

## ORIGINAL ARTICLE

# DOES ADDITION OF ENDOBRONCHIAL ULTRASOUND IMPROVES THE CLASSIFICATION OF SUSPICIOUS LESIONS DETECTED BY AUTOFLUORESCENCE BRONCHOSCOPY?

By

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*Autofluorescence bronchoscopy (AFB) may improve sensitivity at the cost of specificity when it is used to identify suspicious bronchial mucosal lesions not seen by conventional white light bronchoscopy. Thus, it seems useful to study these lesions additionally with endobronchial ultrasound (EBUS) for further classification of dignity of suspicious lesions aiming in improving the positive predictive value (PPV) of AFB. This study aims to answer the following question: Does addition of EBUS improves the classification of suspicious lesions detected by AFB? Fifty five subjects with suspected lung cancer referred for AFB examination for different indications were recruited for this study. Rigid and Fiberoptic bronchoscopic examination were done followed by AFB then EBUS and finally samples from suspicious sites were taken for histopathological examination. The addition of EBUS to AFB improves PPV from 72.7% in AFB alone to 100% in AFB+EBUS in case of benign suspicious lesions detected by AFB with a negative predicted value (NPV) of 60% and with a sensitivity and specificity of 75% and 100% respectively for AFB+EBUS. While EBUS addition to AFB improves PPV from 60.6% in AFB alone to 95.2% in AFB+EBUS in case of malignant suspicious lesions detected by AFB with a NPV of 100% and with a sensitivity and specificity of 100% and 92% respectively for AFB+EBUS. Thus, the addition of EBUS improves the classification of suspicious lesions detected by AFB with its great implication on further patient management and combining AFB (for tumor localization) to EBUS (for depth estimation) may provide a reliable method of staging and choice of treatment modality of lung cancer.*

**Keywords:** autofluorescence bronchoscopy, endobronchial ultrasound, detection of lung cancer.

## INTRODUCTION

Lung cancer is a major cause of death among all tumors death over the world, it is killing over 85% of those it afflicts within 5 years.<sup>(1)</sup> This high mortality was attributed to the late stage of the disease at detection and to aggressive biological behavior.<sup>(2)</sup> Despite the considerable advances that have occurred in the management of many malignant diseases, the outlook for lung cancer sufferers remains depressingly poor.<sup>(3)</sup> According to the World Health Organization (WHO) statistics, Egypt reported the highest incidence, prevalence and mortality rates in North Africa.<sup>(4)</sup> Early lung Cancer is the final stage of a multistep carcinogenic process.<sup>(5)</sup> The intra epithelial stage of neoplastic development typically lasts for a number of years before invasion occurs. Hence the implementation of new techniques for early detection and prevention of lung cancer at this stage acquired a great importance in the past few years.<sup>(6)</sup> In conventional white light bronchoscopy (WLB), detection and localization of dysplasia and carcinoma in situ has been limited to about 30% of the total number thought to be present.<sup>(7)</sup> Fluorescence bronchoscopy (AFB) represents one of several initiatives in the field of early lung cancer detection. By exploiting differences in the fluorescence properties of normal and abnormal bronchial mucosa, it has become possible to detect pre invasive lesions and early micro-invasive carcinomas involving the large air ways, which would otherwise have gone undetected by more conventional white light bronchoscopy.<sup>(8-9)</sup> A relatively low specificity (i.e., too many false positives) remains a problem, however, in both AFB and WLB.<sup>(7-10)</sup> Inflammatory or granulomatous abnormalities, metaplasia, or dysplasia, and also scar formations or any number of other localized changes, can be mistaken for early neoplastic lesions upon inspection.<sup>(11)</sup> This can result in multiple endobronchial biopsies in a given individual, increasing risk and cost of the procedure.<sup>(7)</sup> Bronchoscopic technology that could improve predicting which lesions need to be biopsied could therefore be of significant value. Endobronchial ultrasound (EBUS) allows excellent imaging of the multilayer structure of the bronchial wall and the parabronchial space with

the aim of more precise local staging of lung cancer.<sup>(12-13)</sup> Thus, it seems useful to study suspicious lesions detected by AFB additionally with EBUS for further characterization of these lesions aiming in improving the specificity of AFB.

