REVIEW: ENDOSCOPY

Diagnostic bronchoscopy: state of the art

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ABSTRACT: Since the introduction of the flexible fibreoptic bronchoscope in the late 1960s there have been relatively few technological advances for three decades, aside from the development of a white light video bronchoscope with a miniature charge-coupled device built in its tip replacing the fibreoptics. White light flexible videobronchoscopy with its ancillary devices (forceps biopsy, bronchial brushing, bronchoalveolar lavage, bronchial washings and transbronchial needle aspiration) has long been the only established diagnostic bronchoscopic technique. With the advances in microtechnology over the past two decades, recent technical developments such as autofluorescence bronchoscopy and endoscopic ultrasound allow better evaluation of endobronchial, mediastinal and parenchymal lesions.

KEYWORDS: Autofluorescence bronchoscopy, endoscopic ultrasonography, transbronchial lung biopsy

Bronchoscopy is an invaluable diagnostic tool for many lung disorders and a safe procedure with low (0.1–2.5%) morbidity and very low (<0.05%) mortality. Since the introduction of the flexible fibreoptic broncho-scope in the late 1960s, there have been relatively few technological advances in imaging for three decades, aside from the development of the video charge-coupled device (CCD) replacing the fibre-optics. A conventional transbronchial needle aspiration (TBNA) has probably been the most important innovation to the ancillary diagnostic devices (forceps biopsy, bronchial brushing, bronchoalveolar lavage and bronchial washings), as it created the possibility of sampling beyond the wall of the central airways. The quality of the diagnostic bronchoscopic examination is certainly operator dependent, but the pathological analysis and interpretation of the acquired samples is also of eminent importance in the diagnostic process.

Technological advances in endoscopic imaging over the past two decades, such as autofluorescence bronchoscopy (AFB) and endoscopic ultrasonography (radial transducer at the tip of a miniprobe and curved-linear transducer at the tip of an endoscope), are nowadays playing an important role in clinical practice. We will discuss these advances in endoscopic imaging that contribute to a better evaluation of: 1) endobronchial, 2) mediastinal and 3) parenchymal lesions.

The newest diagnostic imaging techniques, such as high magnification videobronchoscopy, confocal fluorescence laser micro-endoscopy or optical coherence tomography bronchoscopy, are currently available but still require further research to delineate their role and clinical use within the airways.

EVALUATION OF ENDOBRONCHIAL LESIONS: AFB

AFB is an endoscopic instrument that uses an excitation source emitting light in the violet–blue part of the spectrum (400–450 nm). Following light absorption, chromophores in the airway mucosa can emit light, generating a fluorescence image that can discriminate normal from abnormal mucosa due to the difference in fluorescence emission: normal bronchial mucosa emits a green coloured fluorescence and abnormal bronchial mucosa emits a red-brown coloured fluorescence (fig. 1).

Based on a large amount of research during the past decade, AFB added to white light broncho-scopy (WLB) has a role in the routine work-up of patients suspected of having operable lung cancer based on chest imaging, or in patients with newly diagnosed lung cancer planned for resection, along with as a surveillance tool in patients with known central pre-invasive lesions, or in patients who have undergone previous curatively treated lung cancer. Video autofluorescence bronchoscopy systems are nowadays available, allowing easy access to anatomic and functional information at the time of the first bronchoscopy without significantly increasing the procedural time.

FIGURE 1. Autofluorescence bronchoscopy can discriminate normal from abnormal bronchial mucosa due to the difference in fluorescence emission: normal bronchial mucosa emits a green coloured fluorescence and abnormal bronchial mucosa emits a red-brown coloured fluorescence. Autofluorescence image of a patient with a history of lung adenocarcinoma resected 2 yrs prior (b); the arrows point at a moderate dysplastic lesion on a subsegmental carina which is difficult to identify with white light (a).

