Bronchial Epithelial Ki-67 Index Is Related to Histology, Smoking, and Gender, but Not Lung Cancer or Chronic Obstructive Pulmonary Disease

York E. Miller,1 Patrick Blatchford,2 Dae Sung Hyun,6 Robert L. Keith,1 Timothy C. Kennedy,3 Holly Wolf,2 Tim Byers,2 Paul A. Bunn, Jr.,4 Marina T. Lewis,4,5 Wilbur A. Franklin,5 Fred R. Hirsch,4,5 and John Kittelson2

1Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, Denver Veterans Affairs Medical Center, Departments of Preventive Medicine and Biometrics, Medicine, HealthOne, and 4Division of Medical Oncology, Department of Medicine, and 5Department of Pathology, University of Colorado at Denver and Health Sciences Center, University of Colorado Comprehensive Cancer Center, Denver, Colorado; and 6Catholic University of Daegu, Daegu, South Korea

Abstract

Purpose: To determine whether increased bronchial epithelial proliferation is associated with histology, smoking status, gender, age, chronic obstructive pulmonary disease (COPD), or lung cancer.

Experimental Design: Cross-sectional study of 113 subjects undergoing white light and autofluorescence bronchoscopy: 27 never smokers; 27 current or ex-smokers with normal spirometry; 31 current or ex-smokers with COPD; and 28 current, ex-, or never smokers with lung cancer. Ki-67 expression was determined by immunohistochemistry on all evaluable biopsy sites without carcinoma. Relationships between Ki-67 index (percentage of epithelial cells expressing Ki-67), demographic variables, smoking, histology, and the presence of COPD and/or lung cancer were determined.

Results: Results for both maximal and mean Ki-67 index are similar, so only the former are reported. Average maximal Ki-67 index was higher in current smokers than either ex-smokers or never smokers (48.0% versus 30.6% versus 22.6%; \( P < 0.001 \)). Males had higher Ki-67 index than females (39.9% versus 23.6%; \( P < 0.001 \)). Compared with subjects without disease (Ki-67 index = 30.0%), maximal Ki-67 index was not significantly elevated (\( P = 0.44 \)) in subjects with either lung cancer (Ki-67 = 39.1%) or COPD (Ki-67 = 38.9%).

Conclusions: Smoking status, bronchial histology, and gender were significantly associated with Ki-67 index. No increase in Ki-67 index was found in the nonmalignant epithelium of patients with lung cancer or COPD. Although Ki-67 index may provide insight into the short-term effects of chemoprevention agents on cell proliferation, its lack of association with lung cancer or COPD raises question regarding its utility as a lung cancer risk biomarker. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2425–31)

Introduction

Lung cancer is the leading cause of cancer death in the United States and the world (1). Eighty-five percent of lung cancer cases in the United States can be attributed to tobacco smoke. However, only one of nine smokers will develop lung cancer, so determination of lung cancer risk is extremely important. Smoking history, age, the presence of airflow obstruction [indicative of chronic obstructive pulmonary disease (COPD)], exposure to additional respiratory carcinogens, personal history of other smoking-related cancers, and family history of lung cancer are all simple historical or clinical features that can be used to further define risk (2-5). Reliable biomarkers of risk would be of great utility for developing early detection and prevention strategies. Many biomarkers of lung cancer risk have been proposed, but few have been comprehensively validated. Sputum cytology of moderate or worse atypia carries an increased risk, as described in several longitudinal cohort trials, with moderate atypia having a modestly elevated relative risk and severe atypia or carcinoma in situ having a highly elevated relative risk (6, 7). We recently reported that methylation of a panel of genes in the sputum is associated with an approximate 7-fold elevation in risk in an incident case control study (8).