This study aims to answer the following question: Does addition of EBUS improves the classification of suspicious lesions detected by AFB?

## METHODS AND SUBJECTS

This prospective study was collaboration between the National Research Centre (Egypt), Chest department at Ain Shams University (Egypt) and ThoraxKlinik at Hiedelberg University (Germany). The study was conducted at the department for interdisciplinary endoscopy at ThoraxKlinik, Hiedelberg University, Germany.

**Patients:** In a prospective study from October to December 2006, 55 suspected lung cancer subjects referred for AFB examination were recruited. The indications for AFB included: a) High risk of developing lung cancer e. g. patients who are current or past heavy smokers (>20 pack /years). b) COPD with a change in symptoms as recurrent unexplained hemoptysis. c) Patients and workers with asbestos exposures or occupationally exposed workers to carcinogenic substances.<sup>(14)</sup> d) Previously resected for cure lung cancer to search for recurrence in resection margins or second primary.<sup>(15)</sup> e) Preoperative assessment of patients with lung cancer to determine the extent of endobronchial involvement and resection margins.<sup>(16)</sup> f) Suspected bronchogenic carcinoma: (clinical or radiological abnormality, positive sputum cytology for malignant cells, undiagnosed pleural effusion or paraneoplastic syndrome. g) Head and neck cancers.<sup>(17)</sup> The study excluded any subjects with contraindication(s) for conventional WLB<sup>(18)</sup> or presence of any condition(s) that may alter AFB results.<sup>(19)</sup>

**Conventional and Autofluorescence Bronchoscopy:** Conventional white light bronchoscopy and AFB, as well as EBUS, were

performed in the same session. Bronchoscopy was performed under general anesthesia with rigid bronchoscope (Karl Storz GmbH & Co. KG Tuttlingen, Germany) in combination with flexible bronchoscopy. The airway examination began with conventional white light bronchoscopy, followed by AFB examination using the D-Light/AFB (11004 BI, KARL STORZ GmbH & Co. KG, Tuttlingen, Germany). Findings were described according to the following classification: class 1, normal appearance; class 2, nonspecific changes (e.g. scar, granuloma, swelling, anatomic abnormalities, inflammation); class 3, suspicion of malignant change; and class 4, visible tumor.<sup>(11)</sup> After the detection of a class 2 or 3 lesion (Fig. I), EBUS was performed on these lesions, as described subsequently.

**Endobronchial Ultrasound:** Endobronchial Ultrasound EBUS was performed with flexible 20 MHz probes (UM-BS 20-26R with driving unit MH-240 and processor EU-C60; Olympus). The EBUS findings were classified to two classes as seen in Table 1.

**Table 1. The classification of EBUS findings detected by bronchoscopist according to ultrasonographic image.<sup>(20)</sup>**

Class 1	Normal in case of preserved structure of the bronchial wall (no invasion of bronchial wall). (Figs. 2,3).
Class 2	Malignant in case of destruction of the bronchial wall integrity. Which was subdivided into: A) Mucosal /Submucosal invasion (intracartilaginous). B) Cartilage/distant invasion (extracartilaginous). (Figs. 4,5,6).

Details of the technique of EBUS examinations are available elsewhere.<sup>(12-13)</sup>

**Endobronchial biopsy:** Endobronchial biopsies were performed on class 2 and 3 lesion(s) detected by AFB and examined by EBUS. Biopsies were always taken starting by distal lesions before proximal ones and from lower lobe then upper

lobe in order to avoid interference by bleeding. Separate forceps (using karl storz foreceps) were used for each site. Biopsied tissues were collected in 4% formalin solution for staining with hematoxylin and eosin and with Giemsa stain for histopathological examination. Histopathological classification of biopsies was done according to the WHO criteria 1999.<sup>(22)</sup>

**Statistical methodology:** The conventional level of statistical significance ( $P < 0.05$ ) was used for all analyses as follows: Description of quantitative variables as mean, standard deviation and range. Description of qualitative variables as number and percent. Chi-square test was used to compare qualitative variables. Unpaired t-test was used to compare two independent groups as regard quantitative variable. Analysis of data was done by a commercial statistics package (SPSS Inc., Chicago, Illinois, USA, version 11)

