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ORIGINAL ARTICLE

COPD AS AN INDEPENDENT RISK FACTOR FOR LUNG CANCER IN PATIENTS WITH BRONCHIAL SQUAMOUS DYSPLASIA

By

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Background: ***We evaluated airflow obstruction as a possible independent risk factor for lung cancer (LC) in patients with bronchial squamous dysplasia (SD).***

Methods: ***A total of 114 patients (111 men and 3 women) with at least 1 bronchial SD, at least 1 follow-up evaluation and normal baseline chest computed tomography (CT) were evaluated; 58 (51%) were COPD and 56 (49%) were non-COPD patients. Median age was 68 years (range,44–84 yr) and median follow-up duration was 21 months (range,4-98 months). Follow-up included periodic white light and autofluorescence bronchoscopy and chest CT. Expression of iNOS (inducible nitric oxide synthase) and EGFR (epidermal growth factor receptor) in bronchial epithelium biopsy specimens was evaluated by immunohistochemistry (IHC). Diagnosis of carcinoma in situ (CIS) and/or LC were follow-up endpoints.***

Results: ***Expression of iNOS and EGFR was closely related to patient COPD status (p=0.007 and p=0.018, respectively). COPD patients were more likely to have baseline high grade dysplasia (p=0.017), multiple dysplasias (p=0.045) and to develop a new dysplasia during follow-up (p=0.003). Progression to CIS or LC occurred more frequently in patients with COPD (p=0.012), positive EGFR (p=0.019), positive iNOS (p<0.001) and baseline high grade SD (p=0.035). Multivariate analysis showed that risk for progression was closely related to airflow obstruction (RR=0.959; 95% CI=0.923-0.997; p=0.044) and iNOS expression (RR=10.521; 95% CI=2.75-40.3; p=0.001).***

Conclusion: ***In patients with bronchial SD, COPD is closely related to risk of progression to CIS or LC. This study supports the hypothesis that inflammation and oxidative stress promote lung carcinogenesis.***

Abbreviations: ***COPD= Chronic obstructive pulmonary disease. INOS= Inducible nitric oxide synthase, EGFR= Epidermal growth factor receptor, LC= Lung cancer, SD= Squamous dysplasia, SqCC= Squamous cell carcinoma, RR= Relative risk, CI= Confidence interval.***

INTRODUCTION

Lung cancer (LC) is the most common cancer type and is the leading cause of cancer deaths worldwide. Despite therapeutic advances, little gain has been achieved in overall LC survival during the past 30 years, with 5-year survival rates at approximately 15% for all stages combined. Thus, conventional treatment remains an

unsatisfactory means by which to decrease the global LC burden. Chemoprevention is theoretically possible for primary LC prevention, although clinical trials have been disappointing.[1] In order to substantially improve LC outcomes, new strategies of prevention and early detection are required. Detecting radiographically occult intraepithelial bronchial lesions with sputum cytology and autofluorescence bronchoscopy (AFB)[2] could contribute to improved LC survival, as seen with other epithelial malignancies like cervical and colon cancers.[3,4]

All published series that used autofluorescence bronchoscopy for individuals at high risk for LC have found very high frequencies of pre-invasive lesions, typically exceeding 50% of the cases.[5-7] Thus, a major issue is to more accurately differentiate lesions that are at high risk to progress into invasive cancers from those that are not.[6,7]

A number of studies have shown that chronic obstructive pulmonary disease (COPD) is an independent risk factor for LC.[8-10] The incidence of LC is 2 to 5 times greater in smokers with chronic bronchitis or emphysema than in smokers without COPD.[9] In addition, an inverse relationship between the degree of airway obstruction and the risk of LC has been demonstrated.[8-10]

A study by Chien et al. of heavy smokers with asbestos exposures showed that a baseline FEV1/FVC ratio < 0.7 was significantly associated with an increased risk of developing LC, even when baseline FEV1 was > 80%. LC risk among those with baseline airflow obstruction and FEV1 < 60% was 4-fold higher than among those without baseline airflow obstruction and FEV1 >80% ($p<0.001$).[10]

The mechanisms by which the risk of developing neoplastic disease increases with COPD are not clear, although several recent publications suggested that inflammation and associated oxidative and nitrosative stress could account for carcinogenesis in COPD patients. During inflammation, enhanced ROS/RNS production may induce recurring DNA damage, inhibition of apoptosis and activation of proto-ontogenesis by activating certain signal transduction pathways. Thus, it is conceivable that chronic inflammation-induced production of ROS/RNS in the lung may predispose individuals to LC.[11,12] Puhakka et al. showed that nitric oxide synthases are associated with bronchial dysplasia and that strong immunoreactivity for iNOS was more often observed in dysplastic than in metaplastic epithelium ($p = 0.011$).[13]

The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor. Mutations or hyper-expression of these receptors are responsible for oncogenic activation in LC by promoting proliferation, differentiation, apoptosis and angiogenesis. Accumulating evidence suggests that EGFR signalling pathways are involved in the development and progression of LC.[14,15] Also, a direct correlation between the EGFR expression rate and bronchial squamous dysplasia (SD) grade has been observed.[16,17]

This study addressed the associations of COPD status with immunohistochemistry (IHC) expression of EGFR (as a marker of carcinogenesis) and iNOS (as a marker of inflammation), the impact of COPD status on the risk of progression of bronchial SD to LC and the predictive value of airflow obstruction (FEV1/FVC), after controlling for other risk factors.

