Value of autofluorescence bronchoscopy in patients with laryngeal cancer

E ÇETİNKAYA[1], B VEYSELLER[2], Y S YILDIRIM[2], F AKSOY[2], M A ÖZGÜL[1],
A GENÇOĞLU[1], S ALTIN[1]

[1]*Department of Chest Disease, Yedikule Chest Disease and Thoracic Surgery Education and Research Hospital, and* [2]*Department of Otorhinolaryngology and Head and Neck Surgery, Haseki Research and Training Hospital, Istanbul, Turkey*

Abstract

Background: Patients with squamous cell carcinoma of the head and neck constitute a high risk group for synchronous and metachronous tumours.

Objective: This study aimed to investigate the usefulness of white light and autofluorescence bronchoscopy in the evaluation of pre-malignant and early neoplastic lesions in patients with laryngeal cancer, who are at high risk of concomitant lung cancer.

Methods: This prospective, cross-sectional study included 30 patients who had undergone total laryngectomy for squamous cell carcinoma of the larynx. The tracheobronchial system was investigated for the presence of pre-malignant and malignant lesions, using a combination of white light and autofluorescence bronchoscopy. Biopsies were obtained from areas with a pathological appearance, and histopathological studies were performed.

Results: All patients had a permanent tracheostomy. Light and autofluorescence bronchoscopy indicated that the tracheobronchial system was normal in 11 patients. A total of 27 biopsies was taken from the remaining 19 patients, and revealed invasive squamous cell carcinoma in one patient and pre-malignant changes in six.

Conclusion: Bronchoscopy is a valuable and practical tool for screening patients at high risk of lung cancer, and requires minimal intervention especially in patients with a permanent tracheostomy. Of the various bronchoscopic techniques becoming available, autofluorescence bronchoscopy shows promise for the detection of pre-invasive malignant changes of the tracheobronchial system in patients previously operated upon for laryngeal cancer.

Key words: Lung Neoplasms; Autofluorescence; Bronchoscopy; Head And Neck Neoplasms; Carcinoma In Situ; Laryngectomy

Introduction

Lung cancer is the most common malignancy in the world and also the leading cause of cancer-related death, being responsible for 29 per cent of all deaths due to cancer.[1] Survival depends on the size and stage of the tumour at diagnosis, with five-year survival rates of 49 and 2 per cent for early stage and disseminated primary tumours, respectively.[2] Published five-year survival rates for patients diagnosed with carcinoma in situ range from 75 to 91 per cent.[3]

Patients with squamous carcinoma of the head and neck constitute a high risk group for synchronous and metachronous tumours. In such patients, studies have reported a prevalence of second primary tumours of 7 to 21 per cent, with lung cancer being the most common type.[4] Smoking is known to be the most important aetiological factor in malignancies of the respiratory system, head and neck.[5]

Patients diagnosed with head and neck cancer require a thorough physical examination, especially of the upper aerodigestive system. X-ray, chest computed tomography (CT), positron emission tomography (PET) CT and upper aerodigestive tract endoscopy (commonly termed panendoscopy) are helpful in the pre-operative investigation of these patients. The diagnosis of central pre-neoplastic and early neoplastic lesions requires bronchoscopy, which also permits biopsy for histopathological examination.[6]

In a previous study, conventional white light bronchoscopy was found to have a failure rate of 60 per cent in the diagnosis of high grade lesions of the central airways.[7] The inadequacy of this method of detecting early lesions has led to investigation of new methods, and autofluorescence bronchoscopy has emerged as a more sensitive method of detecting pre-neoplastic and neoplastic lesions of the central

airways.[8] Numerous clinical studies have found autofluorescence bronchoscopy to be superior to white light bronchoscopy in the detection of early lesions.[9,10]

Materials and methods

Autofluorescence bronchoscopy

When light of a certain wavelength is applied to tissue, fluorescence occurs.[11] Upon excitation with blue light (wavelength 380–460 nm), abnormal tissue shows reduced fluorescence compared with normal tissue. The difference in the fluorescence of normal versus tumour tissue is explained by greater absorption of light by the thickened epithelium of the abnormal tumour tissue, or by decreased fluorescence stimulation in the submucosa. As a result, malignant tissue appears a dark blue-red under fluorescence, while normal mucosa appears green.[12] Digital technology enables the integration of millions of emitted fluorescent light signals, making possible real-time video imaging of the bronchial mucosa during autofluorescence bronchoscopy.[13]

