Review Article

Newer Advances in Bronchoscopy for Early Diagnosis of Lung Cancer

G Ramya, VV Ramana Reddy, JV Praveen, DS Sowjanya, BK Prithvi, PVV Bharadwaj

Abstract: Lung cancer is the most common cause of cancer deaths in developed countries. The prognosis is poor, with less than 15% of patients surviving 5 years after diagnosis. The poor prognosis is attributable to lack of efficient diagnostic methods for early detection and lack of successful treatment for metastatic disease. Most patients (>75%) present with stage III or IV disease and are rarely curable with current therapies. Screening tests using sputum cytology and chest radiograph have been used with limited success. Value of low dose spiral CT scan as screening tool for lung cancer is being evaluated and its limitations include high costs, need for repeated scanning and necessity to obtain histological confirmation with additional procedures. There have been significant advances in the early diagnosis of lung cancer in high risk patient groups using bronchoscopic methods such as white light bronchoscopy, autofluorescence bronchoscopy, high magnification bronchoscopy, narrow band imaging and endobronchial ultrasound. These techniques appear to be promising tools as they might allow visualizing changes of early lung cancer and also permitting sampling for histological confirmation.

Key Words: Bronchoscopy, Lung cancer, Autofluorescence bronchoscopy, Endobronchial ultrasound, High magnification bronchovideoscopy.

Introduction

Lung cancer is a leading cause of cancer deaths and the incidence is rising\(^1\). By the time patients present to clinicians the condition is fairly advanced and at best only 25-30% of patients can be offered curative resection\(^2\). The case fatality rate remains at 85-90%\(^3\). The overwhelming majority of cases of lung cancer are attributable to cigarette smoking and thus primary prevention should continue to be a major focus of public health campaigns. However, such measures are likely to have only a limited impact on mortality in the short term because of a lag phase in the order of 20 years. Early detection of lung cancer with chest radiographic or sputum cytology screening does not improve outcome, particularly disease specific mortality and a recent meta-analysis\(^4\) has shown that these strategies are not very useful and newer strategies need to be evaluated. Furthermore, newer screening methods have now been proposed such as spiral computed tomographic (CT) scanning. A study by Henschke \textit{et al}\(^5\) detected 27 lung cancers using spiral CT whereas only seven of these were visible on chest radiographs which were obtained at the same time. Another study\(^6\) looked at chest radiograph retrospectively in 44 cases of lung cancer diagnosed by using low-dose spiral CT as a screening tool and found that chest radiograph failed to detect 79% of lesions which were less than or equal to 2 cm. This highlights the importance of spiral CT in detecting small lesions in asymptomatic patients. However spiral CT is not yet the perfect tool for lung cancer screening due to the high cost of scanning and need for follow-up scans, small risk of cancer associated with multiple follow-up scans and the inability to do biopsies at the same sitting.

Bronchoscopic techniques for early detection of lung cancer are a promising tool as they might allow to visualise changes of early lung cancer and also permit sampling for histological confirmation. It is important to detect bronchial carcinoma \textit{in situ} (CIS) since over 40% of these can develop into invasive cancer\(^7\). There is strong evidence from the treatment of cervical dysplasia or CIS, resulting in reduction in the incidence and mortality of invasive cervical cancer\(^8\), suggesting that a similar strategy may be useful in lung cancer. The role of conventional fibreoptic bronchoscopy in the diagnosis and management of lung cancer has been well established over the past few decades. There have been exciting innovations in the last decade in this field which can help with the goal of early detection of lung cancer by bronchoscopic techniques and coupled with new modalities of treatment such as chemoprevention and endobronchial therapies, the prognosis and outcome of these patients can potentially be altered in the near future.

Corresponding author

G Ramya

Postgraduate, Department of Pulmonary Medicine
Maharajah’s Institute of Medical Sciences, Nellimarla Vizianagaram - 535 217, Andhra Pradesh, India.
Autofluorescence Bronchoscopy

Fluorescence bronchoscopy is based on the premise that differences in epithelial thickness, blood flow, and tissue fluorophore concentration cause preinvasive and neoplastic tissues to have diminished red fluorescence and substantially diminished green fluorescence compared with normal tissues when exposed to blue-light excitation of 440 to 480 nm wavelength. The need to look for superficial bronchial mucosa malignancy was first addressed by Kato and Cortese using porphyrin injection, followed by bronchoscopic observation using a laser monochromatic light source.