PATIENTS AND METHOD

Patients: From 335 consecutive patients at risk of LC who underwent AFB examinations at Chiba University Hospital, Chiba, Japan during the period from December 1999 to December 2008, 114 patients (111 men and 3 women) were included in the current study. Patients eligible for this study had at least 1 bronchial SD at baseline endoscopy, at least 1 follow-up evaluation and had normal baseline chest computed tomography (CT). Fifteen (13%) patients with a history of aerodigestive cancer and 99 patients (87%) with abnormal sputum cytology were included. Baseline endoscopy was defined as the first endoscopy in which bronchial SD was detected.

Patients' median age was 68 years (range, 44– 84 yr) at time of first bronchoscopy. Median and mean follow-up times were 21 and 27 months, respectively (range, 4 to 98 months). Follow-up included white light and autofluorescence bronchoscopy every 4-6 months and chest CT after 6 months, and then every 12 months. Diagnoses of carcinoma in situ (CIS) and/or LC were follow-up endpoints (when diagnosed, CIS lesions underwent endoscopic treatment, which may modify their course). All patients underwent spirometry. Patients were considered to have COPD if their post-bronchodilator FEV1/FVC ratio was <70%.[18] A patient was considered an ex-smoker if he/she had stopped smoking for more than 1 year.

Bronchial biopsy specimens were reviewed by 2 pathologists according to the WHO 1999 criteria for pre-invasive bronchial lesions.[19] Biopsies were classified as follows: normal or inflammatory, basal cell hyperplasia (BCH), squamous metaplasia (SM), mild dysplasia, moderate dysplasia, severe dysplasia, CIS or squamous cell carcinoma. For each patient, the most severe bronchial lesion detected at baseline was considered the baseline lesion; patients with baseline severe dysplasia were grouped as high grade and those with mild or moderate SD were grouped as low grade SD. The follow-up course was assessed for each individual according to the highest grade lesion at the last follow-up: progression was defined as the development of CIS or LC; regression to squamous

metaplasia or less severe lesion was considered regression; patients with mild, moderate or severe dysplasia at last follow-up were considered to have stable disease. All participants provided written informed consent before enrollment into the study. The study was approved by the Chiba University ethics committee.

Endoscopy: White light bronchoscopy (WLB) done using flexible video bronchoscope (BF-240, Olympus Optical Corporation, Tokyo, Japan until January 2004, and by BF 6C260, Olympus Optical Corporation, Tokyo, Japan thereafter). WLB was first performed under local anesthesia with sedation by intravenous midazolam and oxygen inhalation. This was followed by AFB using Laser Induced fluorescent endoscopy (LIFE) (Xillix LIFE; Xillix Technologies Corp., Richmond, BC, Canada) which was applied using a fiberoptic bronchoscope (BF40; Olympus) from December 1999 to October 2001, or by autofluorescence imaging (AFI) bronchovideoscope (BF type F260, Olympus Optical Corporation, Tokyo, Japan) thereafter. Biopsy was taken from all sites that appeared abnormal at baseline and/or follow-up white light and/or autofluorescence bronchoscopy. Biopsies were immediately formalin fixed and paraffin embedded.

Immunohistochemistry: The expression levels of EGFR and iNOS were determined by immunohistochemistry using a standard Avidin Biotin Complex method. Sections (5-µm thick) from formalin-fixed, paraffin-embedded bronchial biopsy specimens were deparaffinised in Xylene and rehydrated. Antigen retrieval used an autoclave for iNOS and pepsin digestion for EGFR. All sections were immersed in 3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. Sections were incubated with primary antibodies for iNOS (Polyclonal, Rabbit, 1/100 dilution, Santa Cruz Biotechnology, Inc. sc-651) and EGFR (Monoclonal, Mouse, 1/100 dilution, No. 28-0005; Zymed Laboratories, San Francisco, CA) at 4˚C overnight. The peroxidase reaction was visualized using liquid 3, 3-diaminobenzidine substrate. Some cases were excluded from study, as the bronchial epithelium was lost during preparation (10 cases).

