AUTOFLUORESCENCE BRONCHOSCOPY AND NARROW BAND IMAGING

ABSTRACT

Lung cancer is the most common cause of cancer deaths worldwide and accounts for more deaths than breast, prostate and colon cancers combined. For the majority, lung cancer is diagnosed at the advanced stage, and 5 year survival remains dismal at 16% due to poor responsiveness to chemo-radiotherapy and absence of early detection strategies.

Centrally arising squamous cell carcinoma of the airway develops in a step-wise manner from squamous metaplasia, through increasing grades of dysplasia, carcinoma-in-situ and finally invasive cancer. Identification of its preinvasive precursors that predict with substantial likelihood for invasive cancer represents important targets for detection and treatment. Five year survival for carcinoma-in-situ (stage 0) is 74-91%, which is in stark contrast to 2% when the cancer has spread beyond the primary site.

New bronchoscopy technologies that include autofluorescence bronchoscopy and narrow band imaging are developed with these objectives in mind. This chapter appraises the different techniques and recommends a multimodality approach towards detection and treatment of airway preneoplasia.

INTRODUCTION

Lung cancer is the most common cancer both in incidence and mortality with 1.3 million new cases and 1.2 million deaths occurring each year.[1] Notably, lung cancer accounts for more deaths than prostate, breast and colon cancers combined. Despite advances in radiological imaging (CT and positron emission tomography), surgical techniques and post-operative management, radiotherapy delivery and new chemotherapeutic agents, long-term survival from lung cancer remains poor and has not improved significantly over the last 20 years.[2] Although major potential for lung cancer prevention exists, complete eradication of smoking proves difficult and the risk of lung cancer remains high in former smokers.[3] The key to improving lung cancer survival is to detect it at an earlier stage and a recent CT screening trial reports 10 year survival in excess of 80% for clinically stage I parenchymal tumors if intervened early. However CT for early central airway cancer detection remains poor.[4]

Majority of lung cancers that arise from peripheral airways and lung parenchyma are adenocarcinomas, which can be detected by CT while those arising from the central airways are squamous cell carcinomas and can be accessed using the bronchoscope. Based on necropsy study of smokers, Auberbach and coworkers postulated that squamous cell carcinomas arose from pre-invasive lesions that affected the central airways.[5] The hypothesis that squamous cell carcinogenesis progresses in a stepwise manner where the epithelium changes from normal to hyperplasia, metaplasia, mild, moderate and severe dysplasia and then to carcinoma-in-situ (CIS) is supported by sputum cytology studies where 10% of moderate dysplasia and 40% to 83% of severe dysplasia advance to invasive lung cancer.[6-8] Detection of these pre-invasive lesions that have a strong likelihood to become invasive cancer represents a potential target for improving patient survival if they are intervened early, and conventional diagnostic tools such as CT and white light bronchoscopy (WLB) lack sensitivity since these lesions demonstrate only subtle changes and are generally less than 1.5mm thick.[9]

FLUORESCENCE IMAGING

Early studies showing tissues emit fluorescence when excited by ultraviolet light date back to 1933 and 1943.[10,11] Lycette in 1965 noted a difference in the intensity of autofluorescence between normal and cancerous tissue, as when these tissues were excited at

330 nm wavelength, they demonstrated emission in the 360-600 nm range, but fluorescence intensity of tumor was lower than that of normal tissue.[12]

Tissue autofluorescence depends on the concentration of endogenous fluorophores (flavins and porphyrin) as well as the microenvironments between tumor and normal tissues.[13] As the intensity of tumor autofluorescence is weak, investigators have used hematoporphyrin derivatives that are preferentially accumulated in tumor, and accentuated by endoscopic laser illumination to induce red fluorescence. Notwithstanding that photodynamic diagnosis improves CELC detection, it cannot be used routinely due to cost and photosensitivity.[14]When bronchial surface is illuminated by light, light is absorbed, reflected, backscattered or induce fluorescence. WLB, a form of reflectance imaging, defines structural features of bronchial epithelium and discriminates normal from abnormal. It has led to the detection of early hilar lung cancer, [15] and endoscopic features of dysplasia and carcinoma *in-situ* (CIS) have been described.[16] However, Woolner and coworkers have reported that less than a third of patients with CIS could be identified using this modality of imaging.[17] Autofluorescence bronchoscopy (AF) that exploits differences in fluorescence properties of normal and abnormal bronchial mucosa, can faciltate in the detection of pre-invasive neoplasia which may otherwise be invisible on WLB. Laser induced fluorescence endoscopy (LIFE) (Xilix Technologies, Canada) is a device that detects pre-neoplastic lesions by capturing differences in autofluorescence emitted by normal, pre-neoplastic or early malignant tissue when excited by monochromatic blue light (442 nm) delivered by a helium cadium laser. Normal bronchial tissue emits green fluorescence (500-600nm) when excited by blue light while cancer shows decrease in green fluorescence due to greater epithelial thickness and vascularity (Figure 1). The first LIFE-Lung system uses helium cadmium laser for illumination. LIFE-Lung II employs a filtered xenon lamp to produce blue light with two image-intensified charge-coupled device sensors to capture emitted fluorescence: one in the green region (480-520 nm) and the other in the red region (625 nm). Clinical studies using LIFE system have demonstrated enhanced sensitivity for the localization of airway lesions that correspond to moderate dysplasia and worse on pathology. LIFE is highly sensitive, however difficulties in distinguishing benign epithelial changes such as bronchitis, inflammation or fibrosis from previous biopsy or endobronchial intervention from pre-invasive lesions have led to extensive biopsy and higher health costs, longer procedural time and higher incidence of procedure related bronchitis. In fact up to a third of lesions with abnormal fluorescence represent false positives when correlated with pathology.[18,20]

