Cytological findings of pre-invasive bronchial lesions detected by light-induced fluorescence endoscopy in a lung cancer screening system

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Abstract. Lung cancer is a global epidemic and the number one cause of death among all cancers, with a very high morbidity. A new strategy for the treatment of lung cancer is the detection and eradication of pre-invasive bronchial lesions before they become invasive carcinomas. We conducted a detailed investigation into the use of fluorescence bronchoscopy in the detection of pre-invasive bronchial lesions in patients with sputum cytology suspicious or positive for malignancy. We also studied the distinctive cytological findings in the sputum specimens corresponding to the pre-invasive bronchial lesions. Sputum examinations were performed by mass screening a high-risk group of participants. From 1997 to 1999, 61 participants with sputum cytology suspicious or positive for malignancy were referred to our institute, and were examined with both white-light and fluorescence bronchoscopy. For the cytological findings, the collection of sputum was performed in the early morning. Conventional white-light examinations were first performed, and areas with abnormal findings were recorded for subsequent biopsy. Fluorescence bronchoscopy examinations were then carried out. Biopsy specimens for a pathological examination were taken from all the suspicious or abnormal areas discovered by the white-light bronchoscopy, or fluorescence bronchoscopy examination, or both. The laser-induced fluorescence bronchoscopic examination showed a high sensitivity for invasive carcinoma, carcinoma *in situ*, as well as severe, moderate, and mild dysplasia. In the sputum cytological findings, a thickened cytoplasm and slight hyperchromasia were frequently observed in the mild dysplasias compared with the squamous cells without atypia. Hyperchromasia and an Orange G (OG)-philic cytoplasm of squamous cells were frequently observed in the moderate compared with the mild dysplasias. A thickened cytoplasm, a nuclear pleomorphism, a thickened nuclear rim, a coarse chromatin, an uneven chromatin distribution, and an OG-philic cytoplasm were frequently observed in the carcinomas *in situ* and severe dysplasias compared with the moderate dysplasias. We found that the use of fluorescence bronchoscopy in addition to conventional white-light examination can enhance the detection and localization of pre-invasive bronchial lesions in patients with sputum cytology suspicious or positive for malignancy. Sputum cytology is therefore a potential approach to diagnosing pre-invasive bronchial lesions.

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Introduction

Centrally arising squamous cell carcinoma of the tracheobronchial tree develops in multiple stages, especially in heavy smokers, from squamous metaplasia to dysplasia, followed by carcinoma *in situ*, and finally invasive carcinoma. A new strategy for the treatment of squamous cell carcinoma of the tracheobronchial tree is the detection and eradication of pre-invasive bronchial lesions before they become invasive carcinomas (1). In the near future, early detection can make it possible to control carcinomas using only carbon ion radiotherapy (2).

In this study, we performed a mass screening of sputum cytological examinations for a high-risk group of participants.

Table I. Criteria for cytological diagnosis in mass screening by sputum cytology (3).

Levels	Cytological diagnosis	Recommendation
A	No histiocytes in the sputum (insufficient specimens)	Re-examination
B	Normal Only normal epithelial cells Proliferative basal cells Squamous cells with mild atypia Ciliated columnar cells	Next sputum examination (periodic examination)
C	Squamous cells with moderate atypia Columnar cells with changes of nuclear size, or strong staining	Additional sputum examination and follow-up within 6 months
D	Squamous cells with severe atypia or cells suspicious for malignant tumors	Detailed examinations including a bronchoscopy
E	Cells of malignant tumors	

The highest degree of cellular atypia in the specimen should be assigned for cytological diagnosis.

We then conducted a detailed investigation into the use of fluorescence bronchoscopy in the detection of pre-invasive bronchial lesions in patients with sputum cytology suspicious or positive for malignancy, and studied the distinctive cytological findings in the sputum specimens corresponding to the pre-invasive bronchial lesions.

