PATHOLOGICA 2016;108:59-79

Review

Endobronchial-ultrasound needle aspiration and endoscopic ultrascound-fine-needle aspiration in thoracic diseases

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Key words

EBUS-TBNA • EUS-FNA • Thoracic diseases • Diagnosis • Staging

Summary

EBUS-TBNA and EUS-FNA are minimally invasive techniques rapidly gaining ground in the non-surgical invasive diagnostic approach to thoracic diseases due to their high accuracy and low morbidity and mortality compared to surgical techniques. Moreover, in the diagnosis and staging of lung cancer the combination of the two techniques is superior to either test alone. In this review we focus on the role of EBUS-TBNA and EUS-FNA in both malignant and non-malignant thoracic diseases.

Introduction

EndoBronchial ultra sound guided transbronchial needle aspiration (EBUS-TBNA) and endoscopic (esophageal) ultra sound guided fine needle aspiration (EUS-FNA) (are two invasive, non-surgical, diagnostic techniques, performed respectively through the trachea and main bronchi and through the esophagous in which endoscopy is combined with ultrasound. This allows the visualization of the internal wall of the trachea, of the main bronchi and of the esophagous and at the same time the ultrasonic picture allow the visualization of the adjacent structures and of the vessel when combined with the Doppler imaging. These features enable not only the mere inspection of the organs and of the surrounding structures, but also the possibility of biopsies under ultrasound guidance. This made of EBUS and EUS two key techniques in the diagnostic process of several lung diseases.

Technical considerations

Instruments

Both the EBUS and the EUS scopes provide an endoscopic and an ultrasonic picture at the same time. The EBUS scope, also known as linear EBUS or convex probe EBUS, is flexible scope with a convex transducer at its distal end. Most often a frequency of 7.5 MHz for the transducer is chosen. It scans parallel to the direction of the endoscope generating a 50-degree image. The outer diameter of the insertion tube is 6.7 mm and that of the tip is 6.9 mm. Thus the EBUS-scope is larger than a standard flexible bronchoscope. The EUS-scope, which is larger than the EBUS endoscope, also has a convex transducer, but compared with the EBUS-scope it is generating a larger 180-degree image, allowing a better and broader ultrasonic picture. A dedicated needle is inserted into the working channel of either the EBUS or EUS scope. It consists of a long steel needle, a sheath for protection of the endoscope and a handle for manipulation of the needle. The ultrasonic picture allows real time visualization not only of the target, but also shows the progression of the needle into the target, while the biopsy is taken.

EUS has many advantages compared to EBUS: it is better tolerated by the patient (no cough, less sedation), the ultrasonic picture is larger with a higher resolution, there are no cartilage rings interposed between the needle and

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the target lesion, and the maneuverability of the needle is better [1].

Use of the balloon

The EBUS scope has a disposable balloon mounted over the transducer. The balloon can be inflated with saline to enhance the contact between the probe and the tracheobronchial wall. It results in a better ultrasonic picture in cases where air between the transducer and the target is the problem, but there are no studies that prove that the use of the balloon results in a better diagnostic yield [2]. A similar balloon for the EUS endoscope is also available, but rarely needed: If the operator simply applies suction on the distal part of the endoscope, a satisfactory contact with the target through the thin and soft mucosa of the esophagus most often will be established.

Use of suction on the needle

EBUS-TBNA can be performed either with or without suction on the needle [2]. Two studies demonstrated that the diagnostic yield with the application of suction to needle biopsy was not statistically significant compared to no suction, since there were no differences in adequacy, diagnostic yield or quality in the samples [3 4]. The role of suction during EBUS procedures is under debate, since it theoretically may increase tissue trauma resulting in bleeding and lower yield, conversely it could result in a higher number of aspirated cells. Also the role of suction during EUS-FNA is unclear [5]. A randomized controlled trial from Wallace et al.6 suggested that suction should not be used during EUS-FNA of lymph nodes since no improvement in diagnostic accuracy was demonstrated and furthermore there was a risk of an increase in bloodiness of the specimens. In patients with malignant diseases suction is most often used for both EBUS-TBNA and EUS-FNA, whereas suction may not necessarily be an advance in patients with sarcoidosis [4].

Site of entry

The conventional big EUS endoscope is introduced via the mouth, but the smaller EBUS endoscope can be introduced via the mouth or the nose. The use of the nose may lead to a more stable position of the endoscope, but there are no randomized studies to support this assumption. Moreover, a larynx mask or a tracheal tube may also be used in connection with general anesthesia [2] While for EUS the oral route is the only one possible, EBUS is performed via a variety of entry sites. The EBUS scope can be inserted through the nose or through the mouth but there are insufficient data to support one or another. In bronchoscopy, the entry site depends on sedation, anatomy, scope size and operator's preferences and the most common is the transnasal approach because this approach improves patient comfort, decreases gag, decreases lidocaine dosing, and enhances bronchoscope stability. However, it could be not correct to translate the experience from flexible bronchoscopy to EBUS, given its bigger size and its rigidity at the distal end, as well as the limited bronchoscopic view. The EBUS scope can be also insert through the artifical airways, like laryngeal mask or rigid tubes, but actually the evidences are insufficient to recommend for or against an artificial airway.

EBUS and EUS are complementary to each other

In short, EBUS-TBNA gives access to structures close to the central airways in the mediastinum (stations 2R, 2L, 4R, 4L and 7) and the hilar regions (stations 10, 11 and 12). With EUS-FNA it is possible to visualize and biopsy structures close to the esophagus and the stomach for example lymph nodes in stations 4R, 4L, 7, 8 and 9 and structures below the diaphragm (i.e. retroperitoneal LNs close to the aorta and the celiac trunk, tumors in the left liver lobe and the left adrenal gland). Often station 4R is difficult to reach with EUS-FNA since trachea lies between the transducer and the target and is much easier to biopsy with EBUS-TBNA [7]. With EUS-FNA it is also possible in selected cases to reach LNs in station 5 and 6 [8 9]. Moreover, even if the target lesion is not in contact with the esophageal wall, the EUS scope allows to displace the esophagous in order to reach the target lesion in a easier way (Fig. 1).

Staging of proven or suspected lung cancer with endosonography

Accurate staging of patients with lung cancer is mandatory for planning of the correct treatment. In non-small cell lung cancer (NSCLC), patients with stage IA, IB, IIA, and IIB disease can often benefit from surgical resection while patients with stage IIIB, and stage IV disease rarely meet the criteria for surgery. The therapeutic approaches to stage IIIA are still under debate, but these patients are normally considered beyond surgical reach. Imaging techniques like computed tomography (CT) or integrated positron emission and computed tomography (PET-CT) should precede invasive diagnostic procedures both to allow optimal planning of these procedures and also to prevent unnecessary procedures for mediastinal staging for example if distant metastases are detected. However, CT or PET-CT as a main rule cannot stand alone due to the risk of both false negative and false positive results therefore necessitating EBUS-TBNA and EUS-FNA.

In short, the guidelines [10-12] for combined endobronchial and esophageal mediastinal node staging give the following recommendations when focusing on seven different clinical situations:

- Clinical situation number one: abnormal mediastinum and/or hilar nodes at CT and/or PET in a patient with suspected or proven NSCLC (figure 2). The combination of EBUS-TBNA and EUS with use of gastrointestinal (EUS-FNA) or EBUS Endoscopic (esophageal) ultrasound guided fine needle aspiration using the EBUS scope (EUS-B-FNA) scope is preferred over either test alone. If the combination of EBUS and EUS-(B) is not available, EBUS alone is acceptable. Subsequent surgical staging is recommended when endosonography does not show malignant nodal involvement.

Fig. 1. A) Lung tumor (15x9 mm) in the right upper lobe. B) EUS picture showing an hypoechoic area (13x9 mm) with a posterior ringdown. C) Citology smear showing neoplastic cells aggergated in small acini: adenocarcinoma (Diff Quick, midpower).

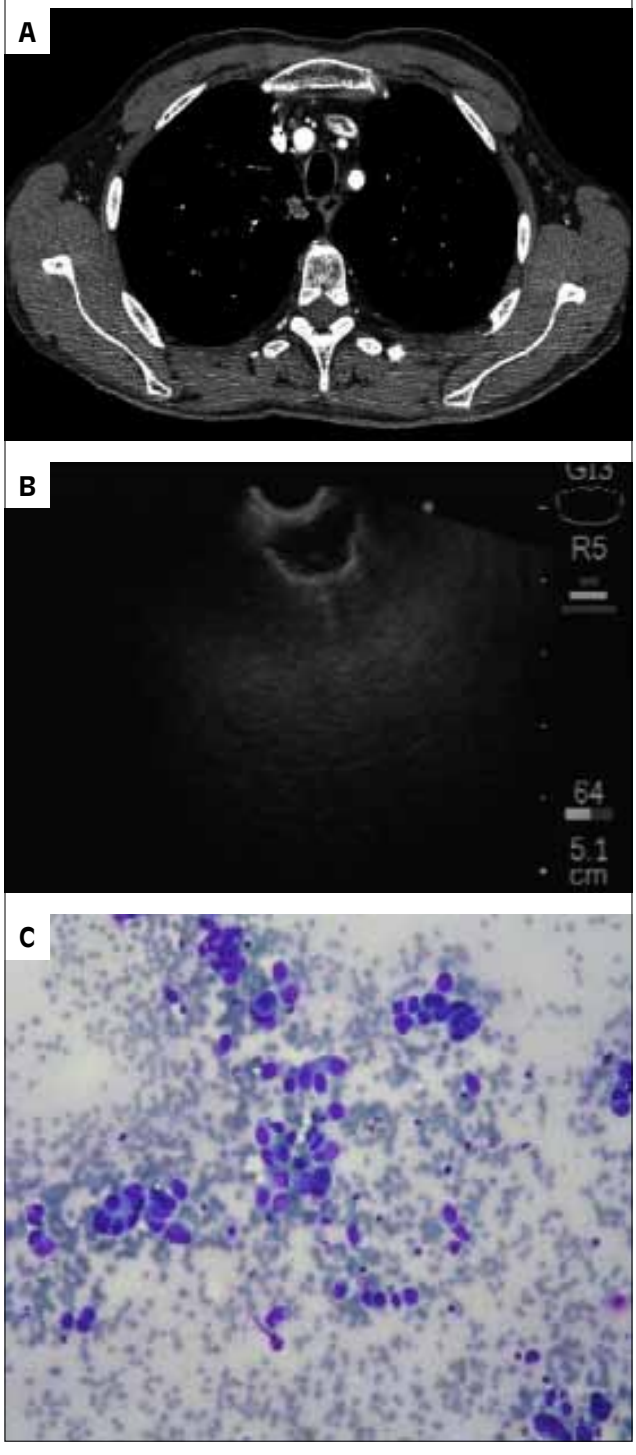


Fig. 2. Clinical situation number one. Abnormal mediastinal hilar lymph nodes. © Paul Clementsen, Denmark.

