Endobronchial ultrasound-transbronchial needle aspiration and its practical application

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Summary

Endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) has emerged over the past decade as one of the most exciting and innovative developments in the field of respiratory medicine. This procedure allows sampling of mediastinal lymph nodes and masses in both malignant and benign disease and overcomes some of the disadvantages associated with mediastinoscopy and blind transbronchial needle aspiration. We describe the clinical use, indications for and limitations of EBUS-TBNA along with several illustrated clinical examples.

Introduction

Historically, mediastinoscopy has been the ‘gold standard’ method by which to accurately stage the mediastinum in lung cancer and in the evaluation of undiagnosed mediastinal lymphadenopathy. However, this procedure involves administration of a general anaesthetic, a neckline incision and short in-patient hospital stay. Moreover, not all hilar and mediastinal lymph nodes are accessible (standard cervical mediastinoscopy can usually sample only lymph nodes at stations 2, 4 and 71 of the Mountain and Dresler classification2), while nodes at the lower aspect of the subcarinal area (station 7) can be difficult to access. Its sensitivity, specificity and sampling strategy can also be variable.3 Although generally safe, mediastinoscopy carries a small but appreciable complication rate,4,5 and it cannot be easily repeated in the same individual. As a result, less invasive strategies such as transbronchial needle aspiration (TBNA) whereby a biopsy needle is inserted ‘blindly’ through endobronchial mucosa into a lymph node have been developed. However, results of this technique are variable and a meta-analysis (n=13 trials) indicated that although blind TBNA was highly specific, its sensitivity was as low as 39%.6 Results of blind TBNA are also dependant upon lymph node size and location, operator, type of cancer, type of needle and speed of cytological examination, with the added anxiety regarding needle location (in relation to vascular structures) as direct vision is not possible.

Endobronchial ultrasound (EBUS)-TBNA is a relatively non-invasive technique—usually performed as a day case—whereby mediastinal lymph nodes can be safely sampled under direct vision and overcomes some of the problems associated with mediastinoscopy and blind transbronchial needle aspiration. It was first described in the early 1990’s7 and since the beginning of the 21st century, its popularity and use has spread across the world. The purpose of this article is to provide general physicians with an insight into the methods by which EBUS-TBNA is performed, its indications and practical application along with a
How is EBUS-TBNA performed?

There are two types of EBUS, namely radial probe and convex probe; this article will deal exclusively the latter as this is the most commonly used method for assessing and sampling lymph nodes and masses. EBUS-TBNA is performed using an ultrasound transducer which is integrated into a flexible fiberoptic bronchoscope which in turn permits biopsies to be taken under direct vision. It is usually performed under intravenous sedation using a short acting benzodiazepine with or without an opiate (it is our practice to use midazolam and fentanyl) in an endoscopy suite with continuous pulse oximetry and heart rate and rhythm monitoring. EBUS-TBNA can also be performed under general anaesthetic, although difficulties can arise passing the bronchoscope through an endotracheal tube or laryngeal mask.

In the conscious sedated patient lying supine, the bronchoscope with integrated ultrasound transducer is passed through the visualized vocal cords and directed to areas of interest; all patients must therefore have had a pre-procedure computed tomography (CT) or positron emission tomography (PET)/CT to indicate which structures are likely to be abnormal in terms of size and/or metabolic activity.

With flexion of the distal end of the bronchoscope by way of a thumb operated lever, the ultrasound transducer comes into contact with the endobronchial mucosa; a balloon at the tip of the bronchoscope can be inflated to improve contact, although adequate images can usually be achieved without it. Once a satisfactory image is obtained, a dedicated biopsy needle whose stylet length has been previously set, is passed through the proximal port of the bronchoscope. It is then inserted, under direct and real-time vision, through the endobronchial mucosa, lymph node capsule and into the node or mass (Figures 1A and B). After removal of an internal sheath, suction, by way of pre-filled vacuum syringe, is then applied to the proximal end of the biopsy needle and the entire needle is then gently moved in and out, while remaining within the lymph node. Once this has been performed, the biopsy needle is completely removed and samples are placed into universal containers containing Cytolyte; when tuberculosis is considered a possibility, a sample should be placed in normal saline. The biopsy needle is then re-inserted and the whole procedure is carried out several more times; it has been suggested that in non-small cell lung cancer (NSCLC), three aspirations per lymph node station provides optimal results. In suspected lung cancer, lymph nodes which would confer the highest TNM stage should be preferentially sampled first, with other nodes sampled thereafter (i.e. N3 then N2 then N1 nodes).

