ORIGINAL

Autofluorescence bronchoscopy, a novel modality for the early detection of bronchial premalignant and malignant lesions

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Abstract : Lung cancer is the leading cause of cancer deaths in developed countries. Recently, autofluorescence bronchoscopy has been reported to improve the early detection of lung cancer in high-risk individuals. In the present study, we evaluated the efficacy of autofluorescence bronchoscopy for the early detection of bronchial premalignant and malignant lesions. From November 2000 through March 2004, 123 high-risk individuals (114 men and 9 women with a mean age of 68 years) were enrolled. Among 282 biopsy specimens, 93 (33.0%) were premalignant or malignant lesions. The sensitivity and negative predictive value for the detection of bronchial premalignant and malignant lesions were significantly higher with the addition of autofluorescence bronchoscopy than white light bronchoscopy alone. Moreover, the sensitivity for the detection of bronchial premalignant lesions was extremely higher with the addition of autofluorescence bronchoscopy than white light bronchoscopy alone, whereas there was no significant difference between autofluorescence bronchoscopy and white light bronchoscopy alone for the detection of non-malignant and malignant lesions. Autofluorescence bronchoscopy is a novel modality for the early detection of bronchial abnormality, especially for bronchial premalignant lesions. J. Med. Invest. 54 : 261-266, August, 2007

Keywords **:** *autofluorescence bronchoscopy, white light bronchoscopy, premalignant lesion*

INTRODUCTION

Lung cancer is the most common cause of cancer death in developed countries (1). Despite advances in the detection and treatment of many cancers leading to improvements in the 5-year survival rate, the survival rate for lung cancer continues to be <15% (2). The main reason for the continued low survival rate for lung cancer patients is that tumors are found at late invasive stages, when the options for treatment are mostly palliative. Thus, to significantly reduce mortality in lung cancer, detection and treatment at earlier stages is essential. Recent advances in endoscopic technology such as autofluorescence bronchoscopy (AFB) have been reported to improve the early detection of premalignant and malignant bronchial lesions in high-risk individuals (3, 4). In this report, we demonstrate the efficacy of AFB which is a novel modality for the early detection of bronchial premalignant and malignant lesions.

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MATERIALS AND METHODS

Autofluorescence bronchoscopy

An AFB system (SAFE-1000®, Asahi Pentax Co., Tokyo, Japan) was used in this study (Fig. 1). This system is composed of a camera unit, fiberoptic bronchoscope, excitation light source, filing system and peripherals. The white light from the xenon lamp is passed through a cut filter and then through an excitation filter which specifically passes 420-480 nm excitation light. The excitation light is then transmitted via a light guide to the target area. Images obtained with an objective lens are transmitted via a fiberoptic image guide back to the eyepiece of the endoscope and guided through the prism to the fluorescence filter which specifically passes a 490-590 nm fluorescence signal. The selected signal is then amplified by the image intensifier and by means of a video camera, it appears as a fluorescent image on the monitor. Abnormal mucosa shows a cold image due to the lack of autofluorescence. It is easy to change between white light and excitation blue light using the changeover switch on the camera unit. This system is also compact and easy to handle.