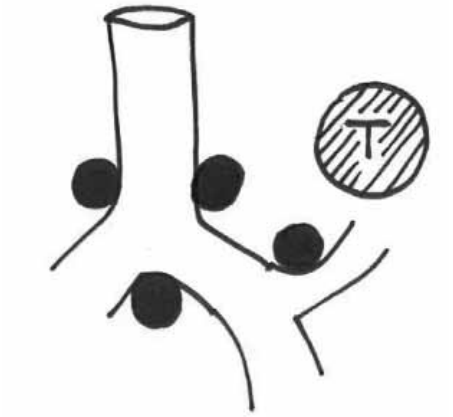
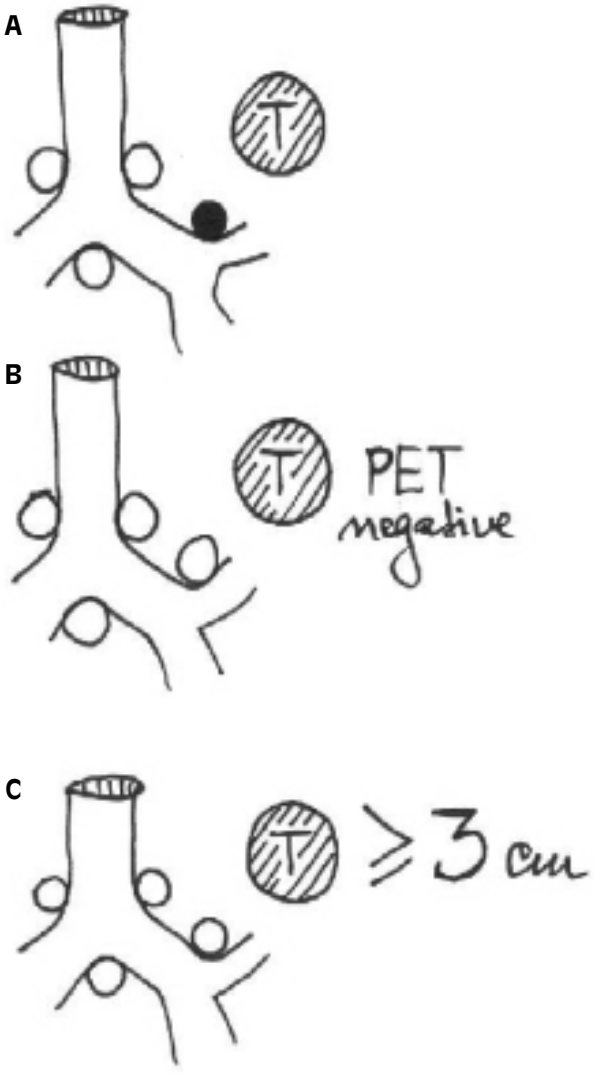
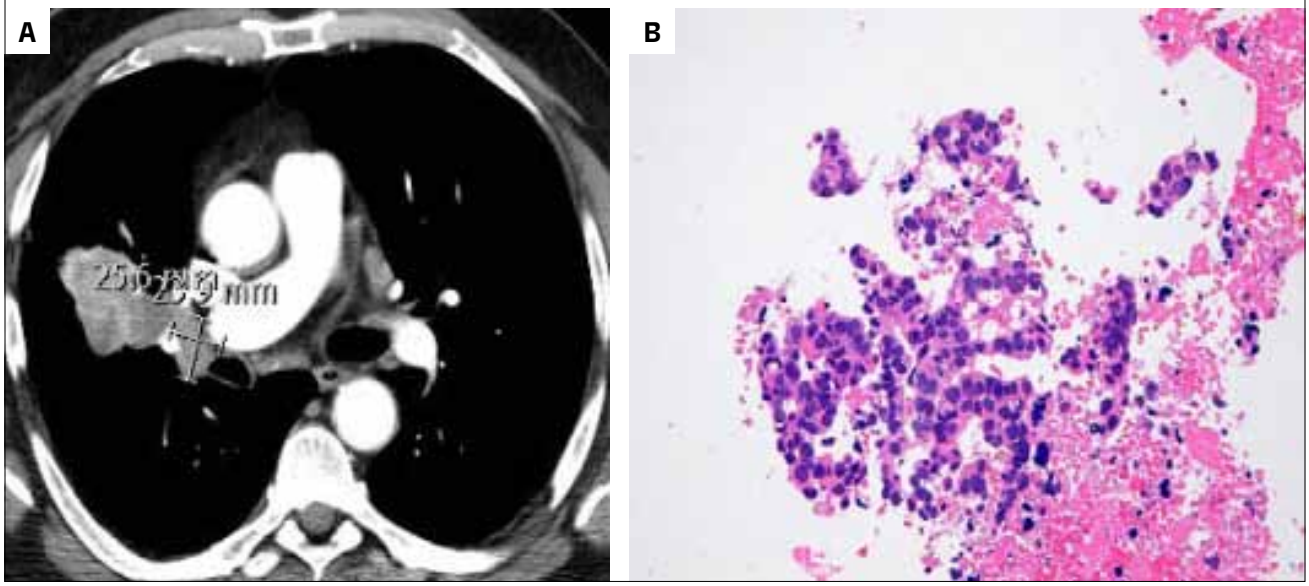


Fig. 3. Clinical situation number two. No mediastinal lymph nodes involment with: A) enlarged or PET positive hilar nodes, B) primary tumor PET negative, C) tumor size larger or equal to 3 cm. © Paul Clementsen, Denmark.



- Clinical situation number two: no mediastinal involvement at CT and/or PET/CT in patients with suspected or proven NSCLC (Fig. 3): EBUS-TBNA and/or EUS-(B)-FNA should be performed provided that one or more of the following conditions are present: (a) enlarged or PET-positive ipsilateral hilar nodes

Fig. 4. A) CT scan shows a mass close to the right hylum, with dysomogeneous contrast enhancement. An omolateral adenopathy is also present in 10R. B) cell block cytology obtained with EBUS-TBNA showing a lung adenocarcinoma (H&E, midopower).



(Fig. 4), (b) primary tumor PET-negative, (c) tumor size larger than or equal to 3 cm. If endosonography does not show malignant nodal involvement, mediastinoscopy should be considered, especially in suspected N1 disease.

- Clinical situation number three: no involvement of mediastinal or hilar node plus lung tumor less than 3 cm in size at CT and/or PET/CT in patients with suspected or proven NSCLC (Fig. 5): initiation of therapy without further mediastinal staging is suggested.
- Clinical situation number four: no involvement of mediastinal or hilar nodes plus centrally located tumor at CT and/or PET in patients with suspected or proven NSCLC (Fig. 6): it is suggested to perform EBUS-TBNA with or without EUS-(B)-FNA. If endosonography does not show malignant nodal involvement, mediastinoscopy may be considered.
- Clinical situation number five: restaging: for mediastinal nodal restaging following neoadjuvant therapy, EBUS-TBNA and/or EUS-(B)-FNA is suggested for detection of persistent nodal disease, but, if this is negative, subsequent surgical staging is indicated.
- Clinical situation number six: biopsy from lung tumor: in patients with a centrally located lung tumor that is not visible with conventional bronchoscopy, endosonography is suggested provided the tumor is located close to the central airways or the esophagus (Figg. 7-8).
- Clinical situation number seven: abnormal left adrenal gland: EUS-FNA is recommended (Fig. 9). EUS-B is still experimental.

Review of the studies

EBUS-TBNA

The sensitivity of EBUS-TBNA for mediastinal lymph nodes staging in lung cancer ranges between 84 and

Fig. 5. Clinical situation number three. Tumor size less than 3 cm. © Paul Clementsen, Denmark.

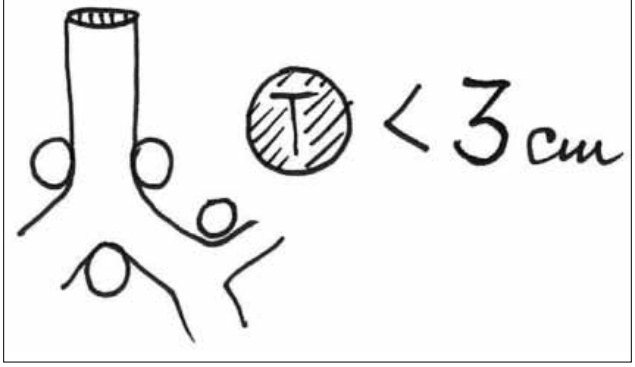


Fig. 6. Clinical situation number four. Centrally located lung tumor. © Paul Clementsen, Denmark.

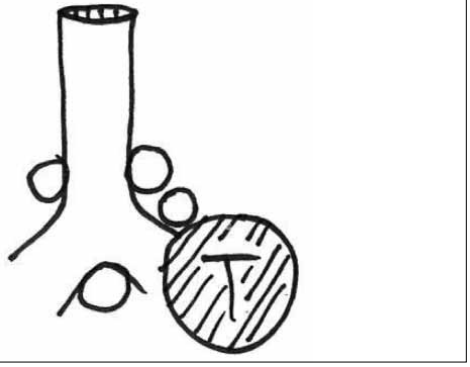


Fig. 7. Clinical situation number six. Tumor not visible with bronchoscopy but close to the airways. Perform EBUS-TBNA. © Paul Clementsen, Denmark.

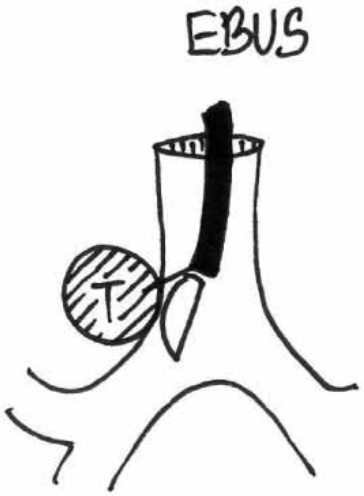
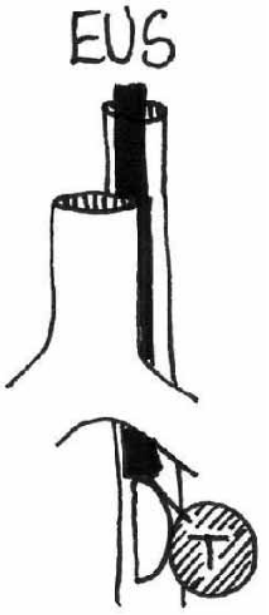


Fig. 8. Clinical situation number six. Tumor not visible with bronchoscopy but close to esophagus. Permorm EUS-FNA. © Paul Clementsen, Denmark.



93% [13-18]. Considering only patients with enlarged/PET positive mediastinal lymph nodes, the sensitivity of EBUS- TBNA ranges between 77% and 94% [13 14], Whilst in patients with normal sized/PET negative mediastinal lymph nodes the sensitivity ranges between 76% and 90%.