When is EBUS-TBNA indicated?

EBUS-TBNA is well established in the staging of mediastinal and hilar lymph nodes in suspected or known NSCLC, diagnosis of suspected cancer when no endobronchial abnormality is present in patients with lymphadenopathy, diagnosis of cancers adjacent to the main airways and evaluation of mediastinal lymphadenopathy of uncertain aetiology (such as suspected sarcoidosis or tuberculosis). Indeed, EBUS-TBNA is advocated as one of the initial diagnostic and staging procedures depending on CT or PET-CT features in recent British
Thoracic Society guidelines and helps facilitate provision of cytological and pathological material which may allow individualized treatment in lung cancer. Other potential indications include restaging of mediastinal and hilar lymph nodes in NSCLC and diagnosis of mediastinal tumours. The lymph nodes most readily and commonly accessible by EBUS-TBNA are those in positions 2 (upper paratracheal), 4 (lower paratracheal), 7 (subcarinal), 10 (hilar) and 11 (interlobar) of the Mountain and Dresler classification. In contrast, blind TBNA is often only used to sample sub-carinal lymph nodes, although other stations can be sampled in skilled hands; standard cervical mediastinoscopy can usually sample lymph nodes at positions 2, 4 and 7.

How are EBUS-TBNA samples processed?

Processing of samples varies among laboratories. Rapid on-site cytology examination in EBUS-TBNA has been shown to confer benefits primarily in the identification of malignant cells, although this practice is not always feasible or possible. EBUS-TBNA specimens can provide both histological and cytological material; in our institution they are therefore processed to allow retrieval of the maximum amount and retain any solid tissue intact. For that reason samples are immediately placed in Cytolyt preservative fluid in the bronchoscopy suite. On arrival in the pathology department, any obvious tissue fragments and clots are removed and made into cell blocks using the thrombin/fibrinogen method; these are then processed to paraffin wax, sectioned and stained with haematoxylin and eosin.

The remainder of the specimen is transferred to a falcon tube and 30–50 ml of Cytolyt together with an appropriate amount of the mucolytic agent dithiothreitol (DTT) are added. The specimen is vortexed and spun until the supernatant is clear. Some of the cell deposit, including any residual solid fragments, is removed, rinsed with phosphate buffered saline and processed to a cell block. A Papanicolaou stained slide is made using the Hologic Thinprep 2000 machine if there is sufficient deposit; a cytopsin preparation is made if the deposit is minimal.

Cases

Case 1

A 79-year-old male, non-smoker with performance status 1, presented with facial swelling and was found to have clinical features of superior vena cava obstruction. Chest radiograph and CT are shown (Figures 2A and B, respectively). No visible endobronchial tumour was evident and EBUS-TBNA of the circumferential tumour/lymph node mass was performed. Cytology is shown in Figures 2C–E; appearances were consistent with small cell lung cancer.

Case 2

An asymptomatic 88-year-old female, non-smoker with performance status 1, was found to have a chest radiograph abnormality (Figure 3A) during pre-operative assessment prior to elective orthopaedic surgery. Past medical history included an anterior resection for Dukes A rectal cancer 8 years earlier. CT is shown (Figure 3B). No visible endobronchial tumour was evident during bronchoscopy and EBUS-TBNA of enlarged subcarinal lymph nodes was performed. Cytology is shown in Figures 3C–E; appearances were in keeping with a primary lung adenocarcinoma and samples were found to exhibit the epidermal growth factor receptor (EGFR) mutation.

Case 3

A 70-year-old male former cigarette smoker with performance status of 1, was found to have an incidental chest radiograph abnormality (Figure 4A). CT is shown (Figure 4B). Bronchoscopy showed abnormal mucosa at the main carina and a necrotic tumour within the right lower lobe; EBUS-TBNA was carried out of enlarged sub-carinal lymph nodes. Cytology is shown (Figures 4C and D) with appearances being consistent with squamous cell lung cancer.