In the current study, we used the Onco-Life autofluorescence bronchoscopy device (Xillix Technologies, Richmond, British Columbia, Canada), comprising a video camera and an endoscopic light source compatible with conventional endoscopes (Figure 1). This device provides real-time images which can be displayed on a monitor. The Onco-Life device has two modes: conventional white light (the colour imaging mode) and fluorescence (blue light is used for this mode). The latter mode enables assessment of tissue fluorescence, facilitating the diagnosis of potentially pre-cancerous or cancerous tissue. Blue light is used to illuminate the tissue, stimulating the fluorophores already present in the tissue. During video imaging, normal areas and areas suspected to be pre-malignant or malignant appear green and brown-red, respectively (Figure 2).

Spectrofluorometric studies have provided objective evidence that the autofluorescence of normal tissue, dysplastic tissue and carcinoma in situ differs.[14–19]

FIG. 1
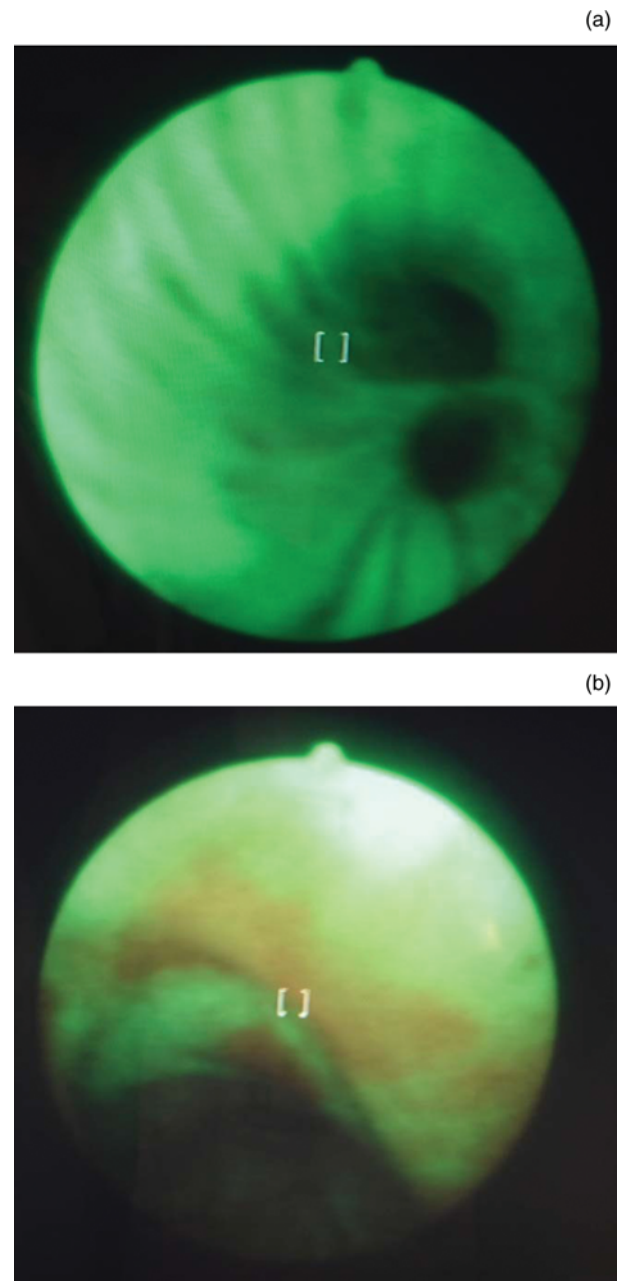The Onco-Life autofluorescence bronchoscopy device.

FIG. 2
Bronchoscopic views showing enlargement and hyperaemia of the right lower lobe superior segment: (a) AFB view; normal (b) AFB view; biopsies were obtained from areas suspected of being premalignant or malignant and found severe dysplasia.