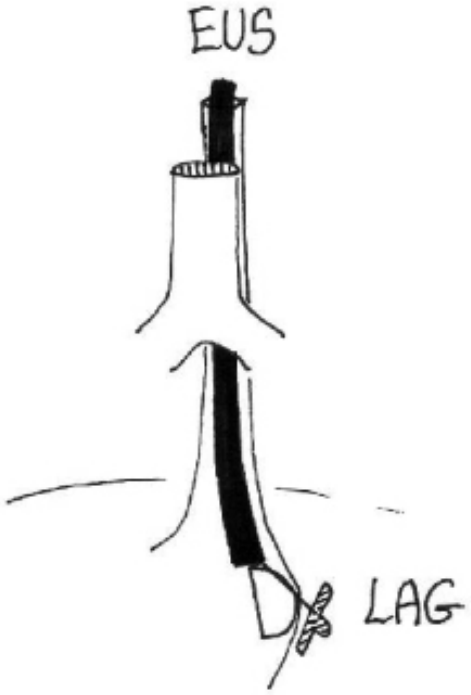
EUS-FNA

EUS-FNA has a sensitivity that ranges between 83% and 88% [19 20] with a sensitivity of 90% when only patient with enlarged/PET positive mediastinal lymph nodes are considered and of 58% in patients with normal sized/ PET negative mediastinal lymph nodes.

The combined procedure

Since EBUS-TBNA and EUS- FNA separately have a good diagnostic accuracy and sensitivity for mediastinal lymph node staging in lung cancer, the combination of the two procedures was proposed in order to increase the diagnostic accuracy. The first randomized clinical trial (RCT) that compared the diagnostic accuracy of both EBUS-TBNA and EUS- FNA with mediastinoscopy was the ASTER study 21. Two-hundred forty one patients were included, 123 underwent EBUS-TBNA and EUS-FNA followed by mediastinoscopy and 118 underwent mediastinoscopy alone. Endosonography resulted in greater sensitivity for mediastinal nodal metastases and fewer unnecessary thoracotomies. The sensitivity for endosonography alone was 85% vs for surgical staging alone was 79% (no significant difference), and for endosonography plus mediastinoscopy 94% (significant). In patients with radiologically abnormal mediastinal lymph nodes, the sensitivity for endosonography was 86%, and 97% when mediastinoscopy was added. In patients with radiologically normal mediastinum, the sensitivity for endosonography was 71%, and did not increase when mediastinoscopy was added. Zhang et al. [22] conducted a meta- analysis with focus on the combination of EBUS-TBNA and EUS-FNA for mediastinal lymph node staging of lung cancer. Eight studies were included in the analysis, for a total amount of 822 patients. The pooled sensitivity for the combination of EBUS-TBNA and EUS-FNA was 86%; in patients with abnormal and radiographically normal mediastinum was 75% and 68% respectively, suggesting that the combina-

Fig. 9. Clinical situation number seven. Abnormal left adrenal gland. Perform EUS-FNA. © Paul Clementsen, Denmark.



tion of the two techniques is more sensitive than EBUS-TBNA or EUS-FNA alone.

An increasing amount of papers propose to combine EBUS-TBNA and EUS-FNA performed exclusively with the EBUS scope both in the airways and in the esophagus (EUS-B-FNA). The rationale is that the EBUS scope is inserted in the trachea first and thereafter in the esophagus. A recent meta-analysis [23] investigated the diagnostic yield of EBUS-TBNA alone and the additional diagnostic gain of EUS-B-FNA over EBUS-TBNA. The sensitivity of the combined procedure was significantly higher than EBUS-TBNA alone (91% vs 80%) in lung cancer staging. In the RCT by Navani et al. [24] 133 patients were enrolled and randomized to EBUS-TBNA (66) or conventional work up (mediastinoscopy, CT guided lung biopsy, conventional TBNA or other procedures not specified). EUS-B-FNA was used if a target node could not be reached with EBUS-TBNA. The primary outcome was the median time to treatment decision and it was found to be shorter with EBUS-TBNA (14 days) than with conventional work-up (29 days) resulting in a hazard ratio of 1·98, (1·39-2·82). EUS-B-FNA was used in two patients for sampling station 5 lymph nodes. Another RCT by Kang et al. [25] randomized 160 patients to receive EBUS-FNA followed by EUS-B-FNA (EBUS-centred group, 60) or to receive EUS-B-FNA followed by EBUS-FNA (EUS-B-FNA centred group, 60). This trial suggested that even if diagnostic values and patient satisfaction were not different between the EBUS-centred and EUS-centred groups, adding EBUS-TBNA to EUS-FNA results in an increasing diagnostic accuracy and sensitivity. Oki et al. [26] found a sensitivity for EBUS-TBNA, EUS-FNA, and for the combined approach of 52%, 45%, and 73%, respectively. Lee et al. [27] found a sensitivity for EBUS-TBNA of 79%, that raised to 100% when EUS-B-FNA was added. Thus, the addition of EUS-B-FNA to EBUS-TBNA is valuable for mediastinal lymph node staging in lung cancer patient and it can be used for lesions that are inaccessible or difficult to access with EBUS-TBNA with a better sensitivity and accuracy compared with the use of EBUS-TBNA exclusively.

Lymph node sampling

In patients where the clinical suspicion of mediastinal node involvement remains after a negative result using a needle technique, surgical staging (eg, mediastinoscopy, video assisted thoracic surgery (VATS), etc) is in general advised. It is not completely clear how many and which lymph node stations should be sampled and which level of thoroughness is necessary for different situations. Sampling of at least three different mediastinal nodal stations (4R, 4L, 7) is suggested in patients with NSCLC and abnormal mediastinum by CT or PET-CT [10].

Lymph node stations 5 and 6 are not easy accessible with endosonography due to the interposition of the aorta and the left pulmonary artery. These two stations can be important to biopsy for correct staging of the patient especially in cases with a left upper lobe lung tumor [28]. These two lymph node stations can be assessed invasively via Chamberlain procedures, VATS, or extended cervical mediastinoscopy [28]. In this case, endosonography was not recommended because mediastinal vascular structures may preclude the access to lymph nodes in the aortopulmonary window. In those cases, a transvascular approach is necessitated. Only few retrospective studies investigated the diagnostic yield of endosonography with fine needle biopsy, where the biopsy neelde is passed through the big vessels (TVNA). A restrospective study by Panchabhai et al. [8], reported 10 procedures with EndoBronchial ultrasound-guided transvascular needle aspiration (EBUS-TVNA) to sample mediastinal lymph node (station 5) and lung lesions inaccessible by standard bronchoscopy or EBUS-TBNA. The final cytopathological diagnosis was obtained in nine patients: five non-small cell lung cancer, one small cell cancer, one metastatic colon cancer, and two cases with the finding of normal lymphoid tissue. In one patient necrosis was demonstrated in the biopsy and consequently required video assisted thoracoscopic surgery where histoplasmosis was diagnosed. Bleeding was not relevant, with no short-term/long-term complications. Von Bartheld et al. [9] analyzed 14 consecutive patients that underwent transaortic EUS-FNA. The diagnosis was made in eight patients without major complications. In two patients, EUS images after biopsy were suspicious for a small para-aortic hematoma but they recovered uneventfully. These results demonstrate that EBUS-TVNA and EUS-guided transaortic are a feasible and probably safe method that results in a diagnosis in the majority of cases, but more trials are warranted to explore their diagnostic potential.

Characteristics of the lymph nodes

The appearance of the lymph nodes can to some extent help to predict the probability of malignancy [29]. Especially suspicious looking lymph nodes should attract attention in respect to biopsy, but no single characteristic can exclude a visualized lymph node from biopsy.

Increasing size of lymph nodes is associated with increasing risk of malignancy. Round shape, distinct margin, heterogenous echogenecity and presence of coagulation necrosis sign are independent predictive factors for nodal metastases.

Important characteristics are:

- Size: in short axis, more or less than 1 centimeter.
- Shape: oval or round. When the ratio of short versus long axis of the lymph node is smaller than 1.5, the lymph node is defined as round.
- Margin: indistinct or distinct; if the majority of the margin (>50%) is clearly visualized with a high echoic border, the lymph node is determined as distinct. If the margin is unclear, it is determined as indistinct.
- Echogenecity: homogeneous or heterogeneous. Central hilar structure (presence or absence) defined as a linear, flat, hyperechoic area in the center of the lymph node.

- Coagulation necrosis sign (presence or abscence) defined as a hypo-echoic area within the lymph node without blood flow.

Restaging of lung cancer

EUS-FNA has a sensitivity in mediastinal restaging that varies between 44% and 75%, a diagnostic accuracy that varies between 60% and 92.3% and a negative predictive value (NPV) between 42% and 91.6% [30-34]. EBUS-TBNA has a sensitivity that varies between 51.9% and 76%, a NPV between 20% and 78% and a diagnostic accuracy between 77 and 81% [35-37].
EBUS-TBNA combined with EUS-B-FNA seems promising also in mediastinal restaging after induction therapy. Szlubowski et al. [37] reported a diagnostic sensitivity and negative predictive value for EUS-B-FNA of 67.3% and 73% respectively, and the sensitivity, accuracy and NPV of EUS-B-FNA were higher when compared with EBUS-TBNA and EUS-FNA alone. However, compared to a surgical techniques like the transcervical extended mediastinal lymphadenectomy (TEMLA), EBUS-TBNA or EUS-FNA has a lower diagnostic yield with a sensitivity of 100% and 64.3% respectively, and a NPV of 100% and 82.1% [34].

Biopsy from lung tumors

Only a few studies have investigated the diagnostic yield and safety of EBUS-TBNA and EUS-FNA from lung tumors. The guidelines suggest that in patients with centrally located lung tumor not visible at conventional bronchoscopy, endosonography should be the next step if the tumor is located immediately adjacent to the larger airways or the esophagus (Figure 10, 11, 12) [10 11]. Vazquez-Sequeiros et al. [38] analyzed 73 consecutive patients with centrally located tumor that underwent EUS-FNA: the overall sensitivity was 96.7% with a diagnostic accuracy of 96.7%. In a prospective study by Annema et al. [39] EUS-FNA provided a diagnosis of malignancy in 97% of the patients (31/32). It is interesting that in 39% of the patients, EUS-FNA also staged patients as having T4 disease. In a retrospective non-comparative study Tournoy et al. [40] demonstrated that EBUS-TBNA is a sensitive tool in the diagnosis of centrally located tumor not visible at conventional bronchoscopy, with a sensitivity of 82% and a NPV of 23%. Verma et al. [41] found a sensitivity for EBUS-TBNA of 91.4% in the diagnosis of parenchymal lesions located close to the airways. Eckardt et al. [42] reported a diagnostic yield for EBUS-TBNA of 72%. Zhao et al. [43] found a sensitivity of 93.7% and a diagnostic accuracy of 93.9%; Nakajima et al. [44] reached a sensitivity and a diagnostic accuracy of 94.1% and 94.3% respectively. Two small retrospective studies [45 46] evaluated the role of EUS- FNA in lung mass: a diagnosis was established in all patients. Finally, Dincer et al. [47] investigated the diagnostic yield of EBUS-TBNA and/or EUS-FNA in pulmonary masses not adjacent to the airways or esophagus, with a median distance from

Fig. 11. A lung tumor located close to the esophagous, seen A) with chest X ray, B) with contrast CT scan, C) with EUS.