AFB for the detection of pre-invasive and radio-occult invasive lesions in high-risk patients

The value of AFB in detecting pre-invasive lesions and/or radio-occult early lung cancer in the large airways of patients in high-risk groups has been evaluated in different clinical and research settings [1–13]. Several studies described that the addition of AFB to WLB resulted in the detection of a significant number of early intra-epithelial pre-invasive lesions that would have been missed with WLB alone; as a result the ratio of sensitivity of (WLB+AFB)/WLB was consistently above 1. However, the sensitivity is ''relative'' as the true prevalence of pre-invasive lesions remains unknown. When considering pre-invasive lesions from moderate dysplasia to radio-occult invasive carcinoma, the addition of AFB improves the relative sensitivity for the detection of pre-invasive lesions in the large airways by a factor of 1.1 to 3.7 compared with WLB alone (table 1). When considering only pre-invasive lesions from moderate dysplasia to carcinoma *in situ* (CIS), the addition of

AFB improves the relative sensitivity for the detection of pre-invasive lesions by a factor of 1.4 to 6.3 compared with WLB alone (table 2). Differences between the studies may be explained by: 1) differences in high-risk profile of the patients studied and thus prevalence of radio-occult lesions; 2) differences in experience between bronchoscopists; 3) differences in observer variability in histopathologic reporting of bronchial biopsy samples; and 4) the growing operator experience over time with WLB resulting in a gradual decrease of the ratio (WLB+AFB)/WLB. The latter is also influenced by the fact that in earlier studies WLB was carried out using a fibreoptic bronchoscope, while more recent studies used a white light flexible videobronchoscope with a miniature CCD built into its tip delivering a clear image. These studies have also pointed out the low specificity of AFB, which is responsible for a high number of false-positive biopsies, and subsequently has an important impact on the cost-effectiveness of this technique. Recent technological developments, such as a

TABLE 1	Test characteristics of white light bronchoscopy (WLB) and autofluorescence bronchoscopy (AFB) in high-grade pre-invasive or radio-occult invasive central airway lesions					
First author [ref.]	Patients n	Publication yr	Prevalence# %	Sensitivity % WLB	Sensitivity % WLB+AFB	Relative factor
LAM [1]	173	1998	43	25	67	2.7
IKEDA [4]	158	1999	27	88	98	1.1
KUSUNOKI [10]	50	2000	32	85	94	1.1
HIRSCH [11]	55	2001	58	22	81	3.7
ERNST [6]	293	2005	NR	44	92	2.1
CHIYO [8]	32	2005	NR	62	85	1.4
CHHAJED [12]	151	2005	NR	72	96	1.3
LAM [13]	62	2006	13	58	92	1.6
EDELL [2]	170	2009	19	56	74	1.3

NR: not reported. #: moderate dysplasia, severe dysplasia, carcinoma *in situ* and invasive carcinoma.

TABLE 2	Test characteristics of white light bronchoscopy (WLB) and autofluorescence bronchoscopy (AFB) in high-grade pre-invasive central airway lesions					
First author [ref.]	Patients n	Publication yr	Prevalence# %	Sensitivity % WLB	Sensitivity % WLB+AFB	Relative factor
LAM [1]	173	1998	14	9	56	6.3
KHANAVKAR [3]	165	1998	NR	32	86	2.7
IKEDA [4]	158	1999	16	62	92	1.5
MORO-SILIBOT [5]	244	2002	5	36	86	2.4
ERNST [6]	293	2005	NR	21	88	4.2
HÄUBINGER [7]	1173	2005	4	58	82	1.4
CHIYO [8]	32	2005	NR	58	83	1.4
IKEDA [9]	154	2006	NR	65	90	1.4
EDELL [2]	170	2009	8	16	56	3.5

NR: not reported. #: moderate dysplasia, severe dysplasia and carcinoma *in situ*.

videobronchoscope that displays a composite image integrating an autofluorescence signal plus reflected green and red light signals, a dual digital video-autofluorescence imaging system and a narrow band imaging bronchoscopy, might have the ability to improve the specificity to detect (pre-)invasive lesions without compromising the sensitivity [14–16].