## RESULTS

Between October to December 2006, 55 subjects underwent AFB according to the indications previously mentioned. The subjects were classified into Group I: AFB class 2 lesions (Non specific changes by AFB). This group included 22 subjects, their ages ranged from 42 to 70 with a mean age  $56.9 \pm 7.9$  and a male /female ratio of 4.5:1.) and Group II: AFB class 3 lesions (Suspicious malignant changes by AFB). This group included 33 subjects, their ages ranged from 46 to 68 with a mean age  $58.5 \pm 5.2$  and a male /female ratio of 3.1: 1). There were no significant statistical differences between group I and group II as regards smoking habits. There were wide variations in the indications for AFB in each group as seen in (Fig. 7).

The Positive Predicted Value (PPV) of AFB alone in group I was 72.7% as noticed from Table 2. And by adding EBUS examination to AFB in Group I, the PPV became 100% and a sensitivity of 75%, specificity of 100% and a negative Predicted Value (NPV) of 60% as recognized from Table 3. And (Fig. 8).

**Table 2. Distribution of lesions detected by AFB in group I with correspondence to the pathological findings.**

Lesions (AFB only)	Pathology Benign No	Pathology Malignant No
Benign Number=22	16	6
Malignant Number =0	0	0

**Table 3. Distribution of lesions detected by AFB in group I and additionally examined by EBUS with correspondence to the pathological findings.**

Lesions (AFB+EBUS)	Pathology Benign No	Pathology Malignant No
Benign Number =12	12	0
Malignant Number =10	4	6

The PPV of AFB alone in group II was 60.6 % as noticed from Table 4. And by adding EBUS examination to AFB in Group II, the PPV became 95.2% and a sensitivity of 100%, specificity of 92.3% and NPV of 100 % as recognized from Table 5. And (Fig. 9). The correct EBUS diagnosis in both groups was 90.9% as observed from Table 6. The detected benign lesions are coded from 1to 5 and the detected pathological are coded from 6 to 9 as noticed in (Fig. 10).

**Table 4. Distribution of lesions detected by AFB in group II with correspondence to the pathological findings.**

Lesions (AFB only)	Pathology Benign No	Pathology Malignant No
Benign Number =0	0	0
Malignant Number =33	13	20

**Table 5. Distribution of lesions detected by AFB in group II and additionally examined by EBUS with correspondence to the pathological findings.**

Lesions (AFB+EBUS)	Pathology Benign No	Pathology Malignant No
Benign Number =12	12	0
Malignant Number =21	1	20

The mean time for AFB was 7.4 minutes with a range of 4.3 - 11.9 minutes. While the mean time for EBUS was 6.3 minutes with a range of 3.1- 14.4 minutes. No recorded complications due to adding EBUS to AFB examination.

**Table 6. EBUS classification of the lesions detected by AFB with correspondence to the pathological findings.**

	EBUS classification							
	Benign (no invasion)		Class 2A (mucosal/submucosal)		Class 2B (Cartilage/ advanced)		Total No	
	No		No		No		No	
	24		7		24		55	
Pathological examination	B 24	M -	B 5	M 2	B -	M 24	B 29	M 26
Correct diagnosis (As regard the pathology)	100%		28.5%		100%		90.9%	

B: Benign, M: Malignant, No: Number.

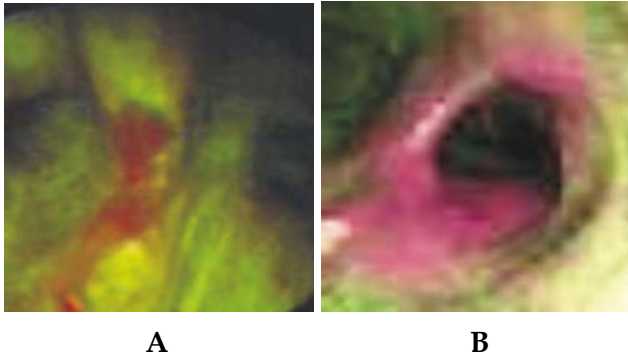


Fig 1. A) Class II Autofluorescence image of moderate Dysplasia between left anterior basal segmental bronchus and left basal segmental bronchus of left lower lobe. B) Class III Autofluorescence image of small cell lung cancer (SCLC) between right medial segmental bronchus and right lateral segmental bronchus of middle lobe.