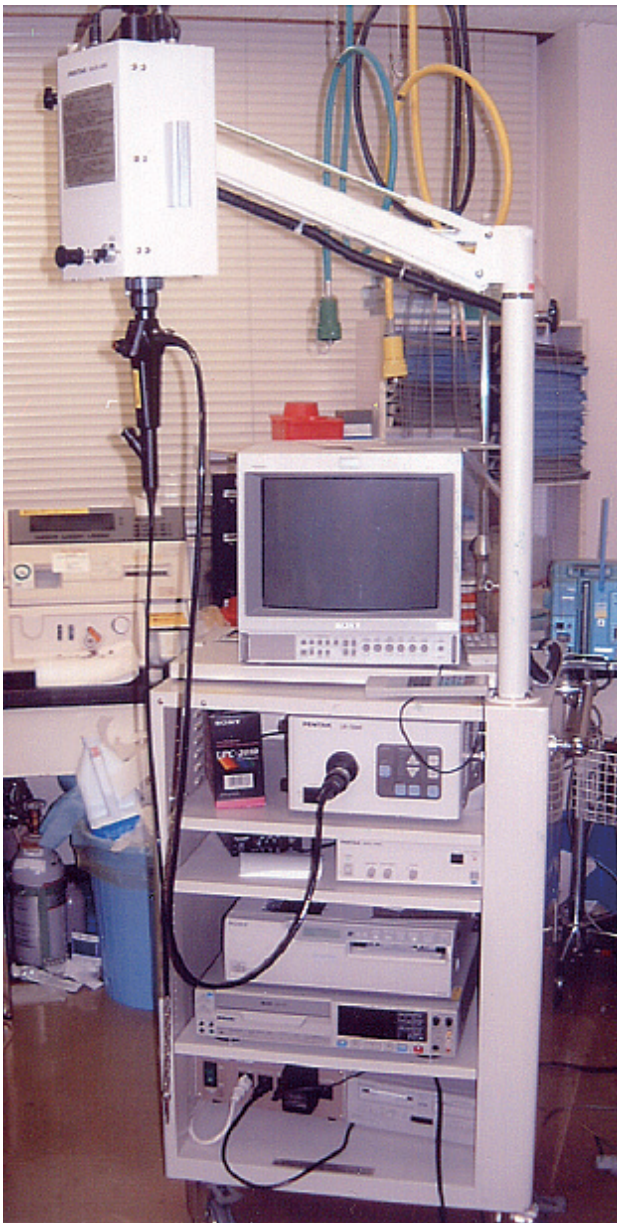

Fig. 1 The autofluorescence bronchoscope, SAFE-1000®.

Subjects

The criteria for enrollment in this study were as follows : 1) patients followed after lung cancer therapy, 2) patients with radiologically suspected lung cancer, 3) heavy smokers with respiratory symptom, 4) patients with sputum cytology suspicious or positive for malignancy without chest abnormal shadow. From November 2000 through March 2004, 123 subjects were evaluated.

Procedure of bronchoscopy

Conventional white light bronchoscopy (WLB) (BF-240, Olympus Optical Co., Tokyo, Japan) was first performed under local anesthesia during which abnormal areas were recorded for subsequent biopsy. Then, AFB was performed and biopsy specimens for pathologic examination were taken from all suspicious or abnormal areas discovered by WLB, AFB, or both. In addition, one or more biopsies were performed in normal areas.

Pathological examination

Bronchial biopsy specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin for histological examination. The biopsy slides were evaluated by a pathologist according to recent World Health Organization criteria. In the present study, lesions in the bronchi were divided into the following three categories according to histopathological diagnosis ; 1) No malignancy : normal epithelium and bronchitis, 2) premalignant lesion : squamous metaplasia and squamous dysplasia, 3) malignancy : carcinoma *in situ* and invasive cancer.

Statistical Analysis

The chi-square test was used to evaluate the significance of differences between the WLB group and WLB+AFB group. P-values of <0.05 were considered statistically significant.

RESULTS

The characteristics of the participants are shown in Table 1. Among 123 patients, there were 114 men and 9 women with a mean age of 68 years (range, 25 to 83 years). Forty-five patients were followed after therapy for lung cancer ; 40 were suspected to have lung cancer based on their symptoms, smoking history and/or chest abnormal shadow ; 23 were heavy smokers with respiratory symptom ; 10 had abnormal sputum cytology without chest abnormal shadow ; 4 were others (Table 2). One hundred fifteen patients (93.5%) were current or former smokers with a mean Brinkmen Index of 1114. A total of 282 biopsy specimens were obtained. The pathologic diagnoses of these specimens were as follows : normal epithelium in 129 (45.7%) ; bronchitis in 60 (21.3%) ; squamous metaplasia in 37 (13.1%) ; squamous dysplasia in 10 (3.5%) ; carcinoma *in situ* in 3 (1.1%) ; invasive cancer in 43 (15.2%). Thus, 93 biopsy specimens (33.0%) were premalignant or malignant lesions (Table 3). Definite diagnoses were yielded in all patients histologically and/or clinically (Table 4). Ninety-five (77.2%) were diagnosed as malignancies, most of which were lung cancer. WLB alone detected 71 of 93 premalignant (squamous metaplasia, squamous dysplasia) and malignant (carcinoma *in situ*, invasive cancer) lesions. With the addition of AFB, 90 of 93 lesions were detected, that is, 19 lesions were discovered only by AFB. The sensitivity of WLB+AFB to detect premalignant and malignant lesions (96.8%) was significantly higher than WLB alone (76.3%). Similarly, the negative predictive value of WLB+AFB (97.2%) was also significantly higher than WLB alone (83.1%) as shown in Table 5. Moreover, the sensitivity of WLB+AFB to detect premalignant lesions (95.7%) was significantly higher than WLB alone (59.6%), whereas there was no significant difference between WLB+AFB and WLB alone to detect non-malignant and malignant lesions (Table 6). These findings strongly suggested the efficacy of AFB to improve the detection rate of premalignant bronchial lesions in high-risk individuals.

