REVIEW ARTICLE

ENDOSCOPIC IMAGING TECHNOLOGIES IN THE DETECTION OF EARLY STAGE LUNG CANCER

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Abstract:

Endoscopic imaging techniques that have been developed for the early detection of bronchogenic carcinoma include auto fluorescence bronchoscopy (AFB), narrow band imaging (NBI), optical coherence tomography (OCT), and probe based confocal laser endomicroscopy (pCLE). Each of these modalities capitalizes on the light absorption properties of the tissues or blood vessels in attempts to identify features consistent with a pre-invasive carcinoma.AFB is based on the principle that dysplasia and carcinoma fluoresce red when exposed to blue light. NBI capitalizes on the light absorption properties of hemoglobin to highlight abnormal appearing vasculature. OCT uses optical light reflections of tissues to construct two and three dimensional images of the bronchial epithelium. Finally, pCLE allows visualization of sub epithelial structures by sectioning tissue based on its natural auto fluorescence. This review discusses these techniques in detail, providing the scientific background, practical application, and review of the literature.

INTRODUCTION

The last several years have seen a significant increase in new endoscopic imaging modalities. While autoflourescence technology has been employed in the tracheo-bronchial tree for nearly two decades, alternative imaging platforms are now available each with their own particular advantages and idiosyncrasies. Herein, each of these imaging modalities: auto fluorescence bronchoscopy, narrow band imaging, optical coherencetomography, and probe-based confocal laser endomicroscopy will be reviewed. The fundamental principles that form the basis of each of the technologies will be discussed followed by an examination of the currently available data and literature.

AUTOFLOURESCENCE BRONCHOSCOPY

Introduction

Autoflourescence bronchoscopy (AFB) is based upon the principle that when illuminated by light in the blue spectrum (380 to 440 nm) dysplasia and carcinoma demonstrate weaker green and red fluorescence than normal tissue[1]. The result is that normal tissue will fluoresce green in this light spectrum, while dysplasia, carcinoma in situ (CIS), and invasive carcinoma will fluorescence red/magenta (Figure 1). It is not clearly understood why this occurs, but there is some speculation that the neovascularization of the abnormal tissue leads to greater blood content and subsequent quenching of the auto fluorescent signal[2].

Background

The first bronchoscopic fluorescence imaging system was reported in 1993 in which it was compared to white light bronchoscopy (WLB). That study demonstrated similar specificity (94%) but 50% greater sensitivity in favor of fluorescence bronchoscopy (72.0% vs. 48.4%)[3]. A study by Lam and colleagues on 173 patients with known or suspected lung cancer demonstrated that the combination of WLB and light-induced fluorescence endoscopy (LIFE) compared to WLB alone had a relative sensitivity of 6.3 for intraepithelial neoplastic lesions and 2.71 for invasive carcinoma[4]. This study was the basis for Federal Drug Administration (FDA) approval of the LIFE device for use in the United States.

Auto fluorescence Systems

The LIFE device (Xillix LIFE-Lung Fluorescence Endoscopy System; Xillix Technologies Corp) utilizes a heliumcadmium laser (442-nm wavelength) to illuminate the bronchial tree and the autofluorescence images are filtered and amplified by the system's camera[4]. The Onco-LIFE system (Novadaq Technologies) has largely replaced the LIFE device. The Onco-LIFE system has a single light source that

can be changed between white light and autofluorescence. In the fluorescence mode the system illuminates the mucosa with a blue light (395-445 nm) mixed with a small amount of red light (675-720 nm). As the red reflectance is not significantly altered by tissue abnormalities it can be used as a reference signal[5]. Olympus Optical Corporation produced an autofluorescence imaging bronchovideoscope (AFI) system that combines autofluorescence with two reflected color light signals. The color tone analysis of three different wavelengths has been reported to improve the ability to differentiate between inflammation and malignancy[6]. The Karl Storz D-light system uses a 300-watt xenon lamp and uses wavelengths in the 380 to 460 nm range for excitation. Like the Onco-LIFE system the bronchoscopist can toggle between AF and WLB at the touch of a button[7]. The SAFE-3000 by Pentax uses a xenon lamp for WLB and a diode laser for the AF mode. In this system the images are digitally processed and AF images can be compared to videoendoscope images in real time[8].