Uncontrolled cellular proliferation is a hallmark of cancer and a biologically plausible risk biomarker for preneoplastic epithelium (9-11). Ki-67 immunostaining has been used as an indicator of cellular proliferation for more than 20 years (12). The Ki-67 antigen is localized to the nucleus, currently incompletely biochemically characterized, and is expressed in all phases of the cell cycle except for G0 (13). The percentage of epithelial cells expressing Ki-67, or Ki-67 index, is progressively
Bronchial Epithelial Ki-67 and Lung Cancer

Subjects gave full informed consent and were enrolled in research bronchoscopies supported by the Veterans Affairs Medical Center and the HealthOne IRB. Research and Development Committee of the Denver HealthOne IRB. Ki-67 index has been characterized in current and ex-smokers by Lee et al. (11). Current smokers exhibited higher Ki-67 index than ex-smokers, and the duration of abstinence from smoking required for this effect was estimated at 1 year or less. Mao et al. (16) have evaluated Ki-67 index as an intermediate end point biomarker in a chemoprevention trial comparing celecoxib to placebo. They reported a decrease in Ki-67 index with celecoxib compared with placebo.

The aerodigestive epithelium is thought to exhibit a field effect, in which histologic, genetic, and epigenetic alterations are dispersed throughout the mucosa of individuals with carcinoma (17, 18). Evidence supporting a field effect includes the relatively common occurrence of multiple primary lung tumors, sharing of chromosomal loss of heterozygosity between tumors and uninvolved epithelium (termed allele-specific mutation), shared gene methylation signatures or epidermal growth factor receptor (EGFR) mutations between resected tumors and adjacent non-neoplastic epithelium, and demonstration of widely dispersed p53 mutant cells within the airway epithelium (19-24). If the field effect results in a widespread increased level of proliferation within the bronchial epithelium of individuals destined to develop lung cancer, then Ki-67 index would be a useful risk biomarker. One would expect that individuals who have developed lung cancer would then exhibit higher Ki-67 index in nonmalignant epithelium than those who have not, after adjustment for factors known to affect Ki-67 index.

We hypothesized that the Ki-67 index would be increased in the airway epithelium of current smokers compared with ex-smokers as well as in ex-smokers compared with never smokers. We also hypothesized that Ki-67 index would be increased in nonmalignant bronchial epithelium of patients with the tobacco-induced diseases COPD and lung cancer compared with subjects without lung disease, after adjustment for smoking and other factors. We carried out a cross-sectional analysis of a subgroup of subjects who were enrolled into research bronchoscopy trials supported by the University of Colorado Specialized Program of Research Excellence (SPORE) in Lung Cancer. To our knowledge, this is the first study that has reported quantitative Ki-67 index in current and ex-smokers compared with never smokers or in subjects with or without the tobacco-induced lung diseases COPD and lung cancer, all of which are highly relevant to the evaluation of Ki-67 index as a risk biomarker.

Materials and Methods

Human Subjects. All protocols for tissue acquisition by bronchoscopy were approved by COMIRB, the Research and Development Committee of the Denver Veterans Affairs Medical Center and the HealthOne IRB. Subjects included both paid volunteers and individuals in whom a clinically indicated bronchoscopy was done. Subjects gave full informed consent and were enrolled before bronchoscopy. Flexible fiberoptic bronchoscopy was done as described with both autofluorescence and white light examination of the airways using either a Xillix LIFE II or OncoLIFE system (25). Biopsies were taken when clinically feasible at all areas suspicious for either premalignant dysplasia or invasive carcinoma, as well as at the following predetermined sites regardless of bronchoscopic appearance: carina between right upper lobe and right mainstem bronchus, carina between right middle lobe and bronchus intermedius, carina between superior segment right lower lobe and right lower lobe bronchus, carina between left upper lobe and left mainstem bronchus, carina between left upper division bronchus and lingula, carina between superior segment left lower lobe and left lower lobe bronchus. Subjects filled out a standard questionnaire, spirometry was done or obtained from medical records, and results were recorded.