On the other hand, previous reports have demonstrated that the specificity of AFB to detect premalignant and malignant lesions was relatively low and these was no significant difference with WLB alone (3, 4). In the present study, the specificity and positive predictive value of WLB+AFB were not signifi-

Table 1. Patient Characteristics.

Gender	male	114	(92.7%)
	female	9	(7.3%)
Age	mean	68	
	range	25-83	
Smoking History	smoker	115	(93.5%)
	non-smoker	8	(6.5%)
Brinkman Index	mean	1114	
	range	0-3120	

Table 2. Patients examined with autofluorescence bronchoscopy.

purpose of examination		
follow up after lung cancer therapy	45	(36.6%)
further examination for chest abnormal shadow	40	(32.5%)
heavy smoker with respiratory symptom	23	(18.7%)
sputum cytology suspicious or positive for malignancy	10	(8.1%)
hemosputum and others	5	(4.1%)
total	123	

Table 3. Pathological diagnosis of the biopsied specimens.

histology		
invasive cancer	43	(15.2%)
carcinoma *in situ*	3	(1.1%)
squamous dysplasia	10	(3.5%)
squamous metaplasia	37	(13.1%)
bronchitis	60	(21.3%)
normal epithelium	129	(45.7%)
total	282	

Table 4. Final diagnosis of the patients enrolled in this study.

diagnosis		
malignancy	95	(77.2%)
lung cancer	86	
esophageal cancer	3	
other malignancies	6	
benign lung disease	15	(12.2%)
chronic obstructive pulmonary disease	6	
interstitial pneumonia	3	
middle lobe syndrome	2	
other benign lung diseases	4	
infectious disease	12	(9.8%)
bronchopneumonia	8	
pulmonary tuberculosis	2	
other infectious diseases	2	
idiopathic bronchial bleeding	1	(0.8%)
total	123	

Table 5. Comparison of diagnostic rate between WLB with WLB+AFB for premalignant and malignant lesions.

statistics	WLB		WLB+AFB	
sensitivity (%)	76.3	(71/93)	96.8*	(90/93)
specificity (%)	57.1	(108/189)	56.1	(106/189)
positive predictive value (%)	46.7	(71/152)	52.1	(90/173)
negative predictive value (%)	83.1	(108/130)	97.2*	(106/109)

WLB : white light bronchoscopy, AFB : autofluorescence bronchoscopy, premalignant lesion : squamous metaplasia, squamous dysplasia, malignant lesion : carcinoma *in situ*, invasive cancer
*$p < 0.05$ compared with WLB group.

Table 6. Comparison of sensitivity for the diagnosis between WLB with WLB+AFB in each group.

pathological diagnosis	WLB		WLB+AFB	
no malignacy	42.9	(81/189)	43.9	(83/189)
pre-malignant lesion	59.6	(28/47)	95.7*	(45/47)
malignancy	93.5	(43/46)	97.8	(45/46)

WLB : white light bronchoscopy, AFB : autofluorescence bronchoscopy, no malignancy : normal epithelium, bronchitis, premalignant lesion : squamous metaplasia, squamous dysplasia, malignancy : carcinoma *in situ*, invasive cancer
*$p < 0.05$ compared with WLB group.

cantly different from WLB alone (Table 5) in accordance with previous reports.

In the present study, the addition of AFB to standard WLB was feasible. There were no adverse events related to the use of this device.