Materials and methods

Sputum examinations by mass screening were performed for a high-risk group of participants. The groups consisted of the following: i) Men or women, >50 years of age, with >30 'pack years', ii) men or women, >40 years of age, who had a bloody sputum within the past 6 months, and iii) men or women at a high risk for occupational disease. The sputum findings were classified based on the criteria of the Japan Lung Cancer Society (Table I) (3).

From 1997 to 1999, 61 participants with sputum cytology suspicious or positive for malignancy were referred to our institute. These participants underwent a detailed examination in the Chiba lung cancer screening system using sputum cytology, and were examined by both white-light and fluorescence bronchoscopy. There were 57 males and 4 females. Their smoking history in 'pack years' ranged from 0 to 215 with a mean of 53.

Conventional white-light examinations were first performed with patients under local anesthesia with sedation by intravenous injection and O_2 inhalation. Areas with abnormal findings were recorded for subsequent biopsy. A fluorescence bronchoscopy examination was then carried out. Biopsy specimens for a pathological examination were taken from all the suspicious or abnormal areas discovered by either white-light bronchoscopy, or fluorescence bronchoscopy examination, or both. All of the biopsied specimens were diagnosed by two expert pulmonary pathologists at our institute. Informed consent was obtained from each patient prior to the investigation.

The bronchoscopic findings were as follows (4): In the white-light bronchoscopy, class 1 revealed a normal bronchial epithelium. Class 2 had edema and/or thickening of the bronchial mucosa. Class 3 had redness and/or necrosis of the bronchial mucosa. In the fluorescence bronchoscopy examination [laser-induced fluorescence bronchoscopy (LIFE)] (5), class 1 showed normal fluorescence. Class 2 showed attenuated autofluorescence. Class 3 showed dark red fluorescence. All lesions diagnosed as class 2 or 3 were biopsied. Some sites diagnosed as class 1 were also biopsied as the control. In this study, pre-invasive lesions included dysplastic lesions and carcinomas *in situ* based on the WHO histological typing (6).

For the cytological assessment, the collection of sputum was performed in the early morning for three days by the cell concentrating method using Dithiothreitol for pooled sputum cytology (7) based on the Saccomanno technique (8). Smear preparations with Papanicolaou staining were made. Of the 61 patients who underwent LIFE, the smears of 57 informative patients were assessed as belonging to one of two categories. This categorization was based on the presence or absence of OG-philic atypical cells, a thickened cytoplasm, a nuclear pleomorphysm, a thickened nuclear rim, hyperchromasia, a coarse chromatin, and an uneven chromatin distribution. The histological findings showed that 8 cases were invasive carcinomas, 2 were carcinomas *in situ*, 7 were severe dysplasias, 6 were moderate dysplasias, 4 were mild dysplasias, and 30 were none of the above.

Table II. Results of the laser-induced fluorescence bronchoscopy and white-light examination.

	LIFE		WL	
	Class 1/2 or 3	Sensitivity (%)	Class 1/2 or 3	Sensitivity (%)
SeD-InvCa	0/19	100	2/17	89.5
MoD-InvCa	2/31	93.9	6/27	81.8
MiD-InvCa	8/46	85.2	15/39	72.2

Sensitivity, class 2 or 3 cases/total case number; LIFE, laser-induced fluorescence bronchoscopy; WL, white-light examination; InvCa, invasive carcinoma; SeD, severe dysplasia; MoD, moderate dysplasia; MiD, mild dysplasia.

Results

In terms of the sputum cytological findings before the bronchial examination, 14 cases showed category E, 25 category D, 14 category C, and 7 category B.

In the LIFE examination, all of the invasive carcinoma cases (9 cases), carcinoma *in situ* cases (2 cases), and severe dysplasia cases (8 cases) were classified as LIFE class 2 or 3 (Table II). The sensitivity rate for these cases was 100% (19 of 19 cases). Twelve cases of moderate dysplasia were classified as class 2 or 3, with a sensitivity rate of 85.7% (12 of 14 cases). Fifteen cases of mild dysplasia were classified as class 2 or 3, with a sensitivity rate of 71.4%.

