PART I

Advances in diagnostic bronchology

CHAPTER 1

Autofluorescence in the detection of lung cancer

Michael J. Simoff, MD

Perception depends upon the detection technique. What we see is the result of the brilliant possibilities of the human eye and human brain.

Martin Leonhard, "New Incoherent Autofluorescence/Fluorescence system for Early Detection of Lung Cancer"

Lung cancer continues to be the leading killer among all cancers. Despite recent advancements in treatment, the 5-year survival rate for lung cancer remains at approximately 15%. In the 25% of patients diagnosed with lung cancer who are offered surgery for curative resection, only one half are ultimately cured of their disease. The greatest hope for patients is the early detection of lung cancer allowing them the opportunity to attempt a treatment course for a cure.

These poor statistics do not reflect on the aggressiveness of treatment, rather on the late diagnosis and frequent recurrence of lung cancer in patients. Finding a solution to the dilemma of how to diagnose lung cancer early remains a goal of many researchers. Chest radiographs and computed tomography screening [1–3] have been and are being looked at to identify this disease earlier in its development.

With only 30% of early endobronchial cancer and/or premalignant lesions identified by white light bronchoscopy (WLB) [4], it would be an understatement to say that we are missing many opportunities for the treatment of early synchronous and metachronous tumors. What is needed is a new modality to detect early forms of the disease, which then have the opportunity to be aggressively treated and potentially cured, some with endobronchial techniques. One such technology for early detection is autofluorescence bronchoscopy (AF).

Autofluorescence is not the answer to the dilemma of the diagnosis of lung cancer, but it may give us another tool for not only diagnosing, but also guiding management decisions [5], thus better allowing us treatment planning and option evaluation for patients with lung cancer. The format of this chapter will be to guide the reader through the whys and hows of AF bronchoscopy prior to discussing the actual clinical use. Only by understanding what information we gain by AF can this tool be effectively used.

The problem

Despite advancements in chemotherapeutic agents, radiation and surgical techniques, the recurrence rate of lung cancer is 3.6–4% per year. Second primaries occur in 17% of patients within 3 years of treatment of their primary disease [6,7]. With 10–20% of patients having a second primary or recurrence, it suggests a more complicated process than a single tumor alone.

The presence of synchronous primary cancers is common. Of the patients who die of lung cancer, 15% have synchronous carcinoma in situ (CIS), with a prevalence of 3.4% among one–two packs per day smokers, and 11.4% among patients smoking greater than two packs per day [8]. Qu *et al.* [9] looked at 225 subjects, including patients with known or suspected lung cancer, patients post

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Table 1.1 Comparison of patients with known or suspected lung cancer. Status: post resection for lung cancer, with head and neck cancer and healthy volunteers for the presence of precancerous and cancerous lesions [9].

Group	*n*	*Moderate dysplasia* (%)	*Severe dysplasia* (%)	*CIS* (%)	*≥2 foci* (%)
I	100	14	11	15	15
II	46	18	4	13	24
III	10	20	10	10	20
IV	67	36	15	5	13

n, number of patients.
I, known or suspected lung cancer.
II, stage I completely resected lung cancer.
III, head and neck cancer.
IV, volunteer smokers.

complete resection for lung cancer, those with head and neck cancer and in healthy volunteer smokers (Table 1.1). In the group suspected of cancer, 25% had moderate to severe dysplasia and 15% had CIS, with 15% of these patients having greater than two foci. In the postoperative group 22% of patients were identified to have dysplasia and 13% with CIS, 24% of which had multiple foci. The patients with head and neck cancer had a 30% prevalence of dysplasia and 10% of CIS, 20% multifocal. And last, the volunteer smokers included 51% with dysplasia and 5% with CIS, 13% of which had greater than two foci.

Carcinogenesis

> The concept of carcinogenesis is a multi-step process, suggesting the possibility of blocking or reversing the progression and thus presents the opportunities for a more effective intervention.
>
> Vogelstein *et al.*,
> "The Multistep Nature of Cancer"

The pattern of multifocal areas of dysplasia and CIS in many ways supports the theory of field cancerization as it applies to cancer of the aerodigestive tract [10]. As they are inhaled, cigarette smoke and/or other irritants thought to be the primary carcinogens for lung cancer, expose the entire aerodigestive tract to potential injury. This diffuse injury to the mucosa of the lung should probably be expected rather than be surprising to us. The initial changes of genomic instability within a morphologically normal epithelium begin the molecular stage of carcinogenesis [9]. These mutation-induced changes could therefore be expected to occur throughout the respiratory epithelium.

The process of carcinogenesis begins with the initial injury to the endobronchial epithelium. The genetic mutations that occur in response to this injury bring about the morphologic findings identified as premalignant changes in the tissue. This process of mutagenesis, from normal tissue through metaplasia and subsequently dysplasia, takes 3–4 years to occur usually [11–14]. Once identified, endobronchial dysplasia is a difficult problem in that it is unclear as to the evolution of disease from this stage of change. There can be an apparent resolution of dysplasia to morphologically normal tissue that has been identified and reported [15]. The gradation of mild and moderate to severe dysplasia have progressively stronger implications of areas of true concern, regarding the development of cancer. The pathologic evolution from severe dysplasia to CIS takes about 6 months [11]. Therefore, from the time of a tissue injury, which induces the pathologic changes that allows the development of a cancer, multiple other areas throughout the epithelium have sustained similar injury and must be at similar risk for the development of cancer.

Several authors have studied the rate of progression from CIS to microinvasive cancer; one group demonstrated a 23% progression rate of CIS to microinvasive cancer, by performing follow-up bronchoscopies every 3 months [15]. Venmans *et al.* [16] followed pathologically confirmed CIS in their patients every 3–4 months with bronchoscopy also. They eventually confirmed that all but one of their patients developed an invasive carcinoma of the airways, which required therapy. The single individual in whom CIS did not evolve into a microinvasive cancer in the study had enough macroscopic changes by WLB alone; hence, therapy was begun despite incomplete evolution of the pathologic changes.

Overall, several authors have also begun to look at the issue of progression of endobronchial pathology. It is suggested by review of data available that 10% of moderate dysplasias, 19–46% of severe dysplasias and 22–56% of CIS will eventually evolve

from their current state to an invasive cancer [15–19].

As is suggested here, not all lesions progress to a more evolved state of disease; some actually spontaneously regress or demonstrate no histologic change over time. The studies available, which have used sequential surveillance bronchoscopy with AF, have all had limited numbers of patients [14,16,20,21]. In two of the studies, precancerous lesions that persisted for 3–6 months were treated with endobronchial modalities limiting the length of follow-up [16,20]. Lam *et al.* did follow endobronchial changes with AF in 17 patients with pre-invasive disease for up to 4 years. Of these patients 5 progressed to an invasive squamous cell carcinoma. The lesions in the remaining 12 patients, on the other hand, remained in a pre-invasive state throughout the 4-year follow-up [21]. Unfortunately, despite the knowledge that some lesions improve with time, we are left in a situation where we do not know, nor do we have the capacity at this time to differentiate, which lesions will progress, stay the same or remiss to normal mucosa. AF gives us new information on the identification of these lesions, but also added questions as to what to do with them.

