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The clinical value of autofluorescence bronchoscopy for the diagnosis of lung cancer

(Running title: autofluorescence bronchoscopy as a clinical tool)

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Abstract

Background: To evaluate the role of autofluorescence bronchoscopy in routine work-up of lung cancer.

Methods: Consecutive patients with atypical cells/suspicious cells in sputum or bronchial aspirate, non-localizing radiological examination, and non-diagnostic white light bronchoscopic examination were recruited. White light (WLB) and autofluorescence bronchoscopy (AFB) examination were performed sequentially in the same session. All abnormal areas detected by WLB, AFB or both were biopsied and sent for histological examination.

Results: Sixty-two patients were recruited within the 32 months study period. Seventeen had no endobronchial lesion detected. Among the 45 patients with endobronchial lesions: 37 had lesions with histopathology grade mild dysplasia or less; For the 8 patients who had lesion with histological grade moderate dysplasia or worse, 5 patients were found to have lung cancer: 2 had invasive lung cancer and 3 had intraepithelial neoplasm (severe dysplasia). Lesions with moderate dysplasia or worse were more commonly found in patients with "suspicious cells" than patients with "atypical cells" in sputum examination. AFB is more sensitive than WLB (91% Vs 58%) in detecting these lesions though less specific (26% Vs 50%).

Conclusion: The combination of WLB and AFB can increase the diagnostic yield of this invasive procedure in patients with abnormal sputum cytology.

Key words: autofluorescence bronchoscopy, clinical tool, lung cancer, pre-invasive lesion

Introduction:

Sputum cytology is commonly employed as one of the investigations in patients suspected to have lung cancer, and it is not uncommon for pulmonary physicians to encounter patients with sputum atypia and further investigation has to be contemplated. Lung cancer arising from the central airways can be detected easily by means of conventional bronchoscopy. However, patients with carcinoma *in situ* (CIS), micro-invasive cancers and preinvasive lesions present a diagnostic challenge, even for experienced bronchoscopists (1). Since the prognosis of lung cancer correlates with the staging of the disease, the importance of early detection followed by early treatment might improve the prognosis of lung cancer. Autofluorescence bronchoscopy (AFB) was developed to enhance the detection of pre-invasive lesions, based on the observation that abnormal tissue showed reduced fluorescence when compared with normal tissue upon excitation by blue light (wavelength 380–460 nm) (2). The value of AFB in detecting early lung cancer/preinvasive lesions has been evaluated in different clinical and research settings (3-5). However, the role of AFB in routine work up for lung cancer has not been extensively evaluated. We, therefore, examined the value of autofluorescence bronchoscopy in patients with atypical cells/suspicious cells in sputum/bronchial aspirate but non-diagnostic white light bronchoscopy (WLB).

Material and Method

Study population and eligibility

We prospectively recruited consecutive patients referred to Queen Mary Hospital, the main teaching hospital of the University of Hong Kong in the Hong Kong Special Administrative Region, China for AFB examination from February 2002 to October

2004. Inclusion criteria included the report of i) atypical cells or cells suspicious of malignancy in sputum or bronchial aspirate, ii) absence of a suspicious lung shadow or infiltrate on plain chest radiographs, and iii) non-diagnostic white light bronchoscopic examination. Atypical cells referred to epithelial cells that showed abnormal nuclear features but which were not suspected to be malignant cells. The nuclear changes included mild nuclear enlargement and raised nuclear to cytoplasmic ratio, coarse chromatin with hyperchromasia, and inconspicuous or absent nucleoli. Suspicious cells referred to epithelial cells which showed severe nuclear abnormalities but for which a diagnosis of malignancy could not be ascertained due to a limited number of such abnormal cells or coexisting degenerative features. (6)

The study was approved by the Institutional Ethics Committee.