To analyze the relationship between Ki-67 index and smoking, COPD, and lung cancer, approximately equal numbers of subjects in the following four groups were selected for Ki-67 analysis: (a) never smokers; (b) current or ex-smokers with normal spirometry; (c) current or ex-smokers with COPD but without lung cancer; and (d) current, ex-, or never smokers with lung cancer. COPD was defined as a forced expiratory volume in one second (FEV1) of 75% predicted or less with a FEV1/forced vital capacity (FVC) ratio of ≤0.75 in either current or ex-smokers. Normal pulmonary function was defined as a FEV1 of 85% predicted or greater with an FEV1/FVC ratio of >0.75. The determination of whether or not subjects had invasive lung cancer at the time of bronchoscopy was made based on biopsy results, review of the clinical history, and matching to the Colorado Central Cancer Registry, the Colorado Department of Public Health and Environment Vital Statistics records, and the National Death Index. Between the initiation of research bronchoscopy trials by the Colorado SPORE in Lung Cancer in 1993 and January 25, 2005, 601 different subjects underwent one or more research bronchoscopies. Only subjects who underwent both autofluorescence and white light bronchoscopy were considered for Ki-67 analysis. Initially, a group of ~30 subjects with invasive lung cancer on bronchoscopy biopsy or a subsequent diagnostic specimen were selected for analysis. All never smokers with normal pulmonary function within the overall bronchoscopy group were also selected for analysis, as well as all current or ex-smokers with normal pulmonary function. A subgroup of the remaining current or ex-smokers with COPD but without lung cancer was then selected with frequency matching on the age and sex of the other categories. Subjects with a prior history of lung cancer, who had received previous chemotherapy or radiation treatment, or who had received chemoprevention agents were excluded from analysis. All subjects were followed both actively and passively for the subsequent development of incident lung cancer.

Tissue Analysis. Biopsies were formalin-fixed and paraffin-embedded, then cut and placed on glass slides. H&E-stained sections were evaluated by the study pathologist and graded according to the WHO criteria for bronchial preneoplasia, which separates lesions into eight different grades, each given a number: 1, normal; 2, reserve cell hyperplasia; 3, squamous metaplasia; 4, mild dysplasia; 5, moderate dysplasia; 6, severe dysplasia; 7,
carcinoma in situ; and 8, invasive carcinoma (26). Bronchial histology was summarized on a per-patient basis as either the numerical average of scores at each evaluable site, the worst (highest) score for a given patient, or the dysplasia index (% scores of 4 or greater). In situations in which multiple biopsies were taken at a specific site, the highest grade was used for analysis. Sites at which an invasive cancer was biopsied were not included in the final analysis because these were considered diagnostic and not appropriate for assessment of a risk biomarker. One biopsy from each site at which evaluable tissue was available was then immunostained for Ki-67 using the mib-1 monoclonal (DAKO) as described (ref. 15; Fig. 1). Photomicrographs of the most histologically advanced lesion within the biopsy were taken, reviewed with the study pathologist (W.A.F.), and 400 cells within that lesion were counted and scored for nuclear Ki-67 expression, with percentage Ki-67–positive cells calculated.

**Statistical Analysis.** The Ki-67 measures from all biopsies in a subject were reduced to a single summary measure in two ways: the average of all biopsy values or the maximum value. The analysis of Ki-67 association with demographic characteristics, disease status, and smoke exposure was then conducted using these patient-level summary measures.

Statistical analyses were based on linear regression methods, including the analysis of covariance for comparing groups after adjusting for potential confounders. Because subjects were selected from the four groups described above, it became necessary to adjust analyses for smoke exposure and/or disease status to obtain inference about the population of all bronchoscopy subjects. In addition, the primary analyses were adjusted for major potential confounders that were know (a priori) to differ between disease and smoking groups. The relationship between Ki-67 and histology was analyzed using a linear mixed-effects model of the biopsy-specific measures rather than the within-patient summary measures. Results are reported as two-sided P values and 95% confidence intervals with statistical significance defined by P < 0.05. Preliminary descriptive analyses showed that the Ki-67 measures were not particularly skewed; thus, it was not necessary to consider data transformations to assure proper statistical operating characteristics.