In the white-light examination (WL) group, all the invasive carcinoma and carcinoma *in situ* cases were classified as class 2 or 3. However, 2 cases of severe dysplasia were classified as class 1 (Table II). The sensitivity rate for these cases was 89.5% (17 of 19 cases). Ten cases of moderate dysplasia were classified as class 2 or 3, with a sensitivity rate of 71.4% (10 of 14 cases). Twelve cases of mild dysplasia were classified as class 2 or 3, with a sensitivity rate of 57.1%.

Compared with the mild dysplasias (Fig. 1), hyperchromasia and an OG-philic cytoplasm of squamous cells were more frequently observed in moderate dysplasias (Fig. 2). A thickened cytoplasm, a nuclear pleomorphism, a thickened nuclear rim, a coarse chromatin, an uneven chromatin distribution, and an OG-philic cytoplasm were more frequently observed in carcinomas *in situ* and severe dysplasias (Fig. 3) compared with moderate dysplasias (Table III).

Discussion

Lung cancer accounts for more deaths than any other malignancy in the developed countries of the world. The five-year survival rates for patients with lung cancer have remained depressingly low at 7-13% (9,10). Surgery provides the best chance for cure if the tumor can be radically resected and when no lymph node or distant metastasis is present. The tumor can be resected in only 25% of cases and, of these, only half are ultimately cured. Even after curative treatment of the primary malignancies, patients remain at risk for local recurrence, distant metastasis and subsequent primaries (10). The poor prognosis of lung cancer is mainly related to the fact that most often, diagnosis is made late in the natural history of the disease, at a stage when treatment is only rarely curative (11).

A longitudinal study revealed the possibility of detecting pre-invasive lesions by the cytological study of the sputum. However, a multicenter trial conducted at the Mayo Clinic, Johns Hopkins Hospital, and Memorial Sloane-Kettering Hospital failed to demonstrate an improved long-term survival in patients who had undergone intensive screening by sputum cytology (9). It had long been hoped that sputum cytology could be a useful screening or case finding tool for the diagnosis of the early stages of lung carcinoma. However, three major studies sponsored by the National Cancer Institute

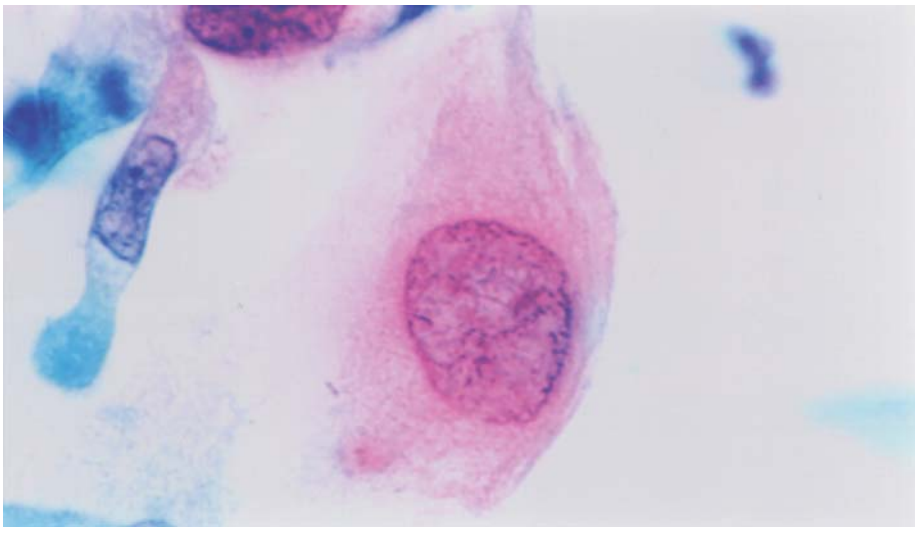

Figure 1. The sputum cytological findings of mild dysplasias showed an even chromatin distribution, and a thin and eosinophilic cytoplasm. (Papanicolaou staining x100).