Literature

There have been multiple studies and two meta-analyses that have reported that AFB combined with WLB improves the sensitivity for detecting airway lesions compared to WLB alone. Chen and colleagues analyzed 14 studies with a total of 1358 patients on which 3612 biopsies were performed. They reported that the pooled sensitivity for AFB and WLB was 0.90 and 0.56 respectively. The specificities were comparable at 0.66 for AFB and 0.69 for WLB[9]. Another meta-analysis of 3266 patients out of twenty-one studies reported that the specificity of AFB + WLB was lower than WLB alone, however the sensitivity of AFB + WLB compared to WLB alone was greater to detect intraepithelial neoplasia and invasive cancer, 2.04 and 1.15 respectively[10]. As such, the American College of Chest Physicians recommends the use of AFB to guide therapy in patients with known carcinoma in situ (CIS) who will be undergoing curative endobronchial treatment. Additionally in patients who have known severe dysplasia or CIS standard AFB should be used every 3 to 6 months for follow-up [11].

Conclusion

AFB appears to be a useful tool for the detection of preinvasive lesions in the central airways. When combined with WLB it has a higher sensitivity than WLB alone and can detect lesions that are not apparent on radiographicimaging. The most appropriate patients for this technology are those that are at high risk for a lung malignancy with sputum atypia. However, there is evidence that AFB also has a role in those patients with known CIS undergoingendobronchial therapy as well as for surveillance in patients with severe dysplasia, CIS, or a history of lung cancer.

NARROW BAND IMAGING

Introduction

Narrow band imaging (NBI) is a bronchoscopic visualization technique that capitalizes on the light absorption properties of hemoglobin. Since early angiogenesis is thought to be an early event in the pathogenesis of bronchogenic carcinoma, the endoscopic identification of abnormal microvasculature in the airways in a high risk population might improve early detection of lung cancer.

Background

Hemoglobin has an optimal absorptive wavelength of 415nm. Conventional Red/Green/Blue (RGB) light sources in current bronchoscopes have wavelengths of 400-500nm, 500-600nm, and 600-700nm, corresponding to blue, green, and red light, respectively. However, with RGB light sources, light is reflected, scattered, and absorbed, thus limiting the ability of conventional bronchoscopes to highlight the vasculature of the mucosa and submucosa. With NBI, there are three wavelength filters: B1 (400-430nm), B2 (420-470nm), and B3 (560-590nm). As a result, there is less scattering of light which allows for better visualization of hemoglobin and, hence, the vasculature of the endobronchial wall[12,13,14]. A normal white light exam and NBI exam are seen in Figure 2a and 2b. Several different vascular patterns have been described to be associated with bronchial dysplasia. In general, thinner blood vessels found close to the surface will be brown in appearance while deeper vessels are thicker and appear more greenish-blue[14]. In addition, as one progresses from carcinoma in situ to invasive carcinoma, the blood vessels will become larger in size. Shibuya and colleagues studied the NBI-histopathological correlations of 79 high risk patients who had suspicious sputum cytology[12]. From this analysis, they proposed a classification system of the NBI vascular appearance of three types of endobronchial dysplasia and invasive carcinoma. First, squamous dysplasia has tortuous vessels and increased vessel growth. Second,

angiogenic squamous dysplasia (ASD) shows a few dotted vessels and tortuous vessels that are increased in diameter as compared to dysplasia.
Third, carcinoma in situ (CIS) has no tortuous vessels, but does have a few small spiral and screw-shaped vessels. Fourth, invasive carcinoma exhibits abundant dotted vessels and larger spirals and screw-shaped vessels (Figure 3). While the diagnostic test characteristics of using this type of classification system are unknown (sensitivity, specificity, positive and negative predictive value, and positive and negative likelihood ratios), it does provide aframework of NBI imaging characteristics in which endobronchial biopsies should be obtained.

NBI System
The NBI mode is currently found on bronchoscopes produced by Olympus (BF-6C260, Olympus Optical Corporation, Tokoyo, Japan). Once an airway examination has been performed with standard white light (WL), NBI is selected on the bronchoscope and an airway exam is repeated. Any abnormal appearance of endobronchial vasculature as outlined above is then biopsied.