**Results**

**Patient Characteristics.** Characteristics of the study population are summarized in Table 1. The mean ages of the never smokers (53.3 years), current or ex-smokers without COPD (55.3 years), current or ex-smokers with COPD (61.5 years), and lung cancer patients (65.9 years) were significantly different from each other (P < 0.001). Gender was also mismatched between the groups, with never smokers, 44.4% male; current or ex-smokers without COPD, 55.6% male; current or ex-smokers with COPD, 77.4% male; and lung cancer patients, 92.9% male (P = 0.001). All groups were predominantly Caucasian. The percent of current smokers also differed significantly across subjects without COPD, with COPD, and subjects with cancer (P = 0.003). Two lung cancer patients were never smokers. Smoke exposure was not different between the no-COPD, COPD, and lung cancer groups in terms of packs per day and years of smoking, but the current or ex-smokers without COPD had fewer pack-years (44.5) than current or ex-smokers with COPD (64.4) or lung cancer (62.1; P = 0.033). Seventy nine percent (22:28) of lung cancer patients had airflow obstruction by spirometry. Never smokers had the lowest histology scores; differences between the other three groups were relatively minor. The lung cancer group was comprised of 19 squamous cell carcinomas, 8 adenocarcinomas, and 1 small cell lung cancer.

**Association between Endobronchial Histology, Demographics, Smoking, COPD, and Lung Cancer.** Average maximum histology scores for subjects categorized by smoking and disease status are shown in Table 2, and the relationships between histology, age, sex, disease, and smoking are shown in Table 3. Current smokers had higher nonmalignant histology than either former or never smokers (4.5 versus 3.3 versus 2.2; P < 0.001 after adjusting for disease, age, and smoking history). Males had worse histology than females (3.9

![Figure 1. Effect of years since quitting smoking on maximal Ki-67 index. Relationship is nonsignificant (P = 0.81).](https://example.com/figure1.png)
versus 2.4; \( P < 0.001 \) adjusted for disease, age, and smoke history). There was no association between maximum histology and either lung cancer or COPD after adjustment for smoking, gender, and age (\( P = 0.79 \)). Similarly, separate analyses in either current or former smoker groups alone failed to reveal any association between average maximum histology and either COPD or lung cancer after adjustment for age and gender.

### Association between Ki-67 Index and Demographic Variables
Analyses of the relationships between Ki-67 index and all demographic, smoking, and disease variables were carried out using either the average Ki-67 index across the biopsies within each subject or the maximum Ki-67 index across the biopsies. Similar results were found with either approach, so only the maximum Ki-67 index is reported. The mean values for the maximum Ki-67 index for subjects categorized by smoking and disease status are shown in Table 4. The adjusted and unadjusted associations between maximum Ki-67 index and age, sex, smoking, and lung disease are shown in Table 5. Males had significantly higher Ki-67 index than females (39.9% versus 23.6%, \( P < 0.001 \)) even after adjusting for age, smoking status, and disease status (either COPD or lung cancer). Neither age nor race was significantly associated with Ki-67 index (data not shown).

#### Ki-67 Index and Smoking
Before adjusting for age, gender, and disease differences, there was a trend for increasing Ki-67 index from never to former to current smokers (22.6% versus 30.6% versus 48.0%). After adjustment, never and former smokers did not differ, but current smokers had, on average, a maximal Ki-67 index that was 20.6% higher than never smokers (\( P < 0.001 \); Table 4). This same pattern (highest Ki-67 index among current smokers) was also apparent within each disease category (Table 5). Ki-67 index was not associated with quantitative parameters of smoke exposure (pack-years, smoking years, and packs per day; data not shown). There was no evidence for a continual decrease in Ki-67 index with increasing time since quitting, suggesting that the major decrease in Ki-67 index with smoking cessation occurs within a relatively short timeframe of months to several years (Fig. 1).

