squamous cell diagnosis. In the remaining 2 patients undergoing conventional TBNA, a negative procedure was followed by a mediastinoscopy that demonstrated mediastinal metastases.

The accuracy of nodal staging is shown in Table 8.8 (page 189). In patients who underwent routine EBUS-TBNA (or EUS-FNA), one patient had the nodal staging underestimated (cN0, pN2). In the CDS arm 5 patients had the nodal stage underestimated. However, in each of these cases, the pathological nodal status was N1 (when the clinical stage was N0) and therefore may not have affected the

decision to operate. The proportion of inaccurate nodal staging was 38% in the CDS arm and 7% in the EBUS-CDS arm (P=0.077).

8.3.6 Patient treatments

Patient treatments are summarised in Table 8.9 (page 190) according to the arm of the trial. There were significantly more patients undergoing chemotherapy in the EBUS-CDS arm (50%) compared to the CDS arm (28%, P=0.028). The number of patients with lung cancer submitted for treatment with radical intent was not significantly different between the groups with 23 (40%) in the CDS arm of the trial and 17 (34%) in the EBUS-CDS arm of the trial (P=0.429). Fewer patients in the EBUS-CDS arm of the trial received palliative radiotherapy or supportive care only but this did not reach statistical significance (Table 8.9).

8.3.7 Economic evaluation

The cost of diagnostic and staging procedures is shown in Table 8.10. Using the base case assumptions, the mean cost per patient of the CDS arm was £2970.61 while the mean costs per patient in the EBUS-CDS arm was £2965.78 giving a small non-significant difference of £4.83 in favour of the EBUS-CDS arm. Univariate threshold sensitivity analysis (which varied the potential cost of EBUS-TBNA) demonstrated that under the conditions of the trial, the EBUS-CDS strategy was less costly than the CDS strategy if the cost of EBUS-TBNA was less than £1387 (Figure 8.5, page 196).

8.3.8 Adverse events

Adverse events from diagnosis and staging were rare and are summarised in Table 8.11 (page 192). One patient in each arm of the trial had a pneumothorax, with the patient in the CDS arm of the trial requiring intercostal drainage and inpatient admission.

Table 8.2: Baseline clinical characteristics in the Lung-BOOST trial

	Conventional diagnosis and staging (n=66)	EBUS-TBNA diagnosis and staging (n=66)
Age: mean (range) in years	67 (44 – 88)	69 (40 – 87)
Gender		
Male	46 (70%)	43 (65%)
Female	20 (30%)	23 (35%)
Ethnicity		
Caucasian	59	51
Asian	2	6
African	2	4
Caribbean	2	3
Other	1	2
Performance Status 0 or 1: number (%)	57 (86%)	60 (90%)
Pack year smoking history: mean (range)	42 (0 – 110)	43 (0 - 138)
FEV1: mean (SD) in litres	1.9 (1.0 – 3.6)	1.9 (1.1 – 3.8)
Mediastinal lymph node ≥10mm in short axis		
Yes	42 (64%)	39 (59%)
No	24 (36%)	27 (41%)
Clinical Nodal Staging on initial CT scan		
cN0	20 (30%)	21 (32%)
cN1	9 (14%)	6 (9%)
cN2	33 (50%)	34 (52%)
cN3	4 (6%)	5 (8%)

Table 8.3: Final diagnosis according to arm of study

	Conventional diagnosis and staging (n=66)	EBUS-TBNA diagnosis and staging (n=66)
Non-small Cell Lung Cancer	50 (76%)	46 (70%)
Adenocarcinoma	21 (42%)	26 (57%)
Squamous Cell	21 (42%)	17 (37%)
Large Cell	3 (6%)	1 (2%)
Adenosquamous	2 (4%)	1 (2%)
Not otherwise specified	3 (6%)	1 (2%)
Small Cell Lung Cancer	7 (11%)	4 (6%)
Extra-thoracic Malignancy	3 (5%)	2 (3%)
Benign lesion	6 (9%)	14 (21%)

Table 8.4: Investigations and outpatient appointments for all patients

	Conventional diagnosis and staging (n=66)	EBUS-TBNA diagnosis and staging (n=66)	P value
For all patients (n=132)			
Number of patients diagnosed and staged with a single investigation	8	30	<0.0001
Number of PET scans (%)	50	33	0.0037
Number of mediastinoscopies (%)	8	7	NS (P=1.000)
Total number of investigations after CT scan until treatment decision (mean and SD of tests per patient)	158 (mean 2.394 SD 0.828)	112 (mean 1.697 SD 0.828)	<0.0001
Number of outpatient appointments to treatment decision (mean per patient)	178 (mean 2.7 SD 1.069)	103 (mean 1.561 SD 1.066)	<0.0001
Total number of inpatient days during diagnosis and staging for all 6 patients (range)	28 (0 – 5) 0.424 SD 0.857	17 (0 – 3) 0.258 SD 0.846	NS (P=0.2648)

Table 8.5: Diagnostic performance of each arm in the Lung-BOOST trial

	Standard techniques of conventional TBNA, PET-CT scanning and mediastinoscopy* (n=66)	EBUS-TBNA** (n==66)	P value
Diagnosis of lung cancer	57	50	NS (P=0.18)
Prevalence of N2/N3 disease (%)	39 patients (68%)	44 patients (67%)	
Sensitivity for detecting mediastinal metastases	100%	98%	
Number of patients with lung cancer undergoing radical treatment	23 (40%)	17 (34%)	
Surgery	13	14	
Radical Radiotherapy	5	2	
Chemo-Radiotherapy	5	1	
Unnecessary thoracotomies***			
Total	8 (62%)	6 (43%)	NS (P=0.269)
pN2	0	1	
pT4	3	0	
pM1a	1	1	
Death within 12 months of surgery	2	0	
Recurrence within 12 months of surgery	2	4	

*investigations were agreed at multi-disciplinary team discussion

** If EBUS-TBNA was negative, subsequent investigations were determined by the multi-disciplinary team

*** median follow-up of 20 months

Table 8.6: Summary of primary and secondary endpoints (intention-to-treat analysis)

	Conventional diagnosis and staging (n=66)	EBUS-TBNA diagnosis and staging (n=66)	P value
Primary endpoint			
Median time to treatment decision	29 days	14 days	<0.0001
Pre-specified Secondary endpoints			
Healthcare costs of diagnosis and staging (£ per patient)	2970.61	2965.78	NS
Mean number of investigations per patient for diagnosis and staging	2.394	1.697	<0.0001
Mean number of outpatient appointments per patient	2.706	1.561	<0.0001
Proportion of patients diagnosed and staged with a single investigation	8 (12%)	30 (45%)	<0.0001
Number of PET-CT scans	50	33	0.0037
Mediastinoscopies	8	7	NS (P=1.000)
Unneccesary thoracotomies	8	6	NS (P=0.269)

Table 8.7: Performance characteristics of EBUS-TBNA in the Lung-BOOST trial

Total number of patients who underwent EBUS-TBNA	64
Number of patient with mediastinal nodes ≥1cm on CT scan	39
Median size of lymph nodes sampled (mm)	12 (range 4 – 45)
Lymph node station sampled	
2R	1
4R	26
2L	1
4L	5
7	23
10R	7
10L	1
Median number of passes per node (range)	3 (2 – 6)
Sensitivity of EBUS-TBNA (TP/TP+FN)	92% (35/38)
Negative Predictive Value of EBUS-TBNA (TN/TN+FN)	90% (26/29)
Diagnostic accuracy of EBUS-TBNA (TP+TN/n)	95% (61/64)

Table 8.8: Accuracy of clinical T and N staging in 27 patients who underwent pathological staging. Shaded rows represent categories of clinical under-staging

	Conventional diagnosis and staging (n=13)	EBUS-TBNA diagnosis and staging (n=14)	P value
cN0, pN0	8	12	
cN0, pN1	5	0	
cN0, pN2	0	1	
cN1, pN1	0	1	
cN1, pN2-3	0	0	
cN1, pN0	0	0	
cT1-3, pT3	10	13	
cT1-3, pT4	3	1	
Inaccurate N Stage	5 (38%)	1 (7%)	0.077
Inaccurate T stage	3 (23%)	1 (7&)	NS (P=0.326)

Table 8.9: Treatment modalities according to arm of trial in patients with lung cancer

	Conventional diagnosis and staging (n=57)	EBUS-TBNA diagnosis and staging (n=50)	P value
Surgery	13 (23%)	14 (28%)	NS (P=0.656)
Radical radiotherapy	5 (9%)	2 (4%)	NS (P=0.445)
Chemo-radiotherapy	5 (9%)	1 (2%)	NS (P=0.211)
Chemotherapy	16 (28%)	25 (50%)	P=0.028
Palliative radiotherapy	12 (21%)	5 (10%)	NS (P=0.184)
Supportive care only	6 (11%)	3 (6%)	NS (P=0.498)

Table 8.10: Mean costs per patient of the randomised strategies for the diagnosis and staging of lung cancer. *Data from Lung-BOOST trial. Unit cost is estimated for EBUS-TBNA. Other unit costs are from the 2010-11 NHS Tariff. All costs are measured in 2010-11 £UK.

	Parameter value (proportion of patients)*			Mean cost per patient; UK£		
	Conven-tional diagnosis and staging	EBUS-TBNA diagnosis and staging	Unit cost	Conven-tional diagnosis and staging	EBUS-TBNA diagnosis and staging	Difference
EBUS-TBNA	0.076	0.970	1382	104.70	1340.54	
EUS-FNA	0	0.030	800	0	24	
Bronchoscopy	0.667	0.030	503	335.30	15.24	
Conventional TBNA	0.076	0	100	7.58	0	
CT guided biopsy	0.439	0.076	450	197.55	34.20	
PET-CT scan	0.758	0.500	1695	1284.09	847.5	
Mediastinoscopy	0.121	0.106	3228	361.58	316.38	
Bone scan	0.030	0	258	7.82	0	
Other major surgical biopsy	0.045	0.015	2983	135.59	45.20	
Inpatient days	0.333	0.258	500	166.50	129.00	
Outpatient appointments	2.70	1.56	137	369.9	213.72	
Total cost per patient				2970.61	2965.78	-4.83

Table 8.11: Adverse events. One patient had a pneumothorax requiring an intercostal drain and inpatient admission.

	Conventional diagnosis and staging (n=66)	EBUS-TBNA diagnosis and staging (n=66)
Related to EBUS-TBNA	None	None
Related to CT guided biopsy	1 Pneumothorax (chest drain required)	1 Pneumothorax (no chest drain required)
Related to bronchoscopy	None	None
Related to mediastinoscopy	None	None
Inpatient days related to complications	2	None
Other	None	None