Literature
Clinically studies supporting the use of NBI as an adjunct to white light bronchoscopy (WLB) and autofluorescent bronchoscopy (AFB) are promising. Shibuya et al first published their use of NBI with high magnification videobronchoscopy in 2003 and showed that there was a significant correlation between the dotted vessels seen on NBI-B1and the histhopathological finding of ASD[13]. Vincent and colleagues showed that in 22 patients with a history of lung cancer who underwent bronchoscopy for either abnormal airways on imaging studies or radiographic evidence of airway obstruction, that NBI had an improved sensitivity and specificity when compared with WLB (1.63 sensitivity ratio, with >1 favoring NBI over WLB, and 64% versus 81% specificity)[15]. In their series, they obtained 42 biopsies from abnormal areas seen on WLB, NBI, or both. Twenty-two control samples were obtained as well. NBI detected 23% of cancers (5 cases) that were not visible under WLB. Finally, Herth et al evaluated the role of NBI in conjunction with AFB and WLB. They found that as compared with WLB, AFB and NBI hadbetter, though similar sensitivities (3.7 and 3.0 sensitivity ratios, respectively, with >1 favoring AFB and NBI over WLB), but NBI had a higher specificity than AFB[16]. This suggests that NBI and AFB might be complementary, rather than mutually exclusive techniques in the evaluation of early stage lung cancer in high risk patients.

Conclusion
NBI as an aide to detect pre-malignant lesions in the airways of high risk patients is promising. Since NBI capitalizes on the wavelength of hemoglobin and filters out unwanted light, the endobronchial vasculature is more easily highlighted allowing for the identification of abnormal regions amiable to biopsy that would not have been seenon WLB. While initial studies are encouraging, questions remain as to the diagnostic test characteristics of NBIand the impact of treating pre-malignant lesions. As such, it cannot be recommended as a routine screening tool to detect early stage lung cancer at this time outside of clinical trials. However, while these questions are unanswered pending further research, the potential for NBI as an adjunct to WLB and AFB in the early detection of lung cancer in the right patient population are hopeful.

OPTICAL COHERENCE TOMOGRAPHY
Introduction
Optical coherence tomography (OCT) is a technique that uses the optical reflections of biologic tissues to create non-invasive cross-sectional imaging. This optical backscattering is performed by low coherence interferometry utilizing a low coherence light source from a superluminescent diode. The difference in the time it takes for light to return from deeper tissues compared to shallow tissues allows the system to reconstruct two dimensional images in a time-domain system. Recently, spectral-domain OCT has been developed that allows for comprehensive microscopic three-dimensional imaging[17].

Background
Since its introduction, the use of OCT in ophthalmology has become routine practice in many disorders[18]. This technology has also found use in gastroenterology, dermatology, and cardiovascular diseases[19-21]. The use of OCT in human lung is largely investigational at this stage although it has shown promise in several different pulmonary pathologies.

OCT Systems
Pentax and LightLab Imaging produce a 1.5 mm diameter probe that can be used with a standard bronchoscope. It is a radially scanning probe that can theoretically achieve an axial resolution of 15 to 20 μm and a lateral/transverse resolution of 21 to 27 μm at a depth of 1-2 mm[22]. Niris Imaging system has a commercially available OCT system that is cleared by the US Food and Drug Administration for imaging of human tissue microstructure. It has a larger outer diameter of 2.7 mm and can generate two dimensional images with an axial resolution of 10 to 20 μm and a transverse/lateral resolution of 20 to 25 μm. The image depth is reported at 2.2 mm[23].

Literature
Studies of resected lungs with OCT delivered via bronchoscopy has demonstrated a uniform layer structure in the normal bronchus, but a loss of layer structure and increased thickening between epithelium and cartilage in thelocation of tumor. It can also discern between areas of inflammation and malignancy. An *in vivo* study of 138 heavy smokers and 10 patients with known lung cancer was performed with OCT. Biopsies were than taken at the same sites and sent for histopathologic examination. The data demonstrated that OCT was able to discriminatedysplasia and carcinoma in situ from normal, hyperplasia, and metaplasia[22]. A recent *in vitro* study used optical coherence tomography to quantify permeability coefficients of 30% glucose diffusion in variants of lung tissue: normal, granulomatous, squamous cell carcinoma, and adenocarcinoma. Compared to normal lung the permeability coefficient increased by 32%, 113%, and 162% for granulomatous, squamous cell carcinoma, and adenocarcinomarespectively and suggested that this may be useful to distinguish between normal and malignant lung tissue[24].