DISCUSSION

Lung cancer has become the most common cause of all deaths from cancer and the mortality rate of lung cancer is still increasing. Diagnosis and resection of lung cancer at an early stage are considered to be effective based on dramatically improved survival rates for resected patients with no surgery (5) ; however, only a minority of patients are diagnosed at these curable stages, because of the limitations of radiographic technology, the lack of specific symptoms at an early stage of the disease, and no currently available validated screening test (6).

Advances in endoscopic technology such as AFB have recently improved the detection of premalignant and malignant bronchial lesions in high-risk individuals (7-9). We have also reported that the precise location of malignant lesions was clearly detected as a defect of autofluorescence by inspection with AFB, whereas it was quite difficult by inspection with conventional WLB alone (Fig. 2). According to a previous report, the sensitivity of the biopsy proved that premalignant lesions detected by white-light bronchoscopy alone was 61.2%, whereas it was significantly increased to 89.8% with the addition of AFB (9). Among AFBs, we have been using the SAFE-1000® system since November 2000. The advantages of this system are as follows : 1) it uses a simple device such as a standard xenon lamp with a filter, rather than a laser, 2) it is more portable than the LIFE ® lung system (10).

In the present study, the sensitivity for the detection of bronchial premalignant and malignant lesions was significantly higher by means of WLB+AFB (96.8%) than WLB alone (76.3%), according to previous reports. Similarly, the negative predictive value of WLB+AFB (97.2%) was also significantly more elevated than WLB alone (83.1%). Moreover, the sensitivity for the detection of bronchial premalignant lesions was extremely higher by means of WLB +AFB (95.7%) than WLB alone (59.6%), whereas there was no significant difference between WLB +AFB and WLB alone for the detection of nonmalignant and malignant lesions. These findings strongly suggested the efficacy of AFB to improve the detection rate of premalignant bronchial lesions in high-risk individuals.

Little is known about the natural history of premalignant bronchial lesions in high-risk individuals. Bota, *et al.* have reported the alteration of premalignant bronchial lesions followed for 2 years with AFB (11). During 2 years, 6 of 36 normal epithelia became dysplastic ; 47 of 152 metaplasia evolved to low-grade dysplasia, two progressed to carcinoma *in situ*, and one to invasive cancer ; 6 of 169 low-grade epithelial lesions progressed to persistent severe dysplasia ; 10 of 27 severe dysplastic lesions and 28 of 32 carcinoma *in situ* progressed. They recommended that low-grade epithelial lesions, such