Figure 8.3a: Lung-BOOST trial flowchart

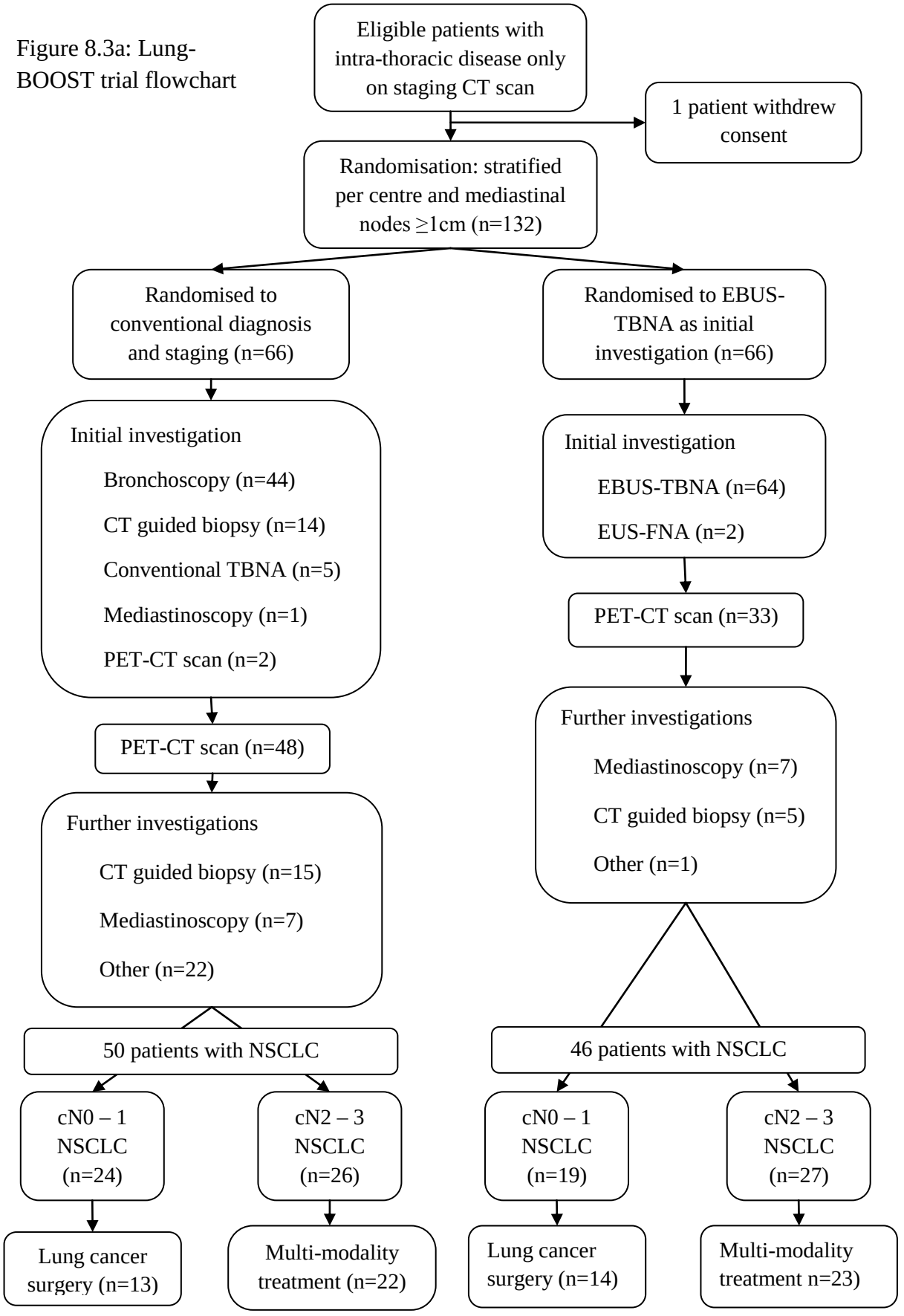


Figure 8.3b: CONSORT diagram

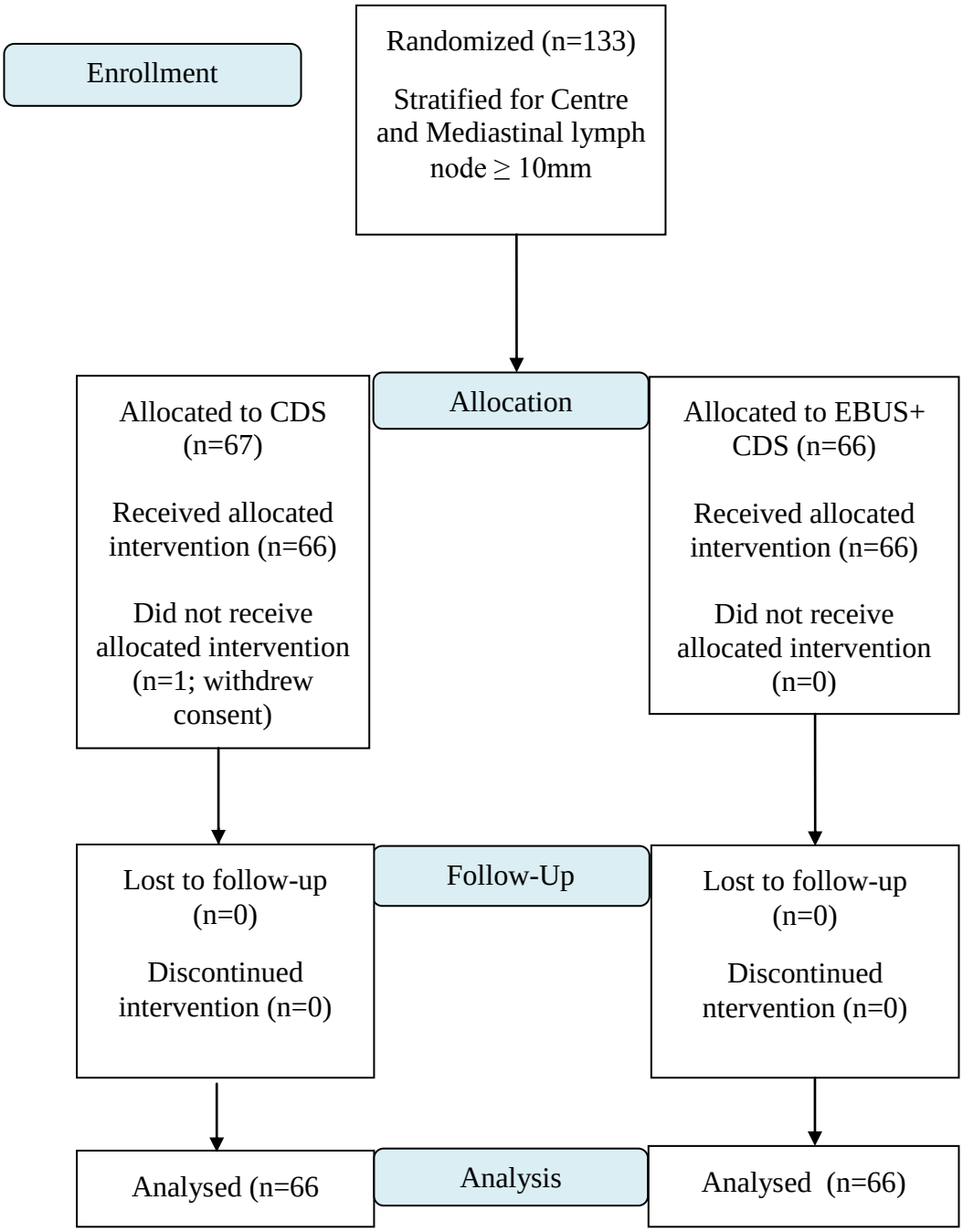
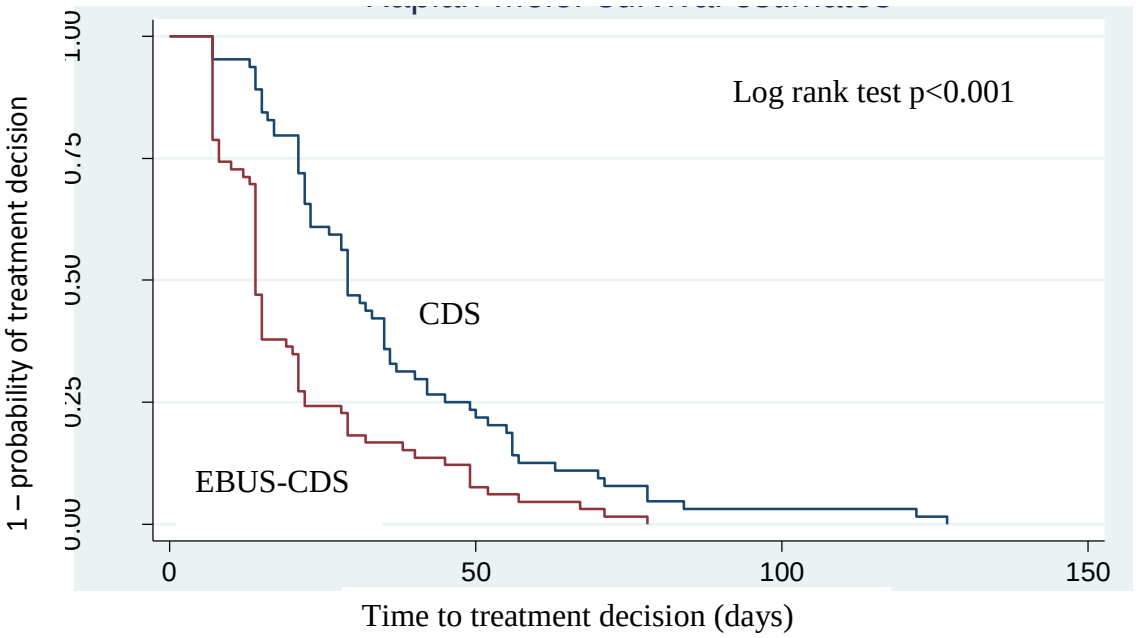


Figure 8.4: Kaplan-Meier graph of time to treatment decision in each arm of the trial (complete case intention to treat analysis).



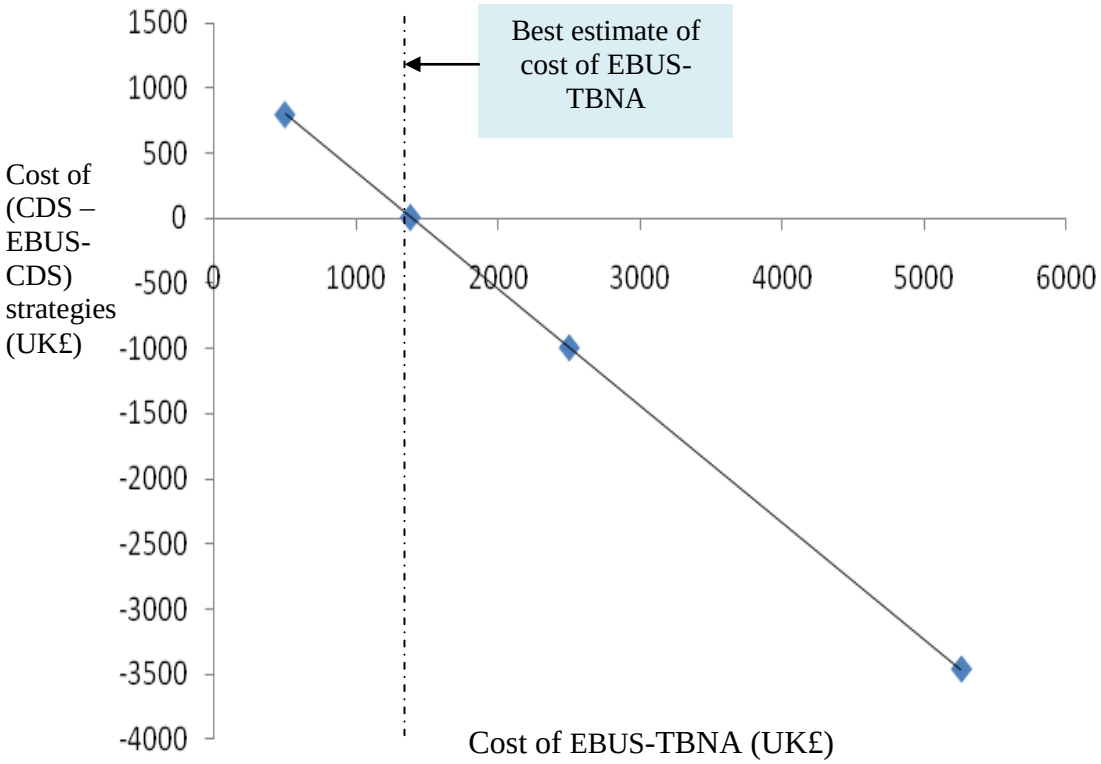
CDS – conventional diagnosis and staging; EBUS-CDS – endobronchial ultrasound followed by conventional diagnosis and staging.

Cox analysis:

_t	Haz. Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
trt	2.023029	.3658469	3.90	0.000	1.419286	2.883596

1 – probability of treatment decision

Figure 8.5: Univariate threshold sensitivity analysis. The costs of the strategy of EBUS-TBNA as an initial investigation minus conventional diagnosis and staging is plotted according to different estimates of the cost of EBUS-TBNA. At the best estimate for the cost of EBUS-TBNA of £1382, the EBUS strategy is approximately cost-neutral. The cost of conventional diagnosis and staging is higher than that for the EBUS strategy as long as EBUS-TBNA costs are less than £1387.



8.4 DISCUSSION

This is the first randomised controlled trial of EBUS-TBNA in patients with suspected lung cancer and shows that routine use of EBUS-TBNA as an initial test after staging CT scan results in a faster treatment decision and utilises fewer investigations and outpatient appointments.

EBUS-TBNA has become an important investigation for patients with lung cancer. However, much of the data demonstrating its utility is based on case series, many of which are retrospective. These cohort studies suffer with problems of selection bias and a further problem with the early EBUS-TBNA literature was that much of the data was from a few expert centres. The randomised design of the current study minimises the risk of selection bias as EBUS-TBNA operators were unable to choose patients for the procedure. Despite this, the sensitivity of EBUS-TBNA in the study remained high at 92% (95% CI 78% – 98%).

Randomised trials of lung cancer staging techniques are rare but provide the highest quality evidence on which to base diagnostic algorithms. The outcome of futile or unnecessary thoracotomies has been used as a surrogate for accuracy of staging, since less accurate staging would result in a higher proportion of futile thoracotomies. Trials of PET and PET-CT demonstrated that PET-CT was able to prevent 1 in 5 futile thoracotomies. Preliminary results of a trial of routine EUS-FNA also suggested that futile thoracotomies could be prevented, however this trial did not routinely employ PET-CT. The recent ASTER trial (Annema et al. 2010) suggested that combining EBUS, EUS and mediastinoscopy (when EBUS/EUS was negative) could prevent 1 in 7 unnecessary thoracotomies. However, in the ASTER study, the relative individual diagnostic merits of EBUS-TBNA and EUS-FNA were

not explored and it is unclear if both procedures need to be routinely performed to obtain this benefit. The definition and concept of futile thoracotomies is controversial. The current Lung-BOOST trial incorporated death or recurrence within 12 months as part of the definition; however the ASTER trial only included death or recurrence within 3 months. This may in part explain the high rate of futile thoracotomies in the trial (63% and 43% in the CDS and EBUS-CDS arms respectively). A statistically significant benefit of EBUS-TBNA in reducing the number of unnecessary thoracotomies was not seen in this trial and this may represent low power of the analysis of this secondary endpoint and also the relatively short length of follow-up (median 20 months).

The primary endpoint of the Lung-BOOST trial was time to treatment decision and the trial demonstrated that routine and upfront use of EBUS-TBNA in the diagnostic pathway can reduce the median time to treatment decision from 29 days to 14 days. UK government initiatives in the NHS Cancer Plan have mandated since 2005 that patients have a treatment decision by 31 days from referral and a further maximum of 31 days between decision to treat and receiving treatment. The time that patients spend undergoing diagnostic and staging investigations is a time of great anxiety for patients and emphasised by the fact the median survival for all patients with lung cancer remains poor at 6.2 months. Therefore, the primary outcome measure in this trial of time to treatment decision is of great importance to patients and the multi-disciplinary teams charged with their care. The trial demonstrates that EBUS-TBNA can provide a diagnosis and inoperable disease stage in 45% of patients so that they require no further investigation before a treatment decision can be made. It is recognised that many patients diagnosed with N2 disease by EBUS-TBNA will still require further investigations, including PET-CT scan if combination chemo-

radiotherapy is being considered, However in this pragmatic trial PET-CT was only necessary in 19% of patients after a positive EBUS-TBNA. Routine use of EBUS-TBNA was able to reduce time to treatment decision primarily by reducing the number of outpatient appointments and investigations (particularly PET-CT scans).

Despite the reduction in investigations and outpatient attendances, overall costs of diagnosis and staging were similar in the two arms. This is due to the current high estimated cost of EBUS-TBNA which may fall in the future as the technology is adopted by more centres. A sensitivity analysis demonstrated that if the cost of EBUS-TBNA was below £1387, the EBUS-CDS strategy would be cost saving. Of considerable interest is the fact that significantly more patients in the EBUS-TBNA arm had a treatment decision of chemotherapy. Longer term follow-up will determine whether this will translate into a survival benefit.

In addition to the short length of follow-up of the trial (which closed to recruitment on the 1st July 2011), other limitations are recognised. The pragmatic nature of the trial meant that a consistent diagnostic and staging algorithm was not observed across the trial centres. However, this design for the study (which was carried out at 2 teaching hospitals and 4 district general hospitals), gives the results strong external validity and potential for reproducibility, which may not be possible with some of the other lung cancer staging randomised trials. A reflection of the pragmatic nature of the trial is that only 8 patients in the CDS arm of the trial and 7 patients in the EBUS-CDS arm of the trial underwent mediastinoscopy. This is a considerably smaller proportion of patients than in other randomised trials such as ASTER and Fischer et al. (2009). The high accuracy of nodal staging in this trial justified the approach of the multi-disciplinary teams. A final limitation is that the economic

analysis in this trial has not taken into account treatment decisions and survival and therefore requires further analysis when longer follow-up is available in the future.

In conclusion, when EBUS-TBNA is utilised as an initial investigation in patients with suspected stage I – IIIA lung cancer on CT scan, the time to treatment decision is reduced, with fewer PET-CT scans and outpatient appointments when compared to a conventional diagnostic and staging strategy.

CHAPTER 9: SUMMARY

This thesis has examined the role of EBUS-TBNA in patients with mediastinal lymphadenopathy. It has demonstrated that EBUS-TBNA has wide applications in patients with mediastinal lymphadenopathy. Data on the learning curve for EBUS-TBNA using CUSUM analysis suggests that approximately 20 procedures are required for training in the procedure. This will however require confirmation from other centres before formal guidance on the procedure can be issued.