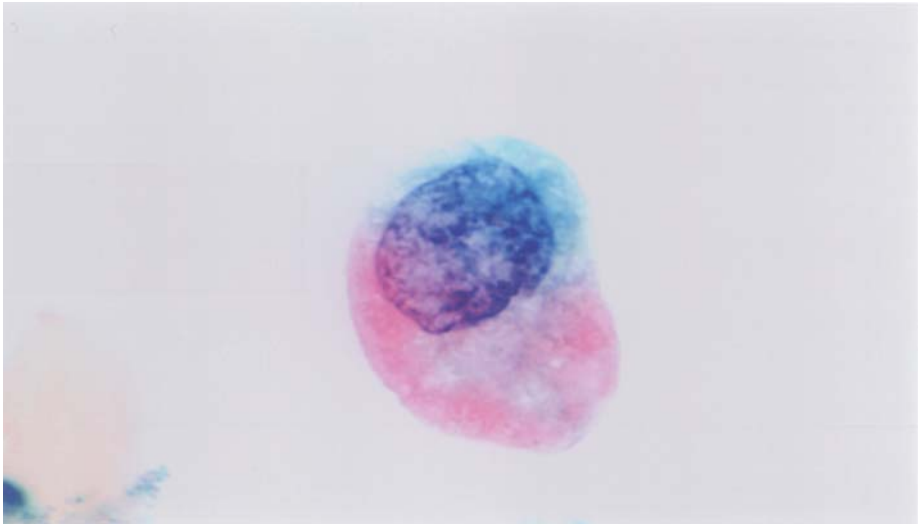

Figure 2. The sputum cytological findings of moderate dysplasias revealed a coarse chromatin and a nuclear pleomorphism, and an eosinophilic cytoplasm of squamous cells. (Papanicolaou staining x100).

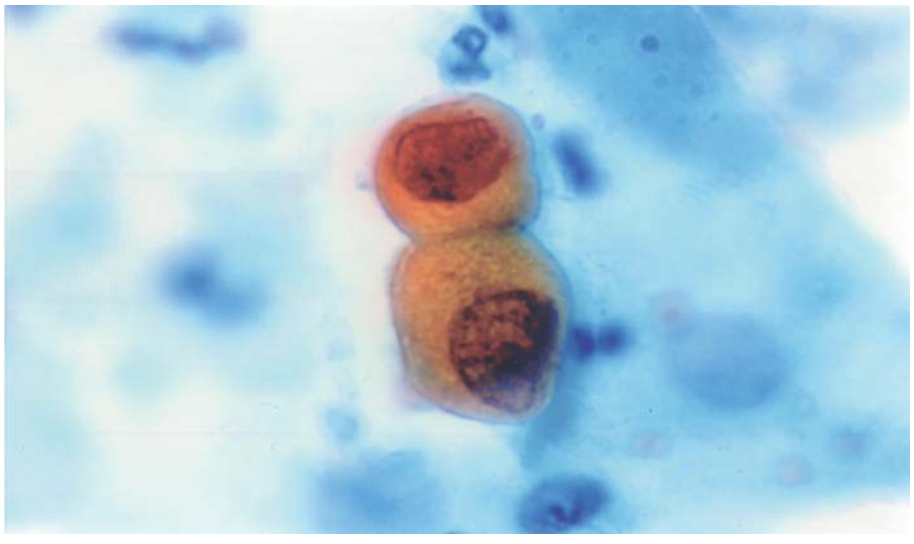

Figure 3. The sputum cytological findings of carcinomas *in situ* showed a nuclear pleomorphism, a thickened nuclear rim, a coarse chromatin, an uneven chromatin distribution, a thickened cytoplasm, and an Orange G-philic cytoplasm. (Papanicolaou staining x100)

Table III. Cytological findings.