Microscopic anatomy of the airways

The airway is a multilayered structure, consisting of the ciliated epithelium (46 ± 3 microns) with the underlying basement membrane. Immediately below the basement membrane is the submucosa (680 ± 20 microns), which consists of mucous glands, collagen, elastin, nerves, lymphatics, and vascular structures. Smooth muscle separates the submucosa from the cartilaginous layer (1.2 ± 0.1 mm) of the airway. The adventitia, a connective tissue sheath containing branches of bronchial arteries and veins and nerve plexi, is the outer most layer of the airway [9,22].

The pathologic changes of dysplasia, CIS and microinvasive carcinoma are very superficial. These changes occur initially in the epithelium, eventually invading through the basement membrane and into the upper submucosa (Figure 1.1). Pathologic evolution of microinvasive cancer usually involves the superficial 70–116 microns of the airway [9].

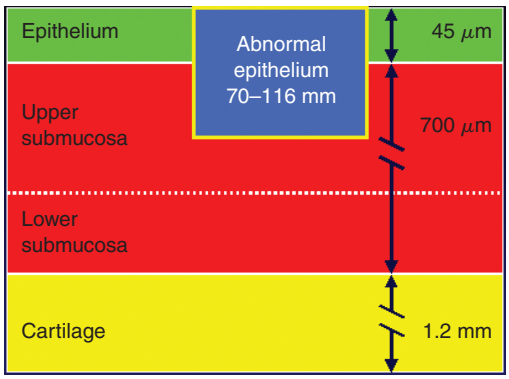


Figure 1.1 Depth of penetration of early cancerous lesions in respect to microscopic anatomy of the bronchus wall.

It is important to understand the process of carcinogenesis as well as the microscopic anatomy of the airway to effectively use the technique of AF.

WLB and the detection of early disease

Due to the intra-epithelial to superficial submucosal development of CIS and microinvasive cancers, it is difficult to diagnose many of these sites with conventional WLB techniques alone. CIS and early cancers are only detected with WLB about 29–40% of the time [4,7,23–25]. This is due to the fact that these early pathologic lesions are only a few cells thick (0.2–1 mm) leading to only minimal mucosal changes. When visualized, these precancerous and early cancerous lesions are superficial, often flat lesions, which are usually less than 5 mm^2 in surface area. Endobronchial changes less than 10 mm^2 are commonly invisible to standard WLB observation. With WLB, many of these lesions present as nonspecific changes of the endothelium such as a pale or a more reddish discoloration of the mucosa. Other epithelial changes observed by WLB examination include a lack of luster or a rough/microgranular appearance of the mucosa [23,25,26]. Mucosal folds and bronchial bifurcations can be swollen or thickened with nodular lesions becoming more evident after they have grown greater than 2 mm in size [23,25].

Bronchoscopic evaluation of the airways can take place in bronchi of the fifth order with modern flexible WLB [27]. As the clarity of images continues to improve with the advancement of bronchoscopic

optics, what will be the role for AF bronchoscopy? I was challenged on one occasion with this very question. The questioner explained that with his newest generation bronchoscope, he could see the vascularity of the bronchial mucosa with great clarity; why then, with such advanced optics, do we need a different tool to look for subtle endobronchial changes when they should be clearly visible. My response was simply: "So do you look?" The changes we are trying to identify are subtle. Having the capability to examine the airway and actually performing such a detailed examination in a breathing, coughing patient is very different. The technology used for AF allows us an improved ability to look for subtle changes throughout the airways of our patients in a relatively straightforward, safe and effective manner.

There have been multiple studies attempting to use conventional WLB to identify early stage lung cancer. One such study used WLB to evaluate the airways of patients with positive sputum cytology for lung cancer. They identified CIS or microinvasive cancer in 61% of patients who were examined, making the diagnosis of an early cancer in 88% of the patients (44 of 55 patients) [28]. Sato and colleagues [29], looked at 180 patients who underwent 527 bronchoscopies. Two hundred occult cancers were identified during the time of the study. To achieve this result though, it required a mean of 29.2 months and an average of three bronchoscopies for each patient to attain a definitive diagnosis. Both groups of investigators identified early stage cancers; the limitations in time to diagnosis and the number of bronchoscopies required make this approach of limited value and less practical for clinical application.

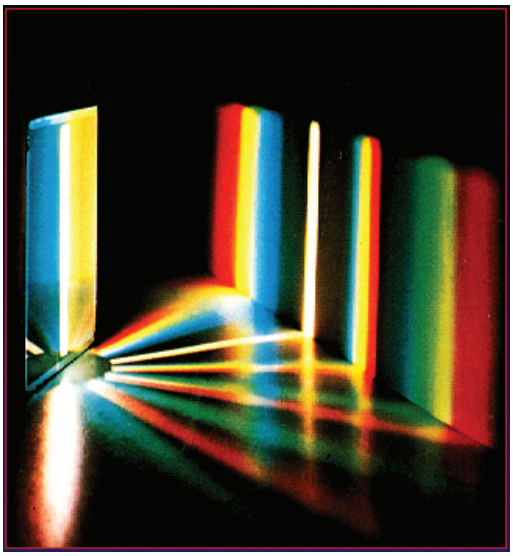


Figure 1.2 White light separated into various wavelengths (colors) through a prism. (Image courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

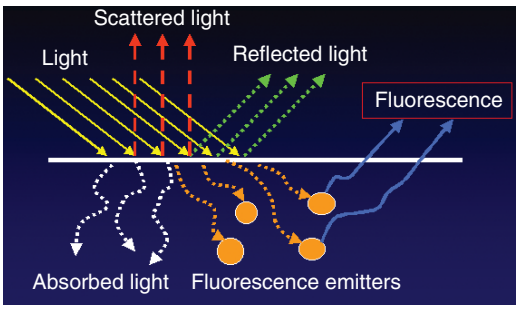


Figure 1.3 Reflectance imaging: the four physical properties of light as it interacts with a surface: absorption, scattering, reflection and fluorescence.

Light

Light is a form of electromagnetic radiation. White light, as in sunlight or incandescent light, is a polychromatic blend of all wavelengths of the spectrum of visible light. White light can be separated into individual wavelengths; each distinct color can be exposed by passing the white light through a prism or as is similarly seen in a rainbow (Figure 1.2). We see in color due to the various light wavelengths and their interactions with objects and/or tissue.