Spectral differences observed among normal, preneoplastic and neoplastic tissues allow for the development of AF-reflectance imaging devices such as Storz D-light (Karl Storz, Germany), Onco-LIFE (Onco-LIFE Endoscopic Light Source and Video Camera; Novadaq Technologies), SAFE 3000 (Pentax) and AFI (Olympus, Japan). D-light and Onco-LIFE are based on fiberoptic technology while SAFE 3000 and AFI are videoendoscopes. AF in clinical studies (Table 1) is 1.3 to 6.4 times more sensitive than WLB for pre-invasive lesions, and is especially helpful in airway evaluation of patients with abnormal sputum cytology.[18-28] As AF demarcates the tumor margins, it provides useful information for pre-operative surgical planning as well as better defines the target for endoscopic treatment (Figure 2).[27,28]

Figure 1: Principles of Autofluorescence: green fluorescence is emitted by normal bronchial tissue when excited by blue light, cancer shows decreased green fluorescence due to increased epithelial thickness and vascularity.

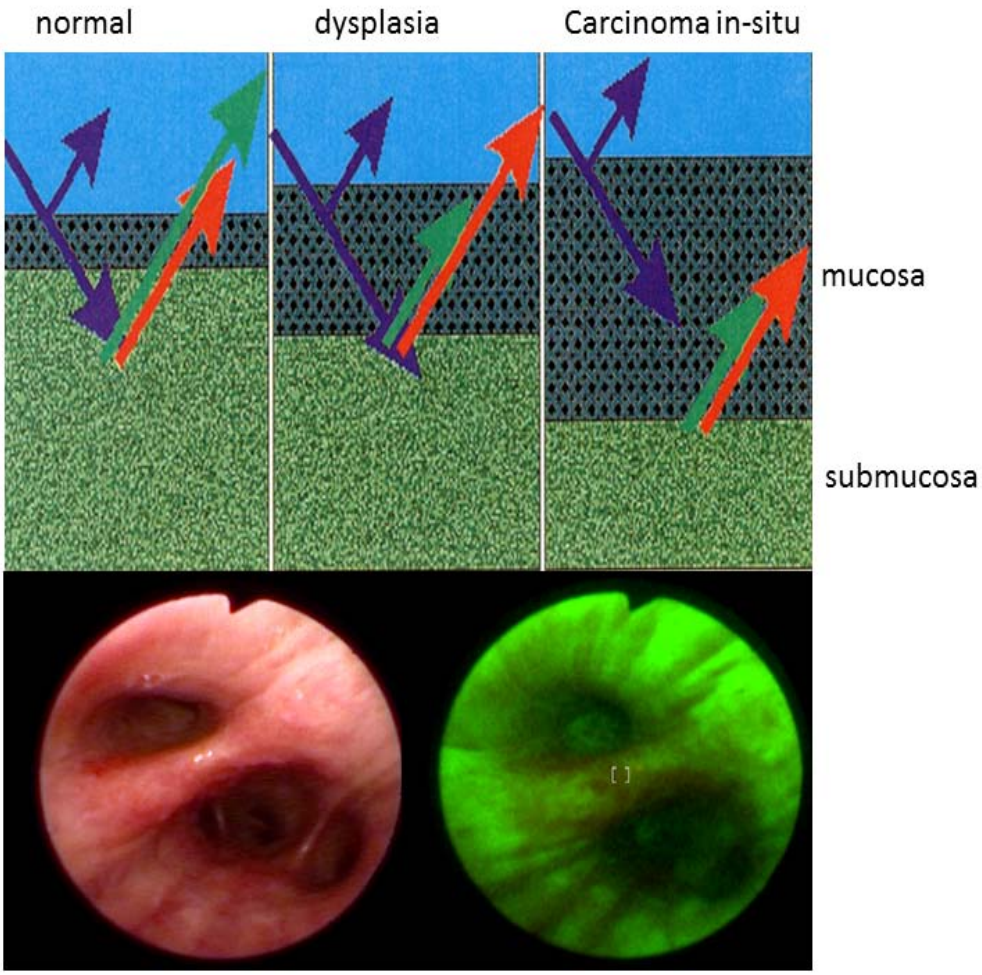
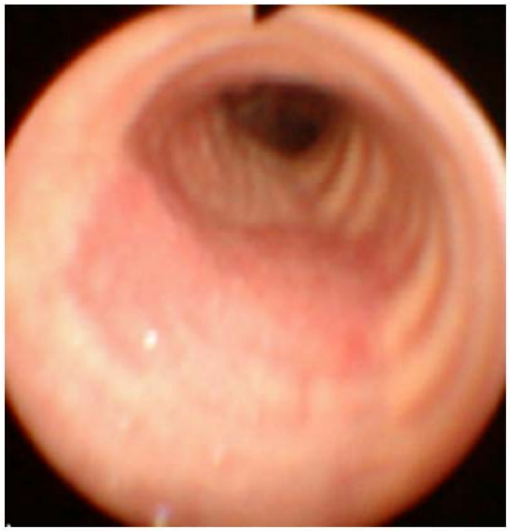