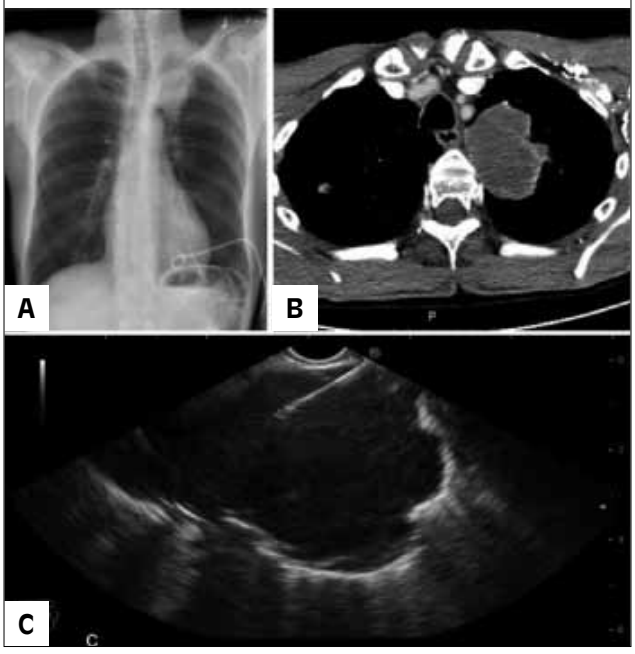


Fig. 10. A) Right upper lobe lung tumor close to the trachea beside the azygos vein. The tumor was visualized by EUS. B) Cytology smear showing neoplastic cells (adenocarcinoma) (Diff Quick, midpower).

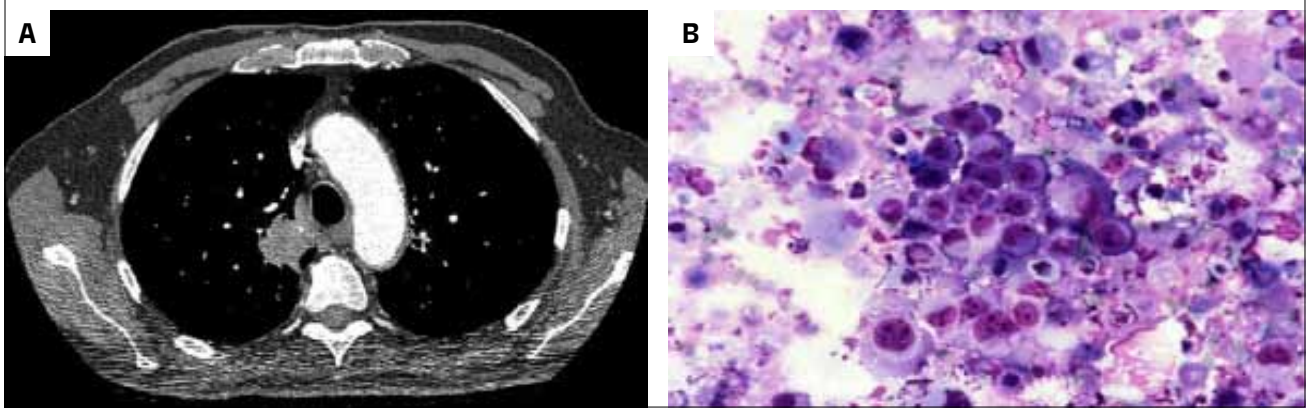
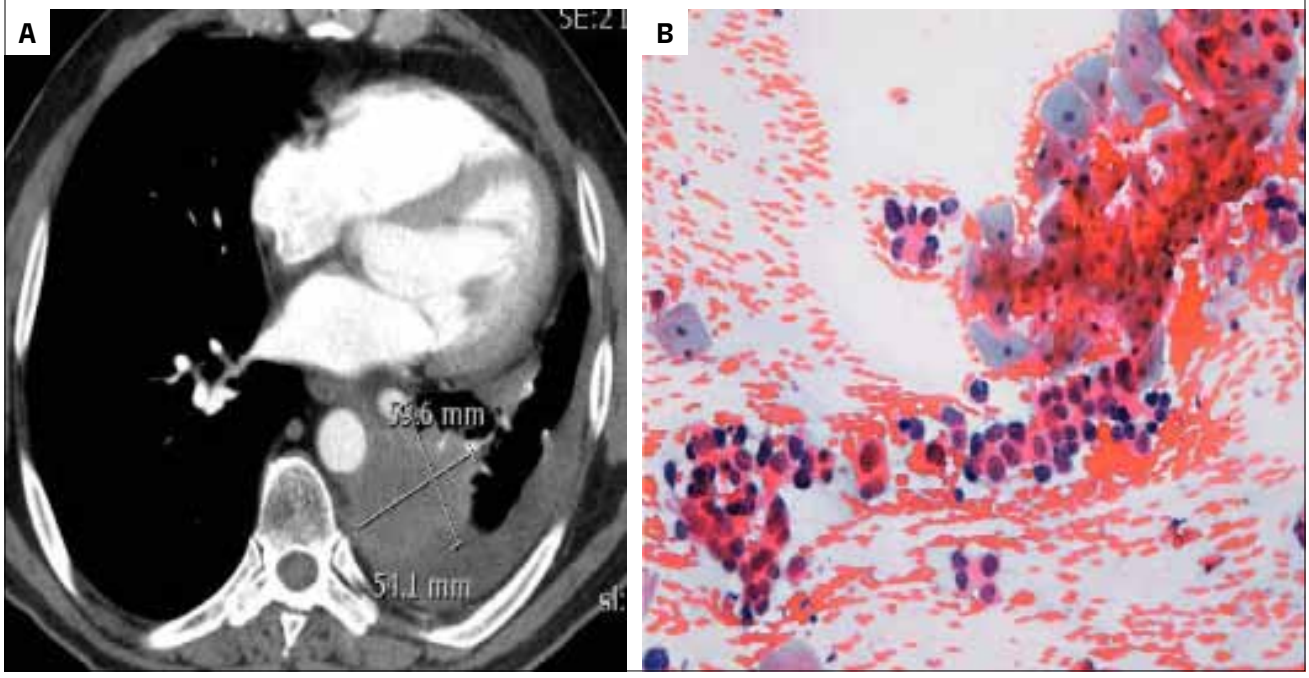


Fig. 12. Contrast enhanced CT scan shows a 5,4x 5.9 cm lesion in the left lower lobe associated with moderate left pleural effusion and mild atelectasis. The lesion is adjacent, without cleavage, to the left pulmonary vein, aorta and esophagous (A). Smear cytology obtained with EUS-FNA shows adenocarcinoma cells admixed to squamous esophageal cells (Papanicolaou, midpower)(B).



airway or esophagus of 19 mm (5-30 mm). A specific diagnosis was obtained in 15 patients (93.8%). In conclusion, EBUS-TBNA and EUS-FNA can be proposed as first diagnostic test in patients with centrally located tumor suspected for lung cancer following a negative bronchoscopy.

Biopsy from the liver

In patients with suspected or proven lung cancer and a lesion in the liver, a biopsy from the liver in most cases is necessary to rule out the suspicion of M1b-disease, which normally excludes surgery with curative intention [48]. Only a few studies evaluated the role of EUS for liver biopsy in these patients. Liver biopsy has traditionally been performed via a percutaneous, transjugular, or surgical approach. EUS-guided liver biopsy, however, has resulted in promising results in terms of tissue yield and procedural safety, producing specimens from the left liver lobe at least comparable and sometimes better than traditional procedures like transjugular, percutaneous and surgical approaches [49]. The right liver lobe cannot be routinely visualized by EUS [48]. In an international survey of 167 cases [48], EUS- FNA provided a diagnosis of primary liver cancer or liver metastases in 138 cases (83%); the ultrasonic features of the lesions like size, echogenicity and edge characteristics were not predictive of malignancy. Complications were reported in 6 out of 167 patients (4%), which means that liver biopsy with EUS-FNA in expert hand is relatively safe. Moreover, in comparison with the CT scan, EUS-FNA has been found to have a higher diagnostic accuracy in detecting the number of metastatic lesions and to be useful to identify the nature of lesions that were too small to be characterized on the CT scan [50 51].

Biopsy from the adrenal glands

EUS-FNA enables detailed imaging and sampling of both adrenal glands, but only biopsy from the left adrenal is considered as a routine procedure. EUS-FNA of the left adrenal gland is safe and accurate and has a very good profile compared with the percutaneous approach because the only organ traversed by the needle is the gastric wall. In contrast, EUS-FNA from the right adrenal gland is rather difficult because of the retrocaval location of the right adrenal gland. EUS identified the left adrenal gland in almost all cases (98%) and the right adrenal gland in only 30% of the cases.

In 150 patients that underwent EUS-FNA for lung cancer staging, the right adrenal gland was visualized in 131 patients (87.3%) and the left adrenal gland was visualized in all patients [52]. Puri et a.l [53] prospectively analyzed 21 patients with adrenal masses in which other imaging methods failed and/or were not feasible. EUS-FNA established a diagnosis in all cases and was able to distinguish between neoplastic and non- neoplastic disease: Ten patients were diagnosed with tuberculosis (shown by the presence of caseating granulomas [n = 10] and acid-fast bacilli [n = 4]). Two patients had EUS-FNA results suggestive of histoplasmosis. The other patients were suffering from metastatic lung carcinoma (n = 6), hepatocellular carcinoma (n = 1), and adrenal lipoma (n = 1) and adrenal myelolipoma (n = 1). Schuurbiers et al. 54 reported a sensitivity and NPV for EUS-FNA of the left adrenal gland in lung cancer of 86% and 70%.

Eloubedi [55] found that malignant masses were more likely to have an altered adrenal gland shape compared with benign masses, whereas a size of 30 mm or larger and hypoechoic nature were not. In the retrospective study by DeWitt et al. [56] the absence of enlargment of the left adrenal mass was related with non-diagnostic biopsies. Botger et al. [57] found that in 40 patients with known or suspected lung cancer a malignant Left adrenal gland (LAG) lesion was found in 28% and it was significantly associated with shorter survival.

Only small studies have reported the feasibility and safety of EUS-FNA of the right adrenal gland. A small study [58] reported the results of EUS-FNA in the 4 patients through the transduodenal approach. Three of the patients were shown to suffer from lung cancer metastasis in the right adrenal gland and in one patient a benign aspirate consistent with angiomyolipoma was obtained. No minor or major complications were seen. Four passes were performed in all cases, and the diagnosis was rendered on the first pass. Sharma et al. [59] reported 2 cases of EUS-FNA from the right adrenal gland in which lung adenocarcinoma was diagnosed without any complications. Six FNA passes were performed from the duodenal sweep with a 22-gauge needle. Eloubeidi et al. [60] described a case of right adrenal gland biopsy performed with EUS-FNA in a patient in which percutaneous biopsy was declined due to estimated high risk of bleeding. The biopsy showed metastatic lung cancer and no complications were observed.