The sensitivity and specificity of autofluorescence bronchoscopy in detecting abnormal lesions are 73–89 per cent and 46–91 per cent, respectively.[14–20] A significant number of abnormal lesions can be detected during conventional bronchoscopy; however, sampling all these lesions prolongs procedural time and increases the workload of the pathologist.[21–24]

Patients with laryngeal cancer have an increased risk of lung cancer. The aim of this study was to detect pre-invasive tracheobronchial lesions before they progressed to invasive cancer, and also to test the efficacy of bronchoscopy as a screening tool, bearing in mind that bronchoscopy is relatively straightforward where such patients have a permanent tracheostomy. This study also aimed to test the sensitivity and specificity of autofluorescence bronchoscopy and white light bronchoscopy in detecting abnormal lesions, by comparing their independent and combined use.

Between November 2007 and December 2008, 30 patients in the department of ear, nose, throat and head and neck surgery of Haseki Education and Research Hospital were included in this prospective study. These patients had undergone total laryngectomy for squamous cell carcinoma of the larynx, and had normal chest X-ray and chest CT results. The patients were referred to the Yedikule Chest Disease and Thoracic Surgery Hospital, where bronchoscopies were performed after the first post-operative month.

Patients with cardiovascular disease, bleeding diatheses and unstable asthma were not included in the study. We also excluded patients with nonlaryngeal head and neck cancer and those with subtotal laryngectomy (because of the safer and easier application of bronchoscopy after total laryngectomy).

Diagnostic bronchoscopy procedure

Informed consent was obtained from all patients before the procedure, and the study protocol was approved by the local ethics committee and institutional review board.

FIG. 3
Bronchoscopy was carried out through the tracheostomy site.

The Onco-Life (Xillix) autofluorescence and white light bronchoscopy device was used, always by the same physician (EC), who was experienced in the procedure. Pre-medication with 0.07 mg/kg midazolam was given before bronchoscopy, and supplemental lidocaine was applied through the working channel of the bronchoscope when necessary. Bronchoscopy was performed through the tracheostomy site in all patients (Figure 3). During bronchoscopy, oxygen was given at 2 l/min through the tracheostomy site via a cannula, and oxygen saturation and heart rate were monitored transdermally. Bronchoscopy commenced at the tracheostomy site and progressed toward the trachea, carina, main bronchi, lobes, segments and visible segmental bronchi, using first white light and then autofluorescence. In both modes, areas suspected to be pre-malignant or malignant were recorded. Finally, white light bronchoscopy and autofluorescence bronchoscopy were used in combination, and biopsies were obtained from all areas suspected to be pre-malignant or malignant. Samples were fixed in formalin, numbered and sent for histopathological examination. During white light bronchoscopy, severe erythema, mucosal thickening or irregularity, and nodular or polypoid changes were considered abnormal. During autofluorescence examination, all areas appearing brown or purple (rather than the normal green) were considered abnormal. To avoid error, friable and/or haemorrhagic fields and areas with dense mucous secretions were re-evaluated after lavage. In order to determine true and false negatives, for the calculation of diagnostic accuracy, patients with reactive changes were accepted as false positives. Biopsy samples deemed inadequate or inappropriate by the pathologist were not included in the evaluation.

Histopathological analysis

Pathology results were divided into the following six stages, according to the World Health Organization classification of lung tumours: one = normal tissue; two = reactive changes, chronic inflammation, basal cell hyperplasia and squamous metaplasia; three = mild dysplasia; four = moderate dysplasia; five = severe dysplasia or carcinoma in situ; and six = invasive squamous cell carcinoma.[25] Stages one and two were evaluated as reactive changes, while stages three to six were evaluated as pre-neoplastic and neoplastic. Of those patients who had undergone biopsy for positive findings during bronchoscopy, those with pre-neoplasia and neoplasia were accepted as true positives, while those with stage one and two lesions were accepted as false positives.

Statistical evaluation

Sensitivity, specificity, positive predictive value and negative predictive value were calculated separately for white light bronchoscopy and autofluorescence bronchoscopy, and also for the combined use of both

modalities. Statistical analyses were performed using MedCalc® version 10.4.0 software.