Case 4

A previously healthy 42-year-old male presented with a 6-week history of bilateral ankle arthralgia and mild exertional breathlessness; there were no other symptoms and no risk factors for immunosuppression. He was taking no medication and there was no history of tuberculosis or foreign travel. Chest radiograph and CT are shown in Figures 5A and B, respectively. No visible endobronchial abnormality was found during bronchoscopy and EBUS-TBNA of enlarged paratracheal and subcarinal lymph nodes was performed. Cytology is shown in Figures 5C and D. Despite sarcoidosis being the most likely diagnosis on clinical and radiological grounds, the patient expressed a desire to have further investigations to confirm this. Cytological and histological appearances were in keeping with sarcoidosis.
Figure 2. (A) Chest radiograph showing a large right paratracheal mass and left hilar mass. (B) Axial CT image showing extensive mediastinal nodal disease with tracheal compression and extensive collateral circulation secondary to superior vena cava obstruction. (C) ThinPrep (Pap) cytology showing typical small cell carcinoma cells presenting as bare nuclei; strands of smeared nuclei give a tangled appearance to cell groups. (D) Histology (H&E) of cell block showing small cell carcinoma. Crush artefact of cells appears on the top right and left. A fragment of cartilage from the bronchial wall is seen bottom right. (E) Histology of small cell carcinoma cells can be difficult to differentiate from lymphoid cells. This slide shows positive staining of cells with neuroendocrine marker CD56, supporting a diagnosis of small cell carcinoma.
Figure 3. (A) Chest radiograph showing a left lower zone mass. (B) Axial CT showing subcarinal lymphadenopathy. (C) ThinPrep (Pap) cytology showing scattered large poorly differentiated malignant cells. (D) Histology (H&E) showing a group non-small cell carcinoma cells surrounded by lymphoid cells (centre right) indicating lymph node sampling. (E) Histology showing TTF1 positive nuclei to the right support a diagnosis of adenocarcinoma. Numerous large carbon-laden macrophages are also present among the lymphoid cells to the left.
Discussion

We have described the uses of and indications for EBUS-TBNA, and by way of several clinical cases, illustrated its application in both malignant and benign disease. EBUS-TBNA overcomes some of the shortfalls associated with non-invasive (CT and PET/CT) and invasive (mediastinoscopy) methods of assessing the benign or malignant nature of mediastinal structures. As a result, it is likely that its popularity will continue to increase in the years ahead and most respiratory departments dealing with patients with suspected lung cancer should have easy access to this investigation and at least one (although preferably two) respiratory physicians within a department should become trained in its use.

In the mediastinum, most studies measuring CT imaging accuracy have used a short-axis diameter of ≥1 cm as the threshold for abnormal lymph node size, although in patients with NSCLC, as many as 40% of nodes considered malignant by size may be benign, while 20% of nodes considered benign may be malignant. In the mediastinum, PET is more sensitive than conventional CT for assessing lymph nodes, although the negative predictive value of PET for mediastinal metastasis is determined by avidity for FDG and tumour location, while non-malignant processes that involve increases in glucose transport—typically infection—may cause false positive 5-fluourodeoxyglucose (FDG) uptake. It is important to note that British Thoracic Society guidelines state that further mediastinal sampling is deemed unnecessary if PET is negative and nodes <1 cm.

Several studies have evaluated EBUS-TBNA vs. radiological staging, with data indicating an important role for the former in the radiologically normal mediastinum. For example, 100 patients with NSCLC without lymphadenopathy or FDG avid lymph nodes, underwent EBUS-TBNA followed by mediastinoscopy to corroborate results. Malignancy was identified in around 10% of nodes considered malignant by size may be benign, while 20% of nodes considered benign may be malignant. In the mediastinum, PET is more sensitive than conventional CT for assessing lymph nodes, although the negative predictive value of PET for mediastinal metastasis is determined by avidity for FDG and tumour location, while non-malignant processes that involve increases in glucose transport—typically infection—may cause false positive 5-fluourodeoxyglucose (FDG) uptake.