When white light is shown onto a surface, and for the purpose of this discussion, specifically a tissue surface, the colors that we see are due to several of the physical properties of light: scattering, absorption, reflection and fluorescence. (Refer to Figure 1.3 for the following discussion.) As light strikes a surface, some of the light is scattered in different directions still as white light. Our observation of this phenomenon is often referred to as glare. As the same light strikes a surface, some wavelengths of light are absorbed into the tissue/structure. These wavelengths of light are absorbed into various

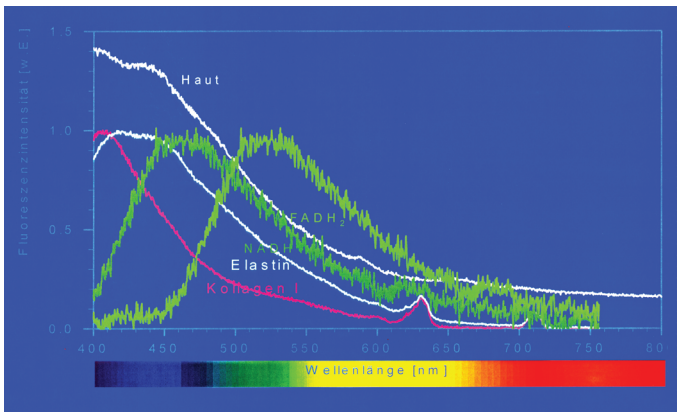


Figure 1.4 Florescence wavelengths of the major tissue fluorophores: $FADH_2$, NADH, elastin and collagen 1. (Image courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

components of the structure (cells, molecules, etc.). This absorption leads to loss of these wavelengths of light. The remaining light wavelengths that are reflected off the tissue/structure surface are blended into the colors that we see objects in. This combination of effects of reflection, back scattering and absorption are known as reflectance imaging. We observe by reflectance imaging when using WLB.

Autofluorescence

As white light strikes a tissue surface, and reflectance imaging occurs, as mentioned earlier, some of the light is absorbed. Certain cells within the epithelium and upper submucosa, known as fluorophores, are stimulated by this influx of energy (Figure 1.1). The most commonly recognized fluorophores in the epithelium and submucosa are collagen I and II, elastin, NADH and $FADH_2$ (Figure 1.4). Fluorophores absorb short wavelengths of light, usually about 390–460 nm (blue light), stimulating electrons from their ground state energy level (E1) to an excited state (E2). Spontaneous decay from the excited state leads to the emission of longer wavelengths of light from the fluorophores that are eventually released from the surface of the tissue (Figure 1.5). These higher wavelengths of light that are released are of 520 nm, which is seen as green, and of 630 nm, seen as red (see Figure 1.6).

Fluorescence or AF is expressed by all tissue surfaces stimulated with white light, or more specifically the shorter wavelength blue light (390–460 nm) within white light. AF is always present, but as it is 10 000 times dimmer than reflected light, it is not visualized with normal viewing. The tissue epithelium is not very biologically active and is responsible for less than 5% of tissue released AF. On the other hand, due to their cellular makeup, the submucosa and cartilage have strong AF potentials. Due to the shallow penetration of blue light into the tissue surface, clinically observed AF is a characteristic of the upper submucosa predominantly (Figure 1.1) [30,31].

The tissue characteristic of AF was first discussed in the literature in 1933 [32]. Historically, AF was pharmacologically augmented by the use of photosensitizers like partially purified hematoporphyrin. With further advancements in 1961, hematoporphyrin was found to have preferential retention in cancer cells [33]. In 1979, hematoporphyrin was used in work pertaining to the early detection of lung cancer by Doiron *et al.* [34]. As our knowledge of photobiology progressed, new pharmacologic agents were developed including hematoporphyrin II in 1979 [35]. Low doses of hematoporphyrin II were used by Palcic *et al.* to clinically identify early stage lung cancer [36].

The next leap in technology was in 1990 with the development of a Lung Imaging Fluorescence Endoscope (LIFE) (Xillix Technologies Corp., Richmond, British Columbia, Canada). LIFE bypassed the need of photosensitizers, rather using low energy monochromatic laser light to stimulate cellular AF. A series of filters and cameras were then used to allow clear visualization of the green and red light generated by AF [37].

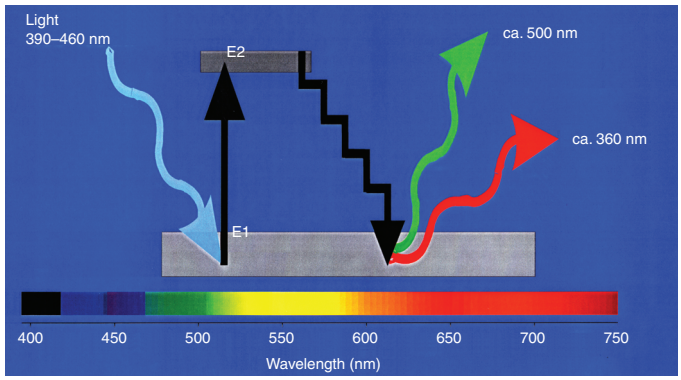


Figure 1.5 Certain wavelengths of light (390–460 nm) excite molecules in fluorophores to higher energy states (E2). Spontaneous decay produces fluorescence with emittance of green (520 nm) and red (630 nm) light. (Image courtesy of Karl Storz of America, Culver City, California USA, with permission.)

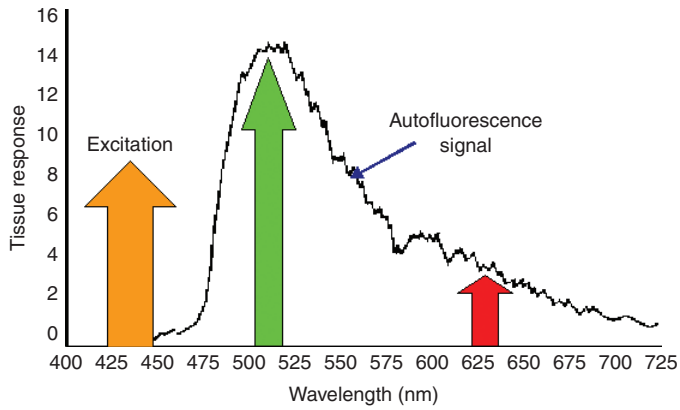


Figure 1.6 Relative release of green and red wavelengths of normal tissue in response to excitation. (Image courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

Autofluorescence bronchoscopy is performed by the stimulation of fluorophores by illuminating them with a monochromatic light source (helium–cadmium laser, filtered xenon or metal halide light sources). Reflectance is then filtered out and with the assistance of filters and specific optical camera systems images in green and red are visualized. With AF normal bronchial epithelium is visualized in green (520 nm), due to the predominate formation of these wavelengths of green light by normal stimulated fluorophores. Areas of the submucosa or epithelial layers that have precancerous changes or have evolved into microinvasive cancers will have a diminishment in the green light released and subsequently increased visibility of red light (630 nm) produced.

The reduction of visualized green light is due to the pathologic changes associated with the cellular evolution into a microinvasive cancer. An early change in the process is thickening of the epithelium, which allows less of the delivered light to pass into the submucosa, overall decreasing the AF that is produced. Second, cancer-induced angiogenesis occurs within the thickened epithelium and upper submucosa as the cancer continues to grow locally. Blood is visualized by the naked eye as red, due to the fact that blood products have an increased absorption of colors other than red, in this case green, leaving red as the predominate color visualized. Thereby the localized angiogenesis of cancer formation increases the red as seen with AF. The pathologic formation of a cancer also includes changes to the extracellular matrix in the epithelium and submucosa by secretion of mefalboproteinase by proliferating cancer cells. These structural changes in the submucosa also reduce the AF produced, but more significantly reduce the green produced from affected areas (Figures 1.7a–c and 1.8) [29,38,39].