AFB for monitoring the evolution of pre-invasive lesions

There exists no standard algorithm for the surveillance of detected pre-invasive lesions or the management of high-grade pre-invasive lesions. Moreover, the understanding of the natural course of pre-invasive lesions such as severe dysplasia or CIS is incomplete, but they are believed to be precursors of invasive squamous cell carcinoma. Almost no low-grade pre-invasive lesions progress to a higher grade or invasive carcinoma (table 3). Even a large number of the individual high-grade pre-invasive lesions do not progress to invasive carcinoma (table 3). One potential reason why dysplasia regresses in up to two thirds of cases after an initial biopsy is complete removal of the pre-invasive lesion at the time of the biopsy. Although high-grade pre-invasive lesions have a high regression rate and low progression rate, these patients are at high risk of developing invasive lung cancer at any site in the lung [17, 18]. This is consistent with the ''field cancerisation'' concept, in which the entire bronchial epithelium is exposed to carcinogens and therefore at risk of developing an invasive carcinoma. Therefore, surveillance is required for the known high-grade pre-invasive lesion and for timely detection and

curative intent treatment of CIS or invasive carcinoma at any site in the bronchial epithelium.

AFB for detection/localisation of synchronous lesions and for post-surgical surveillance

Adding AFB to WLB reveals synchronous multicentricity of pre-invasive and radio-occult invasive lesions in 23% of the patients in whom high-grade pre-invasive lesions or radio-occult invasive lung cancer were detected during a preceding WLB alone [19].

Adding AFB to WLB reveals synchronous multicentricity of pre-invasive and radio-occult invasive lesions in 10% of the patients in whom radiographically visible invasive lung cancer was detected [20, 21].

AFB for staging/guiding treatment of potentially curable central CIS

AFB is important for the correct staging of radio-occult potentially curatively treatable early proximal lung cancer prior to endobronchial therapy, by obtaining a more accurate assessment of the lesion size and margins [22].

EVALUATION OF MEDIASTINAL/HILAR LESIONS: THORACIC ENDOSONOGRAPHY

Thoracic endosonography allows imaging beyond the mucosa into the mediastinum. Two techniques, namely the curved-linear trans-oesophageal ultrasound with real time guided

| TABLE 3	Natural course of pre-invasive central airway lesions		
Pre-invasive lesion	Regression %	Persistence %	Progression to CIS/invasive carcinoma %
Metaplasia	37–42	29	0–9
Mild/moderate dysplasia	64	22	0–11
Severe dysplasia	52–63	16	11–56
CIS	12	70	21–67

CIS: carcinoma *in situ*.

fine-needle aspirations (EUS-FNA) and curved-linear endobronchial ultrasound with real time guided TBNA (EBUS-TBNA), make it possible to obtain cytologic material from hilar and/or mediastinal lesions (figs 2 and 3). Their main indications are the diagnosis and/or mediastinal staging of (lung) cancer and the evaluation of suspected benign granulomatous diseases (such as sarcoidosis and tuberculosis), thereby challenging the current practice of mediastinoscopy and/or conventional TBNA (table 4) [23, 26].

Cervical mediastinoscopy is still considered the gold standard for mediastinal staging of unselected patients with resectable clinical stage I–III nonsmall cell lung cancer (NSCLC), based on a sensitivity of 78% and a negative predicted value of 91% in a very large population with a prevalence of mediastinal metastases of 39% [23].

A conventional TBNA can be performed during the initial flexible bronchoscopy if enlarged mediastinal lymph node(s) are present on computed tomography (CT) of the chest. The technique is in essence a blind aspiration, and therefore has a very variable diagnostic yield of 15–83%, mostly related to the size and location of the nodes and the operator's experience. A blind TBNA can be safely performed in lymph nodes with a short axis >10 mm. A blind TBNA is most often applied to selected lymph node stations. Lymph nodes with clear anatomic landmarks (such as lower right and left paratracheal mediastinal lymph nodes in position 4R and 4L, respectively, as well as the subcarinal lymph nodes in position 7 and hilar lymph nodes in position 11), which are clearly enlarged (largest short axis ⩾15 mm) can be adequately sampled using a needle through the working channel of the standard bronchoscope [27]. A recent meta-analysis reported a sensitivity of 78% and a false-negative rate of 28% for blind TBNA in clinical N2 disease with high disease prevalence of 81% [23, 28]. A blind TBNA is very useful if it leads to proof of N3 disease, but it should not lead to a conclusion of absence of N3 when only N2 disease is found (lack of negative predictive value).