Fig1. AFI system configuration. AFI exploits the characteristics of 2 different wavelengths: (1) autofluorescence (460–690 nm) is attenuated when a neoplastic lesion is irradiated with blue excitation light (390–440 nm), and (2) components absorbed by the blood are reflected when irradiated with green light (540–560 nm).

Normally, when a surface is illuminated by light, the light can be reflected, backscattered, or absorbed. In addition, light also causes the tissue to fluoresce; however, this autofluorescence (AF) cannot be seen during conventional WLB because the intensity of fluorescence is very low and is overwhelmed by the reflected and backscattered light. The major chromophores in the airway mucosa are elastin, collagen, flavins, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide hydrogen (NADH), and porphyrins. Exposure of the chromophores to light of specific wavelengths excites electrons, and fluorescence is emitted when the electrons return to ground level. Accelerated intracellular metabolism in cancer cells decreases riboflavin and flavin coenzymes and NADH caused by overproduction of lactic acid through glycolysis. Tumour drug fluorescence was detected at 630 nm wavelength which was quite distinct from normal fluorescence at 500-580 nm.

Fig2. AFI findings and color distribution. AFI displays normal areas in light green, areas with abundant blood flow and blood vessels in darker green, and neoplastic lesions in magenta.

Though this technology improved sensitivity, the prohibitive cost and photosensitivity reaction meant that it could not be applied in routine screening. Subsequent research by a group in Canada lead to the development of the LIFE (light imaging fluorescence endoscope). This technology uses blue light at 442 nm from a laser light source. Autofluorescence distinctions between normal and malignant mucosa can be made using image intensified cameras in real time. For the diagnosis of early lung cancer, the findings at white light bronchoscopy (WLB) are classified as (1) normal (2) abnormal - areas with increased redness and hypervascularity, swelling or thickening of the bronchial mucosa, focal thickening of subcarina (3) suspicious – nodular or polypoid lesions, irregularity of bronchial mucosa. On autofluorescence, normal bronchial mucosa appears green, abnormal lesions appear slight brown with ill defined margins and areas suspicious for high grade dysplasia or cancer appear brown or brownish-red.

In a multicentre study Lam et al used LIFE as an adjunct to WLB to detect and perform biopsies from areas suspicious of intraepithelial neoplasia as compared to WLB alone. This study involved 173 patients and 700
biopsies were examined. This study showed that LIFE together with WLB had a relative sensitivity of 6.3 for detecting intraepithelial neoplastic lesions and 2.7 when invasive carcinomas were included, as compared to WLB alone. This set the stage for the use of LIFE for detecting early stage lung cancer. However, another smaller study by Kurie et al involving 53 patients did not show any additional benefit of using LIFE. One of the reasons for the difference between the two studies might have been that the study by Lam et al included patients with known or suspected lung cancer whereas the study by Kurie et al included patients with heavy smoking history (>20 pack years) but free of active cancer.

A D-Light fluorescence-reflectance system has been developed. This uses noncoherent ultraviolet to blue 300W xenon filtered lamp (380–460 nm) to excite broad emission spectra of the different chromophores in tissue. Using this system the normal tissues appear bluish and the areas with high grade dysplasia and CIS give darker images. The LIFE system is somewhat bulky and does not allow direct and immediate comparison of white light and autofluorescence images. The D-Light system allows direct comparison of images between the two modalities. A study by Herth et al compared the Light system with the LIFE system in 332 patients and included 1,117 biopsies. Differences between the two systems were observed only in five biopsies which was not statistically significant (p=0.3). This study demonstrates that the D-Light and LIFE are comparable. The examination time by the D-Light system was much less (7.4 vs 11.4 mins, p<0.001) probably due to direct switch between white light and autofluorescence imaging.