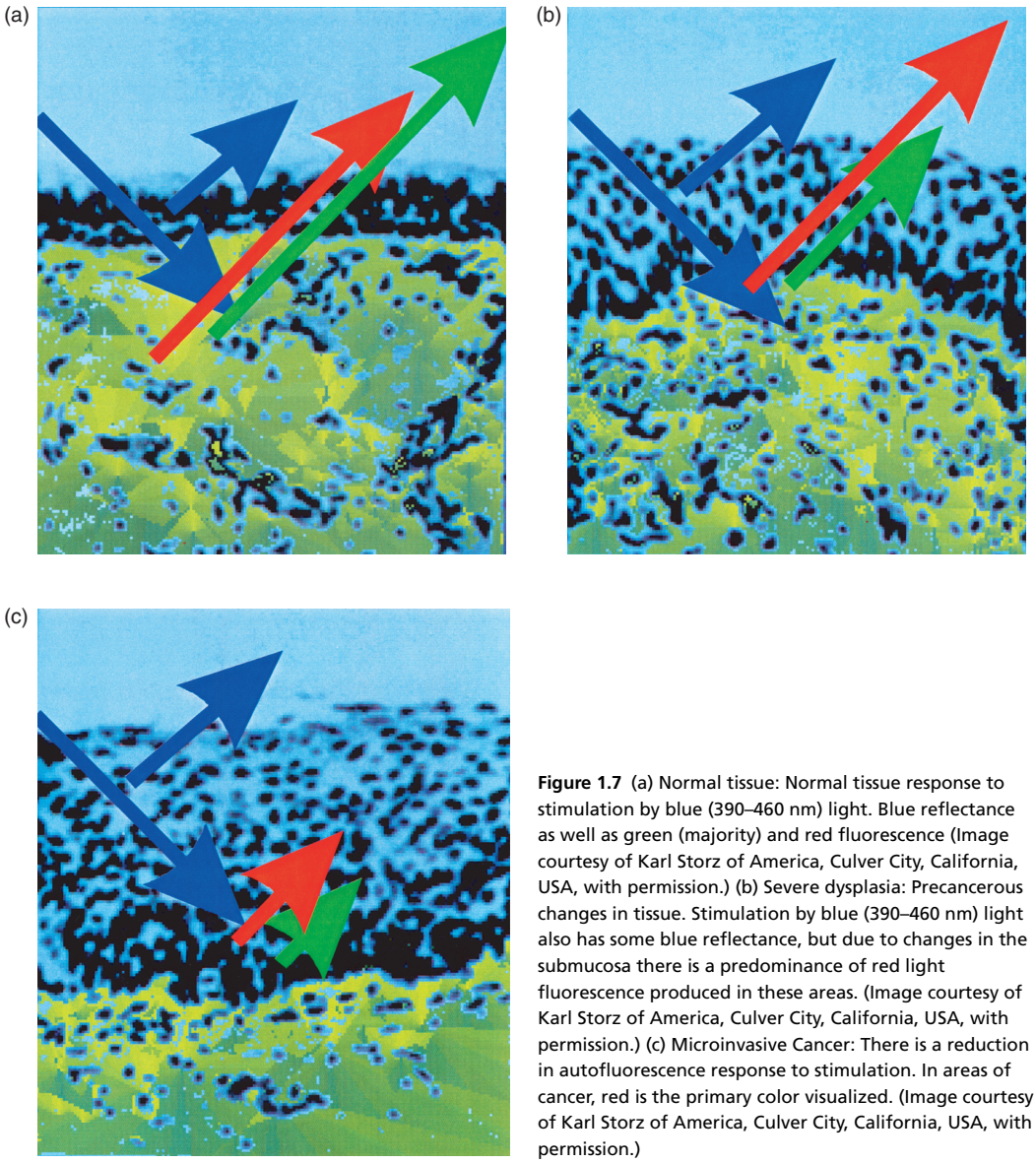


Figure 1.7 (a) Normal tissue: Normal tissue response to stimulation by blue (390–460 nm) light. Blue reflectance as well as green (majority) and red fluorescence (Image courtesy of Karl Storz of America, Culver City, California, USA, with permission.) (b) Severe dysplasia: Precancerous changes in tissue. Stimulation by blue (390–460 nm) light also has some blue reflectance, but due to changes in the submucosa there is a predominance of red light fluorescence produced in these areas. (Image courtesy of Karl Storz of America, Culver City, California, USA, with permission.) (c) Microinvasive Cancer: There is a reduction in autofluorescence response to stimulation. In areas of cancer, red is the primary color visualized. (Image courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

Figures 1.9–1.12 are examples of side-by-side views of the airway with WLB and AF in a normal trachea, with epithelial changes of dysplasia, *CIS* and a microinvasive carcinoma. (The AF images were created by the Storz D-Light system.)

The technology

The initially developed and still commonly used tool for AF bronchoscopy is Laser Induced Fluorescence Endoscopy or LIFE system (Xillix Technologies Corp., Richmond, British Columbia, Canada). The LIFE system uses a low-energy helium–cadmium laser at a wavelength of 442 nm for fluorophore stimulation. Two charge coupled device (CCD) cameras connected through a fluorescence collection sensor and optical multi-channel analyzer are used via an optical broncho-scope. The image is then processed through an image board, which transforms the various light

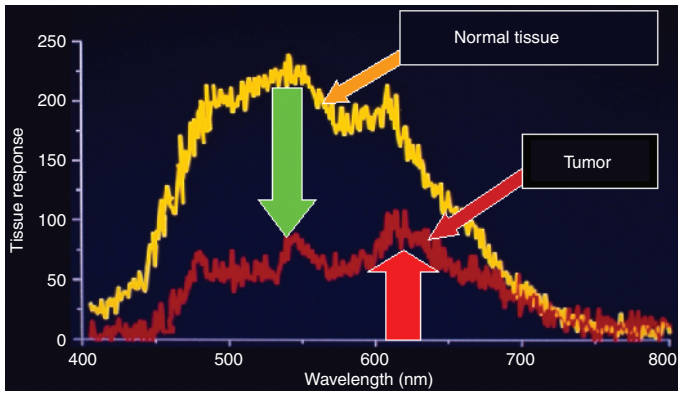
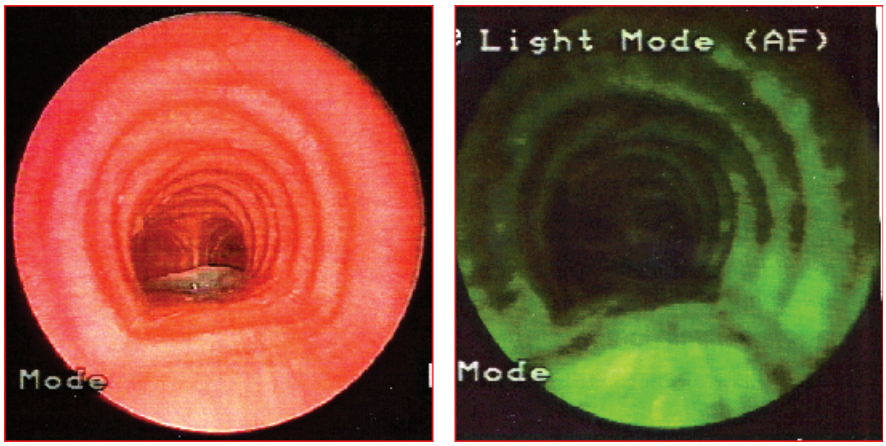