#### Ki-67 Index and Disease
Subjects with cancer or COPD had slightly higher Ki-67 index than subjects without disease (39.1% versus 38.9% versus 30.0%), although these differences were not statistically significant either with (\( P = 0.44 \)) or without adjustment (\( P = 0.17 \)) for age, sex, and smoke exposure. Although there was some evidence that among current smokers, Ki-67 is elevated in COPD and cancer patients relative to nonsmoked subjects (Table 5), even the post hoc test of

### Table 1. Characteristics of the Ki-67 patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Never smokers (( n = 27 ))</th>
<th>Smokers (no COPD) (( n = 27 ))</th>
<th>Smokers (COPD) (( n = 31 ))</th>
<th>Cancer cases (( n = 28 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Mean (SD) or ( n ) (%)</td>
<td>Mean (SD) or ( n ) (%)</td>
<td>Mean (SD) or ( n ) (%)</td>
<td>Mean (SD) or ( n ) (%)</td>
</tr>
<tr>
<td>Age</td>
<td>53.3 (7.9)</td>
<td>55.3 (9.3)</td>
<td>61.5 (10.3)</td>
<td>65.9 (9.1)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 12 (44.4%)</td>
<td>15 (55.6%)</td>
<td>24 (77.4%)</td>
<td>26 (92.9%)</td>
</tr>
<tr>
<td>Race</td>
<td>White 25 (92.6%)</td>
<td>17 (63.0%)</td>
<td>30 (96.8%)</td>
<td>23 (82.1%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Current 0 (0.0%)</td>
<td>20 (74.1%)</td>
<td>9 (29.0%)</td>
<td>11 (39.3%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Former 0 (0.0%)</td>
<td>7 (25.9%)</td>
<td>22 (71.0%)</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Lung function</td>
<td>COPD 0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>2 (7.1%)</td>
</tr>
<tr>
<td>Smoke exposure</td>
<td>Packs/d 0.0 (0.0)</td>
<td>1.3 (0.5)</td>
<td>1.7 (0.6)</td>
<td>1.5 (0.8)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 12 (44.4%)</td>
<td>15 (55.6%)</td>
<td>24 (77.4%)</td>
<td>26 (92.9%)</td>
</tr>
<tr>
<td>Race</td>
<td>White 25 (92.6%)</td>
<td>17 (63.0%)</td>
<td>30 (96.8%)</td>
<td>23 (82.1%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Current 0 (0.0%)</td>
<td>20 (74.1%)</td>
<td>9 (29.0%)</td>
<td>11 (39.3%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Former 0 (0.0%)</td>
<td>7 (25.9%)</td>
<td>22 (71.0%)</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Lung function</td>
<td>COPD 0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>2 (7.1%)</td>
</tr>
<tr>
<td>Smoke exposure</td>
<td>Packs/d 0.0 (0.0)</td>
<td>1.3 (0.5)</td>
<td>1.7 (0.6)</td>
<td>1.5 (0.8)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 12 (44.4%)</td>
<td>15 (55.6%)</td>
<td>24 (77.4%)</td>
<td>26 (92.9%)</td>
</tr>
<tr>
<td>Race</td>
<td>White 25 (92.6%)</td>
<td>17 (63.0%)</td>
<td>30 (96.8%)</td>
<td>23 (82.1%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Current 0 (0.0%)</td>
<td>20 (74.1%)</td>
<td>9 (29.0%)</td>
<td>11 (39.3%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Former 0 (0.0%)</td>
<td>7 (25.9%)</td>
<td>22 (71.0%)</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Lung function</td>
<td>COPD 0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>2 (7.1%)</td>
</tr>
<tr>
<td>Smoke exposure</td>
<td>Packs/d 0.0 (0.0)</td>
<td>1.3 (0.5)</td>
<td>1.7 (0.6)</td>
<td>1.5 (0.8)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 12 (44.4%)</td>
<td>15 (55.6%)</td>
<td>24 (77.4%)</td>
<td>26 (92.9%)</td>
</tr>
<tr>
<td>Race</td>
<td>White 25 (92.6%)</td>
<td>17 (63.0%)</td>
<td>30 (96.8%)</td>
<td>23 (82.1%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Current 0 (0.0%)</td>
<td>20 (74.1%)</td>
<td>9 (29.0%)</td>
<td>11 (39.3%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Former 0 (0.0%)</td>
<td>7 (25.9%)</td>
<td>22 (71.0%)</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Lung function</td>
<td>COPD 0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>2 (7.1%)</td>
</tr>
<tr>
<td>Smoke exposure</td>
<td>Packs/d 0.0 (0.0)</td>
<td>1.3 (0.5)</td>
<td>1.7 (0.6)</td>
<td>1.5 (0.8)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 12 (44.4%)</td>
<td>15 (55.6%)</td>
<td>24 (77.4%)</td>
<td>26 (92.9%)</td>
</tr>
<tr>
<td>Race</td>
<td>White 25 (92.6%)</td>
<td>17 (63.0%)</td>
<td>30 (96.8%)</td>
<td>23 (82.1%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Current 0 (0.0%)</td>
<td>20 (74.1%)</td>
<td>9 (29.0%)</td>
<td>11 (39.3%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Former 0 (0.0%)</td>
<td>7 (25.9%)</td>
<td>22 (71.0%)</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Lung function</td>
<td>COPD 0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>2 (7.1%)</td>
</tr>
</tbody>
</table>