Results

All patients had a history of smoking and had undergone total laryngectomy for squamous cell carcinoma of the larynx, subsequently requiring a permanent tracheostomy. Their mean age was 55.7 years (range 43–74). All patients were male. After the laryngeal operation time to bronchoscopy time was 4.1 months in patients with neoplasia and 4.5 months in all patients. The minimum and maximum intervals between surgery and bronchoscopy were one month and one year, respectively. One patient developed pneumonia two days after bronchoscopy and healed completely with medical treatment. In 11 of the 30 patients, the tracheobronchial system appeared normal on both white light bronchoscopy and autofluorescence bronchoscopy, and no biopsies were taken. In the remaining 19 patients, a total of 27 biopsies was obtained; these revealed invasive squamous cell carcinoma in one patient and pre-neoplastic changes in six.

Eleven patients had pathological bronchoscopic findings on white light bronchoscopy examination, two of whom had positive histological findings (Figure 4). Evaluation of white light bronchoscopy examination alone revealed the following diagnostic parameters: sensitivity 28.57 per cent, specificity 60.87 per cent, positive predictive value 18.18 per cent, negative predictive value 73.68 per cent, positive likelihood ratio 0.73 and negative likelihood ratio 1.17 (Table I).

Upon autofluorescence bronchoscopic examination, 16 patients had pathological findings and 14 appeared normal. Seven of the biopsies showed positive histological findings (Figure 5). Evaluation of autofluorescence bronchoscopy alone revealed the following diagnostic parameters: sensitivity 100.00 per cent, specificity 60.87 per cent, positive predictive value

TABLE I		
DIAGNOSTIC UTILITY OF BRONCHOSCOPY MODES		
Parameter	WLB	AFB
Sens (%)	28.57	100.00
Spec (%)	60.87	60.87
PPV (%)	18.18	43.75
NPV (%)	73.68	100.00
PLR	0.73	2.56
NLR	1.17	0.00

WL = white light bronchoscopy; AFB = autofluorescence bronchoscopy; sens = sensitivity; spec = specificity; PPV = positive predictive value; NPV = negative predictive value; PLR = positive likelihood ratio; NLR = negative likelihood ratio

43.75 per cent, negative predictive value 100.00 per cent, positive likelihood ratio 2.56 and negative likelihood ratio 0.00 (Table I).

When white light bronchoscopy and autofluorescence bronchoscopy were performed together, 11 of the 30 patients appeared normal and 19 had pathological findings. Biopsy samples obtained from the latter group revealed positive pathological results in seven patients.

Discussion

This study was designed to assess patients who had undergone a total laryngectomy, in order to detect pre-neoplastic lesions at an early stage, and also to assess the role of bronchoscopy as a practical investigative technique in such patients. The study aimed to investigate whether increased diagnostic sensitivity could be achieved by combining white light bronchoscopy, a commonly used diagnostic method, with autofluorescence bronchoscopy, a technique which is gaining popularity due to its greater sensitivity to pre-neoplastic lesions.[26]