Figure 1.8 Wavelength production by autofluorescence for both normal tissue and areas of the tumor. The relative reduction in green wavelength production is clearly identified. (Image courtesy of Karl Storz of America, Culver City, California, USA, with permission.)



White light Autofluorescence

Figure 1.9 Normal Tissue: View of trachea with white light and AF light sources. (Image produced with D-Light system, courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

intensities into a real time video image augmenting the green of normal tissue and the red of abnormal tissue (Figure 1.13a,b).

The D-Light system (Karl Storz Endoscopy of America, Culver City, California, USA) uses a xenon light source. The white light produced by the xenon light source is transmitted to a dedicated optical bronchoscope through a liquid light cable. A series of filters are fit into the eyepiece of the bronchoscope, which generates the monochromatic light needed (380–460 nm) for fluorophore stimulation. Additional filters are used to reduce reflectance of the blue light from the tissue allowing only red and green wavelengths to be visualized. The resulting image is seen in green (normal tissue) and red (tissue with pathologic changes). Due to the faint nature of tissue AF a reduced imaging speed (16 images per second versus 60 images per second in normal WLB) is currently used with the D-Light system to enhance light absorption and therefore clarity of the image of the abnormal tissue. The system has a footswitch and switch on the attached camera to allow quick changes from white light to AF modes , thus permitting the operator to choose which light source best fits his or her needs at any time during the examination (Figure 1.14; see Figures 1.9–1.12 for images).

The Diagnostic AutoFluorescence Endoscopy (DAFE) (Richard Wolf Endoskope, Knittlingen, Germany) is another technology using a filtered

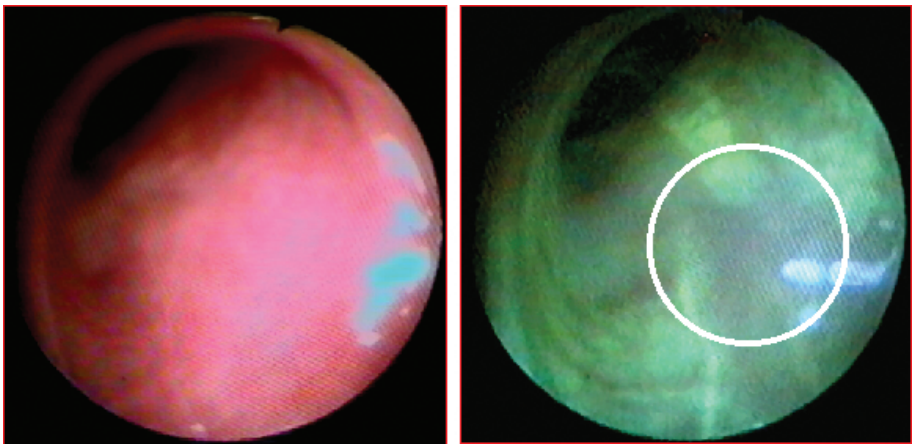


Figure 1.10 Dysplasia: White light and autofluorescence localization of dysplastic tissue. Green identifies normal tissue and red identifies abnormal, precancerous tissue. (Image produced with D-Light system, courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

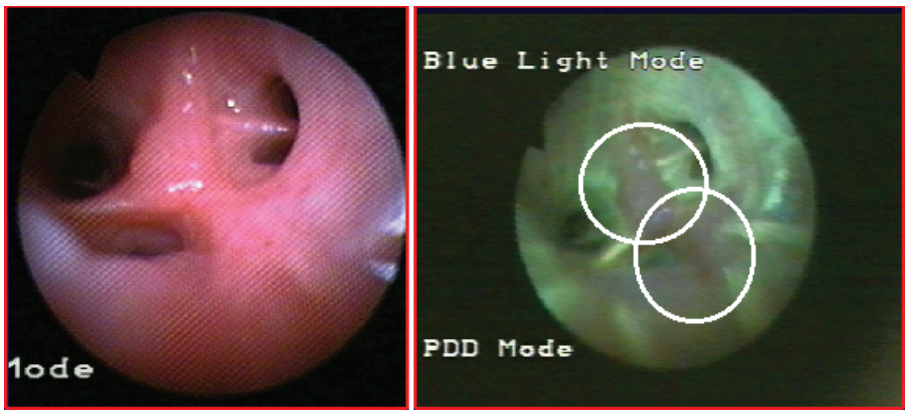


Figure 1.11 Carcinoma in situ: White light and autofluorescence images of carcinoma in situ in a bronchus. (Image produced with D-Light system, courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

xenon light source for cellular excitation. The xenon lamp uses an infrared blocking filter before light is transmitted via a liquid light guide. The image is then generated via a photodetection system using one black and white (B/W) CCD camera with a dual detection range: 500–590 nm and 600–700 nm. This imaging system produces independent green and red imaging, which is overlaid to produce the AF image. The DAFE system attempts to further improve upon AF technology by creating a simultaneous white light image via a color camera driver that has the red and green AF imaging superimposed upon the white light view. This concept allows simultaneous viewing of the airways with WLB and AF [40]. The DAFE system can be used with rigid bronchoscopes or the Wolf, Olympus or Pentex flexible bronchoscope systems (Figure 1.15a,b) [41].