Table 1: Sensitivity and specificity of autofluorescence bronchoscopy for airway dysplasia

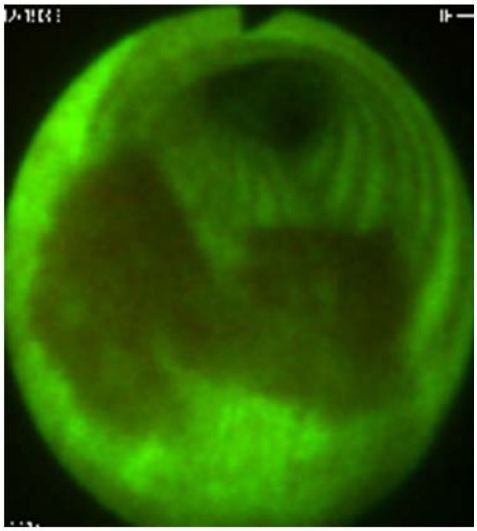
Authors (ref)	Equipment	Sensitivity sequential procedure	Specificity sequential procedure
Haussinger (21)	BF, D-Light	0.82 (WLB + D-Light)	0.58 (WLB + D-light)
Hirsch (20)	BF, LIFE	0.79 (WLB + LIFE)	0.29 (WLB + LIFE)
Lam (18)	BF, LIFE	0.56 (WLB + LIFE)	0.66 (WLB + LIFE)
Vermylen (19)	BF, LIFE	0.93 (WLB + LIFE)	0.21 (WLB + LIFE)
Chhajed (29)	VE, LIFE	0.72 VE/ 0.96 LIFE	0.53 VE/ 0.23 LIFE
Chiyo (23)	VE, LIFE, AFI	0.56 VE/ 0.97 LIFE/ 0.8 AFI	0.5 VE/ 0.37 LIFE/ 0.83 AFI
Ikeda (24)	VE, SAFE	0.65 VE/ 0.90 SAFE	0.49 VE/ 0.47 SAFE
Lee (30)	VE, SAFE	0.86 dual image	0.94 dual image
Edell (22)	BF, Oncolife	0.74 (WLB + Oncolife)	0.75 (WLB + Oncolife)

BF: bronchofiberscope, VE: videobronchoscope

Figure 2 Superficial spreading tumor, margins clearly demarcated by LIFE bronchoscopy



WLB shows suspicious mucosal thickening of bronchus intermedius

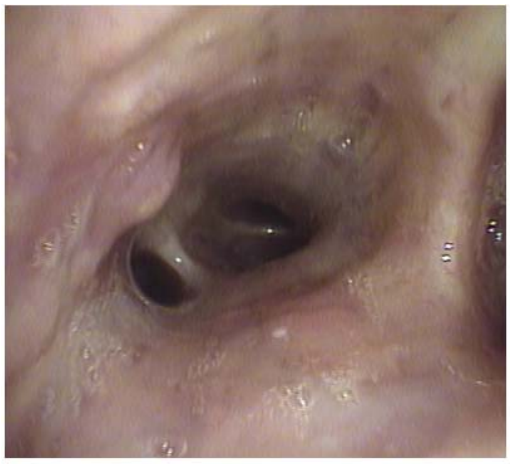


LIFE shows tumor of bronchus intermedius with clear margins

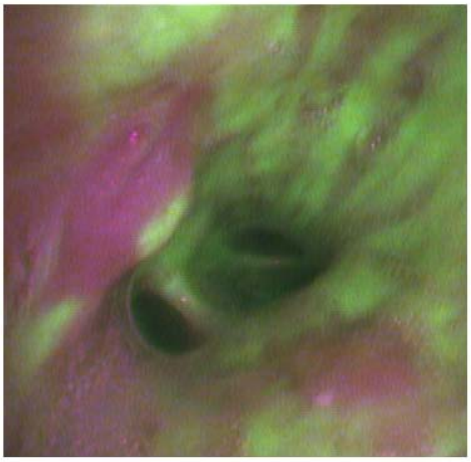
Following the advent of the videobronchoscope that has a miniature charge couple device built in its tip to deliver clearer images, sensitivity for the detection of premalignant lesions has correspondingly improved without compromising its specificity. Chhajed et al. showed that when videobronchoscopy was added to LIFE, the combined modality was better in selecting sites for biopsy.[29] However the procedure required a change of scopes and represented a source of discomfort to the patient as well as inconvenience to the

bronchoscopist. AFI is a video-bronchoscope that displays a composite image by integrating 3 signals: autofluorescence caused by excitation light (395-445nm); green and red light signals by respective green (550nm) and red (610nm) wavelengths. Since haemoglobin absorbs green and minimal red light, areas with high haemoglobin due to increased vascularity which is a feature of dysplasia rather than bronchitis, AFI is more superior than LIFE in the discrimination of pre-invasive and malignant lesions (Figure 3) from bronchitis or hyperplasia where all would appear abnormal with LIFE.[23] Although switching from WLB to AFI involves hitting a button on the bronchoscope, simultaneous comparison of video-endoscopic and AF images cannot be achieved.

