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## REVIEW

# Cytological specimens obtained by endobronchial ultrasound-guided transbronchial needle aspiration: Sample handling and role of rapid on-site evaluation

Prélèvements cytologiques guidés par échographie bronchique : prise en charge du matériel recueilli et rôle de l'examen extemporané

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Cytology

**Summary** Recently developed, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive modality for mediastinal lymph node staging in lung cancer patients as well as for the diagnosis of mediastinal and hilar lymphadenopathy. It has been shown in systematic reviews and meta-analysis that a high diagnostic yield can be achieved with EBUS-TBNA for staging lung cancer. Though still not a standard of practice, this novel technology has attracted physicians and surgeons as an alternative modality to surgical biopsy for the assessment of the mediastinum. Standard cytology, thin layer preparations in liquid medium or cell blocks of cells obtained by EBUS-TBNA can be applicable not only for pathological diagnosis but also for further investigations such as immunohistochemistry and fluorescence in situ hybridization. In addition, samples obtained by EBUS-TBNA can also be used for molecular analysis. The key to a successful EBUS-TBNA is to understand the anatomy of the mediastinum as well as the basic steps of the procedure. Moreover, handling of the sample obtained by EBUS-TBNA is crucial for diagnosis since no amount of skill or interest of the interpreter can make up for a badly prepared sample. The goals of rapid on-site evaluation during EBUS-TBNA include determination of whether sampling of the target has been achieved and more importantly triage of samples to secondary investigations. This manuscript explains the detailed techniques of EBUS-TBNA to master this innovative procedure.

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**MOTS CLÉS**

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**Résumé** Les cytoponctions transbronchiques échoguidées (EBUS-TBNA), récemment développées comme méthodes semi-invasives de la stadification ganglionnaire des cancers bronchiques, se sont rapidement étendues au diagnostic plus général des adénopathies médiastinales et hilaires. De nombreux articles et méta-analyses ont montré la haute rentabilité diagnostique de l'EBUS-TBNA dans la stadification des cancers bronchiques. Bien que ce ne soit pas encore une pratique de routine, cette nouvelle technologie est très attractive pour de nombreux pneumologues et chirurgiens comme méthode alternative aux biopsies chirurgicales dans l'exploration du médiastin. La cytologie standard, les prélèvements en milieu liquide ou les cytoblocs du matériel obtenu au cours des EBUS-TBNA s'appliquent, non seulement au diagnostic cytologique, mais également aux investigations complémentaires telles que l'immunocytochimie, ou l'hybridation in situ par fluorescence. De plus, les échantillons ainsi obtenus peuvent être utilisés pour toutes les techniques de biologie moléculaire. La clé de la réussite des EBUS-TBNA est la compréhension de l'anatomie du médiastin, tout comme celle des différentes étapes de la procédure. La prise en charge rigoureuse et motivée des cytoponctions échoguidées par le laboratoire est cruciale pour le diagnostic et les futures indications thérapeutiques. L'examen extemporané sur place permet de déterminer si la cible ganglionnaire a été atteinte, mais surtout d'anticiper les examens complémentaires utiles en gérant au mieux le matériel obtenu. Ce manuscrit décrit en détails la pratique de l'EBUS-TBNA afin de maîtriser cette technique innovante.

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## Introduction

Accurate staging is mandatory for the management of patients with lung cancer. Staging is initiated by radiological examinations such as chest computed tomography (CT) and positron emission tomography (PET) scan, but mediastinoscopy has been the gold standard for invasive mediastinal staging in patients with lung cancer [1]. Recently, a new bronchoscopic ultrasound technology has emerged as an alternative method for surgical mediastinal staging. The convex probe endobronchial ultrasound (CP-EBUS) allows real-time EBUS-guided transbronchial needle aspiration (EBUS-TBNA) of mediastinal and hilar lymph nodes [2]. EBUS-TBNA has been shown to be an excellent minimally invasive modality for mediastinal staging, and due to its high sensitivity, EBUS-TBNA is increasingly indicated for the initial diagnosis of lung cancer, metastases of non-lung cancers, and certain benign diseases such as sarcoidosis [3–13]. As for any kind of new modality, training is mandatory in order to perform a successful EBUS-TBNA and especially to achieve a high yield. In addition to the basic techniques of EBUS-TBNA, the management of the samples by the cytopathologists and laboratory technicians must be rigorous and constitutes a real challenge. Fine-needle aspiration, generally performed with 22 G or rarely 21 G needles, provides variable quantities of cytological or microbiopsy material, contaminated by blood and bronchial tissues. Even if excellent sampling is achieved, badly prepared samples will minimize the diagnostic yield. This manuscript explains both the technique of EBUS-TBNA as well as the proper handling of the samples obtained by EBUS-TBNA.

## EBUS-TBNA technique

### Equipments

The EBUS incorporates a video-endoscope and a linear ultrasound probe all in one device. The CP-EBUS currently available for clinical use includes a device from Olympus

(BF-UC160F-OL8/BF-UC180F, Olympus Medical Systems, Tokyo, Japan) (Fig. 1) and PENTAX (EB-1970UK, PENTAX Europe, Hamburg, Germany). The ultrasound images are processed using an ultrasound scanner. EBUS-TBNA is performed under real-time ultrasound guidance using dedicated TBNA needles. The 22-gauge and 21-gauge TBNA needle by Olympus (NA-201SX-4022/NA-201SX-4021, Olympus) is dedicated for the CP-EBUS by Olympus. The 22-gauge TBNA needle by Medi-Globe (GUS-21-18-022/GUS-25-18-022, Medi-Globe, Achenmühle, Germany) can be used for both devices. Since there is no clinical data to support the efficacy of the PENTAX EBUS scope for mediastinal staging, this manuscript will focus on procedures using the Olympus system.

### Ultrasound processor

The EU-ME1 is the newest scanner available which provides better ultrasound images compared to the first generation processor EU-C60; the scanning range being from 2 to 24 cm depending on the target. The default setting for the range is 4 cm which is ideal since mediastinal and hilar lymph nodes as well as the major vascular structures are best visualized at 4 cm. Lesions can be measured by the placement of cursors. Gain can be changed for optimal imaging and should be adjusted so that lymph nodes can be easily distinguished from vessels. The EU-ME1 is equipped with the Color and Power Doppler mode, which is helpful for the differentiation of lymph node versus vascular structures, but more importantly to look at the vascularity within the targeted lymph node (Fig. 2A).

### EBUS preparation

The EBUS includes two operating channels: one for inflating a latex balloon coating the probe, the other dedicated to the passage of the needle. Prior to the start of the procedure, the latex balloon is attached to the tip of the CP-EBUS, a 20 ml syringe filled with saline is connected to a stop cock connected to the balloon channel.

The dedicated TBNA needle and the Vaclok syringe should also be set to be ready for use. To avoid damage of the

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