The Onco-LIFE system (Xillix Technologies Corp., Richmond, British Columbia, Canada) is currently not available for sale with only preliminary studies having been performed at

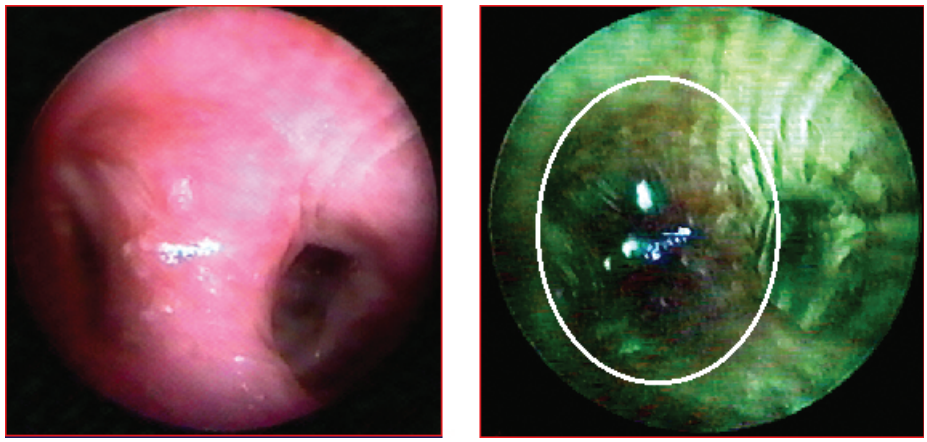
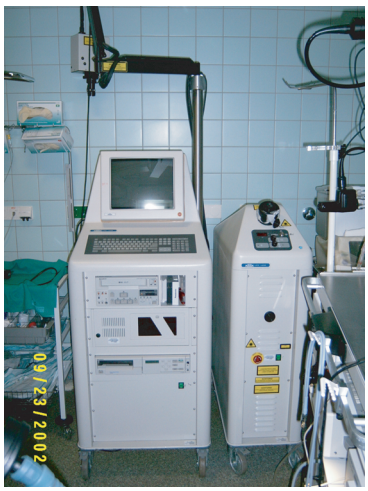


Figure 1.12 Microinvasive carcinoma: White light and autofluorescence visualization of a microinvasive cancer of the bronchus. (Image produced with D-Light system, courtesy of Karl Storz of America, Culver City, California, USA, with permission.)

(a)



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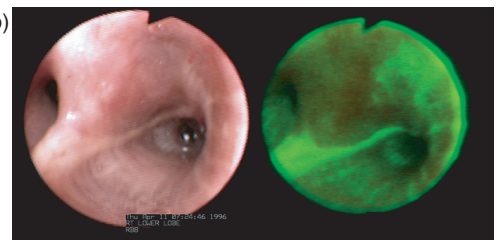

Figure 1.13 (a) The LIFE autofluorescence system. (b) White light and autofluorescence images produced with the LIFE system. (Xillix Technologies Corp. Richmond, British Columbia, Canada, reproduced with permission.)

the British Columbia Cancer Agency. The Onco-LIFE system uses a filtered mercury arc lamp for fluorophore stimulation. It then uses a low light sensor (ICCD) for fluorescence imaging. A color CCD sensor is incorporated into the system for improved white light visualization as well as for imaging of red in AF mode. These combined sensor inputs are put together to create the image visualized. Operators can use a footswitch or switch on the camera. The Onco-LIFE system is developed for use with any endoscope (both rigid and flexible) from Olympus, Pentax, Fujinon, Storz or Wolf (Figure 1.16) [42,43].

The System of Autofluorescence Endoscopy (SAFE) 1000 (Pentex Corporation, Asahi Optical, Tokyo, Japan) uses a xenon light source also, which is filtered to create a light with a wavelength of 420–480 nm. Reflectance filtration is used to improve visualization of AF. An image intensifier is incorporated into the system to improve distinction of the very low light autofluorescent changes. This system creates the distinctive green of typical background of normal mucosa with "cold spots" as areas of abnormality [44].

The D-Light system is currently the only FDA approved, commercially available system in the

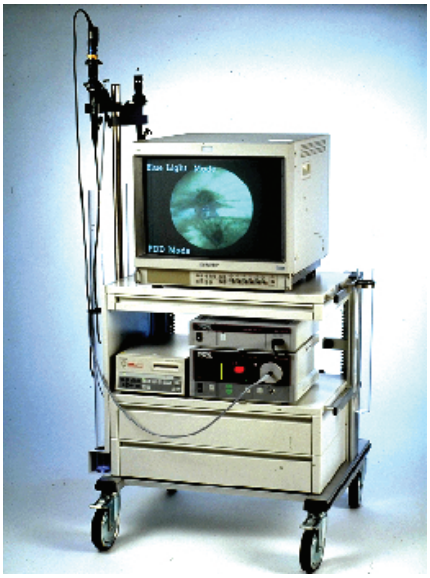

Figure 1.14 The D-Light autofluorescence system. (Karl Storz Endoscopy of America, Culver City, California, USA, reproduced with permission.)

United States. It is also sold and used in the rest of North America, Europe, Africa, South America, Asia and Australia [45]. The LIFE system is no longer available for fresh purchase, but continues to be used worldwide at those institutions that have this equipment. The DAFE system is commercially available in Europe, Asia and Canada; the company is considering further clinical trials [41]. Onco-LIFE is currently not commercially available. Xillix Technologies Corporation states that the first published data will likely be the study carried out as part of the FDA regulatory approval process [42]. No communications were received from Pentex Corporation regarding the availability or plans of clinical trials for the SAFE 1000 system despite multiple attempts at contacting them.

Does it work?

One of the earlier clinical studies by Lam *et al.* [46] looked at 94 subjects, 53 with known or suspected

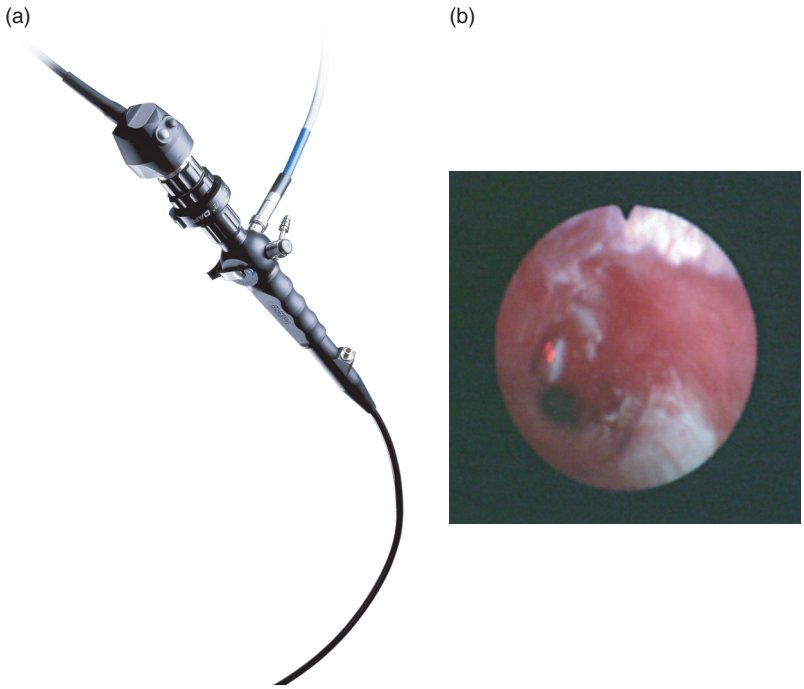

Figure 1.15 (a) The DAFE autofluorescence system. (Richard Wolf Endoscopy Gmbh, Knittlingen, Germany reproduced with permission.) (b) Image produced with DAFE system. Note red area, identifying area of cancerous or precancerous lesion superimposed on a white light view (Richard Wolf Endoscopy Gmbh, Knittlingen, Germany reproduced with permission.)