White light and autofluorescence bronchoscopy

Bronchoscopic examination was performed under local anesthesia in the endoscopy suite of Queen Mary Hospital by the two investigators (BL and SLF) who were experienced in the operation of autofluorescence bronchoscopy. Conventional white-light bronchoscopy was performed first, followed by AFB (SAFE-1000: System of Autofluorescence Endoscopy, Pentax, Asashi Optical Tokyo, Japan). All abnormal lesions detected by white light, autofluorescence bronchoscopy or both were videotaped and biopsies were taken from these lesions. The location of each biopsy in the bronchial tree was precisely recorded using the international bronchial location classification (7). Biopsy specimens were fixed in formaldehyde and embedded in paraffin. One expert pathologist (MW), without knowledge of the bronchoscopic findings, evaluated the specimens routinely stained with hematoxylin and eosin. The

pathological diagnoses were coded according to the World Health Organization Lung Cancer classification (8).

Patients would undergo work up for resectability if histopathology result showed invasive cancer. Endobronchial therapy (cryotherapy, ERBE Elektromedizin GmbH, Tübingen, Germany) would be applied if the histopathology result showed a pre-invasive lesion (severe dysplasia or carcinoma in situ). For subjects who had cryotherapy performed, re-assessment white light and fluorescence bronchoscopy examination would be performed at regular intervals. All new and previously treated lesions were biopsied at re-assessment bronchoscopic examinations. Subjects with moderate dysplasia would be followed up as for those patients who had received cryotherapy. The exact localization of the previous biopsy was deduced from the initial record during re-assessment bronchoscopy. For those patients with no diagnosis reached after AFB , referring doctors were contacted and clinical progress updated.

Informed written consent was obtained from all patients prior to white light and AFB examination.

Statistical analysis

Data are presented as mean values with standard deviation. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of WLB and AFB in detecting moderate dysplasia or worse in the whole group and in different patient groups (based on degree of sputum/bronchial aspirate abnormality) were calculated separately.

Results

Patient characteristics

Sixty-two patients fulfilled the inclusion criteria and their characteristics were shown in Table 1. Twenty patients had underlying lung diseases including a history of pulmonary tuberculosis, silicosis, bronchiectasis, chronic obstructive lung disease, asthma, and resected lung cancer. The indications leading to sputum cytology examination included haemoptysis, chronic cough, imaging finding of lung nodule(s), radiological worsening after several months of anti- tuberculosis treatment etc. Two subjects had a history of lung cancer, one (left pneumonectomy 17 years ago) presented with fever and haemoptysis, the other (right upper lobectomy 6 years ago) had sputum sampled saved during routine follow up. All subjects had normal white light bronchoscopy examination except one who had an endobronchial tumor at the lingular bronchus and swollen mucosa found at the left lower lobe bronchus. The lingular tumor was confirmed to be a squamous cell carcinoma, and biopsy of the swollen mucosa over the left lower lobe bronchus was non-diagnostic. The patient was referred for AFB before lung resection (lobectomy) to rule out synchronous lung cancer. .

WLB had been performed at least once in all 62 recruited subjects and 9 had undergone more than one bronchoscopic examination. The median interval from previous bronchoscopic examination and AFB was 2 months.

Bronchoscopic finding

Seventeen patients had no detectable lesion under both white light and AFB. A total of 91 endobronchial lesions were detected in 45 patients. Four specimens were found to be inadequate for diagnosis and two specimens were not reviewed due to administrative reason. The known lingular tumor was excluded from analysis. For the remaining 84 lesions: 41 were detected only by AFB, 20 were detected only by WLB,

and 23 were recognized by both (Table 2).

Patients referred with sputum atypia

Out of the 43 patients, 10 had normal bronchoscopic examination. A total of 56 lesions were detected in 33 patients: 25 were detected only by AFB, 14 detected only by WLB, and 17 recognized by both (Table 3). For the seven lesions with moderate dysplasia or worse: 3 were detected only by AFB, 1 only by WLB and 3 by both AFB and WLB.

Patients referred with suspicious cells in sputum

Out of the 12 patients, three had normal bronchoscopic examination. A total of 22 lesions were detected in 9 patients: 12 detected only by AFB, 6 detected only by WLB, and 4 recognized by both (Table 3). For the five lesions with moderate dysplasia or worse: two lesions could only be detected by AFB, and the other three lesions by both AFB and WLB.