In patients with suspected sarcoidosis, a prospective study (Chapter 3) has demonstrated that combining the standard bronchoscopic procedures of transbronchial biopsy and endobronchial biopsy with EBUS-TBNA may significantly improve the diagnostic yield of bronchoscopy. The data suggested that transbronchial lung biopsy and EBUS-TBNA were complementary techniques with diagnoses being made with the bronchoscopic techniques in patients with a negative EBUS-TBNA and vice versa. The data showed that combining the procedures in one sitting was safe and efficacious.

Patients with mediastinal lymphadenopathy due to tuberculosis may significantly benefit from EBUS-TBNA (Chapter 4). EBUS-TBNA can provide evidence of tuberculosis in 94% (95% CI 88% - 97%) of cases and be able to provide a positive culture in 47% of patients which may alter the drug regimen, given the rising incidence of drug-resistant cases. Data from this chapter may therefore significantly influence the management internationally of patients with mediastinal lymph node tuberculosis.

Patients with extra-thoracic malignancies often develop mediastinal lymphadenopathy. There is a clinical conundrum of whether this lymphadenopathy is due to the malignancy or another disease process. In the 161 patients included in this study, 68% had a final diagnosis of malignant intra-thoracic lymphadenopathy, highlighting the importance of sampling these nodes and not just assuming they are due to metastatic spread. When the lymph nodes were due to extra-thoracic malignancy, EBUS-TBNA was able to diagnose them in 87% of cases.

Chapter 6 is the first prospective study of EBUS-TBNA in consecutive patients with isolated mediastinal lymphadenopathy in patients who otherwise would have been referred for mediastinoscopy. Eight-seven percent (97.5% CI 78 – 96%) of mediastinoscopies were prevented. These data strongly support the routine use of EBUS-TBNA as an alternative to mediastinoscopy in patients with isolated mediastinal lymphadenopathy. However, if EBUS-TBNA does not provide a definitive diagnosis, mediastinoscopy should still be recommended, given the low negative predictive value of EBUS-TBNA in this setting.

The management of advanced non-small cell lung cancer has undergone significant changes in the last few years, such that a diagnosis of non-small cell lung cancer alone is no longer sufficient to guide a treatment plan. The subtyping and genotyping of non-small cell lung cancer is now important for patient management. Chapter 7 shows that specimens from EBUS-TBNA in routine practice can be used for differentiation of squamous from non-squamous lung cancer and the at EGFR mutation testing is also possible. Finally, the thesis also reports on the Lung-BOOST trial – a pragmatic randomised controlled trial of EBUS-TBNA as a first investigation in patients with suspected lung cancer. EBUS-TBNA is able to provide an inoperable disease stage in a single investigation and also sufficient tissue to

guide clinical practice. Data from the randomised trial shows that patients undergoing EBUS-TBNA as an initial investigation reach a treatment decision in a median of 14 days, compared to 29 days with conventional diagnosis and staging. Economic analysis shows that this significant improvement in time to treatment decision comes at no extra cost to the NHS and that if the price of EBUS-TBNA was below £1387, then the initial EBUS-TBNA strategy would be cost-saving. This is the first randomised data of EBUS-TBNA and despite the lack of selection bias, a high sensitivity of EBUS-TBNA was maintained. The procedure is also a very safe with complication rates that are similar to standard bronchoscopy. Data from this thesis provides a strong evidence base for the utility of EBUS-TBNA in current respiratory medicine.

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APPENDIX 1

Case record forms for data collection in the Lung-BOOST trial

BOOST

A randomised controlled trial of endobronchial ultrasound or oesophageal ultrasound in the diagnosis and staging of lung cancer

Patient Study No.	Patient Initials	Hospital No.	FORM A

- **PATIENT DATE OF BIRTH :** ____/____/_____ *(dd/mm/yyyy)*
- **GENDER : M / F**
- **HOSPITAL AT PRESENTATION :**
- **ETHNICITY :**
- **ARM OF STUDY : RETROSPECTIVE**
  PROSPECTIVE - CONTROL / ACTIVE
- **DATE OF REFERRAL RECEIPT :** ____/____/_____ *(dd/mm/yyyy)*
- **DATE OF 1ST OUTPATIENT APPOINTMENT :** ____/____/_____ *(dd/mm/yyyy)*
- **2 WEEK TARGET MET? : YES / NO**

Date form completed ____/____/_____*(dd/mm/yyyy)* Completed by _____

Signature _____

BOOST / CRFs / Form A / Version 4 November 2010

BOOST

A randomised controlled trial of endobronchial ultrasound or oesophageal ultrasound in the diagnosis and staging of lung cancer

Patient Study No.	Patient Initials	Hospital No.	FORM B

- **NO OF PACK.YEARS :** _____
- **FAMILY HISTORY OF LUNG CANCER :** YES / NO
- **BRONCHOSCOPY TOLERATED :** YES / NO
- **ECOG PERFORMANCE STATUS :** 0 1 2 3 4
- LUNG FUNCTION : **FEV_1 (L)** _____ **% PREDICTED** _____-
- **OUTPATIENT APPOINTMENTS (OPAs) UNTIL AND INCLUDING TREATMENT DECISION**
  - **DATES:** ___/___/_____ ___/___/_____
    ___/___/_____ ___/___/_____
    ___/___/_____ ___/___/_____
    ___/___/_____ ___/___/_____
  - **TOTAL NUMBER OF OPAs :** _____
- **TOTAL NO. OF INPATIENT DAYS :** _____

Date form completed _____/_____/ _____*(dd/mm/yyyy)* Completed by _____

Signature _____

BOOST

A randomised controlled trial of endobronchial ultrasound or oesophageal ultrasound in the diagnosis and staging of lung cancer

Patient Study No.	Patient Initials	Hospital No.	FORM C

▸ **INVESTIGATIONS FOR DIAGNOSIS AND STAGING:**

CT	☐	_____/_____/_____
BRONCHOSCOPY	☐	_____/_____/_____
CONVENTIONAL TBNA	☐	_____/_____/_____
RADIOLOGY GUIDED BIOPSY / ASPIRATE	☐	_____/_____/_____
PET SCAN	☐	_____/_____/_____
MEDIASTINOSCOPY	☐	_____/_____/_____
BONE SCAN	☐	_____/_____/_____
EBUS – FNA	☐	_____/_____/_____
EUS – FNA	☐	_____/_____/_____
OTHER (specify): _____	☐	____/_____/_____

TOTAL NO. OF INVESTIGATIONS AFTER CT SCAN: _____

▸ **COMPLICATIONS OF INVESTIGATIONS:**

NO OF INPATIENT DAYS (related to complications) : _____

CHEST DRAIN : Y / N

Date form completed _____/_____/ _____*(dd/mm/yyyy)* Completed by _____

Signature _____

BOOST

A randomised controlled trial of endobronchial ultrasound or oesophageal ultrasound in the diagnosis and staging of lung cancer

Patient Study No.	Patient Initials	Hospital No.	FORM D

▶ **DIAGNOSIS OF LUNG CANCER : Y / N** _____/_____/_____ *(dd/mm/yyyy)*

▶ **CELL TYPE:** **ADENOCARCINOMA** **SQUAMOUS CELL**
LARGE CELL **ADENO-SQUAMOUS**
SMALL CELL **OTHER NON-SMALL CELL**

▶ **MEDIASTINAL NODE ≥ 1cm ON CT: Y / N**

IF YES, SIZE OF LARGEST MEDIASTINAL NODE ON CT: _____ mm (short axis)

IF YES, LOCATION OF MEDIASTINAL NODE: STATION _____

▶ **IF PROSPECTIVE ACTIVE ARM, FIRST TEST: EBUS / EUS (please circle)**

▶ **EBUS / EUS result: TP / TN / FN (please circle)**

▶ **FINAL clinical TNM STAGE: T ___ N ___ M ___ ▶ STAGE (I-IV A/B):**

Lymph node Station	Size (mm)	No of passes with EBUS / EUS	PET status (SUV if avail)	Pathology
2R				
2L				
4R				
4L				
5				
6				
7				
8				
9				
10R				
10L				

Date form completed _____/_____/ _____*(dd/mm/yyyy)* Completed by _____

Signature _____

BOOST

A randomised controlled trial of endobronchial ultrasound or oesophageal ultrasound in the diagnosis and staging of lung cancer

Patient Study No.	Patient Initials	Hospital No.	FORM E

▸ **DATE OF DECISION TO TREAT :** ____/____/_____
▸ **TREATMENT INTENT :** **CURATIVE / PALLIATIVE**
▸ **TREATMENT : AGREED / REFUSED**
▸ **TREATMENT RECEIVED :** **CHEMOTHERAPY** **RADIOTHERAPY** **RADICAL RADIOTHERAPY** **COMBINATION CHEMO-RADIOTHERAPY** **SURGERY** **BEST SUPPORTIVE CARE**
▸ **DATE OF FIRST TREATMENT RECEIVED :** ____/____/_____
▸ **FINAL PATHOLOGICAL STAGING FOR SURGICAL PTS:** **p T ___ N ___ M ___** **N/A_____**

Date form completed _____/_____/ _____*(dd/mm/yyyy)* Completed by _____

Signature _____

BOOST

A randomised controlled trial of endobronchial ultrasound or oesophageal ultrasound in the diagnosis and staging of lung cancer

Patient Study No.	Patient Initials	Hospital No.	FORM F

▸ **PATIENT STATUS :** **ALIVE / DEAD (please circle)**

IF ALIVE DATE PATIENT LAST SEEN: _____/_____/_____ *(dd/mm/yyyy)*

IF PATIENT DECEASED DATE OF DEATH: _____/_____/_____ *(dd/mm/yyyy)*

▸ **WAS THERE ANY RADICAL TREATMENT?** **Y / N**

▸ **IF YES:**

DID DEATH OCCUR WITHIN 1 YEAR OF RADICAL TREATMENT: Y / N

EVIDENCE OF RECURRENCE WITHIN 1 YEAR OF RADICAL TREATMENT: Y / N

TIME TO FIRST RECURRENCE FROM RADICAL TREATMENT: _____ months

FIRST RECURRENCE SITE: *(please circle)*

RESECTION MARGIN	**MEDIASTINUM**	**LUNG**
BRAIN	**LIVER**	**ADRENAL**
ADRENAL	**BONE**	**SUPRACLAVICULAR NODE**

OTHER (specify): _____

Date form completed _____/_____/ _____*(dd/mm/yyyy)* Completed by _____

Signature _____

BOOST

A randomised controlled trial of endobronchial ultrasound or oesophageal ultrasound in the diagnosis and staging of lung cancer

RANDOMISATION CHECKLIST

v 6 / 08.11.10

PATIENTS INITIALS:	DOB:	Age:	**M** or **F** *(please circle)*	Ethnicity:

Site *(please circle)*:	Princess Alexandra	North Middlesex	University College
	Royal Free	Whittington	Barnet
	Nottingham	Barts and The London	Imperial

Source of patient *(please circle)*:	Hospital Outpatient	Inpatient

Mediastinal node(s) ≥ 1cm in short axis on CT scan: Yes / No

ELIGIBILITY CRITERIA	YES	NO
Does the patient have suspected lung cancer?	☐	☐
Is the patient able to tolerate fibre-optic bronchoscopy?	☐	☐
NO Extra-thoracic disease amenable to biopsy, pleural effusion?	☐	☐
NO presence of any other serious co-morbidity?	☐	☐
Has the patient given written informed consent? *(please keep on site - give copy of consent to patient & one to be filed in the patient notes)*	☐	☐

If any NO box is ticked, patient is NOT eligible for the trial

If eligible phone: 0207 679 0784
for randomisation 9am-5pm Monday – Friday
If the office is unattended, please contact Neal Navani on 07951602494

Phoned in by:		NLCRN use:	Form completed by	Date:
Fax no:			Randomised by	Date:
Randomised to:	Control ☐	Active ☐	**PATIENT UIN/BOOST:** ☐☐☐☐☐☐☐☐☐☐ ***(Given by Randomiser)***	

Please return original completed form to Neal Navani
Dept of Thoracic Medicine, 4th Floor East, 250 Euston Road, NW1 2PG

APPENDIX 2:

REMEDY trial protocol

Lung-BOOST trial protocol

REMEDY: A clinical trial of Endobronchial Ultrasound for the diagnosis of Mediastinal Lymphadenopathy

A clinical trial of endobronchial ultrasound and mediastinoscopy for the diagnosis of mediastinal lymphadenopathy

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GENERAL PROTOCOL INFORMATION

This document describes the trial and provides information about its background, rationale and procedures for entering and managing patients. Every care was taken in its drafting, but corrections or amendments may be necessary.

Problems relating to this trial should be referred to Dr Navani or Dr Janes.