Interpretation of Immunohistochemistry: EGFR staining extent was graded as follows: 0 = no positive immunostaining, 1 = staining of basal epithelial layer, 2 = staining ≤1/3 of bronchial epithelium, 3 = staining of >1/3-≤2/3 of bronchial epithelium, 4 = > 2/3 of bronchial epithelium. EGFR intensity was graded as: 1 = weak, 2 = moderate, 3 = strong. EGFR immunostaining was considered positive if the sum of extent and intensity scores was ≥ 5. For iNOS immunostaining, extent was graded as: 0 = no positive immunostaining, 1 = staining ≤1/3 of bronchial epithelium, 2 = staining of >1/3-≤2/3 of bronchial epithelium, 3 = staining > 2/3 of bronchial epithelium. INOS intensity was graded as: 1 = weak, 2 = moderate, 3 = strong. INOS immunostaining was considered positive if the sum of extent and intensity scores was ≥ 5. Examples of positive and negative EGFR and iNOS IHC expression are shown in (Fig. 1).

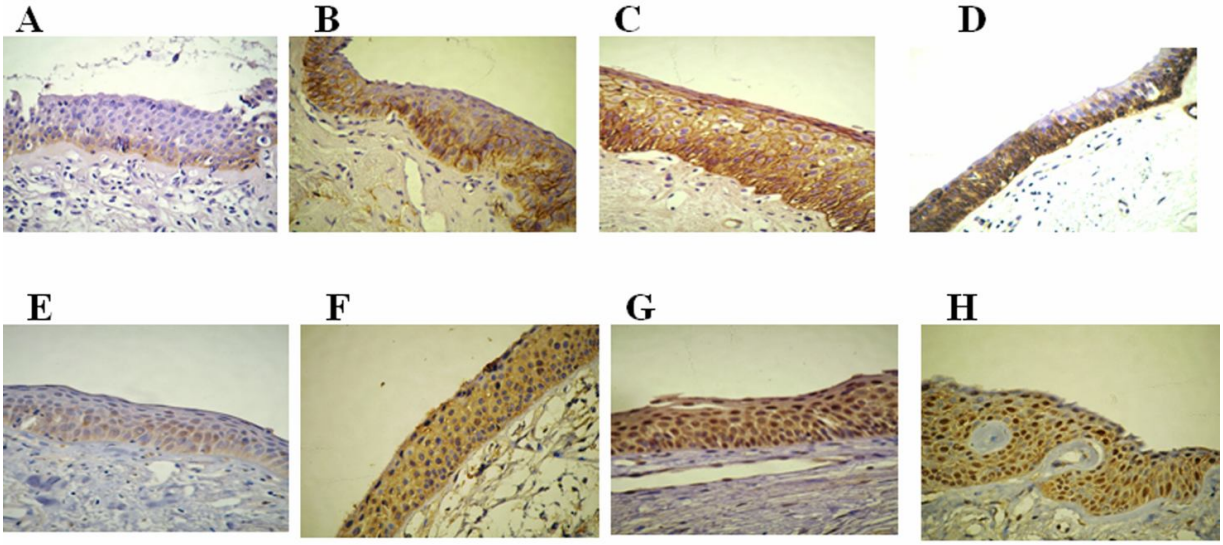

Fig 1. Examples of EGFR and iNOS IHC staining.

- Upper row shows EGFR immunohistochemical staining of bronchial squamous dysplasia. **A:** moderate dysplasia with positive staining of the basal epithelial layer only. **B:** Mild dysplasia with moderate staining intensity of 2/3 of epithelial thickness. **C:** Mild dysplasia with moderate staining

of full epithelial thickness. **D:** EGFR staining pattern in a transitional zone from normal epithelium to mild dysplasia

- Lower row shows iNOS immunostaining of bronchial squamous dysplasia. **E:** Mild SD with mild staining of 1/3 of epithelial thickness. **F:** Moderate dysplasia with moderate staining intensity of full epithelial thickness. **G:** Mild dysplasia with strong staining of full epithelial thickness. **H:** Angiogenic SD (Moderat) with strong immunostaining of full epithelial thickness.

Statistical Analysis: Progression free interval was calculated from the date of first bronchoscopy to the date of CIS or LC diagnosis. Comparisons were made by Chi-square tests and bilateral Fisher test, with Yates correction when required, and by independent sample T test. $P< 0.05$ was considered statistically significant. Cumulative risk of progression and comparison of progression free intervals between groups used Kaplan-Meier plots and a Log Rank test. A Cox proportional hazards model was used for multivariate analysis. Analyses used SPSS software version 12 (SPSS Inc., Chicago, IL).

RESULTS

Of 114 patients with bronchial SD, progression to CIS or LC was detected in 18 (16%) patients. Median and mean follow-up times were 21 and 27 months, respectively (range, 4 to 98 months). The detected progression lesions included 4 CIS (3 as direct progressions from severe dysplasia and 1 as a new endobronchial lesion), 10 squamous cell carcinoma (SqCC) (5 as new endobronchial lesions and 5 as peripheral lesions), 2 adenocarcinoma (peripheral lesions) and 2 small cell lung cancers (one as a new endobronchial lesion and one as a peripheral lesion).