FIGURE 2. Curved-linear trans-oesophageal endoscopic ultrasound with real time guided fine-needle aspiration of a tumoural mass in the left adrenal gland.
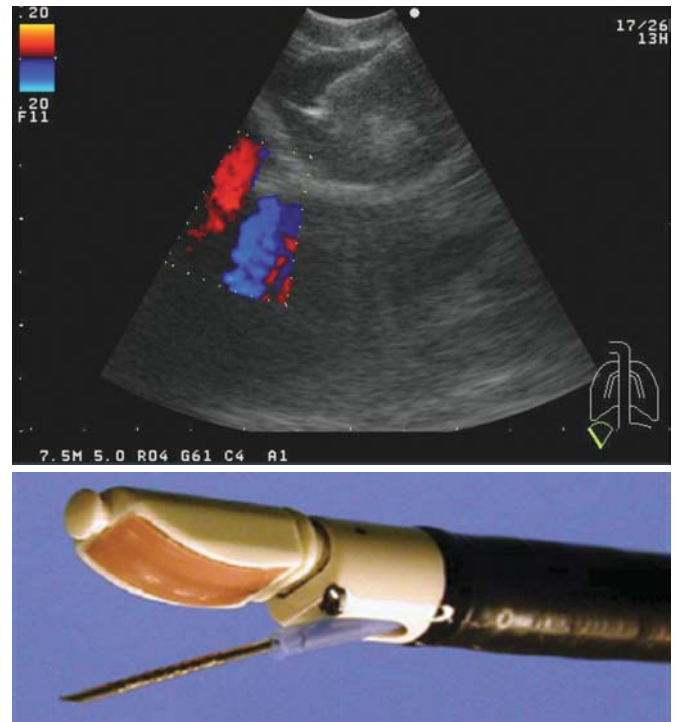
FIGURE 3. Curved-linear endobronchial ultrasound with real time guided transbronchial needle aspiration of a paratracheal lymph node in level four right.

Oesophageal ultrasonography visualises mediastinal lymph nodes in level 4L, and levels 7, 8 and 9, as described on the new International Association for the Study of Lung Cancer lymph node map [29, 30]. Some of these lymph nodes (levels 8 and 9) are not accessible by bronchoscopy or mediastinoscopy. Mediastinal lymph node station 4R is often a blind spot for EUS-FNA because of the interposition of the trachea, and hilar lymph node station 11R and 11L are never accessible by EUS-FNA. However, EUS-FNA can occasionally sample hilar (N1) lymph node station 10R or 10L, in which case one has to be extremely careful not to consider these lymph nodes as mediastinal (N2) lymph nodes. A meta-analysis reported a pooled sensitivity of 90% and negative predictive value of 78% in selected patients with clinical N2 disease (having enlarged mediastinal lymph nodes on CT) and prevalence of malignant N2/3 disease of 73% [24]. Therefore, EUS-FNA could be considered after a negative routine bronchoscopy in selected patients with 18F fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) positive mediastinal lymph node(s) or enlarged mediastinal lymph nodes on CT. In addition, EUS can visualise potential M1 stages such as the left adrenal gland, the left liver lobe and celiac trunk lymph nodes.

EBUS is also performed under local anaesthesia using moderate sedation and enables visualisation of mediastinal lymph nodes at levels 2R/2L, 3P, 4R/4L and 7, as well as hilar lymph nodes at level 10, 11 and even 12, and subsequent performance of a TBNA under real-time ultrasonographic control [30]. The mediastinal lymph node stations accessible for a cervical mediastinoscopy are also accessible with EBUS-TBNA. A recently published meta-analysis on EBUS-TBNA in selected patients reported a pooled sensitivity of 94% for

TABLE 4	Test characteristics of invasive mediastinal staging techniques					
Technique	Clinical stage	Patients n	Sensitivity %	False-positive rate %	False-negative rate %	Prevalence %
Mediastinoscopy [23]	I–III	6505	78	0	11	39
Blind TBNA [23]	III–IV	1339	78	1	28	75
EUS-FNA [24]	III	579	90	0.7	22	66
EBUS-TBNA [25]	III	1036	94	0	30	68

TBNA: transbronchial needle aspiration; EUS-FNA: oesophageal ultrasonography fine-needle aspiration; EBUS: endobronchial ultrasonography.