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Protocol Details

Protocol Version 2

16th February 2009

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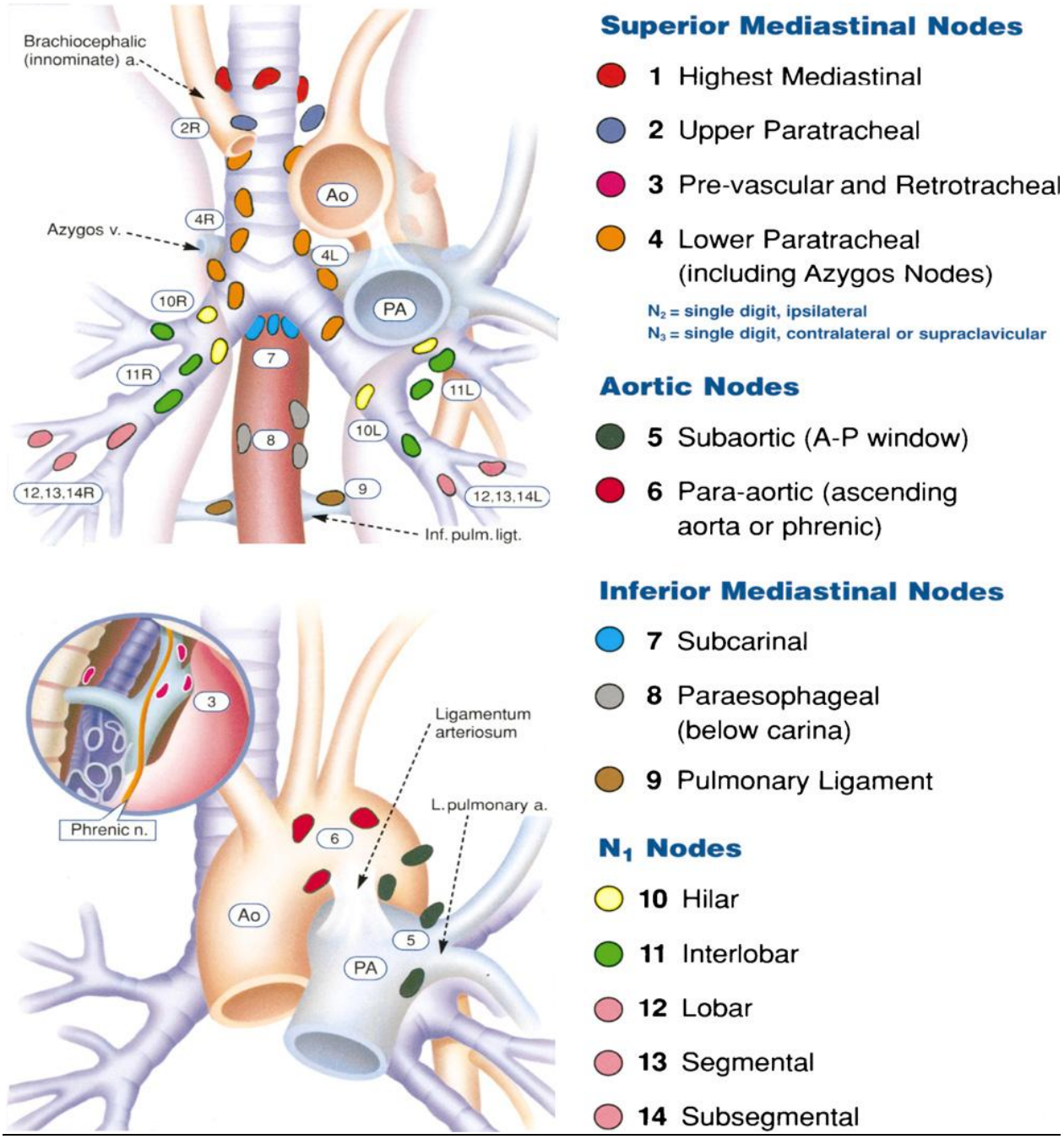
Section 1: Background

1.1 Mediastinal lymphadenopathy: the importance of tissue diagnosis

Mediastinal lymphadenopathy refers to the enlargement of lymph nodes within the mediastinum and determining the diagnosis of mediastinal lymphadenopathy is a common problem faced by respiratory physicians. The differential diagnosis of enlarged mediastinal lymph nodes (MLN) includes neoplasm, granulomatous disease, infection and reactive hyperplasia. Neoplastic causes are most commonly metastatic lung cancer, lymphoma or metastatic disease from the oesophagus, breast, kidney or head and neck. Sarcoidosis and tuberculosis result in granulomatous lymphadenopathy. Fungal infections such as histoplasmosis and coccidiodomycosis may also cause enlarged MLNs. Rarer causes of mediastinal lymphadenopathy include Castleman's disease, angioimmunoblastic lymphadenopathy, chronic berylliosis, Wegener's granulomatosis and chronic mediastinitis.

In UK practice, the most common causes of mediastinal lymphadenopathy are sarcoidosis, metastatic lung cancer, tuberculosis and lymphoma. These four important conditions have vastly different treatments and prognoses. Moreover their symptoms are often non-specific. Fevers, night sweats and weight loss may be a common feature of each diagnosis and does not help with their differentiation. Therefore, a tissue diagnosis of mediastinal lymphadenopathy is critical to allow patient management.

1.2 Mediastinal Lymph Node Map: American Thoracic Society classification



From Mountain and Dresler. The regional lymph node map: Chest 1997.

1.3 Techniques for diagnosing mediastinal lymphadenopathy

Chest radiograph

On the chest radiograph, the ease with which MLN enlargement can be recognized depends on the particular location. Enlargement of the right upper paratracheal nodes causes uniform or lobular widening of the right paratracheal stripe, and an increase in density of the superior vena cava of which the border may become convex to the lung. Enlarged right lower paratracheal nodes push the azygos vein laterally increasing the diameter of the combined opacities of both node and azygos arch. Aorto-pulmonary nodes may cause a bulge in the angle between the aortic arch and the main pulmonary artery. If they are substantially enlarged, the left upper paratracheal nodes induce mediastinal widening. The radiographic features of subcarinal node enlargement include the displacement of the azygo-oesophageal line that becomes convex to the lung, an increased opacity of the subcarinal space on the posteroanterior film and a lack of visibility of the external surface of the medial wall of the intermediate bronchus. Enlargement of the anterior mediastinal nodes may be substantial to be visible on the chest films. In such case, mediastinal widening is frequently bilateral and lobulated in outline. The radiographic signs of enlargement of hilar lymph nodes are hilar enlargement, or a rounded mass in a portion of the hilum.

Computed Tomography

Lymph node enlargement is defined on the basis of a short-axis node diameter exceeding 1 cm. A coalescence of enlarged nodes suggests infection, granulomatous disease or malignancy. Diffuse mediastinal involvement is more typical of lymphoma, large cell undifferentiated carcinoma and acute or chronic mediastinitis. Computed Tomography (CT) can also be used to define the density of lymph nodes. Enlarged nodes may be calcified, or low in density and necrotic in appearance or can enhance following intravenous injection of contrast media. Low attenuation lymph nodes after administration of contrast media, with or without rim enhancement typically reflect the presence of necrosis. This finding is commonly seen in patients with tuberculosis, metastatic carcinoma and lymphoma. Post-contrast enhancement of enlarged hilar and MLNs may suggest Castlemans disease, angioimmunoblastic lymphadenopathy or vascular metastases in particular from renal cell carcinoma. This feature of enhancement may also be found in sarcoidosis and tuberculous lymphadenopathy. Therefore, CT appearances are insufficiently specific to allow a definitive diagnosis and pathological diagnosis remains necessary. CT does however provide accurate anatomical information and acts as a road-map for further investigations.

Positron Emission Tomography

PET enables detection of MLNs with abnormally high functional activity (e.g. tumour metastases), a feature that CT lacks. Because of this advantage and because of the limitations of using size criteria with CT to diagnose malignant MLNs, PET has superior sensitivity, specificity and accuracy in diagnosing mediastinal metastases as compared with CT and chest roentograms. However, inflammatory

mediastinal lymph nodes especially due to tuberculosis or sarcoid may also be positive on PET scanning.

The most commonly utilized radiotracer in PET is 18-fluorodeoxyglucose (18FDG), which detects foci of abnormally high glucose metabolism. The standardized uptake

value (SUV), the measure of metabolic activity detected by PET, is directly related to the degree of metabolic activity within a tissue focus or within an organ and in the context of non-small cell lung cancer provides predictive information regarding treatment response and survival. The maximum SUV (maxSUV) in a region of interest (ROI) has been adopted as an approach to characterize metabolically active lesions. The formula for the

SUV is:

$$\text{SUV} = \frac{\text{mean ROI activity (MBq/mL)}}{\text{dose (MBq)/Body weight (kg)}}$$

The SUV is therefore normalized by body weight. Traditionally, clinicians and radiologists have designated a maxSUV of 2.5 as the upper limit of normal in an attempt to minimize the chance of false-negative results.

This rationale has been best studied in patients with non-small cell lung cancer (NSCLC)[1]. Despite its superiority over CT for detecting mediastinal disease in NSCLC and a high negative predictive value, PET and integrated PET-CT have several distinct limitations. First, any metabolically active tissue may generate a positive PET signal. In patients with NSCLC, 25% of PET positive mediastinal

lesions are false positives[2]. Therefore, current guidelines mandate that PET positive mediastinal (and extra-thoracic lesions) require biopsy confirmation before curative treatment is excluded. Second, the sensitivity of PET for detecting tumour metastasis varies depending on the lymph node's size and location within the mediastinum. The American College of Chest Physicians guidelines suggest that all MLNs greater than 1cm in short axis should be pathologically assessed[3], regardless of FDG uptake. Although PET has excellent sensitivity (80—99%) in detecting metastasis to American Thoracic Society MLN stations 4R, 4L, 10R, and 10L (Fig. 1), its sensitivity at other MLN stations (e.g., stations 5, 6, 7,8R, and 8L) is poor (29—60%)[4].

Scientific data on the role of PET or integrated PET-CT in sarcoidosis is limited. One study suggested that the sensitivity of PET in detecting sarcoid was high for radiographic stages II and III (where enlarged MLNs are a feature) and may predict disease activity and reponse to treatment. However, specificity remains low. SUVmax values between 2 and 15 have been reported in MLNs due to sarcoid[5] and therefore FDG avid mediastinal lymph nodes are non-specific and require pathological diagnosis.

Functional imaging with 18-FDG PET and integrated PET-CT increase the sensitivity and specificity of lymphoma assessment and may also predict outcome and direct future therapies. Once again, however there are no specific appearances on PET images that will preclude the need for pathological diagnosis. Active tuberculosis (TB) infection including asymptomatic and extra-pulmonary disease

may be detected with FDG-PET/CT. It may also be a useful tool in the assessment of latent TB, to exclude active disease prior to treatment. PET/CT has the potential for monitoring response to anti-tuberculosis treatment. Metabolic response may also indicate clinical response and guide duration of anti-mycobacterial therapy[6].

Despite advances in imaging techniques, pathological confirmation of mediastinal lymphadenopathy remains mandatory.

Conventional transbronchial needle aspiration

During standard bronchoscopy, a dedicated transbronchial aspiration needle is introduced into the biopsy channel and blindly punctures the bronchial wall allowing the mediastinal lymph node to be aspirated. This is most accurately done for enlarged lymph nodes in the subcarinal area but lower paratracheal and hilar lymph nodes can also be sampled[7]. A positive result from trans-bronchial needle aspiration (TBNA) may obviate the need for further invasive tests. However, a meta-analysis of patients undergoing TBNA in patient with lung cancer and enlarged MLNs showed a pooled sensitivity that was low at 39%, with a FN rate of 28%, when the prevalence of mediastinal metastases was 34%[8].

One study has examined the utility of conventional TBNA for the diagnosis of isolated MLN[9]. TBNA procedures were performed using a flexible bronchoscope and a 22-gauge Wang needle in 60 consecutive patients with isolated MLN. A diagnosis was reached in 45 of 60 patients (75%). Diagnoses included tuberculosis

(n=21), sarcoidosis (n=21), carcinoma (n=15), and lymphoma (n=3). TBNA had high sensitivity for TB, but diagnosed 1 case (out of 3) of lymphoma[10].

Several other studies have examined the role of conventional TBNA in patients with MLN due to suspected sarcoid and tuberculosis. They have found similar sensitivities of the procedure (75 – 79%)[11;12]. However, overall, the relatively low diagnostic yield and high negative predictive value mean that TBNA is poorly utilized[13] and further tests are commonly necessary in the event of a non-diagnostic or negative sample.

Endoscopic Ultrasound

Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) of mediastinal lymphadenopathy has been available for over a decade[14]. Under conscious sedation, an endoscope is placed in the esophagus and an integrated linear ultrasound probe allows visualization of the mediastinum. Aspiration with a 22-guage needle is performed through the wall of the esophagus under direct vision.

Due to the anatomical location of the esophagus, EUS-FNA is able to sample mediastinal lymph nodes in stations 2L, 4L, 5, 7, 8 and 9 and also the celiac axis nodes. Samples obtained by EUS-FNA with a 22-guage needle are suitable for cytopathological analysis. Occasionally core samples are obtained by EUS-FNA and may be sent for histopathological investigation. Core tissue samples may be more reliably obtained using a 19-guage trucut needle. This method requires that the

mediastinal lymph node be at least 2cm in the direction of the biopsy. Adding trucut biopsy to fine needle aspiration improves the diagnostic accuracy and the adequacy of sampling.

Cohort studies have clearly demonstrated the utility of EUS-FNA in the mediastinal staging of NSCLC[15] and data is now emerging on the utility of EUS for the diagnosis of sarcoid[16]. Currently, there are no reports on its value in patients with MLNs due to tuberculosis or lymphoma.

Although EUS-FNA is a promising tool for the diagnosis of isolated MLNs, there are several restrictions. EUS cannot sample right-sided or hilar lymph nodes stations and these areas are commonly involved in patients with sarcoidosis and tuberculosis (particularly 4R). In addition, EUS does not allow visualization of the endobronchial tree which may provide additional diagnostic information in patients with granulomatous diseases. The equipment and skilled personnel are also not widely available and this has meant that EUS (like conventional TBNA) is underutilized for the diagnosis of isolated MLN.

1.3.1 Mediastinoscopy for the diagnosis of mediastinal lymphadenopathy

Cervical mediastinoscopy is currently considered the best investigation for the diagnosis of mediastinal lymphadenopathy. The procedure is performed under general anaesthesia and provides access to the upper and lower paratracheal lymph nodes and occasionally the anterior subcarinal station. Although rare, complications do occur. One percent of patients experience complications including haemorrhage, vocal cord dysfunction, tracheal injury and pneumothorax[17]. Mortality rate is considered to be 0.1%, usually from damage to major vessels intra-operatively.

The largest published series to date of mediastinoscopy examined 2145 procedures over a nine year period in a single centre. In patients with lung cancer, their false negative rate was 5.5% when the disease prevalence was 23.5% and most patients received an accurate pathological diagnosis[18].

A recent study of 47 patients with isolated MLN examined the diagnostic yield of mediastinoscopy and compared it to the clinical diagnosis[19]. The sensitivity and specificity of the pre-operative clinical diagnosis was 87% and 78% respectively. 1 patient with suspected tuberculosis was revealed to have lymphoma on biopsy. Five out of the 12 patients with a pre-operative diagnosis of malignancy had a final diagnosis of sarcoidosis. Nine cases of isolated MLN were identified incidentally. Of these, 7 had tuberculosis, 1 sarcoid and 1 non-small cell lung cancer. All but one patient had a definitive diagnosis reached at mediastinoscopy.

Another large study of mediastinoscopy of the diagnosis of MLN, prospectively evaluated 271 patients with isolated MLN and 127 patients with a pulmonary or hilar lesion of unknown aetiology[20]. Overall there were 17 false negative results (4.3%). The sensitivity of mediastinoscopy in patients with isolated MLN was 96% and in patients with a pulmonary or hilar lesion the sensitivity was 92%. Interestingly, 76% of the samples were performed in the right latero-tracheal lymph node station (4R), with 12.5% from the subcarinal lymph node station (7) and 7.8% in the left laterotracheal lymph node station (4R). There were no deaths and morbidity was low (2.25%). Importantly, mediastinoscopy altered the pre-operative suspected diagnosis in 74 patients (18.5%)[21].

Mediastinoscopy therefore offers a sensitive and safe technique for the diagnosis of mediastinal lymphadenopathy and is currently considered the gold standard investigation. However, several limitations of the procedure must be recognised. First, standard cervical mediastinoscopy does not allow complete access to the mediastinum. In particular, posterior subcarinal nodes (7), the aorto-pulmonary window (5,6) and inferior lymph node station are inaccessible to the standard technique (8,9). Also, general anaesthesia is required and overnight inpatient stay is still necessary in the UK for the majority of patients. These latter considerations in addition to surgical time are responsible for high healthcare costs associated with the procedure. The current NHS tariff for mediastinoscopy is £2157[22].