Patient COPD status associations with other risk factors: As shown in Table 1, COPD patients were older than non-COPD patients ($p = 0.001$). As expected, COPD patients had a higher smoking index ($p = 0.02$), and were more likely to be current smokers ($p = 0.03$). There were no differences between COPD and non-COPD patients with regard to sex distribution ($p = 0.579$) or indication for AFB ($p = 0.726$). COPD patients were more likely to have baseline high grade dysplasia ($p = 0.017$), multiple dysplasias ($p = 0.045$) and were more vulnerable to develop a new dysplasia during follow-up ($p = 0.003$).

Table 1. Patient characteristics and detected dysplasias according to COPD status.

	COPD (58)	Non-COPD (56)	P value
Age (mean± SD*) yrs	69 ± 7	64 ± 9	0.001
Sex			
Male N (%)	56 (97%)	55 (98%)	0.579
Female N (%)	2 (3%)	1 (2%)	
Indication			
At risk of 2nd primary	7(12%)	8 (14%)	0.726
Abn. sputum cyt.	51 (88%)	48 (86%)	
Smoking status			
Current smoker N (%)	47 (81%)	36 (64%)	0.03
Ex-smoker N (%)	11 (19%)	15 (27%)	
Non-smoker N (%)	0	5 (9%)	
Smoking index (mean± SD*)	58 ± 26	47 ± 25	0.02
SD grade			
High grade	10 (17%)	2 (4%)	0.017
low grade	48 (83%)	54 (96%)	
Mild	20	21	
Moderate	28	33	
Baseline multiple SD			
Yes N (%)	26 (45%)	15 (27%)	0.045
No N (%)	32 (55%)	41 (73%)	
Follow-up new SD			
Yes N (%)	30 (52%)	14 (25%)	0.003
No N (%)	28 (48%)	42 (75%)	
Regression			
Yes N (%)	20 (35%)	39 (70%)	< 0.0001
No N (%)	38 (65%)	17 (30%)	

Abn sputum cyt= abnormal sputum cytology, SD= squamous dysplasia, SD*= sta ndard deviation.

Associations of EGFR and iNOS expression with COPD status and other risk factors: Patients with a history of aerodigestive cancer and those with abnormal sputum cytology showed no significant differences with regard to EGFR or iNOS staining (p=0.707 and p=0.451, respectively; Table 2.

Smoking status and smoking index (pack-year) were not significantly associated with either EGFR (p=0.193 and p=0.479, respectively) or iNOS expression level (p=0.251 and p = 0.177, respectively; Table 2.

COPD status appeared to be a factor that was closely related to EGFR and iNOS expression in bronchial SD. COPD patients were more likely to have positive EGFR (48%) compared to non-COPD patients (27%; p=0.018). COPD patents were also more likely to have positive iNOS (36%) than non-COPD patients (14%; p=0.007). Multiple baseline bronchial SDs and the appearance of a new SD during follow-up were not significantly associated with higher EGFR (p=0.155 and p=0.177, respectively) or iNOS expression (p=0.276 and p=0.215, respectively). Bronchial SD grade was closely related to higher EGFR (p<0.001) and iNOS (p=0.001) expression, as all high grade SD were EGFR positive and 8 (67%) were iNOS positive. In contrast, only 31 (30%) low grade SD were EGFR positive and 21 (21%) were iNOS positive.

Table 2. Associations of EGFR and iNOS IHC with baseline risk factors.

	EGFR			iNOS		
Indication	**Positive N (%)**	**Negative N (%)**	**p value**	**Positive N (%)**	**Negative N (%)**	**p value**
At risk of 2nd primary	5 (33%)	10 (67%)	0.707	5 (33%)	10 (67%)	0.451
Abn. sputum cyt.	38 (38%)	61 (62%)		24 (24%)	75 (76%)	
Smoking status						
Current smoker N (%)	32 (39%)	51 (61%)	0.193	24 (29%)	59 (71%)	0.251
Ex-smoker N (%)	11 (42%)	15 (58%)		5 (19%)	21 (81%)	
Non-smoker N (%)	0	5 (100%)		0	5 (100%)	
Smoking index (mean± SD)	55 ± 28	51 ± 24	0.479	58 ± 30	51 ± 24	0.177
COPD status						
COPD N (%)	28 (48%)	30 (52%)	0.018	21 (36%)	37 (64%)	0.007
Non-COPD N (%)	15 (27%)	41 (73%)		8 (14%)	48 (86%)	
Baseline multiple SD						
Yes N (%)	19 (46%)	22 (54%)	0.155	8 (20%)	33 (80%)	0.276
No N (%)	24 (33%)	49 (67%)		21 (29%)	52 (71%)	
Follow-up new SD						
Yes N (%)	20 (45%)	24 (55%)	0.177	14 (32%)	30 (68%)	0.215
No N (%)	23 (33%)	47 (67%)		15 (21%)	55 (79%)	
SD grade						
High grade	12 (100%)	0	0.0001	8 (67%)	4 (33%)	0.001
low grade	31 (30%)	71 (70%)		21 (21%)	81 (79%)	

Abn sputum cyt= abnormal sputum cytology, SD= squamous dysplasia.