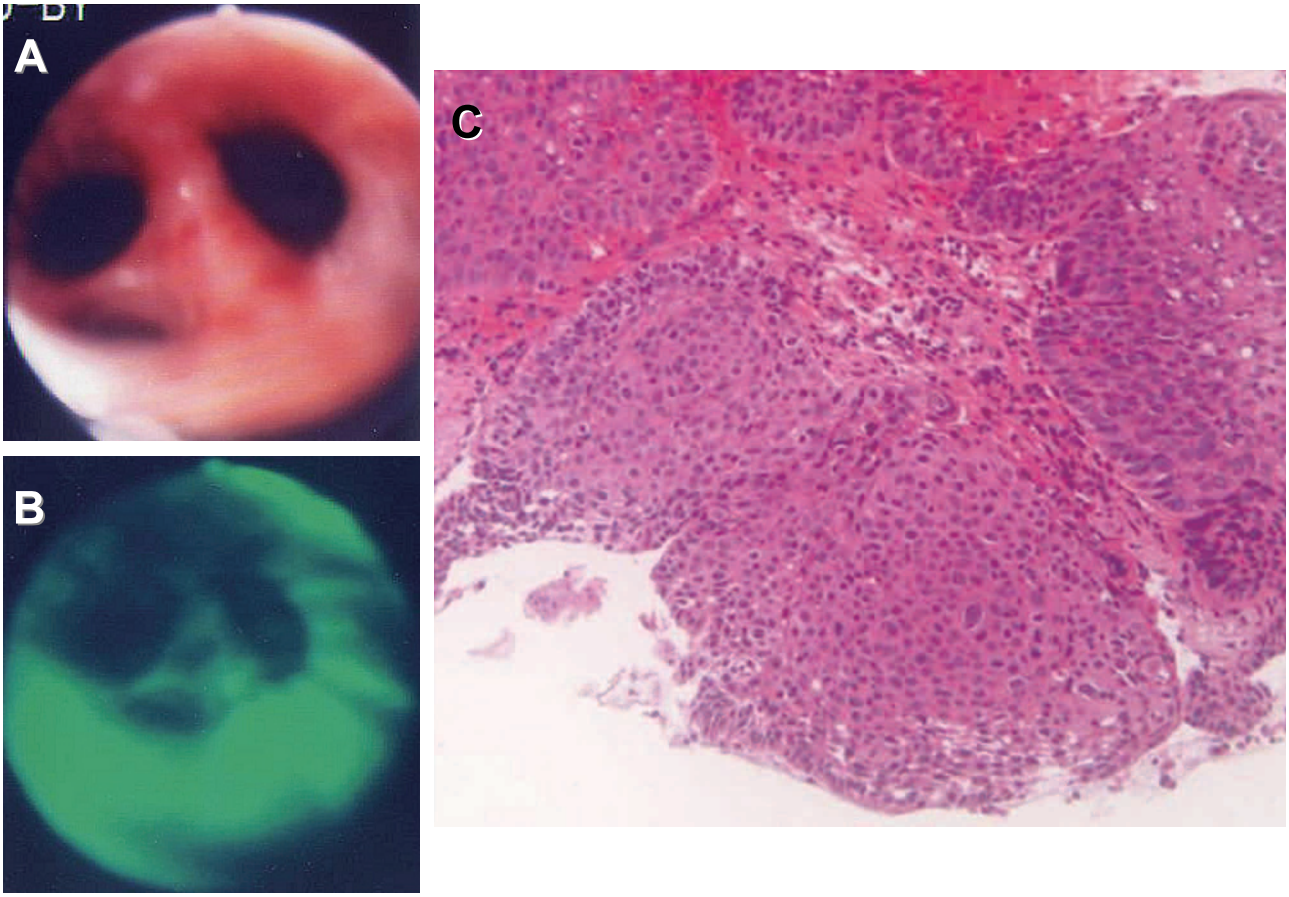

Fig. 2 (A) Conventional bronchoscopic findings. Whereas some spotty, reddened lesions were found at the upper division bronchus of the left lung, the precise location of the tumor was quite difficult to determine. (B) Autofluorescence bronchoscopic findings. The location of the tumor was clearly identified as the defect of autofluorescence by AFB (SAFE-1000®) at the bifurcation between the left B^{1+2} and B^3 bronchi. (C) Histological findings of a transbronchial biopsy specimen obtained at the bifurcation between the left B^{1+2} and B^3 bronchi (H.E stain, ×200). A solid growth pattern composed of polymorphic tumorous cells with large and hyperchromatic nuclei and partial keratinization was found. Histology was squamous cell carcinoma. The tumor invaded beyond the basement membrane.

as squamous metaplasia and mild dysplasia, could be followed carefully with AFB, whereas severe dysplasia which persists at 3 months and carcinoma *in situ* should be treated immediately. According to this recommendation, we are following squamous metaplasia and squamous dysplasia every 3 months with AFB and appropriate therapies have been performed against carcinoma *in situ* and invasive cancer.

The increased thickening of the bronchial epithelium and increases in blood content or vessel growth are thought to be involved in areas of abnormal autofluorescence irrespective of malignancy. As chronic inflammatory bronchial lesions due to bronchial asthma and chronic bronchitis are also known to reveal increased thickening of the epithelium and blood content, defects of autofluorescence are frequently observed by inspection with AFB in these lesions ; therefore, AFB can detect false-positive lesions in patients with bronchial asthma and chronic bronchitis. The relatively low specificity of AFB to distinguish malignancy has been reported as one of the main disadvantages of AFB (3). For example, the specificity of conventional bronchoscopy alone was 78%, whereas it was 42% with the addition of AFB (3). Recently, a high magnification bronchovideoscope has been developed to overcome this disadvantage of AFB (12). At sites of abnormal autofluorescence established by AFB, high magnification bronchovideoscopy detects squamous dysplasia more accurately than AFB alone (12). Thus, the combined use of AFB with high magnification bronchovideoscopy may be able to decrease the false-positive rate detected by AFB alone.