1.4 Endobronchial Ultrasound guided Transbronchial Needle Aspiration (EBUS-TBNA)

EBUS-TBNA, using a linear echoendoscope, was first described in 2003[23]. The procedure allows TBNA with a 22-guage needle under real-time ultrasound guidance. This progress in technology allows the pulmonologist and thoracic surgeon for the first time to sample the majority of the mediastinum in a minimally invasive manner with high sensitivity. Lymph nodes stations 1, 2, 3, 4, 7, 10 and 11 are readily accessible, representing an increased range compared to standard mediastinoscopy. EBUS-TBNA routinely provides samples from the posterior sub-carinal space and hilar areas that are out of reach of cervical mediastinoscopy.

Data now exists that confirms the theoretical benefit of real-time linear EBUS-TBNA over the blind conventional method. A single centre study from the US prospectively examined 138 consecutive patients with suspected or proven lung cancer[24;25]. Each patient sequentially underwent blind TBNA, EBUS-TBNA (and EUS-FNA) and 30% of patients in the cohort had mediastinal metastases. The study demonstrated that linear real-time EBUS-TBNA had a significantly superior sensitivity for detecting mediastinal disease than standard TBNA (69% vs 36%)[26].

The use of EBUS-TBNA has been recommended for the mediastinal staging of non-small cell lung cancer in enlarged PET positive nodes in American[27] and European guidelines[28]. To date, however, it's published use in the diagnosis of sarcoid is limited to under 200 patients[29-31]. There are no reports of its diagnostic properties in TB and a just single cohort study of 11 patients with lymphoma out of whom a diagnosis was reached in 10 with EBUS-TBNA[32].

One of the limitations of EBUS-TBNA is the fact that only a 22G needle is currently available. This generally provides cytological samples only which may make the diagnosis of lymphoma less reliable. However, a newer 21G needle will be available in the near future and mini-forceps have already been employed in one small study with encouraging results[33]. An important limitation of EBUS is the false negative rate of 20% observed with the technique. Therefore all negative samples, that do not provide a definitive diagnosis, should be investigated further with a surgical approach[34].

1.4.1 Advantages of EBUS-TBNA

EBUS has several important advantages over the other techniques for the diagnosis of MLNs. It is a minimally invasive approach that is routinely performed in an ambulatory care setting and only requires conscious sedation. EBUS also allows access to almost the entire mediastinum (except for the aorto-pulmonary LN stations 5,6 and the inferior LNs 8,9), representing a wider range of LN sampling than

mediastinoscopy. In particular bilateral hilar and posterior subcarinal nodes are easily sampled with EBUS but are out of the reach of standard cervical mediastinoscopy.

A further benefit of the bronchoscopic approach for the diagnosis of MLNs is that the complimentary techniques of transbronchial biopsy, endobronchial biopsy and broncho-alveolar lavage may be employed at the same sitting to maximise diagnostic yield. These bronchoscopic techniques are standard procedures for the diagnosis of sarcoid and in isolation have a sensitivity of 75%[35]. Bronchoscopy with washings / broncho-alveolar lavage also has a role in the diagnosis of tuberculosis but is of limited value in the diagnosis of lymphoma. Finally, there have been no major reported adverse events with EBUS-TBNA.

1.5 Why is a trial needed?

The role of EBUS-TBNA for the diagnosis of unselected MLN has not been evaluated. EBUS-TBNA does however have several distinct theoretical advantages over mediastinoscopy for the diagnosis of MLN. We therefore plan to investigate whether EBUS-TBNA, followed by mediastinoscopy if EBUS-TBNA is negative, is an efficacious and cost-effective strategy for the diagnosis of mediastinal lymphadenopathy.

In addition, current published data is from cohorts which are subject to selection bias and therefore do not adequately describe to the clinician, which patients should be referred for EBUS-TBNA. We aim to include consecutive patients with undiagnosed MLN and therefore avoid selection bias.

Section 2 Trial Design

2.1 Hypothesis

In the diagnosis of mediastinal lymphadenopathy, EBUS-TBNA and bronchoscopy will reduce the number of mediastinoscopies and result in healthcare cost savings.

2.2 Objectives

The trial is designed to investigate

1. Whether EBUS-TBNA can reduce the number of necessary mediastinoscopies and inpatient days for patients with mediastinal lymphadenopathy
2. The sensitivity and negative predictive value of EBUS-TBNA (after CT scan) for the diagnosis of mediastinal lymphadenopathy
3. The diagnostic accuracy of EBUS-TBNA for unselected patients with mediastinal lymphadenopathy
4. Whether EBUS-TBNA is a cost-effective technique for the diagnosis of mediastinal lymphadenopathy
5. The prevalence of malignancy in unselected patients with mediastinal lymphadenopathy

2.3 Single-arm trial

This study will conform to the Standards for Reporting of Diagnostic Accuracy statement[36]; therefore the trial will ensure that these standards in study design are met at the outset.

This is a single arm trial to evaluate the effectiveness and cost-effectiveness of EBUS-TBNA in reducing the number of mediastinoscopies, which is considered the gold standard investigation. Comparison of the outcomes (proportion of patients undergoing mediastinoscopy and associated cost) will be made with patients who have all undergone mediastinoscopy. Since the proportion of patients in the control arm to undergo mediastinoscopy is already known (100%), a control arm is not required.

2.4 Bias

Consecutive patients will be recruited and therefore selection bias will be avoided. Pathologists with experience in examining samples from MLNs will report the pathology with knowledge of the clinical scenario, which closely reflects clinical practice. However, they will be blinded to the fact that patient is in a clinical trial, minimising observer bias. Due to the difference in the samples produced from EBUS-TBNA (cytology) and mediastinoscopy (histology) it is not possible to blind the pathologists to the procedure employed.

Section 3 Trial entry

3.1 Recruitment

Consecutive patients with undiagnosed MLN will be recruited from several sites. The centres in the study will be University College London Hospital, Medical University South Carolina, Whittington Hospital, North Middlesex and and Barnet Hospitals. Patients with undiagnosed MLN referred to thoracic physicians or surgeons will be considered for trial entry.

3.2 Patient selection

3.2.1 Inclusion criteria

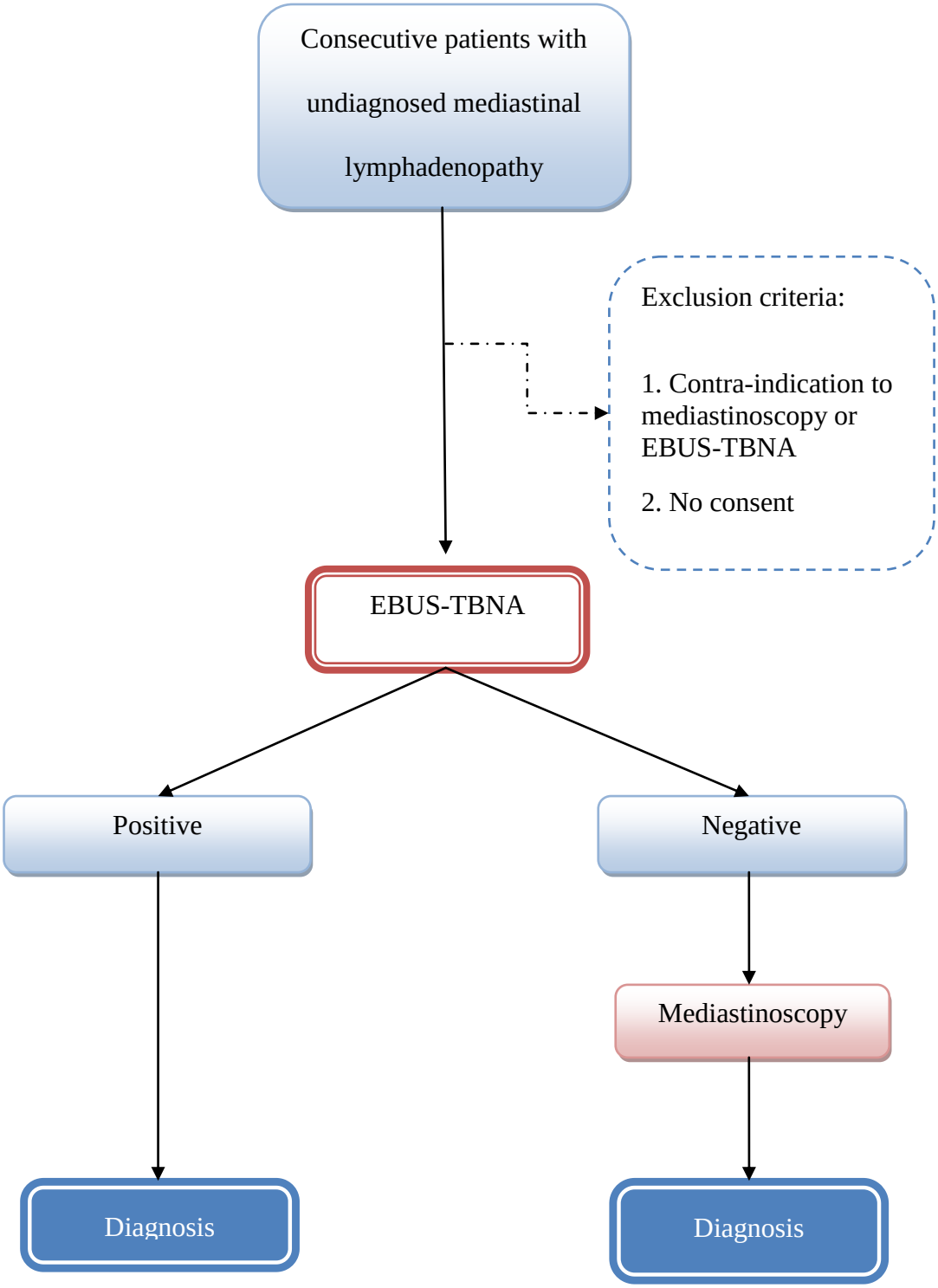
Consecutive patients with undiagnosed mediastinal lymphadenopathy on CT or PET-CT scan will be considered for trial entry. In order to reflect clinical practice as closely as possible and avoid selection bias, all consecutive patients will be included. Patients must have enlarged (>1cm in short axis) MLNs in lymph node stations accessible to EBUS-TBNA. Prior to the trial, these patients would have been referred for mediastinoscopy.

3.2.2 Exclusion criteria

Patients without informed consent, those with anterior mediastinal lesions or with contra-indications to EBUS or mediastinoscopy will be excluded.

Section 4 Patient Management

4.1 Trial Flowchart



4.2 Endobronchial Ultrasound and Bronchoscopy

EBUS-TBNA is performed in the ambulatory care setting by dedicated respiratory specialists with an interest in the procedure (NN, SJ, GS). Intravenous sedation is provided by midazolam and fentanyl. The linear echoendoscope is introduced via the mouth into the trachea. Topical anaesthesia with lidocaine is applied. Mediastinal lymph nodes are visualised and sampled using the standard EBUS technique, as previously described[37]. Vessels are located using the doppler function and are avoided. A 22G or 21G needle will be used and between 3 and 5 passes per node will be performed[38]. Samples will be smeared directly onto slides and air-dried. Any tissue cores obtained will be placed in formalin for histological examination. Needle wash is sent for microbiological examination and culture for tuberculosis and fungi.

Once EBUS-TBNA is completed, the EBUS scope will be replaced with a standard videobronchoscope. Endobronchial biopsies will be taken from any endobronchial abnormalities and transbronchial biopsies will also be performed in patients who are suspected of having sarcoid. The procedure will last in total 30 - 45 minutes. Cytological samples may be examined on-site by a cytologist to assess sample adequacy. Patients are observed for 2 hours after the procedure and then allowed home.

4.2 Mediastinoscopy

Mediastinoscopy is performed by specialist thoracic surgeons at the Heart hospital or University of South Carolina. Under general anaesthesia, the surgeon makes an

incision above the suprasternal notch. A videoscope is then passed behind the sternum and mediastinal structures are directly visualised. Visible lymph nodes are biopsied. At least one biopsy is obtained from lymph node stations 2, 4 and also 7 when possible. Patients are discharged the same day or admitted overnight for observation at the surgeon's discretion.

4.3 Cyto-histological Samples

All samples are examined by pathologists with specialist experience in thoracic specimens.

4.4 Diagnostic criteria

The following diagnoses are applied to pathological results:

Non-caseating granulomas – sarcoidosis

non-caseating granulomas and positive tuberculosis culture – tuberculosis

Caseating granulomas or Granulomas with necrotic material – tuberculosis

Malignant cells – malignancy (immunocytochemistry or immunohistochemistry may be performed to determine the origin of the primary tumour)

Reed Sternberg cells – Hodgkin's Lymphoma

In cases where EBUS-TBNA has failed to provide a definitive diagnosis, the patient will be referred for mediastinoscopy.

In cases where EBUS-TBNA and mediastinoscopy have failed to yield a diagnosis, patients will be followed clinically for a period of 6months - 1 year including

interval CT scans and assessment of MLN size. This will ensure that no cases of malignancy or active tuberculosis are overlooked.

4.5 Management after diagnosis achieved

Once a pathological diagnosis has been reached, the patient's care is continued by the referring physician or surgeon.

Section 5 Potential complications of procedures

5.1 Endobronchial ultrasound and bronchosocpy

No serious complications have been observed in the literature on EBUS-TBNA. At UCLH and MUSC, over 500 procedures have been performed without any adverse events.

5.2 Mediastinoscopy

Mediastinoscopy is the standard procedure for the diagnosis of MLN. It is associated with a mortality rate of 0.1% and morbidity of 1%, most commonly recurrent laryngeal nerve injury, penumothorax or tracheal injury.

Section 6 Healthcare Costs

6.1 Healthcare costs of the new strategy (EBUS-TBNA followed by mediastinoscopy if necessary) will be compared to the standard of mediastinoscopy alone. A decision tree model will be employed.

Section 7 Statistical considerations

7.1 Endpoints

The primary endpoints are the proportion of mediastinoscopies prevented and cost.

Secondary endpoints are length of hospital stay, sensitivity and diagnostic accuracy of EBUS-TBNA.

7.2 Analysis method

Demographic and clinical characteristics of the study population will be summarised using mean, standard deviation, median, or counts and percentages, depending on their type and distribution.

The one sample z test will be used determine if there is a significant reduction in mediastinoscopies due to EBUS-TBNA. The one sample t-test will be used to investigate whether the cost of EBUS-TBNA significantly differs from that associated with mediastinoscopy. We do not anticipate the distribution of cost to be

too skewed. However, a bootstrapping method will be used as a sensitivity analysis (Barber and Thompson, Stats in Medicine 2000).