NOTE: Tests for smoking status and smoke exposure do not include never smokers.

\*P value for testing the equality of variable between all groups.

### Table 2. Patient-level histology by disease and smoking status

<table>
<thead>
<tr>
<th>Disease status</th>
<th>No disease (( n = 54 ))</th>
<th>COPD (( n = 31 ))</th>
<th>Cancer (( n = 28 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>2.1 (1.7)</td>
<td>3.9 (1.7)</td>
<td>3.6 (1.9)</td>
</tr>
<tr>
<td>Former</td>
<td>2.9 (1.9)</td>
<td>4.3 (1.6)</td>
<td>4.2 (1.5)</td>
</tr>
<tr>
<td>Current</td>
<td>3.0 (1.1)</td>
<td>3.0 (1.4)</td>
<td>2.5 (1.2)</td>
</tr>
</tbody>
</table>

NOTE: Results are shown in units of bronchial neoplasia.
differences between disease categories among current smokers is not statistically significant ($P = 0.13$; data not shown).

**Ki-67 Index and Histology.** Figure 2 shows that Ki-67 index and histology are strongly associated. The relationship between Ki-67 and histology does not differ by disease status ($P = 0.1$).

**Discussion**

Ki-67 index is an attractive biomarker of risk for both lung cancer and COPD. Uncontrolled cellular proliferation is a hallmark of neoplasia (9). Lung cancers have increased Ki-67 index, which may also be related to prognosis (27). Other investigators have noted a wide variability in Ki-67 index between different individuals and suggested that this may reflect a differing biological response to tobacco smoke or other injurious agents (11). Furthermore, whereas Ki-67 index increases with increasing histologic grades of preneoplasia, there is a wide range of variation within a given histologic grade, suggesting that Ki-67 index may provide additional prognostic information beyond that of histology. The increase in Ki-67 index in current smokers, compared with ex-smokers, has been previously described, but how this varies in never smokers is unknown.

Only a minority of smokers develop COPD or lung cancer. Several studies have documented an increased risk for lung cancer in smokers with COPD (3, 4). The pathophysiology of COPD is incompletely understood, but involves both the loss of alveolar septae (emphysema) and obstruction of small airways (28). Interestingly, morphometric studies correlating various anatomic parameters with airflow obstruction on pulmonary function testing find the highest correlation between thickness of the epithelium in small airways and airflow obstruction (29). Thus, increased proliferation of airway epithelium in response to tobacco smoke is one attractive mechanism for the development of COPD in susceptible smokers. Genetic mutations are more likely to occur in proliferating tissues, and thus, Ki-67 may be an important marker for lung cancer as well as COPD.