Pleural fluid aspiration and pleural biopsy

Only few studies evaluated the role of EUS-FNA in pleural effusions and in pleural biopsy. One of the first reports was form Chang et al. [61]: in two patients pleural effusion not visible with CT or X-ray of the chest was demonstrated and aspirated with EUS-FNA. Metastatic cells from lung adenocarcinoma was found in the fluid. Lococo et al. [62] evaluated 10 patients in which a pleural effusion was detected and sampled. In 7 out of the 10 cases, the cytological examination of the fluid obtained by EUS-FNA was positive for malignant cells. EUS-FNA can be useful also in cases of pleural effusion secondary to other type of cancer for example endometrial cancer63. Vanderveldt et al. [64] described a case of malignant pleural effusion in pancreatic cancer diagnosed with EUS-FNA. Twine [65] evaluated 49 patients with esophageal cancer in which EUS defined pleural (39), pericardial (8) or ascitic fluid effusions (2).

Biopsy from metastases from extrathoracic malignancy

Yang et al. [66] conducted a meta-analysis concerning the role of EBUS-TBNA in the diagnosis of intrathoracic lymph node metastases from extrathoracic malignancies: 6 studies comprising 533 patients were included. EBUS-TBNA showed a sensitivity of 85% and the overall diagnostic odds ratio was 179.77. However, this meta-analysis has some limitation because included few studies, with a relatively small sized of patient populations.

Mesothelioma

Guinde et al. [67] presented a case of dry-type mesothelioma diagnosed by EBUS-guided needle aspiration of a pleural mediastinal mass and confirmed by a CT-guided needle aspiration of another pleural mass in close contact with the chest wall. A case of epithelioid mesothelioma diagnosed with EBUS-TBNA was reported by Lococo et al. [68]: a pleural mass in the right costovertebral recess, adjacent to the carina, was successfully biopsied with EBUS-TBNA. Kang [69] described a case of a mesothelioma presenting with pleural effusion and at the same time with multiple mediastinal lymphadenopathies, in which a diagnosis was obtained with EBUS-TBNA from after negative repeated thoracentesis, transbronchial lung biopsy, bronchoalveolar lavage, and thoracoscopy. A similar case was reported by Hamamoto [70]: histopathological examination of the lymph node specimens obtained by EBUS-TBNA showed epithelioid-like large atypical cells, immunohistochemically positive for calretinin and cytokeratin 5/6, and negative for Carcino embryonic antigen (CEA) and TTF-1.

Small Cell Lung Cancer

According to the recent ACCP guidelines [71], also in patients with clinical stage I small cell lung cancer (SCLC), who are considered for curative intent surgical resection, invasive mediastinal staging and extrathoracic imaging (head MRI/CT and PET or abdominal CT plus bone scan) are recommended.

Endobronchial ultrasound-guided transbronchial needle aspiration has a high diagnostic yield for the evaluation of mediastinal and hilar lymph node metastasis in SCLC with a sensitivity and a diagnostic accuracy of 96.4% and 97.2%, respectively [72]. Moreover, EBUS-TBNA has the potential role to provide a large numbers of tumor cells suitable for histopathlogic, immunohistochemical and genomic analysis [73]. Murakami et al. [74] found that in 780 patients the overall diagnostic yield of EBUS-TBNA for SCLC was 97%. Rapid on site evaluation (ROSE) was performed at the operator's discretion in 77 procedures. ROSE did not have any impact on diagnostic yield (99% with ROSE vs. 90% without ROSE, $p = 0.1$), but the use of ROSE was associated with fewer lesions (mean 1.1 with ROSE vs. 1.6 without ROSE, $p < 0.01$) or aspirates (mean 2.3 with ROSE vs. 4.0 without ROSE, $p < 0.01$).

EBUS and EUS in the diagnosis of mediastinal and lung rare tumors

There are several descriptions in the literature on the diagnosis of both benign and malign rare tumors with EBUS and EUS.

A case of an anterior mediastinal schwannoma with EBUS was reported by Cifti et al. [75]. Haarmann et al. [76] described a case of mediastinal lymphangioma diagnosed with EBUS in which EBUS was useful also for the therapeutic management. A metastatic chondrosarcoma in the superior segment of the lung left lower lobe was diagnosed with EBUS-TBNA using a 21-G needle without the need for further tissue sampling [77]. The reli-

ability of FNA cytology was confirmed also by Dyhdalo et al. [78]: a mucoepidermoid carcinoma was diagnosed with EBUS from a well-circumscribed nodule in the right lower lobe bronchus. In this case, ROSE revealed a pattern of cells indicating the presence of a low-grade epithelial neoplasm, suggesting a mucoepidermoid carcinoma and this diagnosis was confirmed by both cytology and core biopsy. Moreover, Okamoto [79] described a case in which EBUS allowed a diagnosis of combined thymic epithelial tumor consisting of small cells neuroendocrine carcinoma and thymic carcinoma by biopsy from a 5.5 cm mass in the superior and anterior mediastinum. Moonim [80] reported 3 cases of type B thymoma (one each of B1, B2 and B3 subtypes) and 1 case of thymic carcinoma diagnosed on EBUS-TBNA, using cell blocks, immunocytochemistry and flow cytometry. Finally, Yoshida [81] reported 2 cases of thymomas diagnosed by histopathological specimens obtained with EBUS-TBNA.

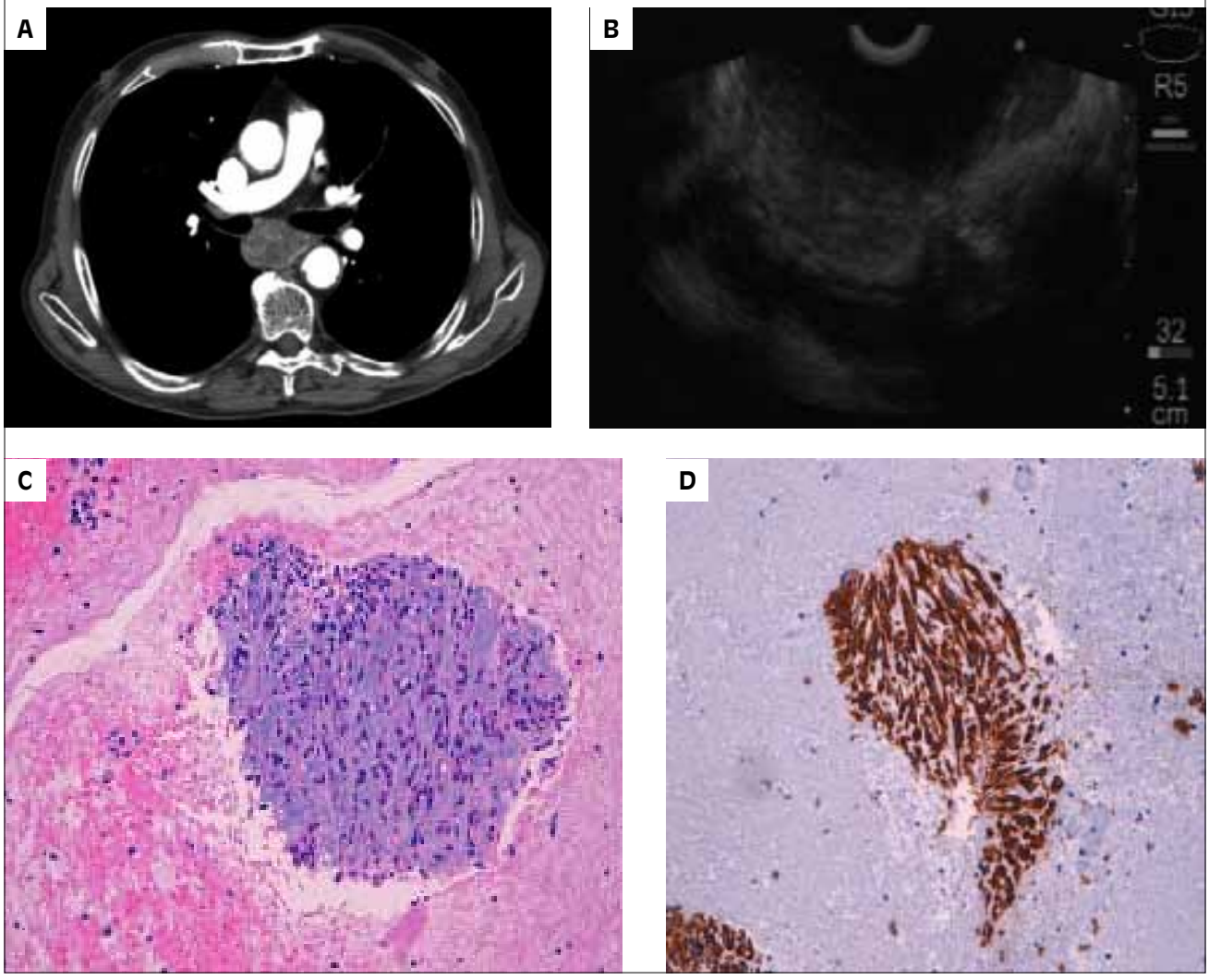
About EUS, compared to EBUS a smaller number of studies are available. Nath et al. [82] described a case of primary pulmonary leiomyosarcoma in the left upper lobe with EUS. A pulmonary inflammatory myofibroblastic tumor with a mediastinal nodal metastatsis was described by Borak [83]. The diagnosis was made with EUS-FNA in conjunction with immunohistochemical, and molecular studies, like fluorescent in situ hybridization for Anaplastic lymphoma kinase (ALK) gene rearrangement.

Thus, several case reports demonstrated the value of EBUS and EUS in the diagnosis of rare mediastinal and lung tumors and also in those case, as it is for lung cancer, ancillary molecular studies help in the diagnosis (Fig. 13).

Specimen adequacy and handling of the samples

The acquisition and preparation of EBUS-TBNA and EUS-FNA specimens have a key role in the procedure

Fig. 13. A) CT scan showing a subcarinal, highly vascularized, mass. B) EUS picture showing the lesion 7. C, D) Cell block obtained by EUS aspiration: c)elongated cells embedded in a bluish extracellular matrix (H&E, low power). D) The elongated cells are clearly positive for CD117 monoclonal antibodies. The final diagnosis was Gastro Intestinal Stromal Tumor (GIST).



performance, since even the most correctly performed biopsy procedure may be thwarted if the handling of the sample is not correct. Recent guidelines [84] have addressed this point in EBUS procedures, in order to standardize the specimen handling, to optimize the procedure outcomes and to provide a practical procedure description. First of all, several aspects of the acquisition technique were assessed: number of aspirates per LN, needle type, use of miniforceps, use of suction, type of sedation, time spent with the needle inside the node and number of revolutions inside the node (needle movements from the proximal to the distal side of the lymph node). Three aspirations per lymph node seem to provide the maximum diagnostic yield. Twenty-two Gauge and Twenty-one Gauge needle are equal for cytological and histological specimens: the needle size does not affect the diagnostic yield or the quality and the quantity of the specimens. The cell block technique employs the retrievial of small tissue fragments from a FNA specimen which are processed to form a paraffin block [85-87]. The use of forceps do not increase the diagnostic yield in lung cancer, but seems to increase the yield in lymphoma [88] and in sarcoidosis [89]. The use of suction does not affect the quality, the quantity and the diagnostic yield [90 91] and neither does the use of general anesthesia vs sedation [78 81] or the number of revolution inside the nodes does not. Ost et al. [92] demonstrated that the diagnostic yield increased in those patients that underwent general anesthesia, and the latter was associated with the possibility to biopsy significantly more and smaller lymph nodes.