Figure 1.16 The Onco-LIFE autofluorescence system. (Xillix Technologies Corp. Richmond, B.C., Canada, reproduced with permission.)

lung cancer and 41 volunteers (17 smokers, 16 ex-smokers, 8 nonsmokers). All patients had WLB and autofluorescence bronchoscopy with a LIFE system immediately following the white light examination. All areas with changes consistent with early lung cancer were biopsied when identified by white light or AF techniques. WLB and AF bronchoscopy identified normal tissue and was also biopsied as a control. A total of 328 biopsy specimens were obtained during the 94 performed procedures. Sixty-four invasive cancers, 29 CIS, 62 areas of dysplasia and 173 normal biopsies were reviewed. The authors reported that for the detection of dysplasia and CIS, they had sensitivities with white light versus AF bronchoscopies of 48.4 versus 72.5% and specificities of 94 versus 72.5% for white light and AF bronchoscopies, respectively [46].

The pattern of improved sensitivity of AF bronchoscopy for the detection of early cancer and precancerous lesions is repeated throughout the literature. I compounded the information available in 11 clinical studies [24,46–54]. Included in these studies were 1084 patients who underwent 1289 bronchoscopies with 3487 biopsies. Matching data as well as was possible, a combined analysis of sensitivity and specificity was performed. The sensitivity of WLB versus AF was found to be 52.4 to 84%, respectively. Specificities for WLB and AF bronchoscopy were 87 and 78%, respectively. The only limitation in these studies that should be pointed out is that the sensitivity referenced in some cases is a relative sensitivity. The most recent review of the use of the LIFE system by Lam *et al.* reports a twofold improvement in the detection of precancerous lesions with AF versus WLB [55]. Currently, there is no gold standard available to identify all possible endobronchial lesions and therefore the actual sensitivity of AF cannot be determined.

Several clinical studies have also been performed using the D-Light system (Karl Storz Endoscopy, Tuttlingen, Germany) in Europe with encouraging statistical results for the identification of precancerous and early cancerous lesions [56–58]. A clinical study was recently completed in the United States with the Storz D-Light system using a very similar research protocol as those performed with the original LIFE studies. The six clinical sites involved reported a white light sensitivity of 10.6% versus the AF sensitivity of 61.2% for abnormal histology. The WLB versus AF specificities was 94.6 versus 75.3%, similar to the specificity relationship seen in previous LIFE studies [59].

Published clinical studies using the DAFE system are currently limited. The study by Goujon *et al.* reports on 20 patients who had WLB and AF performed during the same session, with comparison of identification of precancerous and cancerous lesions. They report a positive predictive value of 75% for AF versus 38% for WLB [40]. These findings with the DAFE system echo those of investigators using various AF systems. Other studies have been performed using the DAFE system for AF evaluation and follow-up of patients, but data

is limited [60–63]. Richard Wolf Endoscopy is, at the time of writing this chapter, in the process of making a decision about a larger clinical trial for their system [41].

The Pentex SAFE 1000 system has been compared to the LIFE system in two clinical studies [64,65]. No sensitivities or specificities are reported, but the authors of both studies suggest similar results were found when comparing the LIFE system to the SAFE 1000 system. Both studies also report a shorter time period involved in the examination with the SAFE 1000 system compared to examination with the LIFE system. The SAFE 1000 system appears to provide results, similar to those of the LIFE and D-Light systems that are being used clinically.

There are currently no clinical studies to compare the Onco-LIFE system to other AF systems or to WLB.

Overall, in these and other studies, AF has improved the diagnostic ability to detect early endobronchial cancer and precancerous lesions. Individual studies can be scrutinized for variances from each other and subtle discrepancies in technique from one another, but this would take us from one of the most important take home points: the technique of AF appears to improve the diagnosis of early cancers and synchronous cancers in patients with more advanced disease. I would emphasize that despite the repetitive comparison of WLB to AF throughout the literature, the most important issue to remember is that it is the combination of white light and AF bronchoscopy data that will be used for the evaluation and treatment of patients clinically. Therefore AF should be thought of as an additive tool to WLB rather than a replacement as is suggested in many studies.

Specificity is repeatedly better with WLB than with AF in all studies. The question that should now be asked is why AF sees this "normal" tissue as abnormal? Wistuba *et al.* have suggested that up to half of these false positive biopsies have some molecular genetic aberrations associated with malignancies despite their normal histology [55]. This concept seems to be supported by the fact that molecular aberrations associated with malignancy have been found in histologically normal mucosal biopsies of smokers in the past [12]. Conceptually, this is an exciting area of consideration. With improved knowledge of molecular clonal abnormalities being developed, it is postulated that the normal histologic findings may be a mask to true pathology [66,67]. Until a better understanding of the implication of genetic changes on the development of and/or natural history of premalignant to cancerous lesions is developed, this additional information remains a question rather than an answer and an area potentially ripe for research.

Clinical application of AF

Autofluorescence improves the way airways are examined, in conjunction with standard WLB airway examinations. Venmans *et al.* looked at their patient population, particularly those at risk for lung cancer who underwent both white light and AF bronchoscopy. They reviewed their data from 114 patients undergoing 224 bronchoscopies. On a per-patient basis the authors concluded that the addition of AF bronchoscopy to standard white light examination alone provided clinically relevant information in 13% of the bronchoscopies performed and/or in 16% of their patients. They defined clinically relevant findings as those biopsy specimens demonstrating moderate dysplasia, severe dysplasia or CIS [54].

Autofluorescence bronchoscopy in conjunction with WLB has been reported by other authors to change the management course in some patients. M.Th.M. van Rens and colleagues evaluated 72 patients with recently diagnosed non-small cell lung cancer or with highly suspicious lesions roentgenographically with WLB and AF. Up to six new high-grade endobronchial lesions were identified in 10 of the patients evaluated. Due to the findings in 3 of these 10 patients, definitive treatment was changed. The combination of WLB and AF techniques may add significantly to patient management by identifying synchronous lesions that would have otherwise gone undetected and therefore unmanaged [68].

Preoperative evaluation of the airways using AF has also been used to modify therapeutic interventions. Forty-three patients who had probable resectable roentgenographically visible lung cancer

had AF performed with 177 biopsies taken. Fifty-six metaplasias, nine dysplasias and four CIS were identified. These findings led to modification of the planned surgery in three patients, two of who received localized therapeutic treatment as the primary therapeutic modality. This study reports 9.3% prevalence of synchronous early lung cancers, metaplasias and dysplasias in the patient population studied [64].

Another study looked at a group of patients ($n = 23$) that had radiographically occult lung cancer and that was being assessed for intraluminal bronchoscopic treatment with curative intent. High-resolution computed tomography (HRCT) and AF bronchoscopy was performed prior to therapy. Of the 23 patients, 19 (83%) had no visible tumor or enlarged lymph nodes on HRCT. With AF bronchoscopy, 32% (6 of 19) of patients evaluated were found to have tumors of total area less than or equal to 1 cm^2 with clear-cut margins. These patients received the planned intraluminal therapy they were being assessed for. AF identified more extensive local tumor infiltration than was originally found with WLB alone in the remaining 13 patients. Of these patients 6 underwent surgical interventions; 7, with tumors that were inoperable, received external beam radiation and intraluminal therapy. Of the patients presenting for intraluminal therapy alone, 70% were identified as having more advanced disease requiring a more aggressive therapeutic program than was pre-AF expected [5].