Figure 3: Superficial spreading tumor with margins clearly demarcated by AFI



WLB shows suspicious mucosal thickening of right lower lobe bronchus

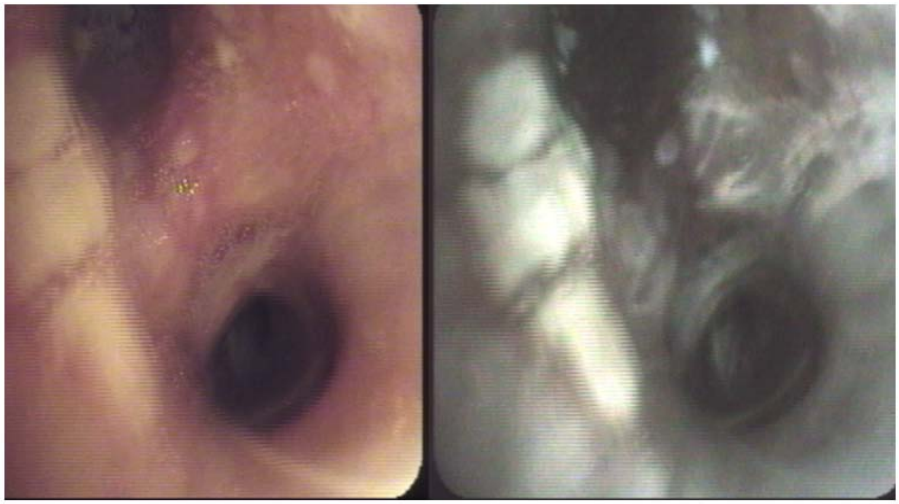


AF shows superficial spreading tumor of right lower lobe bronchus

Dual real-time display of video-endoscopic and AF images is recently made possible with SAFE 3000 (Pentax, Tokyo, Japan). SAFE 3000 is a video-bronchoscope that uses xenon lamp for WLB and allows real-time colour image transmission using the miniature charge couple device that is built into its tip. The AF mode utilizes a diode laser that delivers excitation light to the target from the tip of the scope, and fluorescence from the target is captured by filtering out the wavelength of excitation light with the objective lens. Since both light sources are available, dual real-time display of video and AF bronchoscopic images of the target is achieved. Our study has shown that dual real-time display of videobronchoscopic and AFB images is not only sensitive for detecting pre-neoplastic lesions (0.86), it is highly specific (0.94) as it provides both functional and anatomic information of the lesion simultaneously. By doing so, it aids in targeting biopsy which in turn leads to shorter procedural time, better patient comfort and safety. Good correlation between visual classification (normal, abnormal, suspicious) and pathology is achieved especially in the

identification of previous biopsy site, bronchitis, and airway fibrosis after endobronchial therapy (Figures 4a,b).[30]

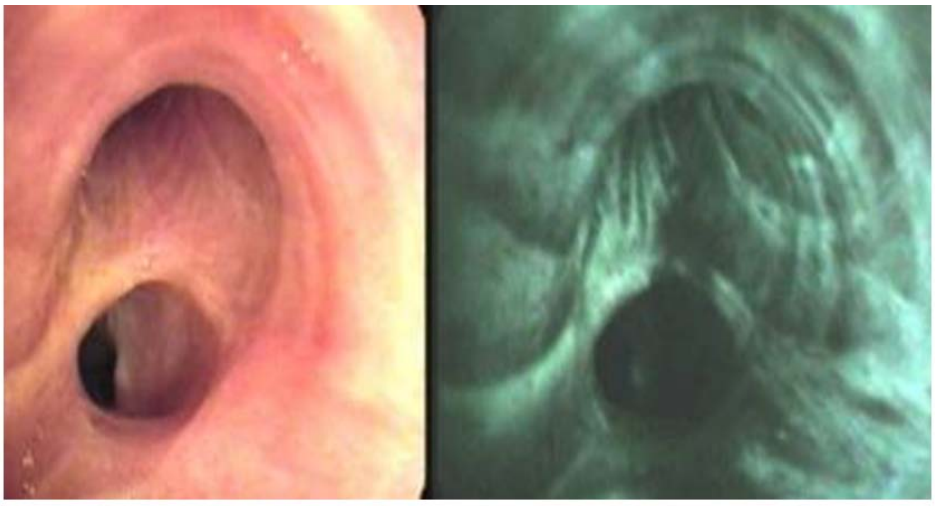
Figure 4a: Dual Images of Carcinoma in-situ with SAFE 3000.