The purpose of the current study was 2-fold; to better understand factors associated with increased Ki-67 index and, subsequently, to determine whether Ki-67 index is increased in two tobacco-associated lung diseases, COPD and lung cancer, after adjustment for confounding factors. We therefore chose subjects in four groups: never smokers; current or ex-smokers with normal pulmonary function and no lung cancer; current or ex-smokers with COPD; and current, ex-, or never smokers with lung cancer.

We analyzed both within-patient mean and maximal Ki-67 index. As results for both were similar, we have only presented the latter, which might represent the most advanced lesion sampled and therefore be a better reflection of lung cancer risk. An unexpected finding was that Ki-67 index is strongly related to gender, with men exhibiting ~12% to 18% higher maximal Ki-67 index than women, even after adjustment for smoking status (current, former, or never), smoking amount (pack-years, years smoked, or packs per day), age, disease, and race. As expected, we confirmed a significant difference between Ki-67 index in current and former smokers (11). Contrary to our initial hypotheses, we found that Ki-67 index in former smokers was not greater than that in never smokers. Although smoking status (current versus former or never) was associated with Ki-67 index, there was no significant correlation

**Table 3. Histology associations with age, sex, disease, and smoking**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>$n$</th>
<th>Mean (SD)</th>
<th>Unadjusted differences</th>
<th>Adjusted differences*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Effect (CI)</td>
<td>$P^+$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P^+$</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>113</td>
<td>3.4 (1.9)</td>
<td>0.01 (0.02, 0.05)</td>
<td>0.386</td>
<td>-0.00 (0.04, 0.03) 0.912</td>
</tr>
<tr>
<td>Sex Male</td>
<td>77</td>
<td>3.9 (1.8)</td>
<td>Reference</td>
<td>&lt;0.001</td>
<td>Reference &lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td>2.4 (1.8)</td>
<td>-1.4 (-2.1, -0.7)</td>
<td>Reference 0.034</td>
<td>0.2 (-0.7, 1.1) 0.794</td>
</tr>
<tr>
<td>Lung disease</td>
<td>None</td>
<td>54</td>
<td>3.0 (1.9)</td>
<td>0.6 (-0.2, 1.5)</td>
<td>Reference &lt;0.001</td>
</tr>
<tr>
<td>COPD</td>
<td>31</td>
<td>3.6 (1.9)</td>
<td>1.1 (0.2, 1.9)</td>
<td>Reference &lt;0.001</td>
<td>0.3 (-0.6, 1.2) &lt;0.001</td>
</tr>
<tr>
<td>Cancer</td>
<td>28</td>
<td>4.1 (1.7)</td>
<td>0.7 (1.4, 3.1)</td>
<td>Reference &lt;0.001</td>
<td>1.9 (1.1, 2.8) &lt;0.001</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Never</td>
<td>29</td>
<td>2.2 (1.7)</td>
<td>Reference</td>
<td>Reference &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Former</td>
<td>44</td>
<td>3.3 (1.8)</td>
<td>1.1 (0.3, 1.9)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>40</td>
<td>4.5 (1.4)</td>
<td>2.2 (1.4, 3.1)</td>
<td>Reference &lt;0.001</td>
</tr>
</tbody>
</table>

NOTE: Results are shown in units of bronchial preneoplasia, where 1, normal, . . . , 8, cancer.

*Adjusted for all other variables shown.
  
$^1P$ value for testing the equality of variables between all groups.

**Table 4. Patient-level Ki-67 by disease and smoking status**

<table>
<thead>
<tr>
<th>Disease status</th>
<th>No disease ($n = 54$)</th>
<th>COPD ($n = 31$)</th>
<th>Cancer (N = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Mean (SD)</td>
<td>$n$</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Never</td>
<td>27</td>
<td>22.9 (21.0)</td>
</tr>
<tr>
<td></td>
<td>Former</td>
<td>7</td>
<td>28.0 (23.8)</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>20</td>
<td>40.3 (24.1)</td>
</tr>
</tbody>
</table>

NOTE: Results are shown as percentage of cells with Ki-67 nuclear expression.
between pack-years, years smoked, or packs per day of smoking history and Ki-67 index. The times since quitting tobacco smoking and Ki-67 index did not show a continuously varying relationship, supporting the previously reported estimate that Ki-67 index decreases rapidly after smoking cessation, over a period of months, rather than years (11) Prospective studies in subjects planning to quit smoking will be needed to further define this time course.

