Study objectives: Autofluorescence bronchoscopy (AFB), when used as an adjunct to standard white light bronchoscopy (WLB), enhances the bronchoscopist’s ability to localize small neoplastic lesions, especially intraepithelial lesions. The current study was undertaken in order to define the population in which the rate of detection is higher using AFB.

Design and patients: Two hundred forty-four consecutive patients, who were symptomatic smokers or patients who previously had been treated for lung cancer or head and neck cancers, underwent WLB and AFB. All patients with endoscopic abnormalities underwent biopsies. Data concerning smoking history were prospectively registered.

Results: We report the prevalence of high-grade or invasive lesions at the time of examination. On a lesion-by-lesion analysis, 92 low-grade lesions, 42 high-grade lesions (ie, moderate dysplasia, severe dysplasia, and carcinoma in situ), and 39 invasive carcinomas were diagnosed. There was no effect of age, gender, and age at smoking initiation on the prevalence of preinvasive or invasive lesions. The 10 patients who previously had undergone surgery for lung cancer and exhibited high-grade preinvasive lesions had a history of carcinoma of the epidermoid histologic type (p = 0.01). These 10 patients displayed multiple lesions in the bronchial tree (mean No. of lesions, 1.8 per patient). In current smokers, the prevalence of high-grade or invasive lesions were both related to the number of pack-years smoking had occurred (p = 0.01) and to the duration of smoking (p = 0.01). In contrast, the prevalence of preinvasive lesions in former smokers was related to a history of epidermoid carcinoma.

Conclusions: AFB should be recommended in patients with a history of epidermoid carcinomas of the lung. Current smokers with a prolonged smoking history appear to comprise a population in which the rate of detection of preneoplastic lesions is high with AFB.

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Key words: autofluorescence bronchoscopy; early detection; occult lung cancer

Abbreviations: AFB = autofluorescence bronchoscopy; CIS = carcinoma in situ; WLB = white light bronchoscopy

Lung cancer is the leading cause of cancer-related death in industrial countries, and cigarette smoking is the main risk factor. Most patients cannot be cured because they present with advanced stages of the disease, and the prognosis remains poor despite therapeutic improvements. Lung carcinoma arises after a series of morphologic and genetic alterations leading to the progression from a normal bronchial epithelium to invasive squamous cell carcinoma. The morphologic changes are thought to progress from hyperplasia to metaplasia, which are rather common reactive lesions, to dysplasia of progressive severity (ie, mild, moderate, and severe) and carcinoma in situ (CIS), which are considered to be true premalignant lesions with a high risk of cancer development. However, all of these lesions are able to regress, including CIS.

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It is thought that multiple intraepithelial lesions develop at various times in patients who have been exposed to carcinogens, which supports the idea that the entire bronchial mucosa is damaged by carcinogens. This phenomenon is referred to as the field cancerization process.

Autofluorescence bronchoscopy (AFB) [LIFE-Lung System; Xillix; Richmond, BC, Canada], when used as an adjunct to standard white light bronchoscopy (WLB), enhances the bronchoscopist’s ability to localize small neoplastic lesions, especially intraepithelial lesions.9 In a North American multicenter study of AFB (using the LIFE-Lung System)9 of 173 subjects who had undergone 700 biopsies, the relative sensitivity of AFB for lesions classified as moderate dysplasia, severe dysplasia, or CIS improved when compared with WLB alone, resulting in a relative sensitivity of 6.3. The false-positive rate (0.34), however, was quite high for the detection of these lesions compared to 0.10 with WLB alone. This study resulted in the approval of LIFE-Lung System for clinical use by the US Food and Drug Administration. Other reports9–11 were in agreement with the conclusions of the current study.

However, contrary results were obtained in a study by Kurie et al12 in which 53 subjects who were enrolled in a chemoprevention trial failed to show increased sensitivity with AFB. The study group had more than 20 pack-years of smoking but lacked additional risk factors for malignancy such as positive sputum cytology findings or airflow obstruction. The bronchoscopic evaluation compared biopsies specimens obtained from six predetermined sites to those from sites of abnormal fluorescence. Only 8 of 245 biopsy specimens (3%) showed metaplasia and/or dysplasia, and there was a poor correlation with suspicious classification by fluorescence. O’Neil and Johnson13 discussed the discrepancies between these two studies and pointed out the importance of carefully defining the patient population as well as providing any further study with comprehensive information about the patient’s characteristics, the pack-years of cigarette smoking, and the duration of smoking cessation. Thus, the current study was initiated in order to define the population in which the rate of detection of preneoplastic lesions would be high with AFB.