Risk factors for progression: Progression occurred in 26% of COPD patients and in 5% of non-COPD patients (p=0.003) (Fig. 2). By comparison, regression to squamous metaplasia or a more benign lesion occurred in 35% of COPD patients and in 70% of non-COPD patients (p<0.0001) Table 1, (Fig. 2). Thus COPD patients were at higher risk for progression and were less likely to show regression.

EGFR and iNOS IHC expression appeared to be closely associated with higher risk of progression. The positive and negative predictive values for EGFR were 28% and 92%, respectively, and the positive and negative predictive values for iNOS were 48% and 95%, respectively. Thus, it would appear that both EGFR and iNOS are good negative predictors for progression in patients with bronchial SD (Fig. 2).

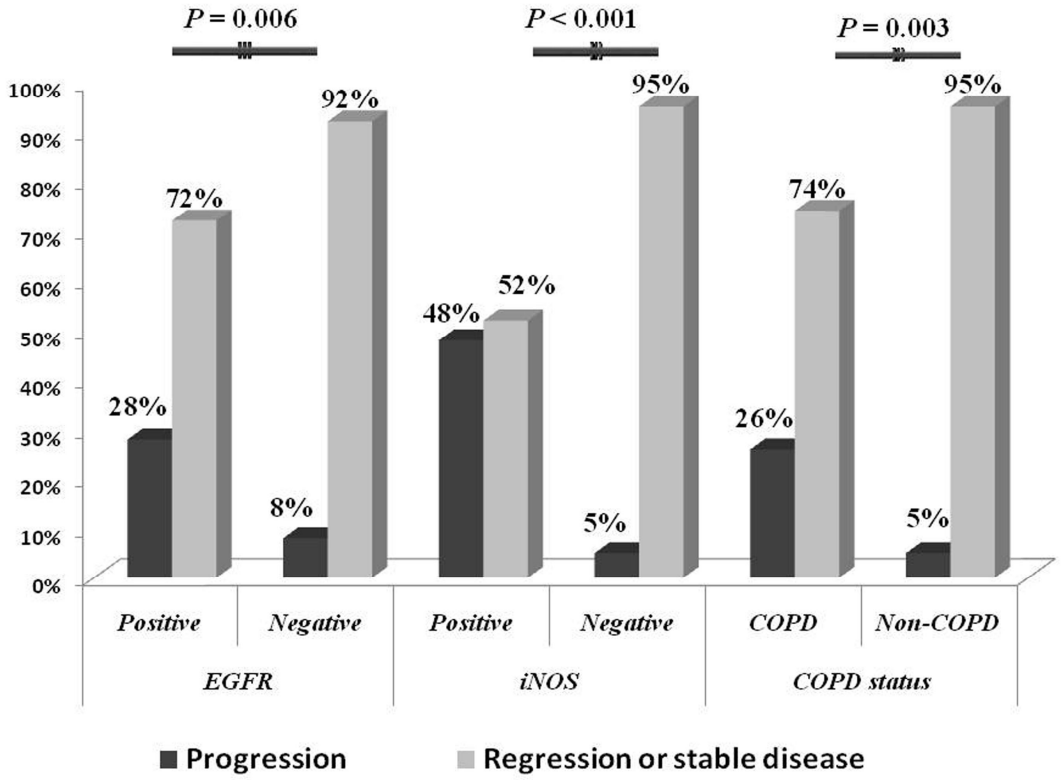

Fig 2. COPD and EGFR and iNOS expression and risk of progression to CIS or LC. COPD patients and patients with positive EGFR or iNOS expression were at higher risk for progression.

Kaplan Meier plots: Kaplan Meier analyses showed significant associations of progression and EGFR IHC expression (p=0.019), iNOS IHC expression (p<0.001), COPD (p = 0.012) and baseline bronchial SD grade (p=0.035) (Fig. 3).

Follow-up duration estimated from time of diagnosis of bronchial SD to time of either CIS or LC diagnosis or the last follow-up.

Multivariate COX regression analysis showed that risk for progression was closely related to airflow obstruction (RR=0.959; 95% CI=0.923-0.997; p=0.033) and iNOS expression (RR = 10.521; 95% CI=2.75 -40.3; p=0.001) Table 3.

To evaluate the risk of progression to SqCC, we excluded 4 cases from analysis (2 adenocarcinoma and 2 small cell lung cancer). By multivariate COX regression analysis, progression to SqCC was closely related to airflow obstruction (RR=0.926; 95% CI=0.876-0.979; p=0.007) and iNOS expression (RR=18.082; 95% CI=3.01-108.5; p=0.002) Table 3.