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Figure 4. (A) Chest radiograph showing a large mass in the right lower zone. (B) Axial CT image showing a large subcarinal necrotic lymph node. (C) ThinPrep (Pap) cytology showing large numbers of necrotic cells and a few poorly differentiated malignant cells. (D) Histology (H&E) showing a fragment of squamous carcinoma with focal keratinization upon a background of necrotic cells.
patients (mean lymph node size 8 mm), suggesting that the sensitivity of EBUS-TBNA for detecting malignancy was 89%, specificity 100% and negative predictive value 99% in a radiologically normal mediastinum. In one study of 102 potentially operable patients with lung cancer, CT, PET (without CT) and EBUS-TBNA were performed prior to surgery to identify possible mediastinal and hilar lymph node metastasis. The sensitivities of CT, PET and EBUS-TBNA for the correct diagnosis of mediastinal and hilar lymph node staging were 77, 80 and 92%, respectively; specificities were 55, 70 and 100%; and diagnostic accuracies were 61, 73 and 98%. In a further study, the accuracy of EBUS-TBNA in sampling nodes ≤1 cm in diameter was evaluated in patients with NSCLC, with all patients having subsequent surgical staging. Malignancy was detected in 19 patients but missed in two, indicating that the sensitivity of EBUS-TBNA for detecting malignancy was 92%, with a specificity of 100% and negative predictive value of 96%. Although a pre-procedure CT or PET/CT is advisable prior to EBUS-TBNA, one study evaluated different ultrasonic features predictive of metastasis in patients (n = 487) with lung cancer whereby 1061 lymph nodes were retrospectively evaluated. Multivariate analysis suggested that a circular appearance, distinct margin, heterogeneous echogenicity and presence of the coagulation necrosis sign were independent predictive factors for metastasis. Two hundred and eighty-five of the 664 lymph nodes (43%) having at least one metastatic feature of the four categories were pathologically proven, and 96% of lymph nodes (381/397) were proven not metastatic when all four categories were absent.

Although the sensitivity and specificity of EBUS-TBNA in patients with NSCLC has been reported to be up to 93 and 100%, respectively, mediastinoscopy should usually still be performed in order to corroborate a negative result. However, this needs to be weighed against the suitability of patients for a further more invasive procedure. Few data have directly compared both techniques, although systematic reviews of the
published literature have suggested that the sensitivity of EBUS-TBNA and mediastinoscopy are generally comparable.21,22,24,25

Recent studies have shown that EBUS-TBNA can be combined with other techniques in an attempt to accurately and conveniently stage the mediastinum in patients with NSCLC. For example, recent data have suggested that EBUS-TBNA can be performed with transoesophageal fine needle aspiration using a single bronchoscope during the same session.26,27 Doing so can increase the accessibility to different mediastinal nodal stations and provide complementary and better diagnostic accuracy than either technique alone. Moreover, in a recent study, 241 individuals with potentially resectable NSCLC were evaluated in whom mediastinal staging was indicated based on radiological grounds.28 Patients were randomized to undergo mediastinoscopy or so-called ‘endosonography’ (combined transoesophageal and EBUS-TBNA), followed by mediastinoscopy when no lymph node metastases were found during endosonography; thoracotomy with lymph node dissection was performed when there was no evidence of mediastinal tumour spread in both groups. Two hundred and forty-one patients were randomized, 118 to surgical staging and 123 to endosonography, of whom 65 also underwent surgical staging. Lymph node metastases were found in 41 patients by surgical staging vs. 56 patients by endosonography ($P=0.11$ for the difference) and in 62 patients by endosonography followed by surgical staging. In the same study, thoracotomy was unnecessary in 21 patients in the mediastinoscopy group vs. nine in the endosonography group ($P=0.02$ for the difference). As a result, the authors suggest that in patients with NSCLC, a staging strategy using endosonography followed—if required—by surgical staging vs. surgical staging alone, conferred greater sensitivity in detecting mediastinal node metastases and resulted in fewer unnecessary thoracotomies.