**Materials and Methods**

**Patients**

Two hundred forty-four consecutive patients with previous, known, or suspected lung cancer who were fit enough to undergo AFB were prospectively entered into the current study. These patients were classified into the following three groups: heavy smokers with respiratory symptoms (group I); patients participating in postoperative follow-up of completely resected lung cancer (group II); and patients participating in follow-up of head and neck cancers (group III).

Data concerning tobacco use were prospectively registered, as follows: age of onset; age of tobacco cessation; number of pack-years; and number of cigarettes smoked per day in the year preceding bronchoscopy. Patients were considered to be lifelong nonsmokers, active smokers, or former smokers if they had stopped >1 year before undergoing the bronchoscopic examination.14

**Bronchoscopic Examination**

The bronchoscopic examination was conducted as an outpatient procedure using topical anesthesia and conscious sedation with parenteral clorazepate, 20 mg, clobutinol, 20 mg, and atropine, 0.25 mg.15 All patients had given their consent to undergo the bronchoscopic examination.

All bronchoscopic examinations were performed by two pulmonologists who were trained in fluorescence bronchoscopy (FA and DM). Conventional WLB was carried out using a fiberoptic bronchoscope (model BF20D; Olympus; Tokyo, Japan). After completion of the WLB examination, AFB was performed (LIFE-Lung System; Xillix).

Lesions were classified visually into one of three clinical categories on each bronchoscopic examination according to the classification system described by Lam et al.8 Using this visual classification system, areas without any visual abnormality were classified as class 1. Under WLB examination, areas with nonspecific erythema, swelling or thickening of the bronchial mucosa, bronchoscopic trauma, anatomic anomalies, or granulation tissue were labeled as class 2. Nodular/polypoid lesions, focal thickening, or irregularities of the bronchial mucosa were classified as class 3. Under fluorescence examination, normal areas appear as green. Areas that were slightly brown with ill-defined margins that could be missed easily were labeled as class 2. Areas suspicious of moderate dysplasia or worse had a definite brown or brownish-red color (class 3). Biopsies were performed on class 2 or 3 areas in WLB light or AFB examination.

**Pathologic Examination**

Bronchial biopsy specimens were fixed in formalin fixative and were embedded in paraffin for conventional histopathologic diagnosis. Preinvasive lesions were classified according to the classic criteria. The recently revised World Health Organization classification16 includes mild dysplasia, moderate dysplasia, severe dysplasia, and CIS. Mild dysplasia is characterized by the presence of mildly atypical crowded cells with pleomorphisms, nuclear irregularities in the lower third of the epithelium, moderate dysplasia with more atypical cells in the lower two thirds of the epithelium, and mitotic figures in the lower third of the epithelium. Severe dysplasia is characterized by the presence of markedly atypical cells into the upper third of the epithelium and mitosis in the lower two thirds of the epithelium. CIS is characterized by the presence of atypia, pleomorphism, and mitosis observed through the entire thickness of the epithelium, and by a lack of progression of maturation from the base to the luminal surface. However, in contrast with invasive carcinoma, no penetration of the subepithelial basement membrane is observed. Invasive carcinoma was classified according to the World Health Organization classification.16
In the present study, subgrouping of lesions was performed as follows: squamous metaplasia and mild dysplasia were considered to be low-grade lesions, whereas moderate dysplasia, severe dysplasia, and CIS were considered to be high-grade lesions. All the lesions were separately interpreted by two trained pathologists and revised by a third pathologist in case of discrepancy (EB, SL, and MHL).

Statistical Analysis

Statistically unbiased estimates of sensitivity and specificity were not possible to obtain because serial sections of the entire tracheobronchial tree would need to be examined after the bronchoscopic procedures were performed to define the true-positive and true-negative standards. Thus, to determine whether the addition of AFB to WLB was better than using WLB alone, we calculated the relative sensitivity, or the ratio of the rate of detection of AFB and WLB compared with WLB alone, as published by previous authors. Following the same methodology, class 3 endoscopic aspects were considered as “endoscopically” highly suspicious of preinvasive lesions and were considered to be “positive.” Class 1 and 2 lesions were considered to be “negative.” Three other estimates, the specificity, the positive predictive value, and the negative predictive value, also were calculated to evaluate the performance. The data were analyzed on a per-lesion basis and on a per-patient basis. For the per-lesion analysis, each biopsy specimen was considered separately. Since preinvasive lesions are often multiple in number, for the per-patient analysis, the highest grade lesion in each patient was considered.