Other systems being used are the system of autofluorescence endoscopy (SAFE) 1000 autofluorescence at 500-580 nm. Fluorescence system using a xenon-lamp (420–480 nm) and a camera with a fluorescence filter and an image intensifier. Another new fluorescence–reflectance imaging system is the ONCO-LIFE with a view to reduce equipment costs and to make the autofluorescence bronchoscopy much easier. All the above studies have used fibreoptic bronchoscopes for the WLB. However, fibreoptic systems are now being replaced by flexible videobronchoscopy (FVB) where the images are clearer and sharper. A study comparing FVB and LIFE in patients at high risk of lung cancer showed that the sensitivity was 72% and 96% and the specificity was 53% and 23% respectively. The relative sensitivity of LIFE over FVB was 1.33. This is in striking contrast to the study by Lam et al which showed a relative sensitivity of 6.3 of LIFE over WLB using fibreoptic bronchoscopy and most likely attributable to better quality of images obtained using FVB.

**High Magnification Bronchovideoscopy**

Although the only abnormality of WLB seen in dysplasia is swelling and redness at bronchial bifurcations, histologically there is neovascularization or increased mucosal microvascular growth. Furthermore, mucosal blood flow is thought to be influenced by vascular and airway pressures, inspired air conditions, and anatomic neurotransmitters. High-magnification bronchoscopy (HMB) enables observation of vascular networks to identify potential areas of increased vascularity in the bronchial mucosa in patients with respiratory diseases, such as asthma, chronic bronchitis, sarcoidosis, and lung cancer. This system can provide information about the bronchial mucosa with a maximum magnification of 110 times. A dense concentration of subepithelial microvessels mainly observed in the inter cartilage portion, indicating an increase in submucosal circulation, suggests that it may be a useful tool for observing and evaluating subepithelial microvessels in large airways in various pathologies, such as lung cancer or asthma. A high magnification bronchovideoscope combining two systems — a video observation system for high magnification observation and a fibre observation system for orientation of the bronchoscope tip, has been reported. The scope is inserted like a normal bronchovideoscope into the tracheobronchial system using the fibre orientation system until the target area of suspicious mucosa is reached and then the mucosa is observed at high magnification on a television monitor. Shibuya et al conducted a study in which they did autofluorescence bronchoscopy in high risk patients for lung cancer followed by high magnification broncho videoscopy and observed vascular patterns in areas of normal and abnormal fluorescence and biopsies were performed at normal and abnormal sites. The areas with increased vessel growth and complex network of tortuous vessels of various sizes on high magnification broncho videoscopy were assumed to be positive for dysplasia and areas with vascular networks with a regular pattern were assumed to be negative for dysplasia. The sensitivity and specificity using this criterion were 71.4% and 90.9% respectively as confirmed by histology.
NARROWBAND IMAGING

As a further improvisation high magnification bronchovideoscopy was combined with narrow band imaging (NBI)\textsuperscript{22}. Microvascular structures are further observed if a new narrowband filter is used instead of the conventional red/green/blue broadband filter. This narrowband imaging (NBI) technique uses a 415-nm blue light, which is absorbed by hemoglobin contained in the capillary network on the mucosal surface and a 540-nm green light that is absorbed by blood vessels located a bit deeper below the capillary layer. Of particular note is the fact that the NBI filter includes the NBI-B1(400-430 nm) filter which includes the 410 nm absorption wavelengths for haemoglobin with perhaps more accurate detection of vessel structures. This would allow focused and detailed observation of bronchial vascular patterns. The optical absorption and scattering properties for tissues are strongly wavelength dependent\textsuperscript{23}. Blue light, which has a shorter wavelength than visible light, reaches into shallow surfaces\textsuperscript{24} which is helpful for detecting the submucosal vessels and patterns of vascularisation. With conventional RGB light delivered through an endoscope, some of the light is reflected from the tissue, some is scattered or absorbed within the tissue and little is detected to form an image viewed on the monitor. However, with the NBI there is less scattering of light and clearer images are viewed on the television monitor.

Compared with WLB, NBI was shown to increase the rate of detection of dysplasia or malignancy by 23\%\textsuperscript{25}. In a recent study, investigators found that NBI has a higher specificity than AF imaging, without significantly compromising sensitivity. Combining AF imaging and NBI did not increase diagnostic yield significantly. NBI might thus become an alternative to AF imaging in the detection of early lung cancers\textsuperscript{26}.

