Endobronchial ultrasound-guided fine-needle aspiration and liquid-based thin-layer cytology

Citation for published version:

Digital Object Identifier (DOI):
10.1136/jcp.2006.038901

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
Journal of Clinical Pathology

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Optimal management of patients with bronchial carcinoma requires histological or cytological confirmation of malignancy, tumour classification and accurate staging to allow treatment planning and the identification of patients who will benefit from potentially curative radical treatment. CT scanning is usually carried out as a first staging procedure and can determine the presence of enlarged lymph nodes, but the predictive value of nodal enlargement for the presence of metastatic disease is recognised to be poor. Although recent developments such as fluorodeoxyglucose positron emission tomography, especially when combined with CT, have shown significant improvements in the accuracy of mediastinal staging, there remains a requirement to confirm the presence of metastatic malignancy by histological or cytological means in order to plan appropriate treatment.

Mediastinal lymph node sampling for this purpose has been largely carried out by mediastinoscopy or thoracoscopy, but these have the disadvantage of being inpatient procedures requiring a general anaesthetic, and in the case of mediastinoscopy only giving access to nodes in the upper anterior mediastinum. Transbronchial and transthoracic lymph node aspiration or biopsy have previously been used to sample mediastinal nodes, but with variable results. The development of endoluminal ultrasound technology however, has, allowed the development of techniques whereby lymph nodes can be aspirated through either the airway (endobronchial ultrasound fine-needle aspiration (EBUS–FNA)) or the oesophageal wall (endoscopic ultrasound fine-needle aspiration (EUS–FNA)).

We and others have previously reported the use of EBUS–FNA in assessing patients with lung malignancy. Such studies from other centres have used standard cytological smears prepared from the aspirated material usually, but not in all cases, with the assistance of a cytopathologist present at the procedure. In this paper, we report our experience of processing and reporting EBUS–FNA specimens using liquid-based thin-layer technology.

**MATERIALS AND METHODS**

**Patients**

During the period between May 2005 and January 2006, 81 EBUS–FNA procedures were carried out on a total of 80 patients (41 men and 39 women, aged 30–86 years) who had been referred to the Respiratory Unit, Royal Infirmary of Edinburgh, Edinburgh, UK. Most patients had a presumed, or proven, diagnosis of bronchial carcinoma with hilar or mediastinal nodal enlargement on CT scan but were not considered to be candidates for surgical resection. To confirm the diagnosis, establish the cell type of the tumour or confirm the stage, EBUS–FNA was carried out as we have described previously. A few patients were, however, also referred with mediastinal lymphadenopathy of unknown cause or were thought to have mediastinal recurrence of carcinoma.

The number of lymph node stations sampled ranged from 1–4. In all, two or three needle passes were performed on each node in the manner we have described previously. All the samples obtained were placed directly into CytoLyte (Cytux UK, Crawley, West Sussex, UK) and delivered to the Pathology Department, Royal Infirmary of Edinburgh.

**Specimen processing**

The specimens were processed using the T2000 ThinPrep System (Cytux UK, Crawley, West Sussex, UK) and the single preparation was stained with Papanicolaou (PAP) stain. Any remaining of 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 H&E stained section was cut. Further sections were cut and used for immunohistochemical staining as required, with a range of antibodies using standard methods and the automated staining machines currently in use in the department.

**Abbreviations:** EBUS–FNA, endobronchial ultrasound fine-needle aspiration; PAP, Papanicolaou; TTF1, thyroid transcription factor 1.
Reporting
All specimens were reported by one of three consultant pathologists with a specific interest in thoracic pathology (WAHW, HMM, DMS) who report both histological and cytological specimens.

RESULTS
The aspirates obtained were of variable cellularity. Evidence of lymph node sampling was assessed by the presence of lymphoid cells or sheets of histiocytes often with associated pigment consistent with the prominent sinus histiocytosis commonly observed in mediastinal nodes. Although some aspirates were relatively sparsely cellular, none were deemed to show evidence of nodal sampling. In addition, all the aspirates contained evidence of varying amounts of endobronchial-derived material in the form of respiratory epithelial cells and alveolar macrophages. The cell blocks again were of variable cellularity, but many contained “mini biopsies” with fragments of identifiable nodal tissue and in some cases cartilage.