Test accuracy will be estimated using sensitivity and negative predictive value (NPV) with 95% binomial confidence intervals.

The length of hospital stay will be summarised using median and interquartile range.

Results from this study will be reported according the Standard of Reporting Diagnostic Accuracy Guidelines[39].

7.3 Number of subjects required for the study

The mean cost for mediastinoscopy is known to be £3226 based on current NHS tarrifs. A small proportion of patients may experience complications and incur additional costs (e.g. staying in the hospital for an additional day). A standard deviation of £1400 is assumed to allow for that. A difference of £500 in cost on average would be considered to be acceptable if EBUS-TBNA significantly reduced the number of mediastinoscopies. A total of 75 patients will be required to detect a mean difference of £500 in cost associated with bronchoscopy, assuming an 80% power and 2.5% significance level (since the Bonferroni correction is applied to adjust for multiple significance testing). This sample size is also sufficient to give the study adequate power to assess whether the proportion of patients undergoing mediastinoscopy is reduced by 40%, assuming the same power and significance level. The sample size has been calculated using the statistical software STATA version 10.

7.4 Recruitment period

Two patients per week are expected to be eligible for the study. At least 90% of these patients will be recruited. Therefore 72 patients should be recruited within 1 year. The trial is scheduled to open in May 2009 and conclude in May 2011.

Section 8 Data Collection

8.1 Data to be collected:

Initials, DOB

Enlarged mediastinal LNs

LN station(s) involved

Parenchymal abnormalities (mass or nodules)

Location of parenchymal abnormalities

Pre-procedure PET?

Positive?

Clinical diagnosis

Date of EBUS-TBNA

Operators

LN station(s) sampled

Size of node

Number of passes

Bronchoscopy: Y/N

Endobronchial biopsies Y/N

Transbronchial biopsies Y/N

BAL: Y/N

Total time of procedure

ROSE: Y/N

EBUS pathology result

Bronchoscopy pathology result

Mediastinoscopy: Y/N

LNs sampled

Complications

Mediastinoscopy pathology result

Total inpatient stay

Culture results

Final diagnosis

Clinical follow-up at 6 months and 1 year if no diagnosis is made

8.2 Data Handling and record keeping

The above anonymised data will be entered into a specifically designed Microsoft Access database. The database will be password protected and kept on a secure computer at the host institution. Data entry will be performed by a clinical trials practitioner (independent to the investigators) with double entry on 20% of the patients.

The Data Protection Act 1988 will be strictly adhered to. Data will be stored for 3 years after trial completion and then destroyed. Drs Navani and Janes and Professor Silvestri retain responsibility for data collection, recording and quality.

Section 9: General issues related to the conduct of the trial

9.1 Regulations and confidentiality of data

Access to the data will be restricted to appropriate trial personnel for the purposes of the research and analyses of results only.

Patient name and address details will be included in the information obtained, but will be kept separate from the medical details. A unique identification number will link the name to the medical details.

Specific personnel at University College London Hospital (UCLH), as trial sponsors, and national regulatory authorities, may access data.

The trial personnel, UCL and any regulatory bodies will keep data confidential. Patient names will not be used in any reports about the study and all data is stored in accordance with the Data Protection Act 1998.

9.2 Data Protection

The trial personnel will act to preserve patient confidentiality and will not disclose or reproduce any information by which patients could be identified. Data will be stored in a secure manner and the study will be registered in accordance with the Data Protection Act 1998 with the Data Protection Officer at UCL.

9.3 Ethical approval

Ethical approval will be obtained and this protocol forms part of the application.

9.4 Declaration of Helsinki and Good Clinical Practice

The study will be conducted according to the recommendation of the Declaration of Helsinki (2000 Edinburgh, Scotland) and in accordance with the ICH principles of Good Clinical Practice.

9.5 Participant informed consent

The Investigator is required to explain the nature and purpose of the study to the participant prior to study entry. A participant information sheet will be given to the candidate and written informed consent obtained before entering in the study.

9.6 Idemnity & Compensation

Non negligent harm: University College London, as sponsor, will provide insurance against claims for compensation for injury caused by participation in this trial (ie non-negligent compensation). Patients wishing to make a claim should address their complaint in writing to the chief investigator in the first instance.

Negligent harm: Where studies are carried out in a hospital, the hospital continues to have a duty of care to a patient being treated within the hospital, whether or not the patient is participating in this trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of employees of hospitals.

9.7 Publication policy

We intend to disseminate findings from the research in peer-reviewed journals. Clinicians and researchers involved in the project will be acknowledged in written papers.

Lung-BOOST: A new pathway with Bronchoscopic or Oesophageal ultrasound for lung cancer diagnosis and staging

A randomised pragmatic clinical trial of endobronchial ultrasound or endoscopic ultrasound as a first test in the diagnosis and staging of lung cancer

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GENERAL PROTOCOL INFORMATION

This document describes the trial and provides information about its background, rationale and procedures for entering and managing patients. Every care was taken in its drafting, but corrections or amendments may be necessary.

Problems relating to this trial should be referred to Dr Navani or Dr Janes.

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Protocol Details

Protocol Version 3

1st May 2008

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Section 1: Background

1.1 The burden of lung cancer

Lung cancer is the biggest cancer killer in the UK, accounting for more than one in five cancer deaths. Over 38,000 new cases are diagnosed each year and in 2004, 24% of cancer mortality in men and 19% of mortality in women was due to lung cancer[1]. Lung cancer continues to have an enormous impact on national mortality and currently accounts for 6% of all deaths and 22% of all deaths from cancer in the UK.

1.2 The importance of accurate staging

Accurate staging of non-small cell lung cancer is a critical step which determines both the treatment modality and the prognosis. This is currently best accomplished via a multidisciplinary approach involving surgical, respiratory, oncology and radiology input in order to establish whether or not curative surgical resection is possible. Preoperative mediastinal lymph node staging separates initial operative versus non-operative status. Patients without mediastinal nodal metastases are considered operative candidates, while patients with central or contralateral mediastinal nodal metastases are treated primarily with chemotherapy and external beam radiotherapy[2].

Staging is used to predict survival and guide therapy. However, even with clinical stage I disease, the 5 year-survival rate after surgery is only 50%. Approximately 60% of cancer recurrences are likely to be from micrometastatic involvement at presentation, which is currently not detectable with existing diagnostic and staging

modalities[3]. Patients with clinical stage II disease (with hilar lymphadenopathy) have a five-year survival rate after surgery of 30%. At clinical stage IIIA, the 5-year survival rate is 17%, and at stage IIIB it is only 5%.[4] These patients are generally treated with combined chemotherapy and radiotherapy. The 5-year survival rate for patients with stage IV disease is virtually nil, and this disease is treated either with chemotherapy and supportive care or with supportive care alone. It is therefore critical to stage patients accurately as the treatment modalities and subsequent patient outcomes vary widely based on stage designation.

Several invasive and non-invasive methods exist to diagnose and stage lung cancer, and most patients require more than one. Inadequate staging or indeed incorrect staging of the mediastinal nodes can have the catastrophic consequences of a missed opportunity to operate or more commonly an inappropriate operation leading to high morbidity and worse outcome.

1.3 Techniques for staging lung cancer

Most centres perform a thoracic and upper-abdominal computed tomography (CT) scan first to assess whether to carry out a fibro-optic bronchoscopy (FOB) or trans-thoracic CT guided fine needle aspiration of the primary cancer. This provides the diagnosis and then patients are investigated for secondary lesions. This often results a in second invasive biopsy after 18-F-deoxyglucose positron emission tomography (PET) scan.

Accurately determining the status of mediastinal nodes is of paramount importance in determining treatment and prognosis. Non-invasive methods for staging include

CT with intravenous contrast administration, PET, as well as simultaneous acquisition CT-PET fusion imaging. Invasive mediastinoscopy has been considered the gold standard for mediastinal evaluation. The advent of the newer techniques of endoscopic ultrasound (EUS) and endobronchial ultrasound (EBUS) provides minimally invasive approaches for accurately staging lung cancer.

1.3.1 **Computed tomography (CT)**

A CT scan is widely available, relatively inexpensive and is always performed as a preliminary step in patients in whom a clinical diagnosis of lung cancer is suspected. It is the most commonly employed modality for assessing the mediastinum in lung cancer. Intravenous contrast is useful for distinguishing vascular structures from lymph nodes as well as delineating mediastinal invasion by centrally located tumours. Various criteria exist to define the malignant involvement of mediastinal nodes. The most widely used criterion is a short-axis lymph node diameter of $\geq$ 1 cm on a transverse CT scan.

A recent systematic review of 35 studies from 1991 to June 2006 analysed the accuracy of CT in diagnosing mediastinal metastases[3]. The combined studies yielded 5,111 evaluable patients and the pooled sensitivity and specificity of CT scanning for identifying mediastinal lymph node metastasis were 51% (95% CI, 47 to 54%) and 86% (95% CI, 84 to 88%) respectively. Therefore CT has only a limited ability to diagnose or exclude mediastinal metastasis. The results from this systematic review mirror those of other large meta-analyses[5,6].

The limitations of CT are highlighted by a false positive rate of 40% in mediastinal lymph nodes and the fact that 5 to 15% of patients with clinical T1N0 (stage I) tumours are found to have positive lymph node involvement at surgery[7]. Therefore CT can overstage and understage and cannot solely determine mediastinal lymph node status in patients with NSCLC. CT does however provide anatomical data that can guide the location and modality to be used for subsequent procedures for accurate staging.

1.3.2 Positron Emission Tomography (PET)

PET scanning is an imaging modality based on the biological activity of neoplastic cells. A standard uptake value of < 2.5 is often used as a threshold for normality, but non-neoplastic processes including granulomatous and other inflammatory diseases as well as infections may also demonstrate PET positive findings. The lower limit of spatial resolution is currently 7 to 10 mm, however, smaller lesions may be detected depending on the intensity of uptake of the isotope in abnormal cells[8]. Additionally, well differentiated low-grade malignancies, particularly bronchoalveolar cell carcinoma are known to have higher false-negative rates[9].

A systematic review of the accuracy of PET for the non-invasive staging of the mediastinum in patients with NSCLC evaluated 44 studies between 1994 and June 2006[3]. The results from 2,865 patients were analysed and pooled estimates of sensitivity and specificity for identifying mediastinal metastasis were 74% (95% CI,

69 to 79%) and 85% (95% CI, 82 to 88%) respectively. PET provides limited spatial resolution and integrated PET-CT scanning may represent an improvement over both techniques separately[10], although the total number of patients evaluated by this hybrid technique is small. PET or PET-CT is certainly an improvement over CT alone and negative predictive values may approach 97% when used in combination. However, given a false positive rate of up to 40%, all positive findings in surgical candidates should be confirmed by biopsy[11].

1.3.3 Surgery

Mediastinoscopy is currently considered to be the gold standard for determining the mediastinal node involvement from cancer. However, it has the limitation of sampling only the left and right para-tracheal, carinal and sub-carinal nodes, leaving nodes in the aorto-pulmonary window to be explored by anterior mediastinotomy. The posterior and inferior nodes are not accessible to surgical techniques. While mediastinoscopy cannot sample all mediastinal nodes, it can however detect microscopic disease even in small nodes. Overall, the sensitivity of mediastinoscopy is 78% and the false negative rate 11% in meta-analysis[12].

1.3.4 Endoscopic ultrasound (EUS)

EUS guided needle aspiration of mediastinal lymph nodes through the wall of the oesophagus is performed with a negligible risk of infection or bleeding as a day-case procedure. EUS provides us with an alternative method of diagnosing and staging

mediastinal nodes and is also able to examine abdominal structures including the celiac axis nodes, the left lobe of the liver and the adrenal glands via the oesophagus. Lymph node stations accessible by EUS include levels 4L, 5, 7, 8 and 9 (figure A). EUS guided needle aspiration is more suited to left sided nodes as compared to the right due to the anatomical location of the oesophagus[13,14]. EUS has been shown to be an accurate and cost-effective technique in mediastinal lymph node staging with a sensitivity of 84%[12]. EUS-FNA has also been shown to decrease the number of subsequent futile thoracotomies[15] but may be underutilized[16]. By performing EUS alone as the first test

in lung cancer patients, Singh et al made the diagnosis in 82% of cases[17]. This method was more accurate at diagnosing metastases in the mediastinum than CT and PET and was also superior to CT at diagnosing extra-thoracic disease. Other studies of patients with lung cancer requiring mediastinal evaluation have also found that EUS was more sensitive and specific than CT and PET[14, 18-21]. Indeed EUS may also be more accurate than mediastinoscopy[22] and one study has shown that 37% of patients with a negative mediastinoscopy had metastatic nodes on EUS[20].

1.3.5 Endobronchial Ultrasound (EBUS)

The endobronchial application of ultrasound was first described in 1992 and has been commercially available since 1999. More recently a video-bronchoscope with a convex linear ultrasound probe has been developed that allows for real-time ultrasound-guided transbronchial needle aspiration as a day-case procedure. EBUS can be used to sample the highest mediastinal, upper and lower para-tracheal,

subcarinal lymph nodes, as well as hilar lymph nodes (figure A). Lymph node station not accessible by EBUS include levels 5,6,8 and 9, which can be reached by EUS. A 22-guage needle is fed through the working channel and multiple passes are made until diagnostic tissue is obtained. The needle allows cores of tissues to be obtained and immunohistochemistry can be performed

EBUS guided needle aspiration is superior to traditional transbronchial needle aspiration (TBNA) in all stations in a large, randomized study[23] and has high sensitivity 90% (range 79-95%), diagnostic accuracy (97.1-98.9%) and negative predictive value (89.5-96.3%) as shown in several case series[24-29]. The average false negative rate was 24%[12]. In one series of 502 patients the range of lymph nodes accessed included levels 2R (n=53), 2L (n= 40), 3 (n=35), 4R (n=86), 4L (n=77), 7 (n=127), 10R (n=39), 10L (n=43), 11R (n=40) and 11L (n=33). Lymphocytes were present (indicating adequacy of specimen) in 94.5% of cases and the diagnosis was established from the lymph node biopsy in 93.5% of cases[30]. Some of these lymph node stations are frankly inaccessible by any other modality. EBUS may be combined with EUS in the same procedural setting[29] and sub-centimeter lymph nodes even as small as 4mm can be reliably sampled[26]. Until the false negative rate is more clearly defined, negative EBUS biopsy results are confirmed by surgical staging modalities.