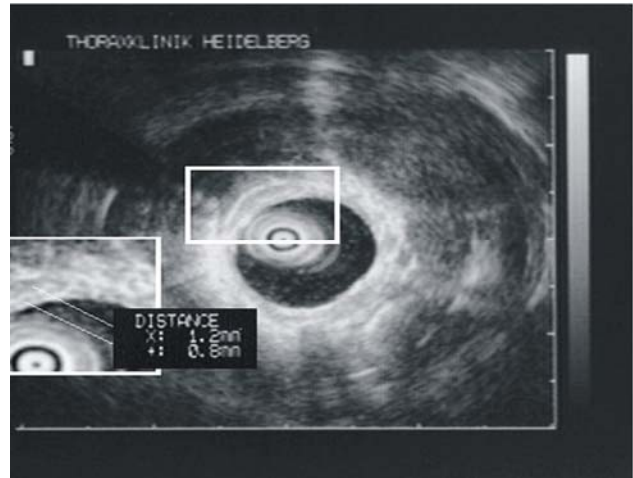


Fig 3. Class I EBUS image at bronchus intermedius.

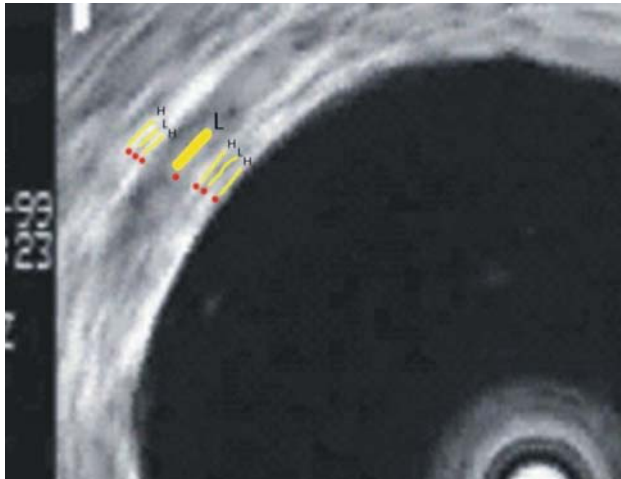


Fig 2. Magnified normal airway structure as shown by EBUS. Shown is the seven-layer structure as visualized with a 20-MHz probe. Identifiable structures from the inside out are mucosa (H), submucosa (L), endochondrium (H), cartilage (L), perichondrial layer (H), connective tissue (L), adventitia (H). L: low-density layer; H: high-density layer.<sup>(21)</sup>

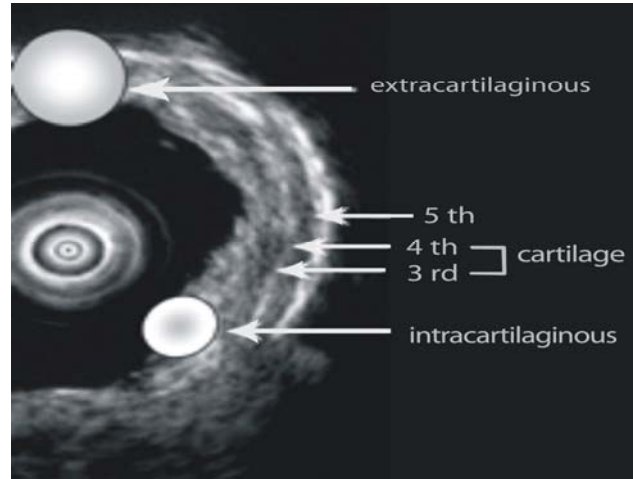


Fig 4. The classification of EBUS findings detected by bronchoscopist according to ultrasonographic image EBUS image showing a layered structure of tracheobronchial wall. Starting on the luminal side of the tracheobronchial wall, the third, fourth, and fifth layers (the hyper/hypo/hyperechoic layers) are induced by the presence of the cartilage. The depth of tumor invasion into the bronchial wall was determined by the cartilage layer and was divided into two categories: intracartilaginous (Class IIA) or extracartilaginous (classIIB).<sup>(20)</sup>