Patients with squamous cell carcinoma of the head and neck are at high risk of developing squamous cell carcinoma of the lung.[27] Chest X-rays and CTs
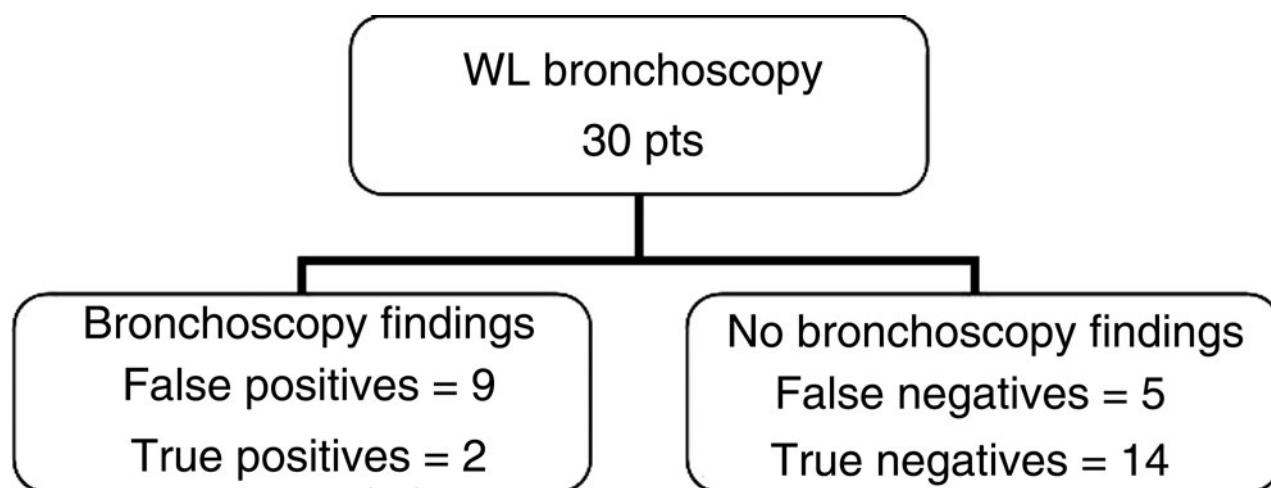

FIG. 4
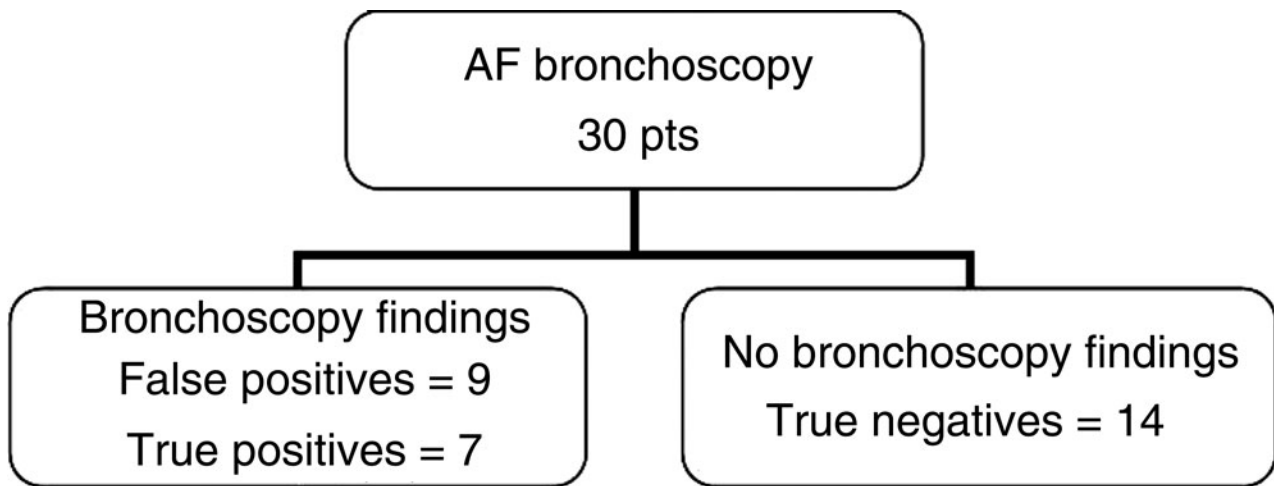Results of white light (WL) bronchoscopy evaluation.

FIG. 5

Results of autofluorescence (AF) bronchoscopy evaluation.

do not have adequate sensitivity to detect early stage neoplasia in such high risk patients.[28] Furthermore, PET CT is expensive and requires biopsy, and sputum cytology cannot diagnose intraepithelial lesions (studies in high risk patients have shown that sputum cytology is inadequate for detecting pre-neoplastic changes, does not help reduce cancer-specific mortality, and requires further bronchoscopic evaluation and histological examination in patients with atypical findings).[29] Therefore, bronchoscopy is the most valuable screening and diagnostic tool for such high risk patients.[30] In addition, bronchoscopic access in such patients may be facilitated by the presence of a permanent tracheostomy.