WLB shows mucosal thickening of right secondary carina RC1

AF shows carcinoma in-situ of right secondary carina RC1

Figure 4b: Dual Images of previous biopsy site with SAFE 3000



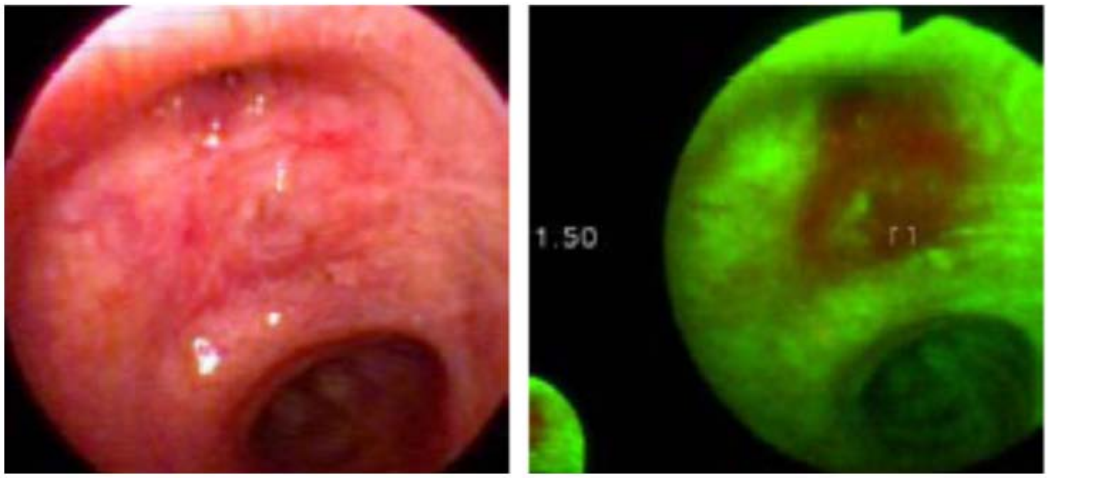
WLB shows previous biopsy site

AF shows abnormal fluorescence from previous biopsy site

The newly developed Onco-LIFE device uses both reflectance and fluorescence light for imaging. Blue light (395-445 nm) and small amount of red light (675-720 nm) from a filtered mercury arc lamp are used for illumination. A red reflectance image is captured together with the green autofluorescence image by non-image-intensified charge coupled device to enhance the contrast between pre-invasive/ malignant from normal tissues. By using reflected infrared red light as a reference, it has an advantage over reflected blue or

green light as it is less absorbed by haemoglobin, and may therefore be less influenced by changes in vascularity associated with airway inflammation thereby reducing its false-positivity.[22] Red light is also uniformly scattered within the tissue and serves as a reference signal that corrects for differences in light intensities from changes in angle and distance of the bronchoscope from the bronchial surface. In addition, Onco-LIFE also allows quantitative analysis of the fluorescence image by providing a numeric representation (R/G ratio) of the combined colors in the central portion of the displayed image. R/G ratio of the 16 by 16 pixel square target defined within the displayed brackets is calculated by dividing the average red reflectance with green fluorescence signals captured by the camera (Figure 5). We performed a study correlating pathology of 3,362 biopsies with their corresponding R/G ratios. R/G ratio 0.54 and greater conferred 85% sensitivity and 80% specificity for the detection of high-grade and moderate dysplasia. When visual score (normal, abnormal, suspicious) was combined with R/G ratio, specificity in diagnosing moderate dysplasia and worse was further improved to 88% suggesting that color fluorescence ratio could objectively guide the bronchoscopist in selecting sites for biopsy with good pathological correlation.[31]

Figure 5: Color Fluorescence ratio R/G of Carcinoma In-situ.



WLB shows suspicious focal mucosal thickening of LB6

AF shows abnormal fluorescence with distinct margin of LB6.

RG ratio of target within the brackets 1.5 is derived by dividing red reflectance with green fluorescence signals

NARROW BAND IMAGING

The conventional RGB sequential video bronchoscope system has a xenon lamp and rotation disk with 3 RGB optical filters. The rotation disk and monochrome CCD are synchronized and 3 band images are generated sequentially. Color images are created by the video processor. Narrow band imaging (NBI) is a novel system that is developed to enhance microvessel structure using a new narrow banding filter on an RGB sequential video bronchoscope system instead of the conventional RGB broad band filter (Figure 6). Wavelengths used by the NBI filter are B1: 400-430nm; B2: 420-470nm and G: 560-590nm which are in contrast to the conventional RGB broad band filter namely; B: 400-500 nm, G: 500-600 nm and R: 600-700 nm. Tissue optical absorption and scattering properties are wavelength dependent. Blue light has a shorter wavelength and reaches into shallow surfaces. The main chromophore in bronchial tissues is haemoglobin which has a maximum absorptive wavelength near 415 nm and is within the wavelength range for NBI-B1. Therefore it is expected that the NBI-B1 filter would detect blood vessel structures more accurately than other filters. When conventional RGB broad band light is delivered onto tissue surface, light is scattered and absorbed by tissue with little light reflected to form an image, however if narrow band light is delivered onto same surface, it causes less scattering thereby enhancing its reflected image. The first publication on NBI combined with high magnification bronchovideoscopy showed significant association between dotted vessels detected by NBI-B1 and pathological diagnosis of angiogenic squamous dysplasia (ASD) (Figure 7a).[32] ASD is a morphological entity of squamous dysplasia of the central airways where collections of capillary blood vessels are projected into dysplastic bronchial epithelium suggesting that angiogenesis is an early event of lung carcinogenesis. Several studies investigating into multi-step model of carcinogenesis have indicated that angiogenesis switch occurs in pre-invasive lesion prior to invasive tumor development.[33] Although Hirsch and coworkers have shown that fluorescence bronchoscopy was able to detect 75% of ASD cases,[20] it cannot distinguish ASD from bronchial squamous dysplasia without ASD. NBI appears to be useful for the detection of capillary blood vessels in ASD lesions at sites of abnormal fluorescence. Studies are underway to determine if patients harboring ASD have greater risk for progression to lung cancer. In a pilot study of 22 patients WLB was performed followed by NBI, NBI identified dysplasia or malignancy not apparent on WLB in 23% of subjects.[34] This was confirmed in a larger trial where 62 patients underwent WLB then randomized to AFI or NBI with the objective of evaluating value of NBI if any to AFI

and WLB.[35] Results showed respective sensitivities and specificities of AFI and NBI were 3.7/ 3.0 and 0.5/ 1.0 relative to WLB. The addition of NBI to AFI did not increase diagnostic yield significantly and the sequence with which AFI and NBI was performed did not impact findings. The authors propose NBI as an alternative to AFI for early lung cancer detection (Figure 7b) since WLB can be converted to NBI by merely pressing a switch button on bronchoscopy systems (EVIS EXERA II, BF-Q180/ BF-IT180, Olympus Medical Systems Corp, Japan) unlike AFI which requires a change of scope (Evis Lucera videobronchoscope BF-F260, Olympus Medical Systems Corp., Japan). Subsequently NBI is found to be useful in early detection of head and neck cancers as well as impacting therapeutic decision in the assessment of tumor extension for centrally located lung cancer.[36,37]

Figure 6: Narrow band imaging enhances microvessel structure by means of narrow banding filter instead of conventional RGB broadband filter.