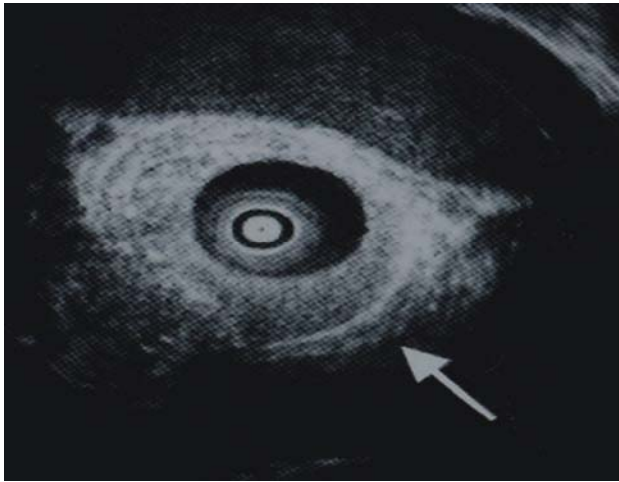


Fig 5. Class IIA EBUS image at right upper lobe bronchus.

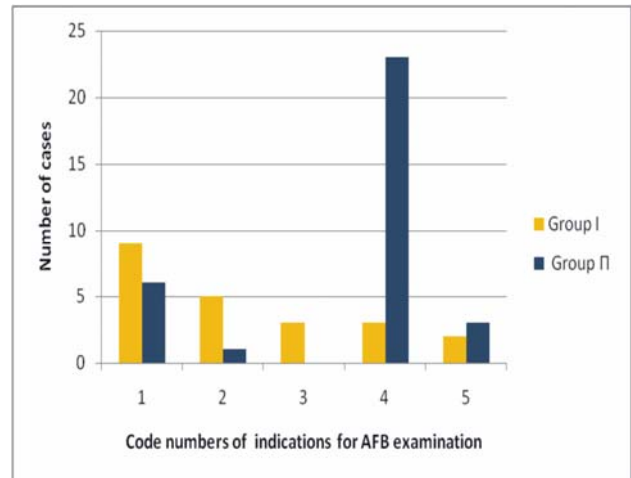


Fig 7. Distribution of indications for AFB examination between Group I and Group II. 1-Individuals at high risk of developing lung cancer.2- Patient with previously resected for cure lung cancer.3- Preoperative assessment of patients with lung cancer to determine the extent of endobronchial involvement and resection margins.4- Patient with suspected bronchogenic carcinoma.5- Patient with head and neck cancers.

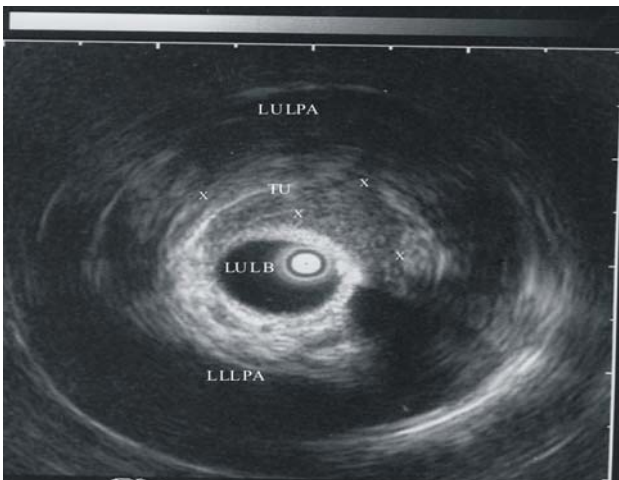


Fig 6. Class IIB EBUS image in left upper lobe bronchus. LULB (left upper lobe bronchus), TU (tumor), LULPA (left upper lobe pulmonary artery), LLLP (left lower lobe pulmonary artery).