From the 81 procedures carried out, metastatic malignancy was identified in 40 cases. Non-small-cell carcinoma (fig 1) was diagnosed in 30 cases (6 squamous carcinoma, 3 adenocarcinoma and 21 unspecified), small-cell carcinoma (fig 2) in 8 cases and combined small-cell carcinoma/non-small-cell carcinoma in 2 cases. In two cases of squamous carcinoma, malignancy was diagnosed solely on the basis of the cell block, with no identifiable tumour in the PAP-stained cytological preparations. In addition, in one of the cases of combined small-cell carcinoma/non-small cell carcinoma only the latter was present in the cytological preparation and the small-cell component was only identified in the cell block.

Immunohistochemistry with CD45, pan-cytokeratin and CD56 was performed in sections obtained from the cell block in the cases of small-cell carcinoma and this was found to show patterns of staining in the tumour present similar to that seen in more traditional histological specimens (fig 2).

DISCUSSION
EBUS–FNA is a relatively new technique that allows aspiration of mediastinal and hilar lymph node groups under direct ultrasound control.10–14 The traditional approach of making spreads from such material may result in large numbers of slides to screen, and assessment can be difficult if the diagnostic material is obscured by blood. The quality of the preparations may be improved if an appropriately trained

Figure 1 Photomicrographs from endobronchial ultrasound fine-needle aspiration samples illustrating the appearances of metastatic adenocarcinoma (A, B) and metastatic squamous carcinoma (C, D) in Papanicolaou-stained ThinPrep preparations and the accompanying H&E stained sections from the cell blocks. (original magnification ×400).
Figure 2  Photomicrographs from endobronchial ultrasound fine-needle aspiration samples illustrating the appearances of metastatic small-cell carcinoma in a Papanicolaou-stained ThipPrep preparation (A) and in a section from a cell block stained with H&E (B). Immunohistochemistry with pan-cytokeratin shows positive staining of the tumour cells (C) whereas no staining with CD45 was identified (D; original magnification ×400).

Figure 3  Photomicrographs of specimens obtained from an endobronchial ultrasound fine-needle aspiration from a woman with a history of breast cancer and a right hilar/paratracheal mass. Groups of malignant glandular cells consistent with origin from an adenocarcinoma were identified both in the Papanicolaou-stained ThipPrep preparation and in the H&E-stained sections from the cell block (A). Immunohistochemistry demonstrated that the tumour cells expressed BerEp4 (B) and showed focal nuclear staining with thyroid transcription factor1 (C). No staining with antibodies to oestrogen receptor was identified (D). A subsequent mediastinoscopy confirmed the presence of adenocarcinoma, which was morphologically different from the breast lesion and consistent with origin from a bronchial carcinoma, (original magnification ×400).
EBUS–FNA cytology

Take-home messages

- Endobronchial ultrasound fine-needle aspiration is a useful tool for the diagnosis and staging of lung cancer.
- The use of thin-layer cytological techniques provides good-quality specimens for diagnostic purposes.
- The routine use of cell blocks adds to the diagnostic yield and provides material for immunohistochemical studies.

Authors’ affiliations

W A H Wallace, H M Monaghan, D M Salter, Department of Pathology, Royal Infirmary of Edinburgh, University Hospitals Division, Lothian Health and Division of Pathology, College of Medicine and Veterinary Medicine, Edinburgh University, Edinburgh, UK

M A Gibbons, K M Skwarski, Department of Respiratory Medicine, Royal Infirmary of Edinburgh, University Hospitals Division, Lothian Health and Division of Pathology, College of Medicine and Veterinary Medicine, Edinburgh University, Edinburgh, UK

Competing interests: KMS has received equipment and educational grants for travel from KeyMed (Medical and Industrial Equipment) WAAHW, HMM, DMS and MAG have no financial or other interests in the material published.

REFERENCES