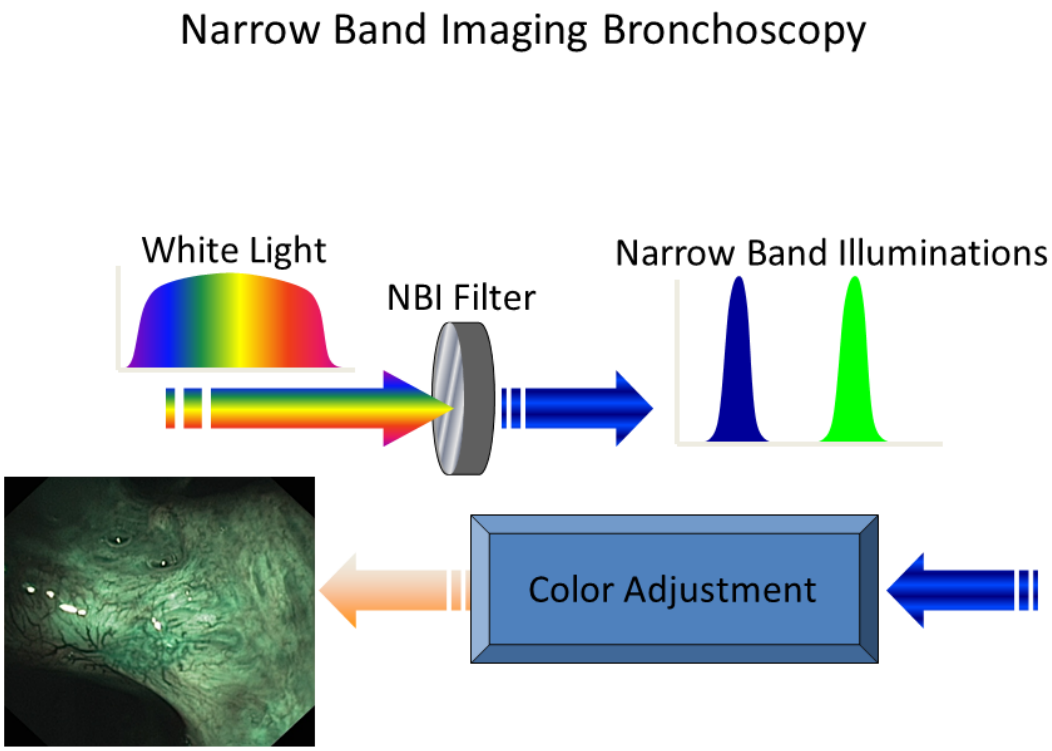


Figure 7a: Narrow Band Imaging shows dotted vessels of angiogenic squamous dysplasia.