Cytological finding	CIS + SeD	MoD	MiD
OG-philic atypical cell	8/9 (88.9)[a]	2/6 (33.3)	0/4 (0)
Thickened cytoplasm	9/9 (100)	3/6 (50)	2/4 (50)
Nuclear pleomorphism	8/9 (88.9)	0/6 (0)	0/4 (0)
Thickened nuclear rim	7/9 (77.8)	2/6 (33.3)	0/4 (0)
Hyperchromasia	9/9 (100)	4/6 (66.7)	0/4 (0)
Coarse chromatin	9/9 (100)	1/6 (16.7)	2/4 (50)
Uneven chromatin distribution	9/9 (100)	1/6 (16.7)	0/4 (0)

[a](%); CIS, carcinoma *in situ*; SeD, severe dysplasia; MoD, moderate dysplasia; MiD, mild dysplasia.

were disappointing - these were studies that included many light smokers (12). Sputum cytology can be useful in identifying lung carcinoma in its early and occult stages, particularly in patients who are at a high risk for this disease (12). In order to facilitate the detection of these lesions, bronchoscopic systems are now being developed that can exploit the differences in the fluorescence properties of normal and abnormal bronchial mucosae (9). Autofluorescence bronchoscopy can be performed under local anesthesia, without systemic sedation in patients at a very high risk for lung cancer (11). The system of auto-fluorescence bronchoscopy is based on the observation that lung cancer tissue fluoresces less than normal tissue. This thereby allows the detection of premalignant lesions and carcinomas *in situ* that can have a normal appearance by conventional white-light bronchoscopy (11).

Lung carcinogenesis is a multistep process. Histological changes associated with lung carcinogenesis include reserve cell hyperplasia, squamous metaplasia, moderate or severe dysplasia, carcinoma *in situ*, and invasive carcinoma (13). The presence of angiogenic squamous dysplasia in high-risk smokers suggests that aberrant patterns of microvascularization can occur at an early stage of bronchial carcinogenesis (13). Invasive squamous cell cancer in the central airways gradually develops from normal mucosa and can be multifocal, so-called field cancerization (10). However, the stepwise progression through this series of morphological changes is rarely observed in single individuals because premalignant airway lesions are less easily recognized and characterized than lesions in organs such as the colon (13). Sato *et al* (14) have shown the difficulty in detecting and localizing carcinoma *in situ* by conventional white-light bronchoscopy. However, Lam *et al* recognized that autofluorescence bronchoscopy enhances the bronchoscopist's ability to localize small neoplastic lesions, especially intra-epithelial lesions including moderate and severe dysplasia, and carcinoma *in situ*.

In this and our previous study (15), the sensitivity of white-light bronchoscopy and fluorescence bronchoscopy was similar when assessing invasive lung cancer and early hilar lung cancer. However, the sensitivity of fluorescence bronchoscopy was higher than that of white-light bronchoscopy when assessing pre-invasive bronchial lesions including mild and moderate dysplasia.

Moreover, based on these results, we compared the sputum cytology with the histological findings of the pre-invasive bronchial lesions, and evaluated the sputum cytological findings in this study. Our results showed that there were significant differences among the sputum cytological findings of carcinoma *in situ*/severe dysplasia, and those of moderate and mild dysplasia. Therefore, our results suggest that we may be able to evaluate cytological pre-invasive lesions preoperatively.

In conclusion, we found that the use of fluorescence bronchoscopy in addition to the conventional white-light examination can enhance the detection and localization of pre-invasive bronchial lesions in patients with sputum cytology suspicious or positive for malignancy. Sputum cytology is therefore a potential approach to diagnosing pre-invasive bronchial lesions.

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