PET- positive and/or enlarged mediastinal lymph nodes on CT with a prevalence of malignant N2/3 disease of 68% [25]. An important issue is that EBUS-TBNA, just as EUS-FNA, has a suboptimal negative predictive value ranging from 60% to 80%, which requires a confirmatory surgical staging procedure in case of a non-malignant echo-endoscopic needle aspiration of a suspicious mediastinal lymph node. It is clear that cervical mediastinoscopy remains the gold standard, but its position can be narrowed from unselected patients to selected patients with: 1) a negative echo-endoscopic needle aspiration of PET-positive and/or enlarged mediastinal lymph nodes and 2) patients with a central primary tumour, or with a hilar lymph node staged cN1 and a normal mediastinum on CT and PET.

The diagnostic yield and reproducibility of the EUS test characteristics are influenced by several aspects, such as a dedicated endoscopist, a dedicated cytopathologist (laboratory techniques, reproducibility of cytopathologists), cancer staging issues and feasibility of its implementation in daily routine. A cytopathologist dedicated to non-gynaecological cytopathology is of utmost importance and has to be familiarised with or experienced in conventional smears and/or liquid-based preparations [31]. Most of the diagnostic criteria used for conventional smears are also applicable to liquid-based preparations, but one has to be aware of the differences in technical and cellular features of both techniques. The sensitivity and specificity of liquid-based preparations is comparable to conventional smears in detecting abnormalities. If one only relies on liquid-based preparations, it is crucial to add cell block preparation for further immunohistochemical and molecular cancer analysis or for the detection of granulomatous lesions. Moreover, the reproducibility of the diagnosis on EBUS-TBNA and EUS-FNA is excellent among pathologists experienced with these types of samples [32]. There is no internationally accepted recommendation regarding mediastinal lymph node staging. The European Society of Thoracic Surgeons working group recommends systematic exploring and biopsy of the right and left lower paratracheal nodes (lymph nodes 4R and 4L, respectively) and the subcarinal nodes (lymph node 7), and also biopsy of the ipsilateral upper paratracheal lymph node [26]. In this context, an advantage of mediastinoscopy over EUS-FNA might be that a more complete mediastinal mapping can be performed, with inclusion of contralateral lymph nodes. Available evidence indeed shows that EUS-FNA alone, compared to mediastinoscopy, can either understage N2-disease or sample significantly fewer lymph node stations [33, 34]. Large-scale

randomised controlled clinical trials further investigating this issue are awaited. Most of the data in literature are produced by the same expert centres. Nevertheless, data on the implementation of EUS-FNA or EBUS-TBNA in routine clinical practice by chest physicians obtained sensitivities and accuracies for mediastinal staging of lung cancer similar to that of the experts with longstanding experience [35, 36].

In conclusion, both invasive surgical staging techniques and minimally invasive endoscopic staging techniques have their role in selected NSCLC patient groups. Conventional TBNA performed during a first standard bronchoscopy is a valuable mediastinal staging tool in selected patients with clearly enlarged (defined as >15 mm in largest short axis) or bulky mediastinal lymph nodes in levels 4R, 4L or 7. EBUS-TBNA and EUS-FNA are valuable staging tools in selected patient groups with any clinical N2/3 disease based on a positive-PET scan on mediastinal lymph nodes or enlarged (defined as >10 mm in largest short axis) mediastinal lymph nodes on chest CT. A cervical mediastinoscopy is indicated in selected patient groups with: 1) a negative echo-endoscopic needle aspiration in clinical N2/3 disease, 2) patients with a central primary tumour and a normal hilum/mediastinum on CT and PET and 3) in patients with a hilar lymph node staged cN1 and a normal mediastinum on CT and PET.

EVALUATION OF PARENCHYMAL LESIONS: TRANSBRONCHIAL LUNG BIOPSY REVISITED
Transbronchial lung biopsies (TBLB) can be useful in diagnosing neoplasia, infections and certain interstitial lung diseases. The likelihood of successful sampling and diagnosis depends on whether we are dealing with focal (including local infiltrates, nodules and masses) or diffuse radiological abnormalities. For the latter it is also important to consider whether the patient is immunocompetent or not. Further limitations of the transbronchial technique are the small size of the samples, the crush artefacts and the fact that transbronchial biopsies only sample the centrilobular area and often fail to penetrate beyond the peribronchial sheath. A histopathological diagnosis and diagnostic yield of TBLB are thus dependent on the topographic distribution and the morphologic specificity of the lesions.