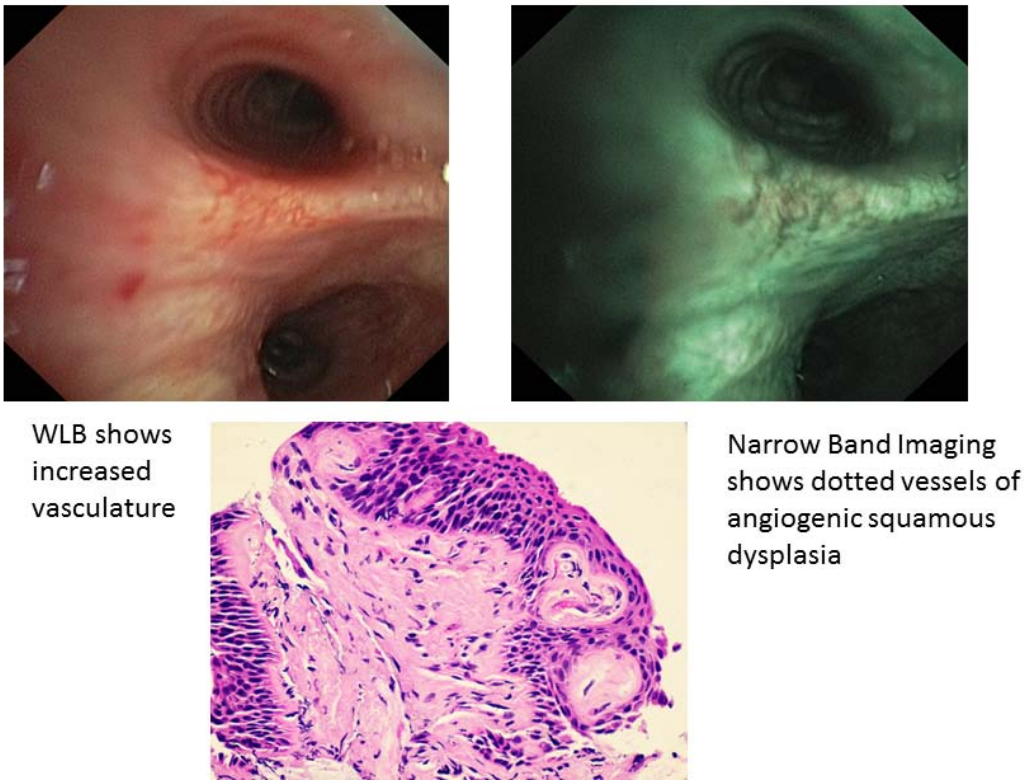
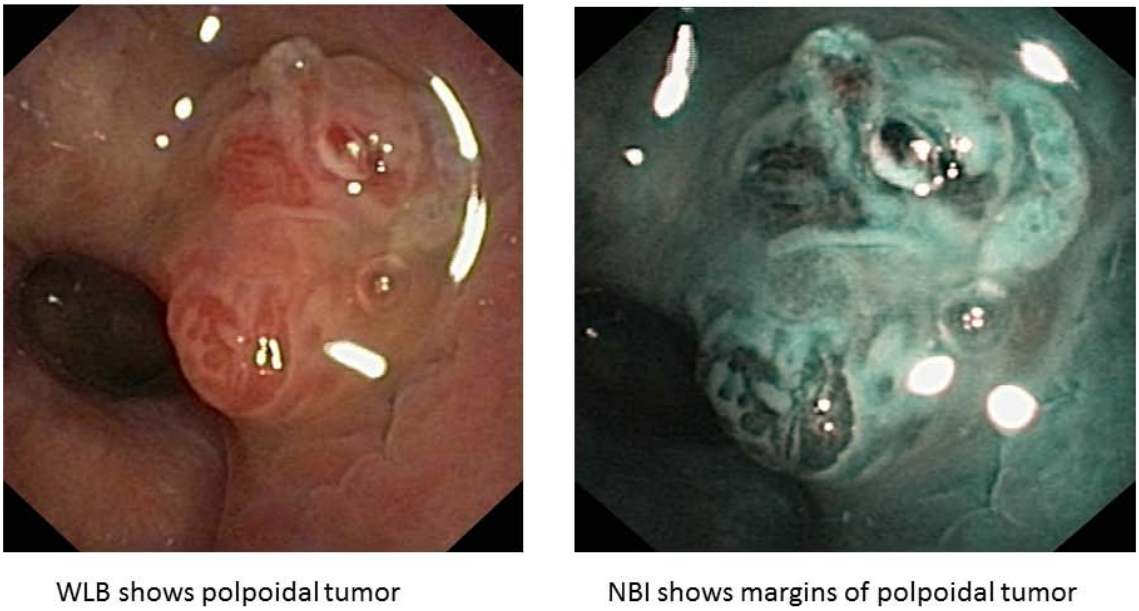


Figure 7b: Narrow band imaging shows tumor margins.



CLINICAL APPLICATIONS

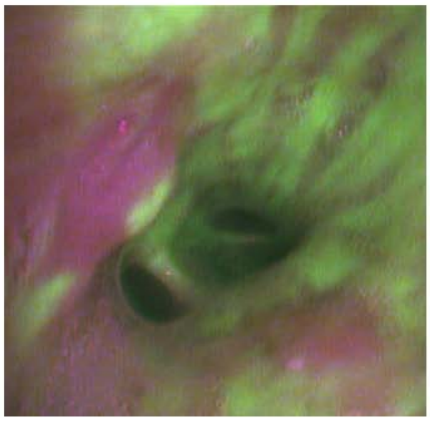
CELC must first be radiographically occult without lymph node and distant metastasis. The squamous cell cancer should measure less than 2cm in greatest dimension with visible distal margin and located in the subsegmental, proximal bronchi or trachea.[38] CELC is classified into 5 categories: polypoid, nodular, thickened, invisible and mixed. Konaka and co-workers have found that tumor dimension strongly correlates with the depth of bronchial invasion. Polypoid or nodular types tend to invade more deeply than the thickened and flat lesions.[39] In fact, polypoid or nodular lesions that measure less than 10 mm and flat lesions 15 mm or smaller tend to be confined within the cartilaginous layer without nodal metastasis.[40] Thus precise evaluation and staging of CELC are important steps towards treatment selection. Surgery is still the treatment of choice for CELC but many of these patients suffer from poor cardiopulmonary reserve due to smoking and are therefore unfit for surgery. Moreover up to 30% of CELC can be multifocal as a result of field effect.[17] Lung sparing techniques such as photodynamic therapy (PDT) or locally applied bronchoscopic treatment such as endobronchial electrosurgery, cryotherapy, and brachytherapy are attractive alternative options to a select group of CELC patients with tobacco-related comorbidities, however these lesions must be confined within the cartilaginous layer of the bronchial wall to enable successful treatment.[41] Therefore a flat lesion measuring 10 mm and less with visible distal margin represents the ideal lesion for bronchoscopic treatment.