The sensitivity success of white light bronchoscopy in detecting early neoplastic changes in the central airways is less than 40 per cent.[31] However, autofluorescence bronchoscopy is now in widespread use.[8,13] Numerous studies have found its sensitivity to be superior to that of white light bronchoscopy.[20,32]

The sensitivity and specificity of white light bronchoscopy and autofluorescence bronchoscopy change with the number of patients, the number of biopsies and the experience of the bronchoscopist. Fuso et al. assessed the use of bronchoscopy to identify pre-cancerous bronchial lesions, and found sensitivities of 43.8 and 82.8 per cent for white light bronchoscopy and autofluorescence bronchoscopy, respectively, and a positive predictive value of 70.2 per cent for the latter.[33] In their multicentre study, Ernst et al. found sensitivities of 28 and 67 per cent for white light bronchoscopy and autofluorescence bronchoscopy, respectively, for the detection of pre-malignant airway change.[34] Venmans et al. reported a sensitivity of 21 per cent for white light bronchoscopy used to detect bronchial intraepithelial neoplasia, compared with 28.57 per cent in our study.[35] In these latter two studies, the sensitivity figures for autofluorescence bronchoscopy were 57 and 60.87 per cent, respectively.

Based on these data, autofluorescence bronchoscopy is a more sensitive technique than white light bronchoscopy in the detection of early neoplasia, and thus should be preferred.

The significance of detecting lesions at an early stage is best illustrated by considering that 10 per cent of moderate dysplasias and 19–46 per cent of severe dysplasias progress to invasive carcinoma within six months, and that 50–60 per cent of squamous cell carcinomas of the lung arise in the central airways and are accessible by bronchoscopy.[31] Patients who exhibit long-term survival are those who were diagnosed early and with completely resectable tumours; however, less than 20 per cent of patients have asymptomatic, stage one lesions at the time of diagnosis. Stage zero and one lesions carry the best prognosis; therefore, early diagnosis of lung cancer in high risk patients is critical for patient survival.

- **Patients with head and neck squamous cell carcinoma are at high risk of lung cancer**
- **Such patients require close post-operative follow up even if pre-operative investigations were negative**
- **In patients with a permanent tracheostomy, bronchoscopy is a simple, comfortable procedure requiring minimum intervention, and is valuable for lung cancer screening**

The pre-malignant lesions discussed above are often seen in smokers, and very rarely in nonsmokers.[36] The risk is 20- to 25-fold higher in individuals with a smoking history of more than one pack per day for more than 20 years.[37] Thus, it is important to screen heavy smokers (i.e. >20 packs/year), symptomatic smokers and patients with a history of upper respiratory tract cancer. Patients with chronic obstructive

pulmonary disease who have smoked more than 40 packs/year have a higher incidence of pre-malignant dysplasia on sputum cytology.[38] Such patients represent an important target population for early diagnosis.

All 30 patients in the present study were heavy smokers, and had undergone total laryngectomy for squamous cell carcinoma of the larynx. Biopsies revealed invasive squamous cell carcinoma of the lung in one patient and pre-neoplasia in six; this total (seven of 30) corresponded to 23 per cent of patients.

Conclusion

Autofluorescence bronchoscopy has greater sensitivity and specificity than white light bronchoscopy in the detection of pre-neoplastic lesions. In patients with a permanent tracheostomy, bronchoscopy is a simple, comfortable procedure which requires minimum intervention, and which is valuable for the screening of patients with cancer of the head and neck and those at high risk of lung cancer. Autofluorescence bronchoscopy shows promise for the detection of pre-malignant changes of the tracheobronchial system in patients previously treated for laryngeal cancer.

These findings need to be supported by larger, multicentre studies.