Patients referred with atypical cells or suspicious cells in the bronchial aspirate

Out of the 7 patients, 4 had no endobronchial lesion. A total of 6 lesions were detected in 3 patients: 4 lesions detected only by AFB and 2 recognized by both (Table 3). None of the lesions were graded histologically moderate dysplasia or worse.

The patient with a lingular tumor was found to have synchronous severe dysplasia at lateral sub-segment of the superior segment of right middle lobe (detected by AFB) and carcinoma-in-situ at lateral sub-segment of the superior segment of lingular lobe (detected by both WLB and AFB). Lesions were also detected by AFB at apical lower segment of left lower lobe and lateral sub-segment of lateral basal segment of left

lower lobe which showed "no significant pathology" and "mild dysplasia" respectively.

The sensitivity, specificity, positive predictive value and negative predictive value of AFB and WLB in detecting moderate dysplasia or worse in different patient groups and in all patients were shown in Table 4.

By using the number of patients as denominator, the diagnostic yield of AFB + WLB for histopathology grade moderate dysplasia or worse was 12.9% (8/62), and

8% (5/62) for histopathology grade severe dysplasia or worse.

Computed tomography (CT) finding

Forty-three patients had a CT thorax performed before AFB and ten had CT performed afterwards. None was found to have radiological evidence of lung cancer. Ten had CT repeated during follow up period, and one was found to have a slowly growing lung nodule

Patient outcome

By excluding the five patients with preinvasive/lung cancer after AFB + WLB examination, the most up-to-date clinical condition of the remaining 57 patients were traced. After a mean follow up period of 22.2 $\pm$ 10.4 months for the remaining 57 patients, one patient (baseline sputum cytology normal, normal WLB and AFB but atypical cell found in bronchial aspirate) was diagnosed to have stage IA lung cancer by percutaneous needle aspiration of the slowly growing right upper lobe nodule, and one patient (sputum showed suspicious cell) was diagnosed to have carcinoma of the vocal cord (vocal cord nodule seen during bronchoscopic examination) .

Discussion

Our study showed that in this group of subjects who presented with abnormal sputum/bronchial aspirate cytology and non-diagnostic white light bronchoscopy, lung cancer/pre-invasive lesions could be detected in 8% of the subjects by means of AFB.

Lung cancer is the leading cause of cancer death throughout the world (9, 10). In order to improve the prognosis, every effort should be made to diagnose lung cancer at its earliest stage. Since high grade pre-invasive lesions have a high chance of progressing to invasive cancer (11), they should be treated if possible, and preferably by endobronchial treatment (12). Currently the only non-invasive way of diagnosing pre-invasive lesion is by sputum examination, and the presence of abnormal cells in sputum cytology or bronchial aspirate is a clear indication for thorough investigation. In this study, we did not select patients based on their risks of developing lung cancer, i.e. smoking history etc., but on the clinical needs.

The two cases of CIS, one case of micro-invasive cancer and one case of severe dysplasia which could not be detected by WLB performed in other hospitals were detected by white light bronchoscopy during the AFB/WLB session under our care. This could be attributed to disease progression after the lapse of time between the two bronchoscopic examinations. It is also possible that bronchoscopists with experience in AFB might have been trained to detect more subtle mucosal changes on white light bronchoscopy.

The detection of synchronous lesions (severe dysplasia and CIS) in the subject pending curative resection led to a change in the management plan (radiotherapy instead of surgery). It is intuitive to suggest that AFB should be considered in the pre-operative assessment for patients with ‘operable’ lung cancer. Actually AFB has been used to detect subtle synchronous malignancy and to delineate the extent of lung

cancer prior to resection (13-15), though the effect of using AFB on prognosis of operable lung cancer is still an issue for future evaluation.