Conclusion
Optical coherence tomography is a promising technology for the diagnosis and evaluation of a variety of pulmonary pathology. Currently its application is limited primarily due to the shallow depth of penetration as well as data that compares the sensitivity and specificity of OCT to histopathology. With improved technology it may be possible that this technology may advance to the point where it can obviate the need for invasive tissue biopsies.

Probed based Confocal Laser Endomicroscopy (pCLE)
Introduction
The principle of confocal fluorescence microscopy has been adapted to microendoscopic devices, where the microscope objective is replaced by a flexible miniprobe[25]. This allows in vivo optical sectioning of tissue basedon their natural autofluorescence (AF) properties. Confocal microscopy relies on both the use of a narrow point source (laser) on the illumination path and of a small aperture on the light detection path, thereby eliminating out of focus images[26]. The illumination and detection source focused on the same plane is termed “confocal”.

Background
Probe based confocal endomicroscopy (pCLE) is a minimally invasive optical tool that can be used during standard bronchoscopy to detect alterations in the airway basement membrane or the acinar elastin network. The technique shows great promise for investigating benign and malignant conditions of the lung. pCLE can be easily perfomed during standard bronchoscopy. The miniprobe is inserted into the 2mm working channel of any standard bronchoscope and advanced to the area to be imaged. The probe tip is applied onto the bronchial mucosa under direct visual control. Care must be taken to avoid tangential positioning. For peripheral scanning, the probe is advanced gently into the distal bronchiole until the alveolar architecture is recognized. Once imaging is acquired the probe is pulled back until contact is almost lost, thus avoiding a compression effect of the probe onto the alveoli[27]. A representative image of healthy appearing bronchial tissue is shown in figure 4.

pCLE System
The only pCLE system that is approved by the United States Food and Drug Administration (FDA) and CE-marked is Cellvizio®. It is commercialized by Mauna Kea Technologies (Paris, France) and allows for imaging of thepulmonary and gastrointestinal tracts during endoscopic applications. Real time confocal images are acquired at 9-12 frames/second using a 1.4mm flexible miniprobe. The probe has a field of

view of 600 μm in diameter, 3.5 μm in lateral resolution, and 0 – 50 μm in depth of imaging. There are two different wavelengths available: 488nm for imaging of the respiratory tract and 660nm for epithelial cell imaging after the topical application of a contrast agent[28-29].

Literature

Ex vivo pCLE has shown that it is possible to look at the subepithelial structures. The high quality images are based on the elastin found in the basement membranes[25]. This led to in vivo studies of the human proximal bronchial tree.From this study it was shown that pCLE is safe, tolerable and can be used to identify abnormalities in both benign and malignant diseases. Normal areas of the airway displayed five distinct reproducible micoroscopic images fromthe trachea to the distal respiratory bronchioles. When applied to 29 high risk patients to detect precancerous lesions it illustrated alterations in the AF microstructure pattern in 80% of those with metaplastic and dysplastic samples and 100% in carcinoma in situ and invasive lesions. pCLE was also able to detect specific basement remodeling alterations in two non malignant diseases. The AF mode used in pCLE cannot separate the different grades of precancerous lesions such as metaplasia, dysplasia, CIS, from each other. This is due to the inability to image the epithelial surface because of the weak AF signal. In order to successfully explore the epithelial layer it requires an exogenous non toxic fluorophore[30], such as methylene blue[28].

Exploration of the distal airways is also possible due to elastin being part of alveolar sacs and capillary microvessels. A study of 88 patients combining navigational bronchoscopy with pCLE for the investigation of peripheral nodules has shown a good correlation for detecting neoplasia when comparing microscopic imaging directly to histopathology[31]. In addition, it has also shown promise as a surveillance tool in monitoring for acute rejection in lung transplant patients. Finally, there are also efforts currently being made to study diffuse peripheral lung disease and mechanically ventilated patients with pulmonary infiltrates[32].