References

1 Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: globocan 2000. *Int J Cancer* 2001;**94**: 153–6
2 Bechtel JJ, Petty TL, Saccomanno G. Five year survival and later outcome of patients with X-ray occult lung cancer detected by sputum cytology. *Lung Cancer* 2000;**30**:1–7
3 Edell E, Lam S, Pass H, Miller YE, Sutedja T, Kennedy T *et al*. Detection and localization of intraepithelial neoplasia and invasive carcinoma using fluorescence-reflectance bronchoscopy: an international, multicenter clinical trial. *J Thorac Oncol* 2009;**4**: 49–54
4 Stoeckli SJ, Zimmermann R, Schmid S. Role of routine panendoscopy in cancer of the upper aerodigestive tract. *Otolaryngol Head Neck Surg* 2001;**124**:208–12
5 Moro-Sibilot D, Jeanmart M, Lantuejoul S, Arbib F, Laverrière MH, Brambilla E *et al*. Cigarette smoking, preinvasive bronchial lesions, and autofluorescence bronchoscopy. *Chest* 2002;**122**: 1902–8
6 Hujala K, Sipila J, Grenman R. Panendoscopy and synchronous second primary tumors in head and neck cancer patients. *Eur Arch Otorhinolaryngol* 2005;**262**:17–20
7 Lam S, MacAulay C, leRiche JC, Palcic B. Detection and localization of early lung cancer by fluorescence bronchoscopy. *Cancer Suppl* 2000;**89**(suppl):2468–73
8 Palcic B, Lam S, Hung J, MacAulay C. Detection and localization of early lung cancer by imaging techniques. *Chest* 1991; **99**:742–3
9 Goujon D, Zellweger M, Radu A, Grosjean P, Weber B-C, van den Bergh H *et al*. In vivo autofluorescence imaging of early cancers in the human tracheobronchial tree with a spectrally optimized system. *J Biomed Opt* 2003;**8**:17–25
10 Lam S, Kennedy T, Unger M. Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy. *Chest* 1998;**113**:696–702
11 Andersson-Engels S, Klinteberg C, Svanberg K, Svanberg S. In vivo fluorescence imaging for tissue diagnostics. *Phys Med Biol* 1997;**42**:815–24
12 Qu J, MacAulay C, Lam S, Palcic B. Laser induced fluorescence spectroscopy at endoscopy: tissue optics, Monte Carlo modeling and in vivo measurements. *Opt Eng* 1995;**34**: 3334–43