Almost all cases of early-stage lung cancer occur in current heavy smokers or ex-smokers, many of whom are not eligible for surgical resection due to synchronous multiple early-stage lung cancers and/or smoking-related complications, such as poor pulmonary function and cardio-vascular diseases ;

thus, these cases seem to be good candidates for bronchial interventions. Photodynamic therapy, brachytherapy, electrocautery, cryotherapy, and Nd-YAG laser therapy are therapeutic options available for the management of endobronchial malignancies. These bronchial interventions have been reported to be effective for both progressive and early-stage lung cancers, especially for the latter (8, 13, 14) ; however, since it is known that early-stage lung cancers even when successfully treated with these bronchial interventions sometimes relapse, multicentric, careful follow-up is required in these patients.

In conclusion, the sensitivity for the detection of bronchial premalignant lesions was proved to be extremely higher by means of WLB+AFB than WLB alone. AFB is a novel modality for the early detection of bronchial abnormality, especially for bronchial premalignant lesions.

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REFERENCES

1. Greenlee RT, Murray T, Bolden S, Wingo PA : Cancer statisitics, 2000. CA Cancer J Clin 50 : 7-30, 2000
2. Fry WA, Menck HR, Winchester DP : The national cancer data base report on lung cancer. Cancer 77 : 1947-1955, 1996
3. Lam S, Kennedy T, Unger M, Miller YE, Gelmont D, Rusch V, Gipe B, Howard D, LeRiche JC, Coldman A, Gazdar AF : Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy. Chest 113 : 696-702, 1998
4. Kusunoki Y, Imamura F, Uda H, Mano M, Horai T : Early detection of lung cancer with laser-induced fluorescence endoscopy and spectrofluorometry. Chest 118 : 1776-1782, 2000
5. Field JK, Brambilla C, Hirsch FR, Hittleman W, Hogan M, Marshall D, Mulshine JL, Rabbitts P, Sutedja T, Watson A, Weiss S : Molecular Biomarkers Workshop. A European strategy for developing lung cancer molecular diagnostics in high risk populations. Lung Cancer 31 : 339-345, 2001
6. Tockman MS, Mulshine JL : Sputum screening by quantitative microscopy : a new dawn for detection of lung cancer. Mayo Clin Proc 72 : 788-790, 1997
7. Lam S, MacAulay C, Hung J, LeRiche J, Profio AE, Palcic B : Detection of dysplasia and carcinoma *in situ* with a lung imaging fluorescence endoscope (LIFE) device. J Thorac Cardiovasc Surg 105 : 1035-1040, 1993
8. Hanibuchi M, Yano S, Edakuni N, Inayama M, Sone S : A case of early-stage lung cancer detected by autofluorescence bronchoscopy. J Med Invest 51 : 234-237, 2004.
9. Kusunoki Y, Imamura F, Uda H, Mano M, Horai T : Early detection of lung cancer with laser-induced fluorescence endoscopy and spectrofluorometry. Chest 118 : 1776-1782, 2000
10. Kato H, Okunaka T, Ikeda N : Application of simple imaging technique for fluorescence bronchoscope : preliminary report. Diag & Ther Endoscopy 1 : 79-81, 1994
11. Bota S, Auliac JB, Paris C, Metayer J, Sesboue R, Nouvet G, Thiberville L : Follow-up of bronchial precancerous lesions and carcinoma *in situ* using fluorescence endoscopy. Am J Respir Crit Care Med 164 : 1688-1693, 2001
12. Shibuya K, Hoshino H, Chiyo M, Yasufuku K, Iizasa T, Saitoh Y, Baba M, Hiroshima K, Ohwada H, Fujisawa T : Subepithelial vascular patterns in bronchial dysplasias using a high magnification bronchovideoscope. Thorax 57 : 902-907, 2002
13. Vonk-Noordegraaf A, Postmus PE, Sutedja TG : Bronchoscopic treatment of patients with intraluminal microinvasive radiographically occult lung cancer not eligible for surgical resection : a follow-up study. Lung Cancer 39 : 49-53, 2003
14. Mathur PN, Edell E, Sutedja T, Vergnon JM : American College of Chest Physicians. Treatment of early stage non-small cell lung cancer. Chest 123(Suppl 1) : 176S-180S, 2003