MULTIMODALITY FLUORESCIN IMAGING

Researchers are investigating a multimodality technique whereby fluorescein imaging, analogous to that used in ophthalmology, in conjunction with high-resolution computed tomography (CT) scanning, bronchoscopy, and four-dimensional spatial reconstructions allow detailed examination of the bronchial microcirculatory system\textsuperscript{27}. The idea is to develop a macro-optical technique that would allow large field visibility of alterations in blood flow to identify focused regions of interest for micro-optical techniques, such as optical coherence tomography (OCT). Fluorescein is a highly fluorescent compound that fluoresces yellow-green after excitation with a blue light. Although a large portion binds to serum protein in the blood stream, much of it remains unbound, and it is this unbound portion that is responsible for the observed yellow-green light emission. Fluorescein stays in the body for about 36 hours before being metabolized by the kidneys. In one study, changes in rabbit trachea fluorescence were noted after the injection of potent vasodilators and vasoconstricting agents, such as bradykinin and cocaine\textsuperscript{28}. In another Interventional Bronchoscopy study, texture mapping and identification of distal airway tumor infiltration beyond an area of initial endobronchial obstruction was possible using simultaneous application of four different imaging modalities, including white light and color bronchoscopy after fluorescein injection and threedimensional multi-detector CT\textsuperscript{29}. 

Figure 3 Squamous dysplasia. Complex networks of tortuous vessels are clearly identified by NBI.
ENDOBRONCHIAL ULTRASONOGRAPHY (EBUS)

Endobronchial application of ultrasound using a miniature probe introduced via a fiberoptic bronchoscope channel was first described in 1992 and Ultrasound and Doppler ultrasound image tissue structure and blood flow, but are limited in spatial resolution to approximately 50 to 200 mm because of their relatively long acoustic wavelengths\(^1\) (Fig. 1A, B). EBUS has been widely used for sampling mediastinal lymph nodes, endobronchial ultrasound - transbronchial needle aspiration (EBUS-TBNA) using both the radial probe as well as the convex probe and for peripheral lung lesions by transbronchial biopsies (EBUS-TBB)\(^3\). Endobronchial ultrasound (EBUS), however, has been used to accurately measure the depth of tumor invasion beyond the cartilaginous layer and to identify the structural layers of the airway wall that are important in defining and understanding various central airway disorders, such as relapsing polychondritis, tuberculosis, tracheomalacia, and lung cancer. It is known that lymph node invasion in radiooccult cancers changes staging and subsequently the treatment and prognosis in non–small cell lung cancer. The general probability is about 5% but very rare in CIS (1%) and not seen in lesions that are less than 3 mm thick, 10 mm in surface area, or those with an invasion index less than 20 mm in the large central bronchi\(^31\), \(^32\). However, significant rates of lymph node invasion have been reported (10%–30%) in case of invasion of the cartilage\(^33\)-\(^35\). Furthermore, 70% of patients presenting with radiographically occult lung cancer were shown to have more advanced cancer using a combination of autofluorescence bronchoscopy and high-resolution CT rather than a conventional initial evaluation\(^36\).

EBUS can be performed using a radial probe introduced through the working channel of a flexible bronchoscope. The working channel needs to be 2.8 mm or more in diameter. A balloon sheath filled with saline is inflated and the transducer is rotated through 360° within this balloon window to form an image of the airway and its surrounding structures. The 20 MHz EBUS probe has a penetration depth of 2 cm, which provides optimum resolution with sufficient airway wall image penetration.\(^37\) A new technique utilizing flexible bronchoscope equipped with a 7.5 MHz convex probe for endobronchial ultrasound (CP-EBUS) that scans parallel to the insertion direction of the bronchoscope has also been developed.\(^38\)

On the basis of EBUS in vitro studies a seven layer ultrasound structure of the cartilaginous portions of trachea or extra/intrapulmonary airways has been described\(^39\), \(^40\). The layers from the lumina outwards are:

1. Mucosa: hyperechoic – this appears as very bright enhanced by the adjacent balloon (first),
2. Submucosa: hypoechoic – clearly distinguishable from the other structures of the bronchial wall (second),
3. Cartilage: appears as three layers;
   (a) hyperechoic – endochondrium (third),
   (b) hypoechoic – internal layer (fourth),
   (c) hyperechoic – perichondrium (fifth),
4. Supporting connective tissue – hypoechoic (sixth),
5. Adventitia – hyperechoic (seventh).

The three-layer structure of the membranous portion of the extra-pulmonary bronchi is:

First layer – hyperechoic – epithelium and initial part of submucosa,
Second layer – hypoechoic – smooth muscle and
Third layer – hyperechoic – adventitia.