Although pCLE provides excellent optical images it is not ready for routine application. This is due to a lack of standardization of the technique for accurate image interpretation. An image atlas describing normal from abnormal pulmonary conditions is currently being compiled.

Conclusion

pCLE is a promising tool that may allow imaging of architecture and morphology of the human airway in real time, with an optical resolution similar to standard histolopathology. The ability to view both epithelial and subepithelial structures will provide invaluable information for clinicians in both benign and malignant pulmonary diseases. In the future, the combination of pCLE with molecular contrast compounds will enable a bronchoscopist to diagnose and assess possible therapeutic outcomes in one procedure.

CONCLUSION

Several new endoscopic imaging platforms are now available. The emergence of these technologies makes thisan intriguing time to be working in these fields. However, the development of different and new imaging platforms also highlights and delineates the conundrum relative to the intrigue and promise of these various systems versusactual clinical utility. Nevertheless, these technologies are intriguing and potentially helpful although the clinical utility remains unknown. Clinical utility, however, is most often an emergent phenomenon and may not be apparent for some time. Given the above then, these approaches should not be dismissed as techniques in search of an application but rather as tools to be developed as in fact these technologies may be complementary.

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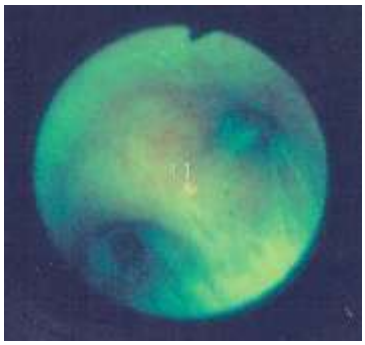

Figure I: Abnormal autofluorescence illustrating reddish discoloration of bronchial mucosa