13 Hung J, Lam S, Le Riche JC, Palcic B. Autofluorescence of normal and malignant bronchial tissue. *Lasers Surg Med* 1991;**11**:99–105
14 Kurie JM, Lee JS, Morice RC, Walsh GL, Khuri FR, Broxson A *et al*. Autofluorescence bronchoscopy in the detection of squamous metaplasia and dysplasia in current and former smokers. *J Natl Cancer Inst* 1998;**90**:991–5
15 Keith RL, Miller YE, Gemmill RM, Drabkin HA, Dempsey EC, Kennedy TC *et al*. Angiogenic squamous dysplasia in bronchi of individuals at high risk for lung cancer. *Clin Cancer Res* 2000;**6**: 1616–25
16 Weigel TL, Yousem D, Dacic S, Kosco PJ, Siegfried J, Luketich JD. Fluorescence bronchoscopic surveillance after curative surgical resection for non-small cell lung cancer. *Ann Surg Oncol* 2000;**7**:176–80
17 Pierard P, Vermylen P, Bosschaerts T, Roufosse C, Berghmans T, Sculier JP *et al*. Synchronous roentgenographically occult lung carcinoma in patients with resectable primary lung cancer. *Chest* 2000;**117**:779–85
18 Sutedja TG, Codrington H, Risse EK, Breuer RH, van Mourik JC, Golding RP *et al*. Autofluorescence bronchoscopy improves staging of radiographically occult lung cancer and has an impact on therapeutic strategy. *Chest* 2001;**120**: 1327–32
19 Kusunoki Y, Imamura F, Uda H, Mano M, Horai T. Early detection of lung cancer with laser-induced fluorescence endoscopy and spectrofluorometry. *Chest* 2000;**118**:1776–82
20 Kennedy TC, Lam S, Hirsch FR. Review of recent advances in fluorescence bronchoscopy in early localization of central airway lung cancer. *Oncologist* 2001;**6**:257–62
21 Beamis JR, Ernst A, Simoff M, Yung R, Mathur P. A multicenter study comparing autofluorescence bronchoscopy to white light bronchoscopy using a non-laser light stimulation system. *Chest* 2004;**125**:148S–49S
22 Jang TW, Oak CH, Chun BK, Jung MH. Detection of pre-invasive endobronchial tumours with D-light/autofluorescence system. *J Korean Med Sci* 2006;**21**:242–6
23 Helfritzsch H, Junker K, Bartel M, Scheele J. Differentiation of positive autofluorescence bronchoscopy findings by comparative genomic hybridization. *Oncol Rep* 2002;**9**: 697–701
24 Venmans BJ, van Boxem TJ, Smit EF, Postmus PE, Sutedja TG. Outcome of brochial carcinoma in situ. *Chest* 2000;**117**: 1572–6
25 Brambilla E, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. *Eur Respir J* 2001;**18**:1059–68
26 Lee P, Brokx HA, Postmus PE, Sutedja TG. Dual digital video-autofluorescence imaging for detection of pre-neoplastic lesions. *Lung Cancer* 2007;**58**:44–9
27 Zargi M, Fajdiga I, Smid L. Autofluorescence imaging in the diagnosis of laryngeal cancer. *Eur Arch Otorhinolaryngol* 2000;**257**:17–23
28 Flehinger BJ, Melamed MR, Zaman MB, Heelan RT, Perchick WB, Martini N. Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Memorial Sloan-Kettering study. *Am Rev Respir Dis* 1984; **130**:555–60
29 Kennedy TC, Franklin WA, Prindiville SA, Cook R, Dempsey EC, Keith RL *et al*. High prevalence of occult endobronchial malignancy in high risk patients with moderate sputum atypia. *Lung Cancer* 2005;**49**:187–91
30 Vermylen P, Pierard P, Roufosse C, Bosschaerts T, Verhest A, Sculier JP *et al*. Detection of bronchial preneoplastic lesions and early lung cancer with fluorescence bronchoscopy: a study about its ambulatory feasibility under local anaesthesis. *Lung Cancer* 1999;**25**:161–8
31 Hirsch FR, Franklin WA, Gazdar AF, Bunn PA Jr. Early detection of lung cancer: clinical perspectives of recent advances in biology and radiology. *Clin Cancer Res* 2001;**7**: 5–22
32 Sato M, Sakurada A, Sagawa M, Minowa M, Takahashi H, Oyaizu T *et al*. Diagnostic results before and after introduction of autofluorescence bronchoscopy in patients suspected of having lung cancer detected by sputum cytology in lung cancer mass screening. *Lung Cancer* 2001;**32**:247–53
33 Fuso L, Pagliari G, Boniello V, Trove' A, Varone F, Longobardi A *et al*. Autofluorescence bronchoscopy to

identify pre-cancerous bronchial lesions. *Monaldi Arch Chest Dis* 2005;**63**:124–8

34 Ernst A, Simoff M, Mathur P, Yung R, Beamis J. D-Light auto-fluorescence in the detection of premalignant airway changes. A multicenter trial. *J Bronchol* 2005;**12**:133–8

35 Venmans BJ, van Boxem TJ, Smit EF, Postmus PE, Sutedja TG. Bronchial intraepithelial neoplastic lesions in head and neck cancer patients: results of autofluorescence bronchoscopy. *Ann Otol Rhinol Laryngol* 2001;**110**:635–8

36 Peters EJ, Morice R, Benner SE, Lippman S, Lukeman J, Lee JS *et al*. Squamous metaplasia of the bronchial mucosa and its relationship to smoking. *Chest* 1993;**103**:1429–32

37 Peto R, Darby S, Deo H, Silcocks P, Whitley E, Doll R. Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case–control studies. *BMJ* 2000;**321**:323–9

38 Kennedy TC, Proudfoot SP, Franklin WA, Merrick TA, Saccomanno G, Corkill ME *et al*. Cytopathological analysis of sputum in patients with airflow obstruction and significant smoking histories. *Cancer Res* 1996;**56**:4673–8

Address for correspondence:
Dr Yavuz Selim Yıldırım,
Haseki Egitim ve Arastirma Hastanesi,
Kulak Burun Bogaz Klinigi,
34089 Fatih,
Istanbul, Turkey

Fax: +90 212 5103701
E-mail: dryavuzselim@yahoo.com

Dr Y S Yıldırım takes responsibility for the integrity
of the content of the paper
Competing interests: None declared