(One paragraph deleted as suggested by Prof. Spiro)

There are several limitations in this study. The original sputum/bronchial aspirate specimens collected before referral were not reviewed due to administrative restraints. It was therefore impossible for us to confirm the reported sputum abnormality. However 2 cases of lung cancer, 1 case of cancer of vocal cord and 1 case of severe dysplasia from the 12 subjects who had suspicious cells in sputum compared to 2 cases of severe dysplasia from the 43 subjects who had atypical cells in sputum would suggest that the baseline reports of sputum cytological abnormality werereasonably accurate. The second limitation is that we could not provide an adequate explanation for all the detected sputum/bronchial aspirate atypia. However, sputum atypia has been reported in a variety of conditions other than lung cancer, including bronchiectasis, airway inflammation and pulmonary infection (16). Since one-third of our patients had an underlying lung problem, we believe that the presence of these conditions might contribute to the detection of the original cytological abnormalities. Another limitation is that we did not randomly biopsy sites which looked normal under both WLB and AFB. Therefore it is not possible for us to calculate the sensitivity and specificity of AFB combined with WLB.

While the role of AFB in lung cancer screening is yet to be determined, we have shown that the combination of AFB/WLB can increase the diagnostic yield of this invasive procedure in patients with abnormal sputum cytology. The diagnostic yield seems to be higher in subjects with “suspicious cells” than “atypical cells” in sputum. Although some extra time is required for the additional use of AFB during bronchoscopy , it is worthwhile, considering the fact that 8% of the subjects could

have lung cancer or a pre-invasive lesion diagnosed without additional risk.

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Table 1. Characteristics of the subjects

Age, mean ± SD (range)	59.9±12.5 (19-83)
Sex, men/women	44/18
Smoking status, ever smoker/non smoker	39/23
Sputum finding	
Atypical cell	43
Suspicious of malignancy	12
Bronchial aspirate cytology	
Atypical cell	5
Suspicious of malignancy	2

Table 2 Histopathological results of the biopsies according to white light and autofluorescence appearance

	Normal	A	B	C	D	E	F	G
AFB (N=41)	25	6	5	2	3			
WLB (N=20)	14	3	2	1				
Both (N=23)	10	3	3	2	1	2	1	1
Total (N=84)	49	12	10	5	4	2	1	1

A: Metaplasia
B: Mild dysplasia
C: Moderate dysplasia
D: Severe dysplasia
E: Carcinoma in situ
F: Invasive cancer
G: Others (endobronchial tuberculosis in this patient)
AFB: Autofluorescence bronchoscopy
WLB: White light bronchoscopy

Table 3. Bronchoscopic finding and number of lesions with moderate dysplasia or above based on degree of cytology abnormality at baseline

	Lesions detected			Histopathology grade moderate dysplasia and above		
Atypical cell in sputum (N =43)						
	WLB	AFB	WLB & AFB	WLB	AFB	WLB & AFB
	14	25	17	1	3	3
Suspicious cell in sputum (N = 12)						
	6	12	4	0	2	3
Atypical cell in BA (N = 5)						
	0	1	0	0	0	0
Suspicious cell in BA (N = 2)						
	0	3	2	0	0	0

BA: Bronchial aspirate
WLB: White light bronchoscope
AFB: Autofluorescence bronchoscopy

Table 4.Sensitivity, specificity, positive predictive value and negative predictive value of white light bronchoscope and autofluorescence bronchoscopy in diagnosing histopathology grade moderate dysplasia or worse in different patient groups and in all patients

	WLB		AFB	
		95% CI		95% CI
Sputum atypia group (56 lesions in 33 patients)				
Sensitivity	57.1%	20-88%	85.7%	42-99%
Specificity	44.9%	31-60%	26.5%	15-41%
PPV	12.9%	4-31%	14.3%	6-29%
NPV	88%	68-99%	93%	64-100%
Sputum with suspicious cells group (22 lesions in 9 patients)				
Sensitivity	60%	17-93%	100%	46-100%
Specificity	58.8%	32-81%	35.3%	15-61%
PPV	30%	8-65%	31.3%	12-59%
NPV	83.3%	57-97%	100%	52-100%
Whole group (84 lesions in 45 patients)				
Sensitivity	58.3%	29-84%	91.7%	60-100%
Specificity	50%	38-62%	26.4%	17-38%
PPV	16.3%	7-31%	17.2%	9-29%
NPV	87.8%	73-95%	95%	73-100%

WLB: White light bronchoscope
AFB: Autofluorescence bronchoscopy
CI: Confidence interval
PPV: Positive predictive value
NPV: Negative predictive value