As a second point, the specimen preparation techniques (cytology slides, core tissue and cell block) were evaluated. Multiple techniques for specimen acquisition and preparation were reported but no direct comparisons of these techniques were performed. Cytology slides are generally adequate for the diagnosis of malignancies and both immunohistochemestry and mutation analysis, though the use of specimen preparation techniques that allow cell block formation in general improve the ability to determine NSCLC subclassification. When needed, the smear used for ROSE can be destained and used for definitive cytological assessment (and immuno- cytochemistry or molecular tests) [93-96].

A close contact with the local pathologists is important to agree on the methods for specimen preparation, since they vary between centers depending on the preference/ expertise of pathologists [97 98]. ROSE offers the possibility to have an immediate feedback on the quality of the obtained specimens and is highly concordant with the final diagnosis but actually evidences are insufficient to recommend ROSE in every procedure. It is still under discussion if ROSE used routinely increases the diagnostic yield. Moreover, ROSE has not been proved to reduce the number of aspirations, the duration of the procedure, the need of additional procedures and the rate of complications [99-101].

Finally, the acquisition techniques, the specimen preparation and ROSE impact on molecular testing was evaluated in several studies. The material obtained by EBUS-TBNA was suitable for molecular testing in the majority of cases, but largely depending on the absolute number of tumor cells, their percentage in the sample, their degree of preservation and the type and sensitivity of the molecular test utilized [102-104]. According to the guidelines, molecular testing is not affected by the acquisition techniques, but 4 aspirations are warranted when the molecular tests are needed [105]. Neither specimen preparation techniques seem to impact on the ability to perform molecular tests. Cell blocks and core tissue represent the best material for genetic analysis and are most always indispensable at the moment to assess ALK translocation, cytological slides can be successfully used to determine the status of Epidermal growth factor receptor (EGFR) mutations, ROS-1 rearrangement, proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene (BRAF) mutations and Kirsten rat sarcoma (KRAS) in cases where cell blocks or core tissue are lacking or feature an insufficient burden of tumor cells. Both smear and cell- block preparations or core tissue can be utilized for molecular testing. A recent retrospective study by Rooper et al. underlined that EBUS-TBNA could subtype NSCLC allowing both immunohistochemistry and molecular analyses in a single procedure without repeating the procedure or more invasive tests. In this study the use of immunohistochemistry did not diminish the ability to perform molecular diagnostic tests on cell block samples, irregardless of tumor site, results of ROSE, the number of passes and other procedure variables [106].

Thus, since adequate specimen acquisition and handling are important to correct diagnose and stage lung cancer, a global unification of the procedures is needed in order to maximize the diagnostic yield of endosonography procedures.

Granulomatous diseases

Tuberculosis

Tuberculous lymphadenopathy is a manifestation of extrapulmonary tuberculosis (TB), but the clinical diagnosis is challenging because of the lack of specific clinical and radiological characteristics. A tissue diagnosis is therefore recommended in order to exclude malignancy or other specific non-malignant diseases like sarcoidosis. It must also be remembered that some patients present with both TB and lung cancer, which further underlines the importance of providing a biopsy. Specimens obtained from endosonography contribute to the diagnosis of TB allowing the identification of the necrosis of the bacterium, polimerase chain reaction (PCR) analysis of Mycobacterium Tubercolosis and the culture of the bacterium. Several studies have investigated the role of EBUS-TBNA and EUS-FNA in the diagnosis of TB and their ability to distinguish between malignancy, sarcoidosis and TB [107 108]. Recently, a meta-analysis by Ye et al. [109] found a pooled sensitivity of EBUS-TBNA for diagnosis the of intrathoracic TB of 80% (range 50%-

95%) and a specificity between 91% and 100%. The addition of EBUS-TBNA at flexible bronchoscopy significantly increased the diagnostic yield in patients with lymphadenopathy [110]: the sensitivity for bronchoscopy alone was 18.1% and raised to 80% when EBUS-TBNA was added. In one of the first studies investigating the role of EBUS-TBNA in the diagnosis of tubercolous mediastinal lymphadenitis, Navani et coll. [111] reported that EBUS-TBNA was provided a diagnosis of TB in 146 out of 156 cases, with a sensitivity of 94%. In 74 patients a positive culture of Mycobacterium tuberculosis was obtained. EBUS samples with necrotic granulomas or necrosis alone were more likely to have a positive culture for TB. Caglayan et al. [112] found a diagnosis of TB in 16 out of 72 patients, with a sensitivity of 84.2% (3 false negative results). The sensitivity increased with the number of lymph nodes stations sampled and with the number of passes. Senturk et al.eagues [113] reported that EBUS-TBNA samples were suitable for PCR testing for detecting TB. Out of 93 patients, TB was diagnosed in 27, with an overall sensitivity of 90%. The sensitivity of PCR was 56.7%, the specificity was 100%, and the general efficiency of the test was 96.4%. Dhooria et al. [114] compared the endosonographic features of tuberculosis and sarcoidosis. Among 165 patients a diagnosis of sarcoidosis was made in 118 cases and of TB in 47. Heterogeneous echotexture and coagulation necrosis were significantly higher in tuberculous lymph nodes. When a positive Tuberculin skin test (TST) was associated with heterogeneous echotexture or coagulation necrosis there was a specificity of 98% and positive predictive value of 91%. Thus, sonographic features of heterogeneous echotexture or coagulation necrosis in the lymph nodes with EBUS are indicative for TB and along with a positive TST, these features strongly suggest a diagnosis of TB over sarcoidosis. Sun et al. [115] prospectively studied 59 patients: 41 had TB, 5 lung cancer, 7 non-specific inflammation, and 6 had sarcoidosis. Pathologic findings were consistent with TB in 80% of patients (33 of 41), and in 27% (11 of 41) the smear was positive. Thirty-seven patients with TB had cultures and of these 17 were positive. The short-axis diameter was an independent risk factor associated with positive pathology, smear, and culture. Additionally, pathology showing necrosis was associated with a positive culture. However, most studies did not report any information about HIV infection status, keeping in mind that TB is more frequently represented in immunocompromised individuals, including those coinfected with HIV [99], Only the study from Navani et al. [101], included 17 HIV-positive patients, and six of them had a positive culture for TB. A previous report has shown that EBUS-TBNA may diagnose also non-TB mycobacterial disease in a patient with HIV116 but further data are required on the utility of EBUS-TBNA in HIV-infected individuals. Puri et al. [117] evaluated the diagnostic yield of EUS-FNA in the diagnosis of intra-abdominal lymphadenopathy. One hundred thirty patients were analyzed: EUS-FNA made the final diagnosis in 90.8% of them, 76.1% were found to have TB, the rest had sarcoidosis, Hodgkin's lymphoma and non-Hodgkin's lymphoma. In 8.4% of patients, nodes were inaccessible because of their retropancreatic location. Also the prospective study from Dhir et al. [118] found that EUS-FNA reached the diagnosis of TB in 35 out of 66 patients with intra-abdominal lymphadenopaties, with a sensitivity of 97.1%. Manucha et al. [119] highlighted the dilemma of tuberculosis versus sarcoidosis in regions with high prevalence of TB. They found that out of 281 aspirates, EUS-FNA was diagnostic of granulomatous lymphadenitis in 206 cases, 76 TB and sarcoidosis in 7 cases only. In remaining 123 cases, the etiology of granulomatous lymphadenitis could not be established and clinical correlation was suggested. A retrospective study by Puri et al. [120] evaluated symptoms, endoscopic features, EUS features, pathological yield, and response to treatment in patients with esophageal TB. EUS showed lymph nodes adjacent to esophageal pathology in all cases. Subcarinal region was the most common site of lymphadenopathy and they were matted, heterogeneous with predominantly hypoechoic center. The primary symptom was dysphagia, and endoscopy ulcers were showed in 18 out of 32 cases and extrinsic bulge in 20 in middle one third of esophagus. Histopathology of endoscopic biopsy of ulcers and EUS-FNA of lymph nodes provided the diagnosis of tuberculosis in 27 patients. As in lung cancer staging, EUS-FNA can be used also for regions other than lymph nodes, like the liver, the left adrenal gland and the pleura, in the suspicion of extrapulmonary TB, but only few and small studies are present in the literature. Itoi et al. [121] described a case of a woman with a 7 cm multilocular and multiseptate cystic lesion around the head of pancreas and caudate lobe of the liver. EUS-FNA confirmed the diagnosis of liver abscess and an EUS-guided liver abscess drainage was carried out, with a culture positivity for TB. Macias-Garcia [122] described a case of tuberculosis at the porta hepatis diagnosed with EUS-FNA. Larghi et al. [123] described a case of a woman with diffuse right pleural thickening and subcentimetric mediastinal lymph nodes that underwent EUS-FNA. A biopsy taken from both the pleura and lymph nodes showed TB granulomas. Puri et al. [124] confirmed that EUS-FNA is a safe and effective method for evaluating adrenal masses; out of 21 patients, 10 patients were diagnosed with TB shown by the presence of caseating necrosis. In conclusion, EBUS-TBNA and EUS-FNA are valuable tools for the diagnosis of TB due to their less invasiveness and their high diagnostic yield and sensitivity. Samples are suitable for histology, cytology, culture and PCR. Sonographic features like heterogeneous echotexture or coagulation necrosis are specific for TB, especially when associated with other test like a positive TST. Finally, endosonography, like in lung cancer, could detect TB not only in the mediastinal lymph nodes but also in the intra-abdominal lymph nodes and in other organs like liver, adrenal glands and pleura.