The statistical comparison of qualitative values was performed using contingency tables and the Fisher exact test. The comparison of quantitative values was performed using the Mann-Whitney U test (for two groups of patients) or the Kruskal-Wallis test (for more than two groups of patients). Statistical analyses were performed using computer software (StatView, version 4.1; SAS Institute; Cary, NC). A p value of < 0.05 was considered to be statistically significant.

RESULTS

From May 1998 to July 2000, 244 patients were examined with WLB and AFB (354 biopsies were performed). Patient characteristics are summarized in Table 1.

In group II (patients participating in the postoperative follow-up of completely resected lung cancers), the mean time interval between prior surgery and bronchoscopy was 27.5 months (median, 19 months; interquartile range, 9 to 39 months).

Using a lesion-by-lesion analysis, 92 low-grade lesions, 42 high-grade lesions (ie, moderate dysplasia, severe dysplasia, and CIS), and 39 invasive carcinomas were diagnosed. The results of WLB and AFB are shown in Table 2. The addition of autofluorescence resulted in a greater relative sensitivity over WLB alone in high-grade lesions (relative sensitivity, 2.4) and in low-grade lesions (relative sensitivity, 4.7) but not in invasive cancers (relative sensitivity, 1.04).

In patients with class 3 lesions that had been detected with AFB, 161 biopsies were performed.

Pathologic examination findings were normal or showed inflammation or fibrosis in 48 cases (30%) and showed either low-grade lesions, high-grade lesions, CIS, or invasive cancer in 113 cases (70%).

In the current series of patients, the specificity of AFB for detecting individuals with moderate dysplasia, severe dysplasia, or CIS was 55%, the positive predictive value was 23%, and the negative predictive value was 96%.

The pathology results of this per-patient analysis are reported in Table 3. There was no influence of age, gender, or age at smoking initiation on the prevalence of preinvasive or invasive lesions. High-grade lesions were most frequently found in group II (patients participating in postoperative follow-up of completely resected lung cancer) [Table 3 and 4; p = 0.0005 by Kruskal-Wallis test]. Nine of the 10 patients with high-grade lesions that were identified in this group belonged to the group of 42 patients who had prior squamous cell carcinomas of the lung.
The last patient had a history of completely resected adenosquamous carcinoma (which was classified as adenocarcinoma). The mean number of low-grade and high-grade lesions in patients with prior squamous cell carcinomas was 1.8 (minimum, one lesion; maximum, five lesions). These high-grade lesions were found in the contralateral lung in 40% of cases and in the same lung in 60% of cases.

In group II, the following characteristics were equally balanced between the different histologic types of the previous tumor: age (p = 0.08); gender (p = 0.08); years of smoking (p = 0.15); and number of pack-years smoked (p = 0.15). In group II, there was no influence of the time interval between surgery and bronchoscopy on the prevalence of high-grade or invasive lesions (p = 0.72 [Kruskal-Wallis test]).

In current smokers (included in group I to III), the number of pack-years smoked and the duration of smoking both influenced the occurrence of high-grade or invasive lesions (number of pack-years smoked, p = 0.01; duration of smoking, p = 0.01) [Fig 1, 2]. In current smokers, recent smoking intensity (ie, the number of cigarettes smoked per day in the year before undergoing bronchoscopy) did not influence the occurrence of preinvasive lesions.

In former smokers, the majority of high-grade lesions were diagnosed in patients with a history of previously resected lung cancers (group I, 1 of 52 patients; group II, 7 of 49 patients; group III, 0 of 13 patients). This difference was statistically significant (p = 0.02 [Fisher exact test]).

The prevalence of preinvasive lesions did not change substantially in former smokers (p = 0.6) and also did not change for > 10 years after the cessation of smoking (p = 0.84) [Fig 3]. Neither years of smoking (p = 0.65) nor pack-years (p = 0.7) influenced the prevalence of preinvasive lesions in former smokers.

### Discussion

Some reports have demonstrated that AFB is an important tool in localizing premalignant and early malignant lesions in the large central airways, particularly when applied to high-risk patients. The end point of the current study aimed at defining high-risk individuals among a large population that had a history of smoking or had experienced a prior smoking-related cancer.