In interstitial lung disease, TBLB are recommended in lung disease with prominent bronchocentric involvement, such as lymphangitis carcinomatosis or sarcoidosis. TBLB is not recommended in suspected idiopathic pulmonary fibrosis, except to ensure an alternative diagnosis, such as sarcoidosis

or hypersensitivity pneumonitis. TBLB is equally unhelpful in the diagnosis of idiopathic interstitial pneumonia, except to confirm cryptogenic organising pneumonia or hypersensitivity pneumonitis, in which TBLB are not diagnostic in isolation but in combination with clinical and CT findings.

In immunocompromised patients with a pulmonary infiltrate, routine TBLB is not recommended, due to safety issues and the fact that the clinical value of the information obtained by TBLB is not well established in all indications. Once the diagnostic strategy has been directed to bronchoscopy with broncho-alveolar lavage (BAL), TBLB can be considered in addition to BAL in selected patients as additional information can be obtained in up to 30% of the patients. In non-neutropenic patients scheduled for diagnostic bronchoscopy, TBLB should be combined with BAL in the initial diagnostic work-up if factors other than infectious causes have to be considered to avoid sequential procedures (provided there is no contra-indication for TBLB). In neutropenic patients scheduled for bronchoscopy, TBLB provided additional information in <5% of patients and should therefore not be performed in addition to BAL.

In peripheral pulmonary lesions (PPLs), flexible bronchoscopy with TBLB has a variable and often poor diagnostic yield in cases of normal endobronchial examination at bronchoscopy, as these lesions are often difficult to locate without a guidance technique. The sensitivity of bronchoscopy for detecting malignancy in PPLs depends on the size of the nodule, the proximity to the bronchial tree and the prevalence of cancer in the study population. CT-guided percutaneous transthoracic fine-needle aspiration biopsy may be considered the gold standard in very small easily accessible peripheral lesions. Its