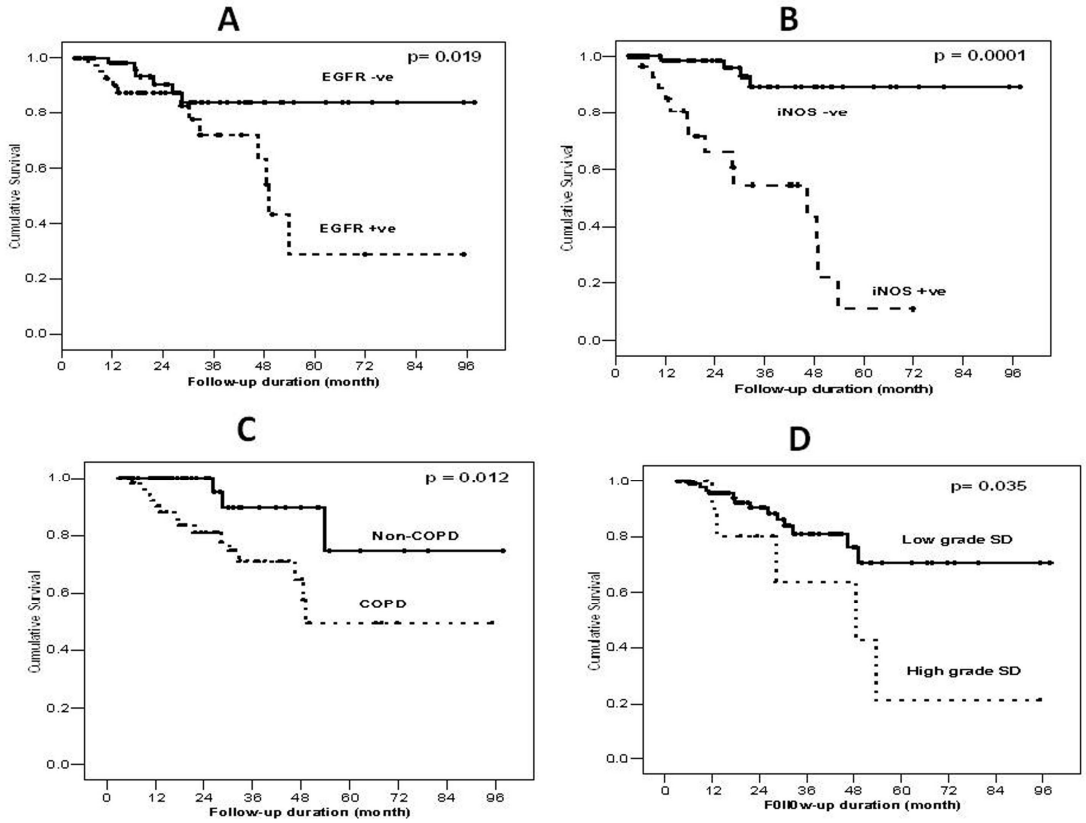

Fig 3. Kaplan Meier plots showing the higher risk of progression in patients with EGFR positive SD (A), iNOS positive (B), COPD (C) and patients with baseline high grade SD.

Table 3. Multivariate Cox regression analysis for all patients and for patients at risk for SqCC.

	All patients		Patients at risk for SqCC	
	RR (95% CI)	**p value**	**RR (95% CI)**	**p value**
EGFR	1.329 (0.34 -5.1)	0.681	3.279 (0.388 -27.74)	0.276
iNOS	10.521 (2.75 -40.3)	0.001	18.082 (3.01 -108.5)	0.002
Age	1.058 (0.963 – 1.16)	0.237	1.072 (0.909 - 1.26)	0.408
Indication	0.802 (0.178 -3.61)	0.774	0.55 (0.077– 4.01)	0.560
>52 pack-year SI	0.986 (0.299 – 3.25)	0.981	0.928 (0.186 – 4.63)	0.927
multiple baseline SD	1.464 (0.484- 4.42)	0.500	1.533 (0.348 - 6.76)	0.572
New SD during follow-up	1.524 (0.5 - 4.64)	0.456	3.811 (0.615 – 23.6)	0.150
Bronchial SD grade	1.071 (0.284 – 4.03)	0.919	1.491 (0.306 – 7.27)	0.621
FEV1 / FVC	0.959 (0.923 - 0.997)	0.033	0.926 (0.876 - 0.979)	0.007

DISCUSSION

Previous studies have shown that most bronchial SD lesions will regress; therefore, a major issue is to more accurately differentiate those lesions that are at high risk to progress into invasive cancers from those that are not.[14,20,21] In the current study, of 114 patients with bronchial SD, progression to CIS or LC was detected in 18 (16%) patients. COPD patients were more likely to have positive EGFR and positive iNOS expression, multiple SDs, high grade SDs and to develop a new SD during follow-up. COPD patients were less likely to show regression and they were at higher risk for progression, even after controlling for other risk factors.