1.4 Diagnosis and staging in a single test

Traditionally, the management of the suspected lung cancer patient begins with techniques to confirm the diagnosis and to differentiate small cell from non-small cell lung cancer. Once the diagnosis and histological type has been established (e.g. with CT guided percutaneous biopsy for a peripheral lung lesion) attention turns to evaluating the stage, which determines treatment options for the patient. Staging of non-small cell lung cancer focuses on defining the disease stage of the mediastinum, as almost all patients with extra-thoracic metastases will have metastatic mediastinal nodes[31]. Approximately 80% of patients in the UK present with inoperable disease. Therefore obtaining tissue from the mediastinum as a first test after CT scan would provide sufficient histological and staging data to guide treatment decisions in 80% of patients. The ultrasound guided techniques of EUS and EBUS offer a minimally invasive, day-case, relatively inexpensive and sensitive method of sampling the mediastinum.

In the operable patient with no extra-thoracic disease, NICE guidelines suggest that FDG-avid mediastinal lesions on PET require histological confirmation before surgery is precluded. Despite this, mediastinoscopy appears to be under-utilized in clinical practice[32]. However, ultrasound techniques would improve the staging of patients with lung cancer and should render mediastinoscopy redundant in most cases. Mediastinoscopies are time consuming and expensive, cause delay with surgical transfer, have some morbidity and a lower sensitivity than ultrasound techniques. When EBUS is combined with EUS guided needle aspiration, the range

of nodal stations amenable to needle biopsy is extended to include all stations except prevascular (level 6) lymph nodes and sub-centimeter lymph nodes even as small as 4 mm can be reliably sampled.

Accurate diagnosis and staging of lung cancer in a single test, by ultrasound guided aspiration of mediastinal nodes, would obviate the need for further and more expensive investigations like PET scan. In addition the number of mediastinoscopies and futile thoracotomies would also be drastically reduced. By reducing the number of outpatient attendances and unnecessary investigations and operations, ultrasound guided diagnosis and staging would represent significant saving in healthcare costs, one study demonstrating $11,000 per patient saving in North America[33].

1.5 Why is a clinical trial needed?

The recent development of mediastinal ultrasound investigation has been quickly incorporated into the patient pathways across Europe and the United States. The evidence discussed has determined that it is safe and accurate. Yet few centres in the United Kingdom currently use these investigations and then after other investigations such as traditional bronchoscopy and PET. We believe introducing an ultrasound investigation as a first test after CT will speed the patient pathway and reduce the number of tests, mediastinoscopies and futile thoracotomies required in these patients, hence improving patient care and considerably reducing costs. To

date, a study comparing patient pathways with and without EBUS / EUS has not been carried out.

We wish to clearly demonstrate that using EBUS / EUS as a first test after CT scan is superior to current practice so that both Hospital Care Trusts and Primary Care Trusts can see the financial rewards of this new pathway as well as the improved service for patients. This should then encourage a wider uptake of these methods across the UK.

Section 2: Trial Design

2.1 Hypothesis

EUS (endoscopic ultrasound) or EBUS (endobronchial ultrasound guided transbronchial needle aspirate) as a first test after CT scan in the diagnosis and staging of lung cancer will result in a reduction in the time from first outpatient referral to treatment decision, a reduction in the total number of PET scans, mediastinoscopies and futile thoracotomies, fewer outpatient attendances and a reduction in NHS healthcare costs.

2.2 Objectives

We aim to prove this hypothesis by:

1. Assessing the time from first consultation to decision to treat and the number of tests and outpatient attendances required in patients receiving standard care compared to patients in the new diagnostic pathway containing EBUS / EUS as a first test.

2. Prospectively diagnosing and staging 80% of consecutive lung cancer patients with a single procedure (EUS or EBUS), after an initial CT.

3. Comparing NHS healthcare costs of diagnosing and staging lung cancer in patients who have received current standard care and those patients who have had EUS or EBUS as their first investigation.

We aim to show that EUS or EBUS after a neck, thoracic and upper abdominal CT is a safe, sensitive and efficient first diagnostic test for lung cancer patients with intra-thoracic disase. It has the advantage over current techniques of being able to accurately determine the histological diagnosis and stage the patient's disease in a single procedure. We envisage that this will significantly reduce the need for further expensive investigations such as PET and mediastinoscopy. In addition, as it is a

single day case procedure, we will aim to demonstrate that a lung cancer patient pathway involving EBUS and EUS will require fewer outpatient attendances and overall will result in substantial savings in healthcare costs.

We also aim to calculate the sensitivity and negative predictive value of EBUS and EUS guided mediastinal aspiration in the diagnosis of lung cancer, since all negative results will be followed by functional imaging or surgical biopsy, the current gold standard of care.

Section 3: Trial entry

3.1 **Recruitment**

The retrospective arm will include 40 consecutive patients from each of 5 North London Cancer Network Hospitals who were referred with suspected lung cancer dating backwards from June 2007. The institutions participating in the retrospective arm are The Royal Free Hospital, The Whittington Hospital, The North Middlesex, Barnet Hospital and University College Hospital.

The prospective arm will be comprised of consecutive patients recruited from the 1st April 2008. All patients referred with suspected lung cancer to University College Hospital, The North Middlesex Hospital, The whittington and other North London Cancer Network hospitals will be recruited, until 168 patients in total are included.

3.2 Patient selection

This will be as follows:

3.2.1 Inclusion criteria

- Consecutive patients suspected of lung cancer on CT scan
- Written informed consent
- Able to tolerate fibre-optic bronchoscopy, mediastinoscopy and thoracomtomy if neccessary

3.2.2 Exclusion criteria

- Evidence of severe or uncontrolled systemic disease that makes it undesirable for the patient to participate in the trial
- Any disorder making reliable informed consent impossible
- Patient unlikely to tolerate bronchoscopy
- Patients with extra-thoracic disease, supraclavicular lymphadenopathy or pleural effusion

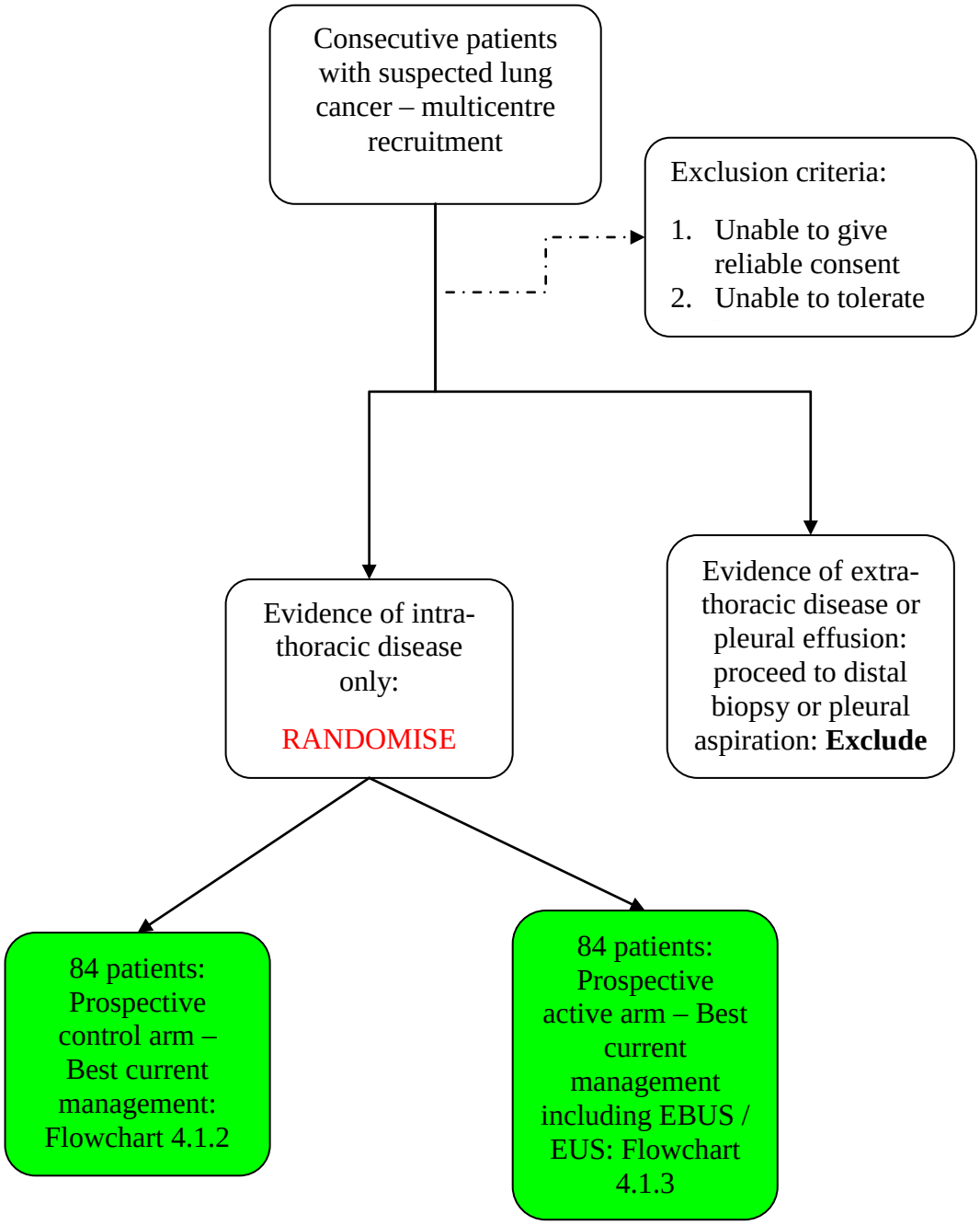
3.3 Randomisation

Block randomisation will be performed. The arms will be stratified for recruiting site and the presence or absence of mediastinal nodes ≥1cm. Patient registration, recording and collection of data will be the responsibility of the PI. The patient will

be allocated to the control or active pathways and given a unique identification number. This number will be quoted on all subsequent forms and samples from the trial.