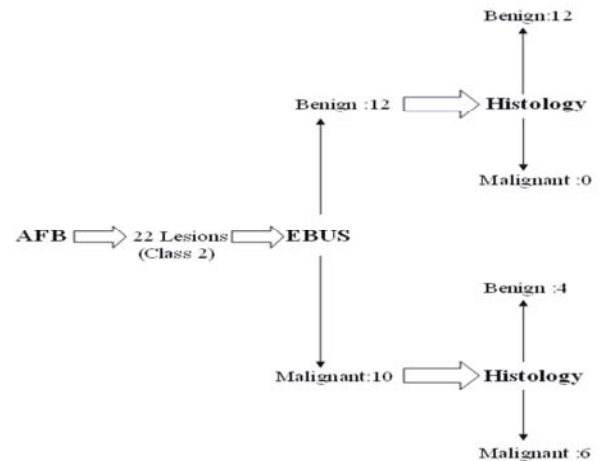
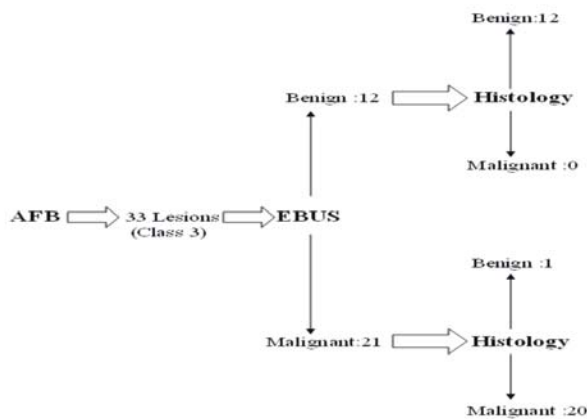
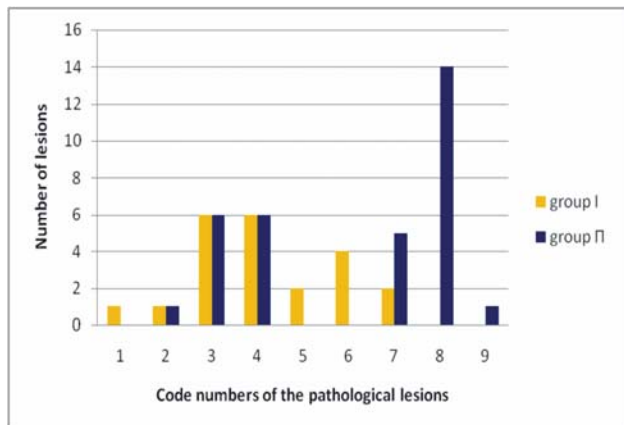


Fig 8. Distribution of lesions detected by AFB in group I and additionally examined by EBUS with correspondence to the pathological findings.



**Fig 9.** Distribution of lesions detected by AFB in group II and additionally examined by EBUS with correspondence to the pathological findings.



**Fig 10.** Distribution of the pathological lesions in group I and group II.

1-Normal. 2-Inflammotry. 3-Mild dysplasia. 4-Moderate dysplasia. 5-Scar. 6-Squamous Cell Carcinoma. 7-Adenocarcinoma. 8-Small cell Lung Cancer. 9-Metastesis.

## DISCUSSION

This study concluded that adding EBUS to AFB improves classification of suspicious lesions detected by AFB.

The addition of EBUS to AFB improves PPV from 72.7% in AFB alone to 100% in AFB+EBUS in case of AFB class 2 lesions (Group I) Table 3. With a NPV of 60% and with a sensitivity and specificity of 75% and 100% respectively for AFB+EBUS. These results are in congruence with the results of Kurimoto and co investigators<sup>(23)</sup> who published in 1999 a paper on EBUS for determination of the depth of tracheobronchial tumor invasion. They found exact correspondence in 23 of 24 lesions with a PPV of 95.8%. Our results were in consistency with the study of Herth and his colleagues in 2003,<sup>(20)</sup> whose aim of work and study procedures were similar to the current study, but the study population (known bronchial carcinoma or follow up after initial therapy) were different. They found that adding EBUS to AFB in benign lesions (class 2) improved the PPV from 55% in AFB alone to 92% in AFB+EBUS. Lam and his group 1998<sup>(11)</sup> stated that class 2 lesions are only a few cell layers thick and a few millimeters in surface diameter. This may explain the relative low NPV (60%) of AFB+EBUS in class 2 lesions in the current study.