We present in the current study the clinical and bronchoscopic data for 244 patients who were examined with WLB and AFB. Our data confirmed those of prior studies demonstrating that AFB was effective in the detection of moderate-to-severe dysplasia and CIS. The increase in the relative sensitivity of AFB compared to WLB was comparable to what has been observed in previous studies.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Endoscopic Aspect</th>
<th>Low-Grade Lesions (n = 92)</th>
<th>High-Grade Lesions, Including CIS (n = 42)</th>
<th>CIS (n = 19)</th>
<th>Invasive Cancer (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLB Class 1 and 2</td>
<td>82</td>
<td>27</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>WLB Class 3</td>
<td>10</td>
<td>15</td>
<td>7</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>WLB Rate of detection</td>
<td>10/92</td>
<td>15/42</td>
<td>7/19</td>
<td>29/39</td>
<td></td>
</tr>
<tr>
<td>AFB Class 1 and 2</td>
<td>45</td>
<td>6</td>
<td>3</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>AFB Class 3</td>
<td>47</td>
<td>36</td>
<td>16</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>AFB Rate of detection</td>
<td>47/92</td>
<td>36/42</td>
<td>16/19</td>
<td>30/39</td>
<td></td>
</tr>
<tr>
<td>Relative sensitivity</td>
<td>4.7</td>
<td>2.4</td>
<td>2.3</td>
<td>1.04</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3—Preinvasive and Invasive Lesions Described by a Per-Patient Analysis

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Group I (n = 136)</th>
<th>Group II (n = 79)</th>
<th>Group III (n = 29)</th>
<th>Total (n = 244)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>97</td>
<td>52</td>
<td>19</td>
<td>168</td>
</tr>
<tr>
<td>Low-grade</td>
<td>17</td>
<td>12</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>High-grade</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>20</td>
<td>5</td>
<td>4</td>
<td>29</td>
</tr>
</tbody>
</table>

### Table 4—Group II Patients With a History of Lung Cancer and Corresponding Lesions Detected During AFB or WLB

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Epidermoid Carcinoma</th>
<th>Adeno- carcinoma</th>
<th>Large Cell Carcinoma</th>
<th>Malignant Neuroendocrine Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24</td>
<td>20</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Low-grade</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>High-grade</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>25</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>
questionnaires were sometimes difficult to complete. Thus, the term *age of initiation of smoking* often was misunderstood, with some patients answering by giving the age at which they smoked their very first cigarette and some patients answering by giving the age at which their regular tobacco use began. The duration of smoking, the date of smoking cessation, and the calculation of the number of pack-years were easier to complete but also were based on individual recollections of approximate usage.

In the current study, the occurrence of invasive or high-grade lesions was associated with prolonged cigarette smoking ($p = 0.01$) and the quantity of cigarettes smoked (i.e., the number of pack-years; $p = 0.01$). However, recent smoking intensity (i.e., the number of cigarettes smoked per day in the year before undergoing bronchoscopy) did not influence the occurrence of preinvasive lesions. This last point is not in agreement with previously published reports.$^{4,17}$

Peters et al$^{17}$ in 1993 performed flexible fiberoptic bronchoscopies on 106 heavy cigarette smokers. Six bronchial biopsy specimens, which were obtained from the carina and five major bronchi, were screened for squamous metaplasia in each patient. This study demonstrated an association between the intensity of tobacco use (i.e., the number of packs per day) and the metaplasia index. In 1979, Auerbach et al$^4$ established that the incidence of dysplasia and CIS increased in frequency according to the number of cigarettes smoked per day. One should note that Auerbach et al$^4$ and Peters et al$^{17}$ used the number of cigarettes smoked per day instead of the number of pack-years smoked as their unit of measurement. We believe that the number of cigarettes per day is a quantitative parameter that lies in between the number of pack-years smoked and the number of cigarettes smoked per day in the year before bronchoscopy, which was used in our study. We suggest that the differences between the results of our study and those of Auerbach et al$^4$ and Peters et al$^{17}$ might be explained partly by slight differences in the parameters used in those studies and partly by the reduction in cigarette tar yields and other changes in cigarette composition in the last 2 decades.

Our results suggest that prolonged cigarette smoking is probably one of the main causative factor of preinvasive lesions in current smokers. This point already has been established for invasive carcinomas.$^{14,18}$

In former smokers, the prevalence of preinvasive lesions did not change substantially for $>10$ years. This also has been observed by Lam et al.$^{19}$ How-
ever, in contrast with current smokers, neither the duration of smoking (p = 0.65) nor the quantity of smoking (ie, the number of pack-years; p = 0.7) influenced the occurrence of preinvasive lesions in this group of former smokers. In former smokers, preneoplastic changes may arise in bronchial mucosa altered by tobacco carcinogens even after several years of tobacco cessation, which indicates that molecularly abnormal clones persist in the bronchial epithelium and have the ability to become preinvasive lesions. Wistuba et al\textsuperscript{20} and Mao et al\textsuperscript{21} have demonstrated that genetic changes similar to those found in lung cancers can be detected many years after smoking cessation in the nonmalignant bronchial epithelia of former smokers. In the current study, there was no correlation between the prevalence of preinvasive lesions and smoking history in former smokers. An explanation could be that tobacco carcinogens acted as promoters of epithelial transformation, and that after tobacco cessation the epithelium was repaired to a certain degree. The level of remaining abnormalities could be explained by other factors such as individual patient susceptibility or, as suggested by some authors,\textsuperscript{22} age at smoking initiation.