sensitivity for detecting malignancy in lesions <20 mm or <30 mm in diameter has been reported to be up to 90%, but with a significant pneumothorax rate of up to 25%. Traditionally, a diagnostic TBLB sample can be obtained during flexible bronchoscopy with the auxiliary use of radio-graphic fluoroscopic guidance but, even then, lesions <20 mm in diameter remain hard to detect with a diagnostic yield <30%, and in the absence of radiographic fluoroscopic guidance it can often be difficult to diagnose PPLs >20 mm. New steering mechanisms have a significant impact on the detection rate and diagnostic yield of TBLB in PPLs. Ultrathin bronchoscopy, conventional flexible bronchoscopy with EBUS-miniprobe, CT-assisted electromagnetic navigation broncho-scopy with extended working channel, and CT-guided ultrathin bronchoscopy are available, but well-designed com-parative effectiveness research is awaited. Radial transducer EBUS-miniprobes can be introduced through the biopsy channel of a standard bronchoscope to perform EBUS (fig. 4). These radial EBUS miniprobes can guide the sampling of more peripheral pulmonary lesions and increase the diagnostic yield of TBLB without fluoroscopic guidance. A prospective randomised trial comparing conventional TBLB without fluoroscopic guidance to EBUS-guided TBLB found a signifi-cantly higher diagnostic yield of 31% *versus* 75%, respectively [37]. In multivariable analysis, probe location (within the lesion *versus* adjacent to the lesion) and lesion size (diagnostic yield clearly drops for lesions <20 mm) were factors related to the diagnostic yield of EBUS-guided TBLB [38, 39]. An improve-ment in diagnostic yield has been observed for lesions detected adjacent to the airway with EBUS-miniprobe if a conventional TBNA is performed in these lesions instead of a TBLB [40]. An improvement in detection rate and diagnostic yield has been observed for lesions <20 mm using electromagnetic naviga-tion techniques for the detection of a small lesion (fig. 5) and/ or EBUS-miniprobe guided sheaths for TBLB sampling [41, 42]. Electromagnetic navigation series suggest that the diagnostic yield for small peripheral nodules (average size 20–25 mm)
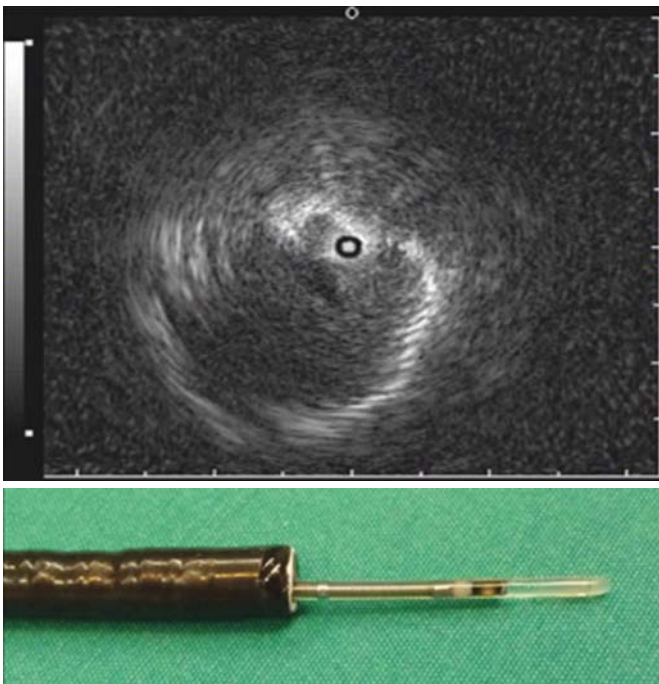
FIGURE 4. A radial transducer endobronchial ultrasound miniprobe can be introduced through the biopsy channel of a standard bronchoscope to perform endobronchial ultrasonography of a peripheral pulmonary lesion.
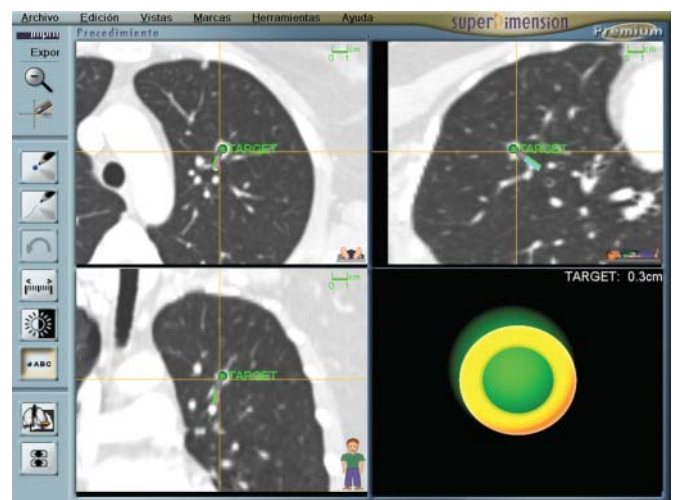
FIGURE 5. Electromagnetic navigation image: the icon representing the locatable guide (arrow) can be seen 3 mm away from the centre of a small pulmonary nodule in a patient with a history of colon cancer. Cytopathologic assessment confirmed metastatic disease.

increases up to 70% with a risk for pneumothorax which is significantly less than for CT-guided percutaneous transthoracic fine-needle aspiration biopsy.

CONCLUSION

Both AFB and endoscopic ultrasonography are macroscopic imaging techniques, which are considered a clinical useful addition to the armamentarium of the chest physician. AFB is finding its role in surveillance after lung cancer surgery, early detection of pre-invasive airway lesions in suspected or known lung cancer and staging of CIS or radio-occult invasive carcinoma. EBUS has become a standard bronchoscopic procedure because of its improved diagnostic yield in mediastinal diagnosis or staging, and in peripheral pulmonary lesions. Nowadays, the diagnostic techniques are entering the era of microscopic imaging (such as fibred confocal fluorescence microscopy and optical coherence tomography) in order to target a suspicious area and take an optic biopsy. These microscopic techniques still require further research to delineate their role and clinical use within the airways.

STATEMENT OF INTEREST

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