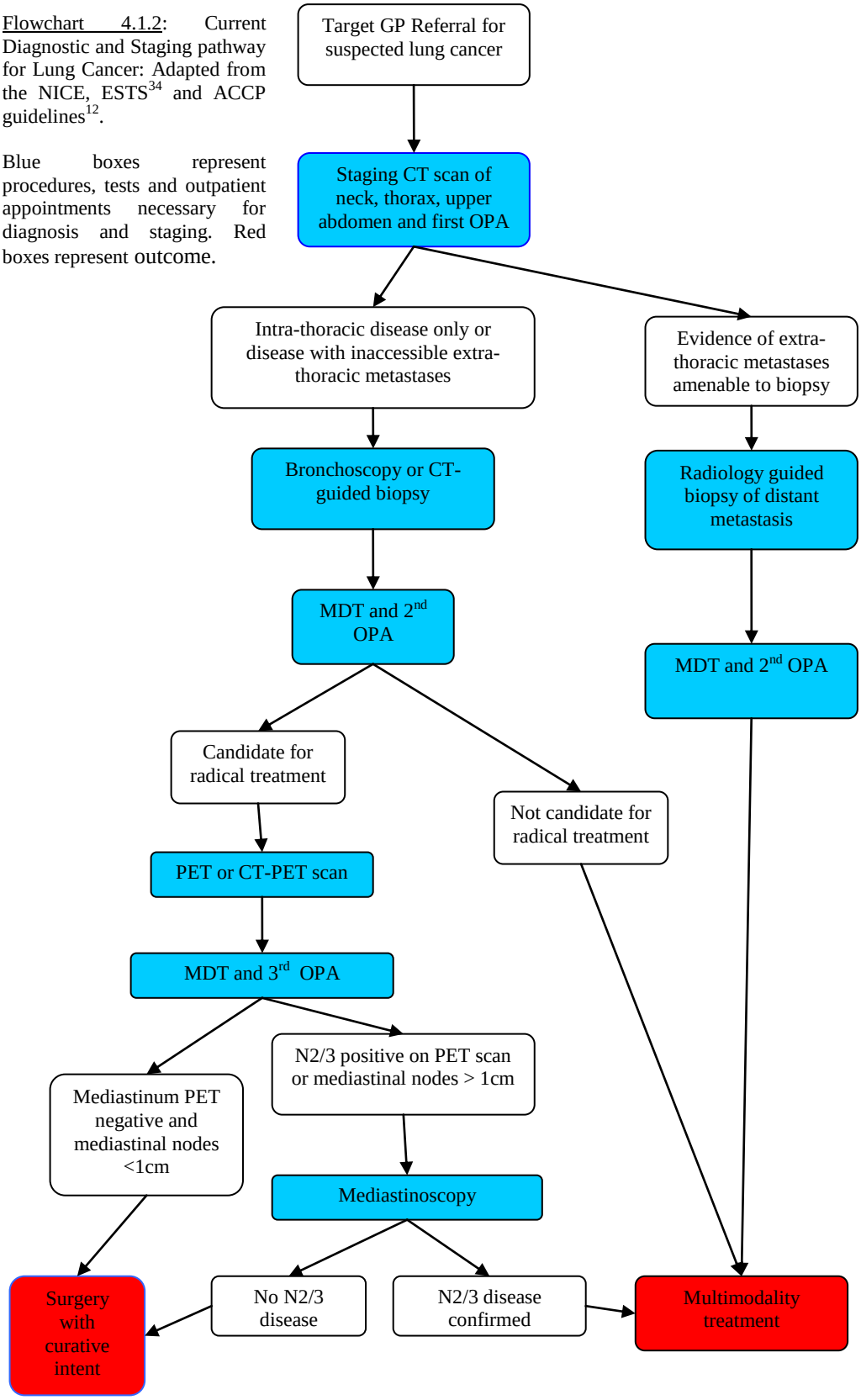
Section 4: Patient Management

4.1.1 Trial Flowchart

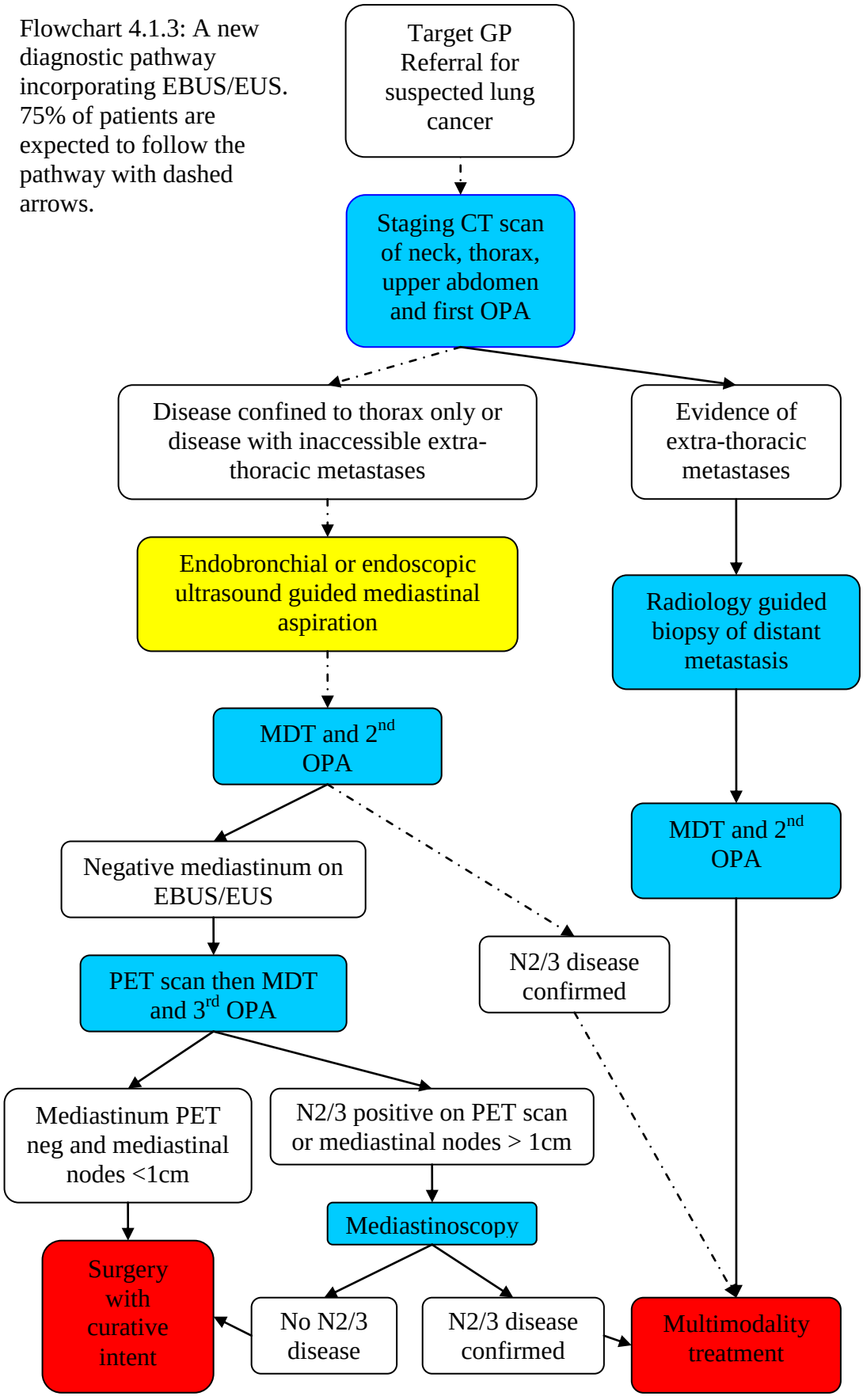


Flowchart 4.1.2: Current Diagnostic and Staging pathway for Lung Cancer: Adapted from the NICE, ESTS[34] and ACCP guidelines[12].

Blue boxes represent procedures, tests and outpatient appointments necessary for diagnosis and staging. Red boxes represent outcome.



Flowchart 4.1.3: A new diagnostic pathway incorporating EBUS/EUS. 75% of patients are expected to follow the pathway with dashed arrows.



4.2 The retrospective arm

Data will be collected from 40 consecutive lung cancer patients in each of 5 of the North London Cancer Network hospitals (University College Hospital, Royal Free Hospital, Barnet Hospital, Whittington Hospital and North Middlesex Hospital). The patients will have been diagnosed with lung cancer dating backwards from June 2007. There will therefore be a total of 200 patients in the retrospective arm.

For each patient, the following data will be collected:

- Name, Hospital number, *Unique Identification Number*, Date of birth, Gender, Ethnicity
- Date of 1st appointment
- Pack.year history
- Occupational Risk
- Family history of lung cancer
- Significant co-morbidity (the presence of an illness which would normally be recorded on a death certificate)
- Would tolerate bronchoscopy
- Performance status (WHO-ECOG)
- FEV_1 (% predicted)
- Source of referral
    - GP, urgent / emergency / suspected lung cancer
    - GP, routine
    - Other consultant
    - Radiology
- First appointment within 2 weeks of receipt of referral?
- Dates of outpatient appointments until diagnosis and stage
- Number of days until diagnosis and stage
- Number of tests required to determine diagnosis and stage
    - CT scan
    - Bronchoscopy
    - CT guided biopsy (?Complications ?Inpatient stay)
    - PET scan
    - USS liver / abdomen
    - Bone scan
    - CT / MRI Brain
    - Mediastinoscopy

- Proven histological diagnosis: cell type
- Stage (+ size and location of mediastinal nodes if available)
- Number of days from first appointment to treatment
- Any active treatment (within 6 months of diagnosis)
- Treatment with curative intent (Surgery, Radical radiotherapy)
- Time from diagnosis to death (if applicable)
- Reason for exclusion

4.3 The prospective control arm

Patients recruited into the control arm will be managed according to the usual practice of the hospital lung cancer multidisciplinary team (MDT). Flowchart 4.1.2 provides a template for best current practice, as suggested by the American College of Chest Physicians, European Society of Thoracic Surgeons and NICE. However, we do not expect that this flowchart is strictly adhered to in lung cancer MDTs. The trial is designed to change current clinical practice and therefore management in the control arm will reflect current clinical practice, as determined by the MDT, including the use of conventional TBNA where available. Drs Janes and Navani may be present but will not influence the investigations performed. This is in order to avoid any bias. Data on each patient, as described in section 4.2, will be collected.

4.4 The active arm: The new diagnostic pathway incorporating EBUS & EUS

Patients randomised into the active arm will be managed according to the pathway shown in flowchart 4.1.3. Patients with intra-thoracic disease only will have EBUS or EUS guided mediastinal aspiration as an initial investigation after CT scan.

Patients with enlarged anterior, hilar or subcarinal nodes will proceed to EBUS. Those with enlarged posterior mediastinal nodes will proceed to EUS. Patients who have no evidence of enlarged mediastinal nodes (≥ 1 cm) will still undergo EBUS or EUS as a first investigation as ultrasound guided aspirations may detect metastatic disease in up to 40% of patients with a radiologically normal mediastinum[26,33]. The pattern of lymph node metastases is predictable and therefore either EBUS or EUS can be selected based on the site of the primary lesion (see Table 1).

Site of primary lung tumor	Most common site(s) of MLN metastasis	Minimally invasive technique for MLN diagnosis
Right upper lobe	4R	EBUS-TBNA
Right middle lobe	4R, 7	EBUS-TBNA[b]
Right lower lobe	4R, 7	EBUS-TBNA[b]
Left upper lobe	5, 6	EUS-FNA[c]
Left lower lobe	5, 6	EUS-FNA[c]

Table 1: Site of mediastinal lymph node metastases and initial investigation according to site of primary lesion, when initial staging CT scan shows no enlarged mediastinal nodes.

4.5 The practical aspects of endobronchial and endoscopic ultrasound

EBUS and EUS are very similar to standard bronchoscopy and endoscopy respectively. Patients randomised to the active arm will be asked to remain nil by mouth for 4 hours prior to the procedure. EBUS or EUS will be carried out by

dedicated specialist physicians with an interest in the procedures. The investigations are carried out under conscious sedation. Where endobronchial disease is directly visualised, biopsies will be taken through a fibre-optic bronchoscope as well as ultrasound guided mediastinal aspirations. The EBUS or EUS procedure will last approximately 30 - 40 minutes. They will be discharged the same day and seen in clinic at the next available appointment with the results of the procedure and MDT discussion.

4.6 Cyto-histological processing

As agreed with Dr Mary Falzon (Consultant Histopathologist at UCH), samples obtained by ultrasound guided mediastinal aspiration will be on smeared slides and processed according to routine laboratory protocols. Where available, samples will also be placed in liquid cytology solution e.g. cytolyte, allowing tissue blocks to be made and immunohistochemical stains performed.

Each patient undergoing an ultrasound biopsy will be consented to have two samples stored for research. The samples will be used to examine the quality of the cytological preparations and determine whether these samples can be used for RNA and DNA work. Subsequent samples will have their DNA or RNA stored as per laboratory protocol for bronchial samples for future research. Dr Navani will carry out these tests in the laboratory of Dr Sam Janes in the Centre for Respiratory Research. If the samples taken for the clinical aspects of patient management are

insufficient, then the samples taken for research purposes would be analysed for patient benefit.

4.7 Management after diagnosis and staging complete

Once a tissue diagnosis is obtained and TNM staging is completed, patients will continue to be managed by the MDT of the hospital to which the patient was referred. Guidance on further management is provided by the NICE guidelines[2].

Section 5: Adverse event reporting

Adverse events will be recorded for all patients from time of randomisation to treatment decision. Serious adverse events (which require reporting) are death or prolonged hospital stay.

5.1 Endoscopic and endobronchial ultrasound

There are no common complications specifically related to endobronchial ultrasound or endoscopic ultrasound guided needle aspiration. All procedures will be carried out by specialist physicians with a dedicated interest in EUS, EBUS and bronchoscopy

and the British Thoracic Society guidelines for bronchoscopy will be strictly adhered to.

Section 6: Cost

6.1 Economic analysis

The procedures, investigations and outpatient appointments will be costed at the time of the study closure as per the national tariffs or best estimates of procedure cost. These costings will be applied to the retrospective, prospective control and active arms and decision tree analysis.

Section 7: Statistical considerations

7.1 Endpoints

The primary endpoint is the time from first outpatient appointment to decision to treat.

Secondary endpoints are:

- The health care costs of diagnosing and staging lung cancer
- The number of tests and outpatient visits a patient requires to be diagnosed and staged with lung cancer
- The proportion of lung cancer patients that are diagnosed and staged with a single test after CT scan
- The time from first outpatient appointment to treatment
- The number of mediastinoscopies
- The number of futile thoracotomies

The sensitivity and negative predictive value of EBUS and EUS in lung cancer will also be calculated. In addition, endpoints will also be analysed according to the presence or absence of enlarged mediastinal lymphadenopathy

7.2 Number of subjects required for the study

The retrospective arm will contain 40 patients from each of 5 hospitals within the North London Lung Cancer network (University College Hospital Trust, North Middlesex Hospital Trust, The Whittington Hospital Trust, Barnet Hospital Trust, and The Royal Free Hospital Trust), totaling 200 patients.

The prospective control arm and active arms will each comprise 84 participants (168 in total). In the retrospective arm and the prospective control group, we would expect that patients will require 2 or 3 investigations and outpatient appointments before a diagnosis and stage is reached, taking 30 days. This study aims to show that patients will only require 1 investigation and subsequent outpatient appointment, by using the newer techniques of EBUS and EUS and therefore reduce the time from 1st appointment to treatment decision to 14 days. We anticipate that 66% of patients will be diagnosed and staged with one test in the active arm, compared to 33% in the control arm. Based on 99% power and 2-sided test of significance, we would require a total of 168 patients (84 patients in each arm). The sample size calculation was carried out by Richard Stephens in the MRC Clinical Trials Unit, London.

Section 8: General issues related to the conduct of the trial

8.1 Regulations and confidentiality of data

Access to the data will be restricted to appropriate trial personnel for the purposes of the research and analyses of results only.

Patient name and address details will be included in the information obtained, but will be kept separate from the medical details. A unique identification number will link the name to the medical details.

Specific personnel at University College London Hospital (UCLH), as trial sponsors, and national regulatory authorities, may access data.

The trial personnel, UCL and any regulatory bodies will keep data confidential. Patient names will not be used in any reports about the study and all data is stored in accordance with the Data Protection Act 1998.

8.2 Data Protection

The trial personnel will act to preserve patient confidentiality and will not disclose or reproduce any information by which patients could be identified. Data will be stored in a secure manner and the study will be registered in accordance with the Data Protection Act 1998 with the Data Protection Officer at UCL.

8.3 Ethical approval

Ethical approval has been obtained from the Charing Cross Research Ethics Committee on behalf of the National Research Ethics Service. REC reference number 07/H0711/127.

8.4 Declaration of Helsinki and Good Clinical Practice

The study will be conducted according to the recommendation of the Declaration of Helsinki (2000 Edinburgh, Scotland) and in accordance with the ICH principles of Good Clinical Practice.

8.5 Participant informed consent

The Investigator is required to explain the nature and purpose of the study to the participant prior to study entry. A participant information sheet will be given to the candidate and written informed consent obtained before entering in the study.

8.6 Quality control and quality assurance

A pilot study using EBUS and EUS in the diagnosis and staging in lung cancer will be performed to ensure the techniques employed match national standards. During the trial there will be meetings every 2 months to ensure these standards are maintained. The lack of blinding in the study will mean that no bias is introduced.

8.7 Idemnity & Compensation

Non negligent harm: University College London, as sponsor, will provide insurance against claims for compensation for injury caused by participation in this trial (ie non-negligent compensation). Patients wishing to make a claim should address their complaint in writing to the chief investigator in the first instance.

Negligent harm: Where studies are carried out in a hospital, the hospital continues to have a duty of care to a patient being treated within the hospital, whether or not the patient is participating in this trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of employees of hospitals.

8.8 Publication policy

We intend to disseminate any findings from our research in peer-reviewed journals. All clinicians and researchers involved in the project will be acknowledged in written papers.

APPENDIX 3:

Publications arising from this thesis as of 1st September 2011

1. Navani N, Spiro SG, Janes SM. Mediastinal staging of NSCLC with endoscopic and endobronchial ultrasound. *Nat Rev Clin Oncol*. 2009 May;6(5):278-86.

2. Navani N, Nankivell M, Nadarajan P, Pereira SP, Kocjan G, Janes SM. The learning curve for EBUS-TBNA. *Thorax*. 2011 Apr;66(4):352-3.

3. Navani N, Booth HL, Kocjan G, Falzon M, Capitanio A, Brown JM, Porter JC, Janes SM. Combination of endobronchial ultrasound-guided transbronchial needle aspiration with standard bronchoscopic techniques for the diagnosis of stage I and stage II pulmonary sarcoidosis. *Respirology*. 2011 Apr;16(3):467-72.

4. Navani N, Molyneaux PL, Breen RA, Connell DW, Jepson A, Nankivell M, Brown JM, Morris-Jones S, Ng B, Wickremasinghe M, Lalvani A, Rintoul RC, Santis G, Kon OM, Janes SM. Utility of endobronchial ultrasound-guided transbronchial needle aspiration in patients with tuberculous intrathoracic lymphadenopathy: a multicentre study. *Thorax*. 2011 Aug 3. [Epub ahead of print].

5. Navani N, Nankivell M, Woolhouse I, Harrison RN, Munavvar M, Oltmanns U, Falzon M, Kocjan G, Rintoul RC, Janes SM. Endobronchial ultrasound-guided transbronchial needle aspiration for the diagnosis of intrathoracic lymphadenopathy in patients with extrathoracic malignancy: a multicenter study. *J Thorac Oncol*. 2011 Sep;6(9):1505-9