It should come as no surprise that habitual cigarette smokers frequently develop LC and COPD. This occurs so frequently that, in the US, these 2 diseases represent the second and fourth leading causes of death, respectively,[22] and are epidemic worldwide. That both diseases often arise in the same person has been traditionally ascribed to bad luck and having one too many cigarettes, with little thought that the 2 diseases might be linked by more than smoking alone.[23] A number of studies have shown that COPD is an independent risk factor for LC.[8-10]

Lam et al. found that men with high grade lesions had statistically significant lower lung functions than those with mild dysplasias or more benign pathologic types; FEV1/FVC was 71% versus 67% (p=0.016).[18] However, Breuer et al. found no significant difference in the rate of progression to CIS or SqCC between COPD versus non-COPD (p = 0.54).[5] In our analysis, we found that COPD was closely associated with progression and that airflow obstruction was predictive for progression, even after adjusting for other risk factors. The reasons for these differences may be attributed to the larger sample size or the method of follow-up that we used (both chest CT and AFB) to detect progression.

Inflammation and oxidative stress-induced carcinogenesis and DNA damage have been suggested mechanisms underlying the COPD and LC association.[11] Soini et al. showed higher expression of manganese superoxide dismutase antioxidant enzyme in bronchial dysplasia than in metaplasia.[24] In addition, iNOS has been associated with the development of human and animal cancers in vivo. It has also been shown that in patients with cancer, nitric oxide has a role in maintaining tumour blood supply.[25]

Increased expression of iNOS in bronchial SD can enhance cellular proliferation and promote carcinogenesis by oxidant mediated mechanisms; this hypothesis is in full agreement with the importance of oxidative stress and inflammation in carcinogenesis and cellular proliferation.[25,26] Puhakka et al. studied the association of nitric oxide synthase with bronchial dysplasia and found that bronchial dysplasias more frequently showed strong iNOS expression (5/9) compared to metaplasia (1/15).[13] Similarly, in our study, iNOS expression was correlated with bronchial SD grade. To the best of our knowledge, the current study is the first to address the prognostic importance of iNOS IHC expression for progression of bronchial SD when adjusted for other risk factors.

Accumulating evidence suggests that EGFR signalling pathways are involved in the development and progression of LC.[14,15] In addition, Merrick et al. demonstrated a direct correlation between EGFR expression levels and bronchial SD grade.[17] Meert et al. demonstrated that the EGFR expression rate changed with the grade of the bronchial lesion, by increasing from its expression in normal epithelium to carcinoma in situ and micro-invasive tumours, with a statistically significant difference between mild versus severe dysplasia.[16] Recently, Massion et al. found that EGFR copy number, as estimated by FISH, was not significantly associated with bronchial SD grade, but was moderately associated with the presence of malignancy (OR= 3.55; 95% CI =1.06-11.86).[27] Similarly, in our analysis, EGFR was associated with SD grade and progression to LC, although when adjusted for other risk factors, it was not predictive for progression.

With regard to association of bronchial SD grade with the risk of progression to cancer, the results of previous studies are not consistent. Some studies showed that the higher the SD grade, the higher was the risk of progression.[6,21] Other studies, however, showed that risk of progression was not associated with bronchial SD grade.[5,28,29] These differences may be attributed to the study populations, follow-up end points and follow-up methods.[20]

In our analysis, we studied the IHC expression levels of EGFR and iNOS rather than their mutation status, although this could be a limitation. However, Suzuki et al. showed that EGFR expression status in LC correlated with its mutation status.[30]

Taken together, the results of the current study support the hypothesis that inflammation and oxidative stress can induce lung carcinogenesis. Inflammation and inflammation-induced oxidative stress (higher iNOS expression) could account for the higher risk of progression in COPD patients. COPD patients with bronchial SD are at high risk to develop LC. Additional evaluations are needed to support the use of anti-inflammatory, anti-oxidant or anti-EGFR drugs in chemoprevention trials.

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REFERENCES

1. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol. 2006;24:2137-50.

2. Hirsch FR, Prindiville SA, Miller YE, et al. Fluorescence versus white-light bronchoscopy for detection of preneoplastic lesions: a randomized study. J Natl Cancer Inst. 2001;93:1385–91.

3. Anderson GH, Boyes DA, Benedet JL, et al. Organization and results of the cervical cytology screening program in British Columbia, 1955–85. Br Med J (Clin Res Ed). 1988;296:975–8.

4. Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, et al. and The National Polyp Study Workgroup. Prevention of colorectal cancer by colonoscopic polypectomy. N Engl J Med. 1993;392:1977–81.

5. Breuer RH, Pasic A, Smit EF, et al. The natural course of preneoplastic lesions in bronchial epithelium. Clin Cancer Res. 2005;11:537–43.

6. Bota S, Auliac JB, Paris C, et al. Follow-up of bronchial precancerous lesions and carcinoma in situ using fluorescence endoscopy. Am J Respir Crit Care Med. 2001;164:1688–93.