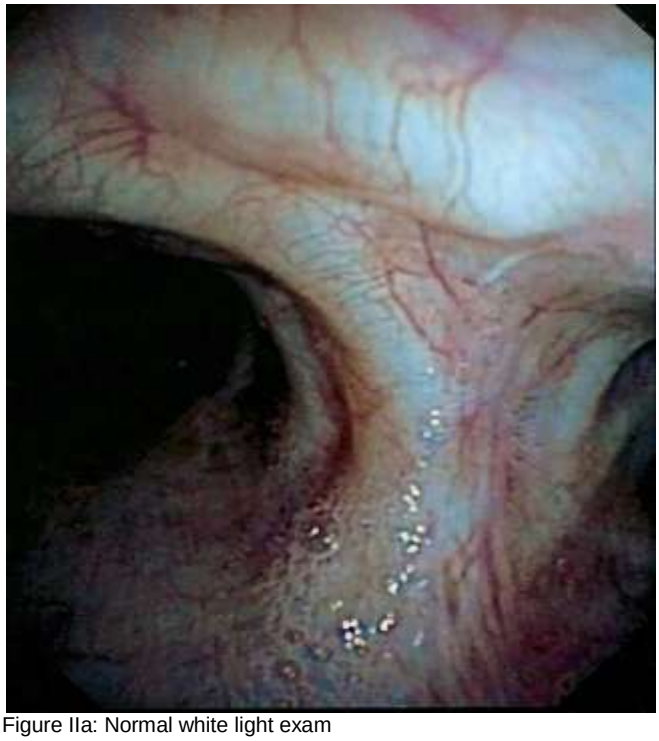

Figure IIa: Normal white light exam

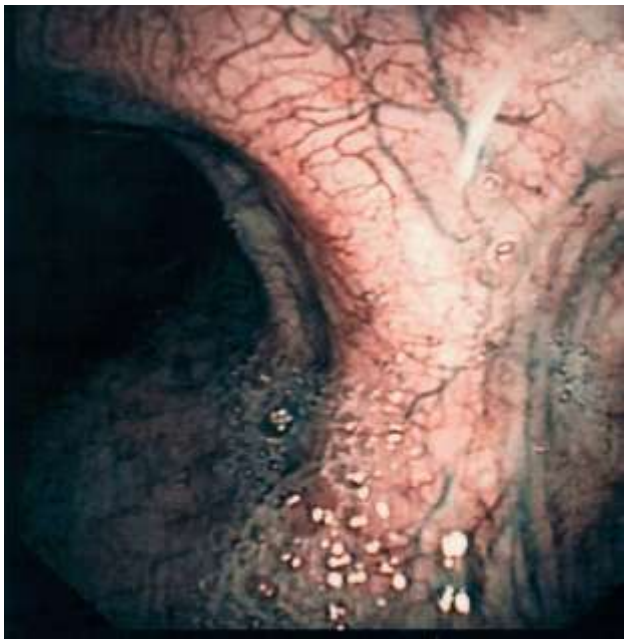

Figure IIb: Normal narrow band image exam

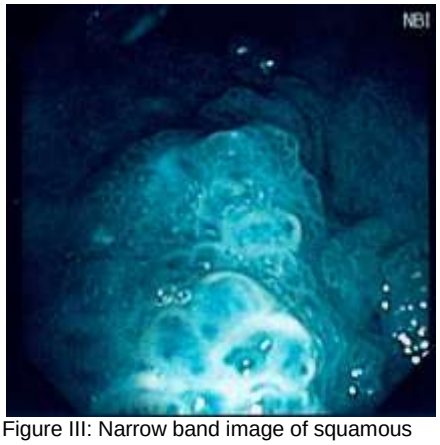

Figure III: Narrow band image of squamous cell carcinoma demonstrating dotted and spiral shaped vessels

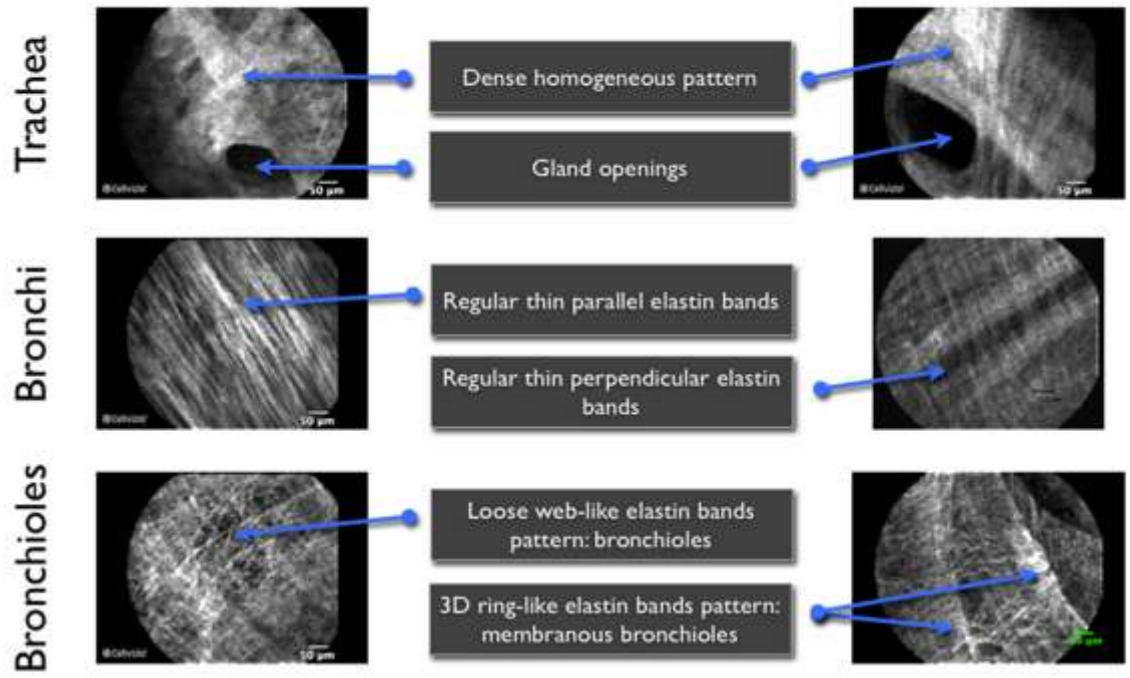

Figure IV: pCLE image of healthy bronchial tissue