Radial endobronchial ultrasound allows visualization of layered structure of the bronchial wall and can be a good tool in determining peribronchial tumor invasion. Herth and co-workers compared depth of tumor invasion by EBUS and high resolution CT with pathology of resected lungs in 105 patients with CELC. All CELC with EBUS evidence of tumor invasion were confirmed on pathology. EBUS underestimated tumor invasion in 6 patients thereby achieving 89% sensitivity and 100% specificity for invasive cancer.[42] Studies incorporating EBUS as part of staging report complete response with PDT if intracartilaginous CELC are treated.[43,44]

Optical coherence tomography (OCT) is similar to EBUS but uses light instead of acoustic waves. In ultrasound, imaging is made possible by measuring the delay time (echo delay) for the ultrasonic pulse to be reflected back from tissue, and because velocity of sound is relatively slow, echo delay can be measured electronically. Since OCT uses light which travels 200,000 times faster than the speed of sound, low coherence interferometry is used. OCT is able to obtain high resolution, cross-sectional micro-images of tissue which can

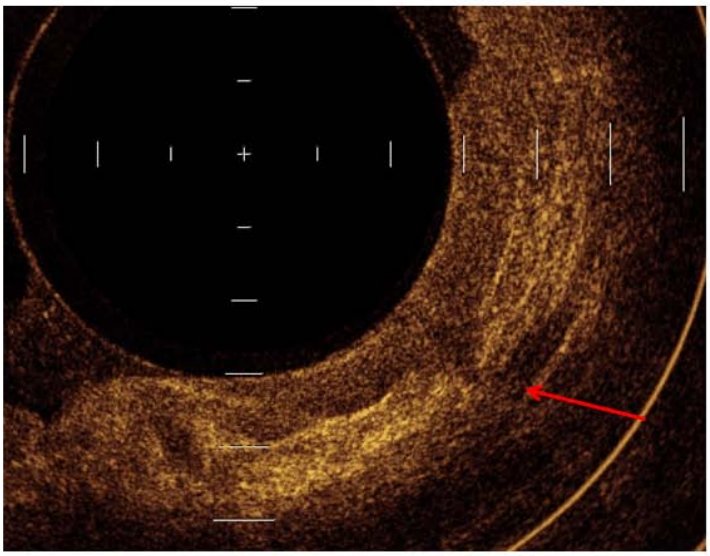
potentially permit optical biopsy in place of conventional excisional biopsy in the future (Figure 8).[45,46]

Figure 8: OCT shows microinvasive carcinoma beyond cartilaginous layer (red arrow).



AF shows suspicious RB6 lesion

OCT shows microinvasive carcinoma with invasion beyond cartilaginous layer (red arrow)



FUTURE DIRECTIONS

Invasive squamous cell carcinoma of the central airways is believed to develop through a stepwise process where the epithelium changes from normal to hyperplasia, metaplasia, mild, moderate and severe dysplasia and CIS.[9] Squamous dysplasia and CIS are categorized as pre-invasive lesions by the recent histological classification by the World Health Organization.[47] This stepwise model is supported by studies where serial sputum cytological examinations in uranium miners and smokers showed that invasive lung cancer develops through mild, moderate and severe atypia, CIS and finally invasive cancer.[48,49] However sputum can come from different parts of the tracheobronchial tree and progression of sputum atypia to cancer may reflect field cancerization rather than progression from the same lesion and the concept of stepwise progression of preinvasive lesions to invasive carcinoma was recently challenged.[50] In attempt to clarify the natural history of preinvasive lesions, longitudinal studies using serial AF and biopsy were performed. About 59% of severe dysplastic lesions regressed spontaneously while 41% persisted or progressed

to CIS or invasive cancer.[51-53] Majority of CIS progressed to invasive cancer within a median of 30 months, recurred despite bronchoscopic treatment or persisted.[52,54,55] George and co-workers followed patients with severe dysplasia or CIS and they found that 5 patients with 6 CIS lesions progressed to invasive cancer within 15 months, of which 3 patients had progressive disease despite radical therapy or PDT. The authors concluded that since majority of CIS progressed and could become incurable by local therapy, treatment was preferable instead of surveillance with repeat AF and biopsies.[56] Spontaneous regression is more common for mild/moderate dysplasia, Hoshino and co-workers found that only 1 of 88 lesions with low grade dysplasia progressed to invasive carcinoma,[53] similarly none of the low grade dysplastic lesions progressed to CIS or invasive cancer over 12-85 month follow up.[56] Therefore a major hurdle in any early lung cancer detection program is to select with accuracy airway lesions that are at high risk of progression to invasive cancer for treatment than those that do not. With advances in molecular biology and micro-dissection techniques, genomic changes responsible for malignant bronchial transformation such as inactivation of tumor suppressor genes, activation of oncogenes, loss of heterozygosity (LOH) and amplifications of chromosomes are detected in pre-invasive lesions. By analysing genomic patterns in pre-invasive lesions (moderate dysplasia to CIS) using fluorescent in-situ hybridization (FISH), 4 probes (TP63, MYC, CEP3 and CEP6) were found to correlate with progression to invasive cancer with 85% sensitivity and 58% specificity.[57] Loss of heterozygosity of 3p allele was also strongly associated with endobronchial treatment resistant as well as progressive to invasive cancer CIS and severe dysplastic lesions.[58] Over-expression of p53, cyclin D1/cyclin E and Ki-67 detected by immunohistochemistry increased in accordance to the histological grade of bronchial dysplasia.[59] Micro-RNA expression profiling was also found to be highly predictive of the histological grade of pre-invasive lesions identified by AF and by recognizing these characteristic trends of expression can lead to early diagnosis of lung cancer.[60]

Emerging data indicate that molecular analysis can not only aid in more precise histopathologic grading of pre-invasive lesions, its incorporation into a molecular based management decision tree may also allow the clinician to identify and target treatment on the airway lesions that harbor molecular signatures which signify high risk of progression to invasive lung cancer.

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