EBUS addition to AFB improves PPV from 60.6% in AFB alone to 95.2% in AFB+EBUS in case of AFB class 3 lesions (Group II) Table 5. With a NPV of 100% and with a sensitivity and specificity of 100% and 92% respectively for AFB+EBUS. Herth and his colleagues,<sup>(20)</sup> found out that adding EBUS to AFB in class 3 lesions lead to increase in the PPV from 69% with AFB alone to 97% to AFB+EBUS, and these results are similar to the results of this study. In 2003 Takahashi and co investigators,<sup>(24)</sup> published a prospective study on assessment of depth of invasion in early bronchogenic squamous cell carcinoma, they observed confinement of the lesion within the mucosal/sub mucosal layer and not invading the cartilage (class A of EBUS classification) in 22 lesions of squamous cell lung cancer. They compared the EBUS assessment (before intervention) with that of the histology after surgical removal. The PPV of their study was 85.7%. EBUS classification in relation to histopathological examination was 100% correct in

the benign lesions (no invasion to the bronchial wall), and 28.5% correct in class A lesions (i. e. malignant with invasion to mucosa or submucosa), and 100% correct in Class B (i. e. malignant with invasion to cartilage or more advanced invasion), with total accuracy of 90.9%. In this study, no surgical correlation with EBUS classification was done. Determination of the PPV on histological anatomy was out of reach. As regards the relative low accuracy in class A of EBUS assessment this could be attributed to the small number of specimen in this group (7 lesions in both groups I and II), in relation to the other two classes (24 lesions in the benign class and 24 lesions in class B). Also this relative low accuracy reflects the difficulty of assessment lesions in this class and may show wide variation between bronchopists depending on their experiences. Miyazu and coworkers,<sup>(25)</sup> investigated the role of AFB and EBUS in the choice of appropriate therapy for lung cancer, especially with regards to early stages. By using the same sonographic definition of the laminar structure of the tracheobronchial wall and 20 MHz ultrasound probe, Miyazu et al. demonstrated that it was difficult to detect all the layers of normal bronchial wall and consequently the level of the depth of tumor invasion by EBUS in a clinical study. As the cartilage layer was clearly more evident than any other layer, the depth of tumor invasion could be categorized into two levels, whether the cartilage is involved or not. This categorization seemed practicable when applied to 3 resectable invasive lung cancers (cartilage involved). The histopathological findings after resection were congruent with the EBUS evaluation. In two other patients with early lung cancer (cartilage not involved) a decision for photodynamic therapy was made. EBUS results determined the choice of therapy.

Studying the sensitivity and specificity of AFB was not the core of this study, as several researches regarding these indicators were thoroughly done before as in 2006, Osman<sup>(26)</sup> studied the value of adding WLB to AFB, Osman results showed out that the sensitivity and

specificity of AFB alone were 92.3% & 92% respectively. Adding the WLB, the sensitivity and specificity were 100% & 98.6% respectively, yet still with relatively low PPV of AFB of 75%. These results are in congruence with reports of other authors such as Metwally in 2001<sup>(27)</sup> and El Assal in 2002<sup>(28)</sup> at national level and internationally there were similar results by different workers.<sup>(8,9,29)</sup> This research highlighted the positive predicted value of adding EBUS to AFB.

This study concluded that adding EBUS to AFB improves the positive predicted value of AFB and hence the classification of suspicious lesions detected by AFB with its great implication on further patient management, also combining AFB (for tumor localization) to EBUS (for depth estimation) may provide a reliable method of staging and choice of treatment modality of lung cancer.

This study recommends to apply combined AFB+EBUS technique on a larger scale and to correlate EBUS assessment of degree of the bronchial invasion with the histopathological results after surgical excision. Evaluation of the role of combined AFB and EBUS in the choice of appropriate therapy for early lung cancer is also recommended to be furtherly studied.

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