Wiencke et al\textsuperscript{22} studied DNA adducts in normal lung tissue from 143 patients and in blood mononuclear cells. This study aimed to correlate smoking histories and the levels of DNA adducts. The study found that the impact of smoking variables on DNA adduct levels may be different in current and former smokers. In current smokers, recent smoking intensity (ie, the number of cigarettes smoked per day) was the most important variable. In former smokers, the age at smoking initiation was inversely associated with DNA adduct levels.

Wiencke et al\textsuperscript{22} proposed that smoking during adolescence might produce physiologic changes that could lead to increased DNA adduct persistence or that young smokers may be markedly susceptible to DNA adduct formation and have higher adduct burdens after they quit smoking than those persons who start smoking later in life.

Unfortunately, our data do not confirm any prognostic value for age at smoking initiation, but, as we have mentioned before, there might have been some differences in the way patients completed the tobacco questionnaire, which may have led to a certain degree of imprecision.

High-grade lesions were found more frequently in patients who were observed after undergoing complete resections of lung cancer (group II) than in those in other groups (ie, groups I and III). Despite a statistically significant difference, we believe that this might be partly explained by a selection bias. Thus, these patients were more closely observed, with some patients having been referred to our center after the diagnosis of preinvasive lesions that were found in follow-up bronchoscopy and others having been referred because preinvasive lesions were found adjacent to lung cancer in the resected lung. However, all patients in this group had a history of resected epidermoid carcinoma of the lung (epidermoid carcinoma, nine patients; adenosquamous carcinoma, one patient). A history of epidermoid carcinoma influenced both the prevalence of high-grade lesions (p = 0.01) and the multiplicity of these lesions, in contrast to subjects with other histologic types of previously diagnosed lung cancer. This point already has been suggested in a more limited series of patients\textsuperscript{23,24} and seems to have been confirmed in the current study.

In our opinion, since all histologic types of nonsmall cell lung cancer require similar follow-up periods, the selection bias mentioned before does not explain the data observed for patients who had experienced prior epidermoid carcinomas.

These data are in agreement with the phenomenon referred to as field cancerization\textsuperscript{25} and with the existence of multiple molecular abnormalities in the bronchial epithelia of smokers and former smokers.

In contrast, preinvasive lesions were not found in patients with prior adenocarcinomas, large cell carcinomas, or neuroendocrine carcinomas. However, we should stress that there were very few patients in the last two groups (large cell carcinomas, three patients; neuroendocrine carcinomas, nine patients). We propose the following two explanations for these results: (1) differences in individual susceptibility to tobacco carcinogens and induction of other molecular pathways of carcinogenesis; and (2) differences in smoke inhalation, tar content, and the type of cigarettes smoked.\textsuperscript{26,27} Thus, low-yield filter cigarettes tend to be inhaled more deeply than high-yield cigarettes in order to satisfy a craving for nicotine. The peripheral part of the lung, where most adenocarcinomas arise, thus is exposed to a disproportionately higher number of smoke carcinogens, whereas patients with proximal epidermoid carcinomas have exposed the main bronchus to smoke carcinogens.

Surprisingly, we did not find any preinvasive lesions in patients with a history of head and neck cancer, which is in contrast with the report of Venmans et al.\textsuperscript{28} In that study of 24 patients, bronchial intraepithelial neoplastic lesions were found in a considerable percentage of head and neck cancer patients (25%). This group of patients needs further study with a careful evaluation of risk factors such as smoking history, alcohol abuse, presence of tracheostomy, and history of head and neck radiotherapy.
CONCLUSION

AFB is an invasive investigation and cannot be considered as a screening approach for asymptomatic patients. However, our findings suggest that AFB should be recommended in the follow-up of patients who have experienced a prior epidermoid carcinoma or prior proximal bronchial carcinoma with an epidermoid component. Despite our data, we think that these conclusions should be extended to patients with a history of epidermoid head and neck cancer, since these patients have approximately the same smoking history and have undergone the same phenomenon of field cancerization. Current smokers with a prolonged cigarette-smoking history and respiratory symptoms are at high risk for preinvasive lesions and might benefit as well from AFB. We recommend additional studies in former smokers who have no history of epidermoid carcinomas, in whom there is a low prevalence of preinvasive lesions and for whom it remains unclear whether AFB is of any use.

REFERENCES