7. Lam S, le Riche JC, Zheng Y, et al. Sex-related differences in bronchial epithelial changes associated with tobacco smoking. J Natl Cancer Inst. 1999;91:691–6.

8. Wilson DO, Weissfeld JL, Balkan A, et al. Association of radiographic emphysema and airflow obstruction with lung cancer. Am J Respir Crit Care Med. 2008;178:738-44.

9. Wasswa-Kintu S, Gan WQ, Man SF, Pare PD, Sin DD. Relationship between reduced forced expiratory volume in one second and the risk of lung cancer: a systematic review and meta-analysis. Thorax. 2005;60:570-5.

10. Chien JW, Au DH, Barnett MJ, Goodman GE. Spirometry, rapid FEV1 decline, and lung cancer among asbestos exposed heavy smokers. COPD. 2007;4:339-46.

11. Lawless MW, O'Byrne KJ, Gray SG. Oxidative stress induced lung cancer and COPD: opportunities for epigenetic therapy. J Cell Mol Med. 2009 Jul 7. [Epub ahead of print

12. Brody JS, Spira A. State of the art. Chronic obstructive pulmonary disease, inflammation, and lung cancer. Proc Am Thorac Soc. 2006;3:535-7.

13. Puhakka AR, Harju TH, Pääkkö PK, Soini YM, Kinnula VL. Nitric oxide synthases are associated with bronchial dysplasia. Lung Cancer. 2006;51:275-82.

14. Lantuéjoul S, Salameire D, Salon C, Brambilla E. Pulmonary preneoplasia--sequential molecular carcinogenetic events. Histopathology. 2009;54:43-54.

15. Hosokawa S, Toyooka S, Fujiwara Y, et al. Comprehensive analysis of EGFR signaling pathways in Japanese patients with non-small cell lung cancer. Lung Cancer. 2009 Jan 30. [Epub ahead of print] doi:10.1016/j.lungcan. 2009.01.005

16. Meert AP, Verdebout JM, Martin B, Ninane V, Feoli F, Sculier JP. Epidermal growth factor receptor expression in pre-invasive and early invasive bronchial lesions. Eur Respir J. 2003;21:611-5.

17. Merrick DT, Kittelson J, Winterhalder R, et al. Analysis of c-ErbB1/epidermal growth factor receptor and c-ErbB2/HER-2 expression in bronchial dysplasia: evaluation of potential targets for chemoprevention of lung cancer. Clin Cancer Res. 2006;12:2281-8.

18. Rabe KF, Hurd S, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am. J. Respir. Crit. Care Med. 2007;176:532–55.

19. Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E. Histological typing of lung and pleural tumours with contributions by pathologists from 14 countries. In: World Health Organization international histological classification of tumors, XIII, 3rd ed. Berlin/Heidelberg: Springer-Verlag. 1999.

20. Banerjee AK. Preinvasive lesions of the bronchus. J Thorac Oncol. 2009;4:545-51.

21. Salaün M, Sesboüé R, Moreno-Swirc S, et al. Molecular predictive factors for progression of high-grade preinvasive bronchial lesions. Am J Respir Crit Care Med. 2008;177:880-6.

22. Kung HC, Hoyert DL, Xu J, Murphy SL. Deaths: final data for 2005. Natl Vital Stat Rep. 2008;56:1-120.

23. Houghton AM, Mouded M, Shapiro SD. Common origins of lung cancer and COPD. Nat Med. 2008;14:1023-4.

24. Soini Y, Kaarteenaho-Wiik R, Paakko P, Kinnula V. Expression of antioxidant enzymes in bronchial metaplastic and dysplastic epithelium. Lung Cancer. 2003;39:15-22.

25. Ng QS, Goh V, Milner J, et al. Effect of nitric-oxide synthesis on tumour blood volume and vascular activity: a phase I study. Lancet Oncol. 2007;8:88-9.

26. Crowell JA, Steele VE, Sigman CC, Fay JR. Is inducible nitric oxide synthase a target for chemoprevention? Mol Cancer Ther. 2003;2:815-23.

27. Massion PP, Zou Y, Uner H, et al. Recurrent genomic gains in preinvasive lesions as a biomarker of risk for lung cancer. PLoS One. 2009;4:e5611.

28. Ponticiello A, Barra E, Giani U, Bocchino M, Sanduzzi A. P53 immunohistochemistry can identify bronchial dysplastic lesions proceeding to lung cancer: a prospective study. Eur Respir J. 2000;15:547-52.

29. Hoshino H, Shibuya K, Chiyo M, et al. Biological features of bronchial squamous dysplasia followed up by autoFluorescence bronchoscopy. Lung Cancer. 2004;46:187–96.

30. Suzuki M, Shigematsu H, Hiroshima K, et al. Epidermal growth factor receptor expression status in lung cancer correlates with its mutation. Hum Pathol. 2005;36:1127-34.