As we have highlighted in Case 4, EBUS-TBNA can be a useful technique to obtain cytological material in patients with suspected sarcoidosis,29 especially in those with stage 1 disease (mediastinal lymphadenopathy alone) in whom endobronchial or transbronchial biopsy are considered less likely to be helpful. In a randomized controlled study, the diagnostic yield of EBUS-TBNA was compared to blind TBNA in 50 patients with features suggestive of sarcoidosis. With blind pathology review, the diagnostic yield was 73% vs. 96% in favour of EBUS-TBNA group ($P=0.05$ for the difference).30 A further study of 50 patients suggested a sensitivity of 85% in patients undergoing EBUS-TBNA for suspected sarcoidosis,31 while the combination of EBUS-TBNA plus transbronchial biopsy have been shown to provide diagnostic accuracy of 100%.32 However, it is important to note that sarcoid-like reactions may occur with NSCLC and other conditions,33 one study suggested that such changes were observed in 4% of all patients with NSCLC, although metastatic involvement by NSCLC was not found in lymph nodes exhibiting sarcoid-like granulomatous changes.34 Furthermore, non-specific collections of macrophages, which would not deserve classification as granulomatous disease, are not uncommon in benign reactive mediastinal lymph nodes, meaning that care needs taken in the identification and interpretation of ‘granulomas’ in a cytological as opposed to histological, ‘intact tissue’ setting. EBUS-TBNA can also increase the diagnostic sensitivity of suspected tuberculosis,35 when used alongside other methods.

Limitations do exist with the use of EBUS-TBNA. Clinicians need to be trained in the technique, with experienced bronchoscopists varying in the speed at which the plateau of the learning curve is reached. For example, in a retrospective review of the first 100 procedures performed in five different centres (across three countries), the pooled sensitivity was 67%.36 Moreover, correct use of the biopsy needle is vital in sampling and obtaining adequate specimens for microscopic examination. The bronchoscope has a direction of view of around 35° which means that passing through the vocal cords can be challenging as the upper trachea needs to be entered relatively ‘blindly’. Although the proximal endobronchial tree can be visualized, passage into lobar bronchi is often impossible due to the greater diameter of the bronchoscope and the more proximal flexion point when compared to conventional flexible fibreoptic bronchoscopes. Furthermore, although bronchial lavage can be carried out using the EBUS bronchoscope, endobronchial bronchial brushes and biopsies are not possible, which in turn means that during the same session, a traditional flexible fibreoptic bronchoscope needs passed following an EBUS-TBNA procedure. EBUS-TBNA generally takes slightly longer than conventional bronchoscopy and it is associated with a greater running costs. The amount of tissue obtained is considerably less than that following a mediastinoscopy which means that false negative EBUS-TBNA results may occur. For this reason, it is advisable that in patients with potentially operable NSCLC, those having a negative EBUS-TBNA result should have this confirmed at mediastinoscopy. EBUS-TBNA is generally regarded as not the investigation of choice with patients with suspected lymphoma and a mediastinoscopy should therefore be arranged in such patients.
It is important to highlight the fact that EBUS-TBNA has gained popularity as a primary diagnostic tool (and not solely as a staging procedure) in suspected lung cancer. If used in this way, operators should therefore bear in mind that the tissue yield is often less than would be obtained by conventional bronchoscopic biopsy sampling or transthoracic core biopsy (although bronchial biopsies can also be taken during the same EBUS-TBNA procedure, albeit with the insertion of a traditional fiberoptic bronchoscope thereby prolonging the procedure). This potential reduction in the amount of diagnostic tissue must be set against an increasing need for tumour material to carry out an expanding range of diagnostic testing required in the expanding era of ‘personalized’ medicine in lung cancer. While mediastinoscopy still plays an important role—especially in validation of negative EBUS-TBNA results in patients with radically treatable NSCLC—this may diminish in the future, especially with the increasing range of diagnostic testing required in the expanding era of ‘personalized’ medicine in lung cancer. Some centres with considerable EBUS-TBNA experience have reverted to using this tool as an additional (rather than sole) sampling procedure in the work up of patients with lung cancer.

In summary, EBUS-TBNA is an innovative procedure which is increasingly used within respiratory departments across the world in the diagnosis of both malignant and benign disorders, with its main role being in the assessment of mediastinal lymphadenopathy and masses. Although extra costs are involved in running an EBUS-TBNA service, the principal way by which savings can be achieved is by reducing the number of mediastinoscopies performed. While mediastinoscopy still plays an important role—in particular in the validation of negative EBUS-TBNA results in patients with radically treatable NSCLC—this may diminish in the future, especially as expertise in and availability of EBUS-TBNA increases. Moreover, recent data have indicated that EBUS-TBNA may be combined with oesophageal ultrasound and provide an effective means of accurately staging the mediastinum in patients with NSCLC.

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References