SARCOIDOSIS

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology; the diagnosis needs a compatible clinical picture and histological or cytological demonstration of noncaseating granulomas.The exclusion of other diseases is mandatory, as noncaseating granulomas can be found in other diseases like lung cancer or malignant lymphoma [125]. The granulomas can be found in almost any part of the body, but occur more often in the lungs, lymph nodes, eyes, skin, and liver. The most common intrathoracic manifestation of sarcoidosis is mediastinal lymphadenopaty, usually in station 4R, 4L, 7 and station 10 and 11. Chest CT scan can help in rendering the diagnosis more or less likely, giving information about the presence of mediastinal lymphadenopathies, the presence of lymph node calcification, lung masses, micronodules or fibrosis [126]. Bronchoscopy is the most often invasive technique used to confirm the diagnosis, allowing bronchoalveolar lavage, Trans bronchial lung biopsy (TBLB) and conventional TBNA (cTBNA). Mediastinoscopy is currently the 'gold' standard for sampling mediastinal lymph nodes but it is not routinely available at all centers, and is associated with morbidity and mortality. Thus as in other thoracic disease, endosonography, due to its minimally invasiveness and its safety compared to surgical procedures like mediastinoscopy and due to its better diagnostic accuracy when compared to cTBNA, plays a role in the diagnostic workup of sarcoidosis. Agarwal et al. [127] conducted a meta-analysis of the efficacy and safety of EBUS-TBNA in the diagnosis of sarcoidosis. Fifteen studies (nine prospective, six retrospective) with a total amount of 553 patients were included in the analysis. In the majority of the studies paratracheal, subcarinal, hilar and interlobar lymph nodes were sampled with four passes in each node. In all studies a 22G dedicated EBUS-TBNA needle was used. Five studies included additional rapid on-site cytology, and two studies included liquid-based cytological technique for diagnosis. The diagnostic yield of EBUS-TBNA (number of diagnosis of sarcoidosis with EBUS-TBNA/ number of patients with confirmed sarcoidosis) ranged from 54 to 93% with a pooled diagnostic accuracy of 79%. The diagnostic yield was significantly higher in prospective studies (83.9%) vs. retrospective studies (74.3%), reflecting a higher heterogeneity in the retrospective study design. The use of ROSE did not increase the diagnostic yield. Only five minor complications were reported (minimal pneumothorax, minor bleeding, airway edema/hypoxemia, prolonged cough).The GRANULOMA study [128] was a randomized multicenter study (14 centers in 6 countries) in which 304 consecutive patients with suspected pulmonary sarcoidosis (stage I/II) were randomized to receive bronchoscopy with TBLB and Endo bronchial lung biopsy (EBLB) (n = 149) or endosonography (EBUS-TBNA or EUS- FNA) with aspiration of intrathoracic lymph nodes (= 155). All patients also underwent bronchoalveolar lavage. Significantly more granulomas were detected in the endosonography group (114 vs 72 patients; 74% vs 48%) with a diagnostic yield of 80%, while for bronchoscopy the diagnostic yield was 53%. However, the study has some limitations: firstly only stage I and II sarcoidosis patients were included and secondly blind TBNA from the lymph nodes was not performed in the bronchoscopy group. Based on CD4/CD8 ratio, the sensitivity of the bronchoalveolar lavage was 54% for flow cytometry analyses and 24% for cytospin analyses. Another recent randomized trial [129] compared TBNA with EBUS-TBNA and EUS-FNA in stages I and II of pulmonary sarcoidosis. In patients with negative biopsy results, a second procedure was performed: EBUS-TBNA in case of a negative TBNA and EUS-FNA and EUS-TBNA in case of negative EBUS-TBNA. If both tests were negative, patients in stage I were scheduled for mediastinoscopy and those in stage II for TBLB. Sensitivity and accuracy of TBNA, EBUS-TBNA and EUS-FNA were 62.5% and 64.7%, 79.3% and 80%, and 88.6% and 88.9%, respectively. In 14 patients with negative results of standard TBNA and in 7 patients with negative results of EBUS-TBNA, EUS-FNA was performed and demonstrated granulomas in 9 patients, while in 5 patients with negative results of EUS-FNA, EBUS -TBNA was performed and did not reach the diagnosis in any patients. The authors concluded that EUS- FNA is the method of choice in granulomas detection. Li et al. [130] compared the diagnostic yield of TBNA and EBUS-TBNA in patients with suspected stage I and II sarcoidosis: patients underwent the same number of needle aspiration lymph nodes and the same lymph nodes needle aspiration times. The overall diagnostic yield for cTBNA was 64% and 93% for EBUS-TBNA. The diagnostic yield of cTBNA was similar to EBUS-TBNA if the lymph nodes were located on station 4 and 7 or if the shortest diameter was greater than 15 mm. Gupta et al. [131] in a randomized controlled trial with 130 patients found that EBUS-TBNA compared to cTBNA had an higher diagnostic yield indemonstrating noncaseating granulomas (74.5% vs 48.5%), but it should be combined with TBLB (when required) for the optimal yield. The diagnostic yield of cTBNA (plus EBLB and TBLB) is similar to EBUS-TBNA plus TBLB. A prospective trial from Tournoy et al. [132] demonstrated that EUS-FNA could be a valuable tool for diagnosing sarcoidosis after negative flexible bronchoscopy results. EUS-FNA was able to avoid a surgical procedure in 47 patients out of 80, with a sensitivity (following negative flexible bronchoscopy results) of 71%. Annema et al. [133] investigated the role of EUS-FNA in 51 patients with suspected sarcoidosis: 36 patients underwent a prior nondiagnostic bronchoscopy and a diagnosis of noncaseating granulomas was made in 41 patients. Von Barthel et Coll134 evaluated 101 consecutive patients who underwent EUS-FNA of mediastinal LNs. The 55% of those patients had previously had a non diagnostic bronchoscopy. The sensitivity of EUS in detecting granulomas was 87% (cytology and cell-block analysis together) (stage I, 92%; stage II, 77%). In 33% of cytology negative patients (n = 6), granulomas were present in the cell block. The optimal

yield for granuloma detection was reached with four needle passes. One patient developed mediastinitis after EUS-FNA. The study from Iwashita et al. [135] suggested that FNA histology is better suited than FNA cytology for the diagnosis of stage I sarcoidosis, and EUS-FNA with a 19-gauge needle plays an important role in this process [125]. Histopathological examinations of FNA samples showed non caseating granulomas in 34 out of 36 cases, while cytological examination was able to make a diagnosis only in 28 cases. Interestly, Michael et al. [136] investigated the role of EUS-FNA in the diagnosis of intrabdominal sarcoidosis. Twenty- one consecutive patients with sarcoidosis and predominant mediastinal and/or intra-abdominal lymph nodes or masses underwent EUS-FNA. EUS-FNA was diagnostic for granulomas in 18 of 21 patients (86%). Out of 21 patients, 7 had intra-abdominal lymph nodes and/or masses, and EUS-FNA was diagnostic of sarcoidosis in 4 cases (57%). Imai et al. [137] evaluated the features of mediastinal lymph nodes with sarcoidosis. Out of 34 patients, 64.3% of the lymph nodes had a round shape, 71.4% had a distinct margin, and 88.1% exhibited homogeneous echogenicity. A germinal center structure was observed in 71.4% of the cases. In the context of shape and margin, no significant difference could be observed between sarcoidosis and lung cancer metastasis. However, homogeneous low echogenicity and the presence of a germinal center structure were observed in sarcoidosis more frequently than in lung cancer. Similar results were found from Annema [123]: a specific ultrasound features of clustered, well demarcated isoechoic lymph nodes were observed in sarcoidosis patients. Dhooria et Coll. [138] found that sonographic features of heterogeneous echotexture or coagulation necrosis in the lymph nodes on EBUS are fairly specific for TB. Determining factors in diagnosis of sarcoidosis with endosonography are the disease stage, short-axis diameter and more than one needle pass per lymph node. Serum angiotensin converting enzyme level, number of lymph node stations sampled per patient, ROSE, or the total number of passes performed per patient were not associated with a better diagnostic yield [139]. Thus, to obtain a higher diagnostic yield of EBUS-TBNA in pulmonary sarcoidosis without ROSE, operators should select the largest mediastinal or hilar LNs accessible and puncture with 3 to 5 passes. In conclusion, endosonography has proved to have a good diagnostic yield, higher than cTBNA even if the latter has a high diagnostic yield in stations 4 and 7 and in lymph nodes bigger that 1.5 cm. Endosonography has also a low complication rate and can provide both cytological and histological specimens. The ultrasonic pictures could distinguish sarcoidosis from other granulomatous disease but is unreliable in distinguish benign precesses from malignancy. Moreover, endosonography can be used not only in the mediastinal lLNs but also in intra-abdominal LNs.

Lymphoproliferative disorders

The diagnosis and classification of malignant lymphomas are based on the cytomorphologic findings, histological pattern, and immunophenotype. The usefulness of FNA cytology for establishing a diagnosis of metastatic carcinoma is commonly accepted but its use for the diagnosis of lymphoma is debated because not always allows a precise typing for a correct therapeutic approach. The most cited drawbacks of the use of FNA cytology is the low volume tissue samples, for its unsuitability in ancillary studies and for the loss of tissue architecture. However, there is a growing evidence that standard cytology, thin layer preparations in liquid medium or even better cell blocks of cells can be applicable not only for pathological diagnosis but also for further investigations such as immunohistochemistry and fluorescence in situ hybridization and molecular analyses [140 141]. Small samples seem to identify small cell lymphomas with highly distinctive immunophenotypes, including small lymphocytic, mantle cell, and T-lymphoblastic lymphoma; on the other hand, in case of follicular lymphoma and marginal zone lymphoma the diagnosis with a small biopsy could be difficult [142-145]. Due to its good diagnostic yield, its good safety and a less invasiveness compared to surgical techniques, endosonography is prosoped has been proposed as initial procedure in patients with mediastinal lymphadenopathies suspected for lymphoma [132 135]. Endosonography could avoid more invasive procedures like mediatinoscopy, especially in patients with high risk surgical risk and in those with masses in inaccessible sites for mediastinoscopy146. Moreover, it has been shown that endosonography may play a role not only in the first diagnosis of lymphoma but also in the re-staging of the disease, especially when the mediastinoscopy was the first diagnostic procedure. Infact, a second mediastinoscopy could be difficult due to adhesions and fibrotic changes formed after the first mediastinoscopy or after radiotherapy [35 147]. The reported sensitivity of EBUS for lymphoma ranges between 57%134 and 100% [148-150]. One of the first studies that retrospectively evaluated EBUS in the diagnosis of lymphoma was the study of Kennedy et al. [151]. They reported 25 patients with suspected lymphoma in which EBUS allowed adequate tissue sampling in 24 cases, with a diagnosis of lymphoma in 10 patients. The sensitivity for lymphoma was 90% (1 false negative results). The method of diagnosis of lymphoma by EBUS-TBNA was a combination of cytology, immunohistochemistry with and without flow cytometry in 6 and 4 patients, respectively.