Fig4. (A) Ultrasound image formation. The transducer sends out a brief pulse of high-frequency sound (1) that penetrates and propagates (2) through various substances (referred to as medium). Ultrasound waves attenuate (3) as they propagate through a medium and get reflected (4) from tissue boundaries and interfaces back to the transducer, which serves as the sensor and the source of signal (5). (B) Endobronchial ultrasound image obtained with radial scanning using a 20 MHz probe for a normal airway (left main bronchus). The cartilaginous rings and the posterior membrane layers are visualized. (C) OCT image formation. Light is emitted from the source, shined into tissues, and reflected off internal structures. The longer the distance traveled, the longer the delay in returning to a detector. The delay in the returning light from deeper structures compared with shallow structures is used to reconstruct images. (D) OCT image of the trachea using a longitudinal scanning direction showing the mucosa and the upper part of the cartilaginous rings.
Kurimoto et al performed EBUS in 45 specimens of normal human trachea or bronchi that had previously been excised for non-neoplastic indications and corroborated them with microscopic findings on histology. They found a good correlation between microscopy and EBUS images and on this basis proposed a five layer EBUS appearance for the tracheobronchial wall. In a further 24 lung cancer resected specimens, comparison of the determination of the depth of tumour invasion on the basis of EBUS and histopathologic findings showed that the findings were the same in 23 lesions (95.8%) and different in only one lesion (4.2%), in which lymphocytic infiltration protruding between the cartilages was misinterpreted by EBUS as tumour invasion. A number of other studies have shown that there is good correlation between EBUS findings and histology.

OPTICAL COHERENCE TOMOGRAPHY

Optical coherence tomography (OCT) is an imaging technology similar to ultrasound. Instead of measuring the intensity of back-reflected sound, OCT uses an infrared light to obtain cross-sectional images of tissue. Compared to ultrasound, the resolution of OCT within the airways is significantly higher, which enables a more detailed evaluation of depth of invasion in endobronchial disease. OCT resolution, between 4 and 20 nm in the airway, is approximately 25 times higher than that of other available modalities. It is also an optically based technologically. This means it does not require direct contact with tissue for transmission of a signal and can therefore easily be used within the airways. OCT’s ability to assess the microstructure of the eye has an established role in ophthalmology and has only recently been adapted to the airways. Its potential to produce in vivo images or “optical biopsies” of the microstructure of the lung without the risk of tissue biopsy could be a very useful modality in such fields as interstitial lung disease and lung transplantation. As in the other modalities discussed above, further studies are needed to before its true clinical efficacy can be determined.

CONFOCAL FLUORESCENCE MICROENDOSCOPY

Alveoloscopy or confocal laser fluorescence microendoscopy (CFM) is a newer technology that enables in vivo microscopic observation of the airways and alveoli. The technology was introduced in 1957 but only in the last decade has the device been adapted for use with bronchoscopy. Similar to autofluorescence, the device uses a blue laser, which has been adapted within a small probe that can be advanced through the working channel of the bronchoscope into the distal airways and alveoli to induce tissue fluorescence. Optical slices of the observed tissue are obtained and in vivo magnified images of the alveoli can be observed.

Figure 6 Confocal microendoscopy showing alveolar septae and alveolar macrophages.

Although the technology will theoretically enable histologic interpretation of in vivo tissue, several technical issues remain. These need to be resolved before the technology can be put in widespread use. Currently, to adequately fluoresce this live tissue, a contrast dye must be administered within the pulmonary parenchyma and, given the limits of standard bronchoscopy and live imaging, the placement of the confocal probe within the correct area of study cannot yet be accurately controlled. The current data within the pulmonary literature remain limited to studies by Thiberville and colleagues. In 2007, their group performed in vivo CFM bronchoscopically on 29 patients at high risk for lung cancer. The investigators recognized several microscopic patterns that may help in the recognition of dysplastic tissue. In 2009, the same group performed confocal fluorescence microscopy in 41 healthy subjects, including 17 active...
smokers. In vivo acinar microimaging was obtained from multiple lung segments (Fig. 5). The investigators reported that alveolar macrophages were not detectable in nonsmokers, whereas a specific tobacco tar–induced fluorescence was observed in smoking subjects. Although this technology does appear to have promise, given the currently limited data, its accuracy in detecting lung pathology has yet to be defined.

References
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