As was previously mentioned, the progression of endobronchial pathology is recognized with, e.g. the conversion of severe dysplasias to a more advanced invasive cancer 19–46% of the time [15,16,69–71]. Bota *et al.* [20] investigated the natural history of precancerous lesions using AF. One hundred and four high-risk subjects had a baseline and follow-up AF bronchoscopy performed for evaluation of their airways. If, at the time of the initial AF bronchoscopy, the highest-grade lesion identified was a mild dysplasia or a lower grade lesion, follow-up AF bronchoscopy was performed in 1 year. If the highest-grade lesion identified was moderate dysplasia, follow up bronchoscopy was performed in 6 months, and a 3-month follow-up AF bronchoscopy was scheduled when severe dysplasia or CIS were found. Patient follow-up for the study was 24 months. The investigators found that 6 of the originally evaluated 36 normal epitheliums developed dysplastic lesions at the 1-year follow-up bronchoscopy. Metaplastic lesions were also followed at 1 year and 47 of the 152 initial metaplastic lesions evolved into dysplastic lesions, with 2 progressing to CIS and 1 to an invasive cancer. Of the original 169 low-grade dysplastic lesions 6 progressed to persistent severe dysplasia. Lesions that were initially found to be severely dysplastic progressed or persisted in 10 of 27 patients, with 28 of 32 CIS doing the same. The authors concluded by recommending a 2-year follow-up examination for patients with low-grade epithelial lesions. They also recommended that patients with high-grade, severe dysplastic lesions should be reevaluated in 3 months. If there is progression or persistence of the lesion, treatment should be instituted. Finally they suggest that CIS be treated immediately.

If we find early disease

> Early diagnosis and localization of lung cancer is an essential precondition for curative therapy.
>
> Vogelstein *et al.*,
> "The Multistep Nature of Cancer"

As techniques in interventional pulmonology continue to advance, the idea of intraluminal bronchoscopic treatment with curative intent is not as far fetched as it may have been in the past. In one small study, six patients were identified by AF to have endobronchial disease. These six patients then went on to have endobronchial ultrasound performed to evaluate each lesion as to the depth of tumor invasion. Of the six patients studied, two were found to have tumor only within the mucosa and submucosa, but not outside of the cartilage, and were treated with photodynamic therapy. Three of the patients with more advanced disease went on to surgical resection, with the final patient undergoing combined chemo and radiation therapy [72]. Although this approach may seem far from the norm now, as we look toward the future, this has the potential to become a more routine practice.

Various tools currently used in interventional pulmonology including neodymium, YAG

laser (0.7–3.0 mm tissue penetration), photodynamic therapy (1–2 mm depth of penetration) and cryotherapy (5–8 mm diameter of tissue destruction) can be considered for endobronchial therapy of more superficial precancerous/cancerous lesions [25,73,74]. Electrocautery, argon plasma coagulation and brachytherapy are other modalities that are also immediately available for endobronchial management. Furthermore, as our understanding of dysplastic evolution continues, advancements such as in chemoprevention may offer additional approaches to the management of early primary or synchronous lesions in patients at high risk and/or with lung cancer [69,75,76].

As advancements in optics and other endobronchial diagnostic technologies develop (i.e. microconfocal scanning microscopy endoscopic optical coherence tomography, endoscopic magnetic resonance tomography and doppler sonography) identification of earlier lesions may become vastly more important in the evaluation and management of our patients' early lesions that are found with AF. Concurrently, as our ability to look at the specimens we are collecting improves (histologic and molecular methods), it may further increase our ability to identify those biopsy samples not only with cancer, but also with a high degree of cancer potential. Park *et al.* [66] have demonstrated that molecular abnormalities in histologically normal bronchial specimens can be quite extensive. Of the samples reviewed by the authors, 68% had at least one abnormality among the chromosomal regions analyzed. The natural history of these genomic changes, as they are understood, will help with our planning and treatment.

Park *et al.*'s study also reported heterogeneity in the molecular changes seen, while endobronchial specimens identified and biopsied with the use of LIFE, which were histologically normal, demonstrated a high percentage of molecular abnormalities that were more homogeneous for the allele specific losses being investigated [66,67]. This type of data suggest that AF positive lesions that are sampled and are now being identified as histologically normal may in fact have precancerous molecular changes that we have not yet been able to clinically consider; yet, they are changing the cellular AF potential early.

Patient selection

Screening for lung cancer is still an area of great uncertainty. Attempts of screening with high-resolution CT, chest radiographs and sputum cytology continue to be looked at to find "the" ideal test. Bronchoscopy may give a tremendous amount of information of diseases of the central airways, but as a screening tool it is too invasive, expensive and would therefore be impractical. AF bronchoscopy should instead be considered an adjunct technique used in those situations where a fiberoptic bronchoscopy needs to be performed for more established indications. Patients who are undergoing bronchoscopy for the diagnosis and/or staging of a radiographically evident lesion should have AF bronchoscopy added to their airway evaluation. Those patients who are to have surgical resection should, be considered for AF evaluation of their airways preoperatively to potentially diminish the recurrence of cancer at the site of the surgical stump. Patients found to have abnormal sputum cytology should also have AF bronchoscopy as part of their evaluation in that the lesions leading to the abnormality may be small and not readily evident. High-risk patients who present with hemoptysis and require airway examination should also have AF bronchoscopy used in their evaluation to improve upon our diagnostic accuracy as to the source of the bleeding. The presence of precancerous lesions as well as synchronous and metachronous cancers is becoming better understood. AF is a tool to identify these lesions in patients, improving upon our opportunity for early diagnosis of lung cancer and possibly bettering our ability to treat this disease.

Conclusion

Autofluorescence is a technique currently available for clinical practice. The technology has been demonstrated to provide improved sensitivity in study after study for the detection of pre-neoplastic and early cancerous lesions. Much of the skepticism associated with the use of AF up to now has been in the interpretation of the data acquired, for instance, what do you do with a dysplastic lesion? As our understanding of how both the natural history and molecular changes influence the progression

of metaplasia to dysplasia to CIS continues to grow, the clinical importance of AF will certainly become more evident.

The technique of AF requires developing skills in looking and interpreting the color variations on the screen. A learning curve for the detection of early cancers is as common with a technique such as AF as in many procedures. In one study, the authors demonstrated improved sensitivities with AF in patients 49 through 95 (sensitivity 86%) versus the original 48 patients (sensitivity 67%) [52]. Once the skill and understanding of using AF bronchoscopy is acquired, it adds little time to a standard examination and the information gained can influence the overall management and very possibly long-term outcomes on our patients.

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