In the study of Grosu et al. [152], out of 75 patients with lymphoma EBUS-TBNA was able to establish a diagnosis of lymphoma in 63 cases (84%)and was able to establish a diagnosis and subtype in 67% of patients with de novo lymphoma and 81% of patients with relapsed lymphoma. Senturk and coworkers [132], prospectively evaluated 68 patients with isolated mediastinal lymphadenopathies. A minimum of 3 needle passes was performed, and cells blocks and immonohistochemistry were done for each patients, flow citometry was not used. Out of 68 cases, 15 patients (22%) had lymphoma as a final diagnosis: 3 follicular center cell, 2 large B-cell primary

and 10 Hodgkin lymphomas (9 primary and 1 recurrent). EBUS-TBNA provided a definitive pathological diagnosis and histological typing were achieved in thirteen of fifteen (86.7%) patients and the two false negative results were two cases of follicular center lymphoma. The sensitivity, the NPV and the diagnostic accuracy of EBUS-TBNA for lymphoma were respectively 86.7%, 96.4% and 97%. One-hundred cases of de novo or relapsed mediastinal lymphoma were investigated with EBUS by Moonim et al. [138]. Classical Hodgkin lymphoma was diagnosed on EBUS- TBNA aspirates and high-grade B-cell non-Hodgkin lymphoma were diagnosed on morphology and immunohistochemistry on EBUS- derived cell block. The diagnosis of low-grade B-cell non-Hodgkin lymphoma was based on morphology and by identifying a light-chain restricted B-cell population either by flow cytometry or cell block immunohistochemistry. Further subclassification into chronic lymphocytic leukemia or small lymphocytic lymphoma, follicular lymphoma, mantle cell lymphoma, and marginal zone lymphoma was made on the basis of morphologic criteria with the demonstration of a specific immunophenotype. Sensitivity, negative predictive value, and accuracy were 89%, 83%, and 91%, respectively while sensitivity in subtyping lymphomas into high-grade non-Hodgkin lymphoma, low-grade non-Hodgkin lymphoma, and Hodgkin lymphoma was 90%, 100%, and 79%, respectively, indicating that EBUS is sensitive in subtyping Hodgkin lymphomas. Ko et al. [139] demonstrated that EBUS-TBNA provides sufficient sample for definitive primary diagnosis and classification of malignant lymphoma and granulomatous inflammation in patients with mediastinal lymphadenopathy. Out of 38 cases, 3 Hodgkin lymphomas and 7 non-Hodgkin lymphomas (1 small lymphocytic lymphoma, 1 small lymphocitic lymphoma with scattered Reed-Sternberg cells, 1 marginal zone lymphoma, and 4 large B cell lymphomas). Immunophenotyping and immunohistochemistry was done in six cases, and FISH in five cases provided necessary information for subclassification. Steinfort et al. [134] reported the value of EBUS-TBNA in mediastinal isolated lymphadenopathies: lymphoma was identified in 16 out of 21 patients, with a lower sensitivity compared with other studies (57%). Four patients required surgical biopsy was required to diagnose specific lymphoma subtypes not readily amenable to diagnosis with low volume specimens, so they criticized the use of EBUS-TBNA for some lymphoma subtypes, such as marginal zone lymphomas or hypocellular variants. Interestly, Ariza-Prota et al. [153] described a case of an anaplastic large cell lymphoma relapsed, diagnosed on tissue fragments obtained by EBUS-TBNA with the particularity of using a 22 G histological needle. Also Furukawa et Coll [154] presented a case of Hodgkin lymphoma presenting as isolated mediastinal adenopathy that was definitively diagnosed with EBUS using a 22 G coring needle in which cellular and histologic specimens were obtained, allowing the core biopsy to be fixed in formalin and treated as a surgical specimen. About EUS-FNA, Ribeiro et al. [155] showed that EUS- FNA has a lower yield in classifing Hodgkin lymphoma and low-grade lymphoma compared with high-grade diffuse large B-cell lymphoma. The diagnosis was reached in 19 out os 24 patients (79%) and subclassification was determined in 16 patients (66.6%). Flow cytometry correctly identified B-cell monoclonality in 95% (18 out of 19). In 1 patient with marginal-zone lymphoma the diagnosis was changed to hairy cell leukemia after a bone marrow biopsy. EUS-FNA had a lower yield in nonlarge B-cell lymphoma compared with large B-cell lymphoma. Yasuda et al. [156] assessed the yield of EUS-FNA biopsy using a 19 G needle in patients with mediastinal and intra-abdominal lymphadenopathy of unknown origin, especially in relation to subclassification of the lymphomas. The overall accuracy of EUS-FNAB for unknown lymphadenopathy was 98%; lymphomas were classified in 88% of cases in accordance with the World Health Organization classifications. Korenblit et al.157 demonstrated that EUS-FNA had a sensitivity and a diagnostic accuracy for lymphoma of 89.7% and 93.5%, with one false positive and 5 false negative cases. In a recent study by Talebian et al. [158] endosonography made the diagnosis of lymphoma in 33 out of 49 patients with suspected primary (n = 32) or recurrent (n = 17) lymphoma. Sensitivity and negative predictive value of endosonography in diagnosing primary versus recurrent mediastinal lymphomas were 55% and 57% versus 88% and 90%, respectively, concluding that endosonography could have some limitations in assessing a primary lymphoma diagnosis. Moreover, EUS-FNA could be of value also in other lymphoproliferative disorders. Conti et al. [159] reported a case in which EUS-FNA of the LAG allowed the diagnosis of lymphomatoid granulomatosis both with cytology and cell block, underlying the importance of the use of this procedure in identifying lymphoproliferative diseases. The use of ROSE in the diagnosis of lymphoma is unclear. In the study of Moonin et al.138 a consultant pathologist performed a real-time evaluation of the aspirates in order to maximize the diagnostic yield, to make a decision on the number of passes and to triage aspirates for ancillary techniques like immunohistochemistry, flow cytometry, cytogenetics, or molecular tests. The authors attributed their high diagnostic accuracy to the use of ROSE. Kennedy et al. [141] used ROSE to evaluate the adequacy of the specimes: 24 out of 25 specimens were considered adequate and the final diagnosis was reached in all the 24 cases. The number of passes varied from 2 to 5 [141 147 138]. Also Ko et al. [139] evaluated the usefulness of the ROSE during EBUS-TBNA as triage of sample for multiple ancillary techniques and ROSE is proposed as a valuable tool for appropriate assignment of sample to ancillary studies. Ultrasonic picture, as in lung cancer, are not reliable of malignancy. This was demonstrated in the study of Korenblit [147], in which lymph node morphologic features of roundness, echogenicity, and homogeneity on EUS were not a predictor of lymph node malignancy [147].

Thus, endonography seems to be promising in the di-

agnosis of malignant lymphomas as in proliferative disorders, with or withour ROSE, but in case of negative results surgical or other diagnostic techniques are mandatory due to the high risk of false negative results. The diagnostic yield is higher when combined with the flow cytometry or cell blocks. The ability to generate cell blocks from FNA obtained with endosonography allows cytologic material to be treated as histologic sections and this could overcome the problem of small biopsy specimens. Although most of the cells within the cell block are disaggregated, small fragments of tissue or slender cores are often identified; this can allow immunocytochemistry, molecular and ancillary tests to be interpreted in the context of the architecture, which is important in the lymphoma diagnosis.

Vascular disease

Due to its ability to outline thoracic anatomy, endosonography can be used beyond the conventional indications to evaluate vascular abnormalities, both of thrombotic and nonthrombotic origin. It is challenging to demonstrate the aetiology of filling defects in the pulmonary vessels on CT since it is not possible to distinguish a vascular tumor from a thrombotic embolus. PET-CT scan may help to differentiate malignant growth from benign emboli, but cytopathologic confirmation remains essential to confirm the diagnosis. EBUS-TBNA may be a useful tool to identify and sample endovascular abnormalities.

Pulmonary embolism

In cases of pulmonary embolism close to the central airways, EBUS may be useful for diagnostic purposes. A prospective multicenter pilot study [160] investigated the feasibility of detecting pulmonary embolism by EBUS. EBUS detected 96% of the emboli detected with the contrast CT scan, whilst the remaining patients had emboli in the middle lobe and in the left upper lobe artery. This study suggested that EBUS could be proposed in the diagnostic algorithm of pulmonary embolism in patients with contraindications to contrast agents, hemodynamic instability preventing transport and radiation exposure. Small case series [161] [162] addressed the role of EBUS in pulmonary embolism. An incidental diagnosis of pulmonary embolism was described by Le Rouzic et al. [163]: during an EBUS-TBNA procedure for mediastinal staging of a right upper lobe tumor a hypoechoic image was seen in the right pulmonary artery and a diagnosis of right pulmonary embolism was suggested and subsequently confirmed with CT scan. Thus, EBUS-guided imaging diagnosis of thrombi could perhaps be an alternative in hemodynamically stable, in patients with poor clinical conditions and in patients with contraindications to contrast agents. Moreover, EBUS can visualize the central vasculature and allow a biopsy from the emboli, enabling vascular malignant diseases to be easily differentiated from PE [164].

Non thrombotic lesions

It was already suggested that endosonography could be useful in the assessment of vascular infiltration (T4 disease) in patients with lung cancer [39], so in the same way it may be useful in the evalutation of primary vascular tumor. Modi et al. [165] reported a case of filling defects in the right pulmonay artery on CT scan that continued to worsen despite anticoagulation. EBUS-TBNA was performed and a diagnosis of metastatic leiomyosarcoma was done. EBUS-TBNA reached the diagnosis in a patient with bilateral pulmonary embolism of unexplained origin: the cytologic analysis of the cell aspirate was compatible with endovascular metastatic sarcoma [166]. Also Al-Saffar et al. [154] demonstrated that EBUS is useful to evaluate the nature of an endovascular lesion. Out of 12 selected cases, EBUS-TBNA was done in 10 patients and reached the diagnosis in 9 cases. The final diagnoses were: sarcoma (n = 6), lung cancer (n = 2), thyroid cancer (n = 1), renal cell cancer (n = 1), melanoma (n = 1), and pulmonary embolism (n = 1). Moreover, an endovascular lesion was incidentally noted in the pulmonary artery during EBUS for evaluating lymph nodes (n = 2). Shingoyoi and Park [167] [168] reported two cases with masslike lesions in the pulmonary artery discovered by EBUS: lesions were sampled and a diagnosis of pulmonary artery sarcoma was established. Hara et al. [169] proposed EUS as a valid tool to diagnose vascular invasion of cancer, especially hepatic hilus cancer. Mhoyan et al. reported a case of epithelioid hemangioendothelioma of the lung diagnosed by EUS-FNA [170]. Even if EBUS and EUS seem promising in the diagnosis of vascular diseases, its use routinely is not recommended. It is important to state that a diagnosis of acute thromboembolic disease is based on clinical evaluation and imaging techniques, such as ventilation/perfusion lung scan, chest CT and pulmonary angiography. Moreover, physicians should be aware of the potential complications of EBUS-TBNA in patients with pulmonary vascular tumor or with pulmonary thromboembolic disease [171]: a large proportion of patients with proximal pulmonary artery chronic obstruction by sarcoma or thromboembolic material may present with pulmonary hypertension, a condition associated with a high risk of complication following TBNA. Indeed, it has been clearly demonstrated that proximal obstruction of pulmonary arteries may be associated with hypertrophy of systemic bronchial arteries, increasing the risk of haemorrhage from transbronchial needle aspiration.

How to learn endosonography

The European guidelines [10] recommend that new trainees in endosonography follow a structured training curriculum consisting of simulation-based training followed by supervised practice on patients. A systematic training should be based on firstly theoretical knowledge [172], secondly training in simulators [173], and thirdly supervised performance on patients [174]. Ideally each of these three steps should be followed by a validated test and the learning curves should be monitored by specific tools assessments [175]. In a randomized study with performance on real patients as an outcome parameter

it was shown that simulator trained novices scored significantly higher than novices who had trained on real patients supervised by experts [176]. There are two virtual-reality simulators commercially available for EBUS, the GI Bronch Mentor™ (Simbionix, Cleveland, Ohio, USA) and the AccuTouch Flexible Bronchoscopy Simulator™ (CAE Healthcare, Montreal, Que., Canada). EUS or EUS-B simulators for the diagnosis and staging of lung cancer are not yet available. The classical approach when learning endosonography is to learn the six basic landmarks for EBUS and for EUS in a systematic order [177]. To avoid both complications in connection with the procedures and secondly to avoid incorrect staging of lung cancer patients, basic competency must be ensured before trainees are allowed to perform independent procedures.

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