We hypothesized that Ki-67 index would be increased in subjects with lung cancer or COPD. Contrary to our original hypothesis, Ki-67 index was not increased in subjects with lung cancer or COPD, after adjustment for smoking, gender, age, and race. We had expected that Ki-67 index would be increased in subjects with lung cancer as a reflection either of increased susceptibility to injurious and carcinogenic agents in tobacco smoke or a field effect of genetic damage to the airway epithelium. The rapid reversion of Ki-67 index to that of never smokers with smoking cessation is in contrast to the reduction of lung cancer risk with smoking cessation, which occurs gradually over many years and never completely normalizes. These findings raise concerns as to whether Ki-67 index will be a useful biomarker of lung cancer risk as well as with regard to its utility as an intermediate end point for chemoprevention studies, although it may be useful to monitor the effects of agents on bronchial epithelial proliferation. It is possible that a marker of apoptosis would be more informative as a risk biomarker.

We analyzed the relationship between Ki-67 index and histology on both a subject and a biopsy level, confirming previous reports that Ki-67 index increases with increasing levels of histologic dysplasia (14, 15). There was no significant difference in the relationship between histology and Ki-67 index for either lung cancer, COPD, or no disease groups.

There are several limitations to our analysis. First, the gender distribution in the subjects with COPD and lung cancer was predominantly male, reflecting the fact that many of those enrolled for clinically indicated bronchoscopy came from a Department of Veterans Affairs hospital. The size of the study population was limited, both by the numbers of appropriate subjects within our bronchoscopy cohort and by economic and time constraints in doing Ki-67 counts. The risk of a type II statistical error is elevated in a small study; however, in this study, we feel that the observed Ki-67 differences between disease groups are quite small (8% or less), and that clinically useful differences have been adequately ruled out (the confidence interval rules out differences larger than 20.4%). This is a cross sectional analysis. Only

### Table 5. Ki-67 associations with age, sex, disease, and smoking

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Unadjusted differences</th>
<th>Adjusted differences*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Effect (CI)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P*</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>113</td>
<td>34.7 (25.3)</td>
<td>-0.02 (-0.47, 0.44)</td>
<td>0.945</td>
<td>-0.16 (-0.63, 0.32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>77</td>
<td>39.9 (24.9)</td>
<td>Reference</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>36</td>
<td>23.6 (22.8)</td>
<td>-16.3 (-26.0, -6.6)</td>
<td>0.171</td>
</tr>
<tr>
<td>Lung disease</td>
<td>None</td>
<td>54</td>
<td>30.0 (23.6)</td>
<td>8.9 (-2.4, 20.1)</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>COPD</td>
<td>31</td>
<td>38.9 (27.5)</td>
<td>9.1 (-2.5, 20.7)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td>28</td>
<td>39.1 (25.4)</td>
<td>Reference</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Never</td>
<td>29</td>
<td>22.6 (20.3)</td>
<td>8.1 (-3.0, 19.1)</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>Former</td>
<td>44</td>
<td>30.6 (23.5)</td>
<td>25.4 (14.1, 36.7)</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>40</td>
<td>48.0 (25.1)</td>
<td>Reference</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NOTE: Results are shown as percentage of cells with Ki-67 nuclear expression.
*Adjusted for all other variables shown.
†P value for testing the equality of variables between all groups.

**Figure 2.** Relationship between Ki-67 index and histology, adjusted for gender, age, and smoking status. Displayed means are for a 60-yr-old male, never-smoker. Mean trends do not differ between disease groups. $F_{13,383} = 1.53; P$ value = 0.104.
a prospective longitudinal study will be able to fully confirm that Ki-67 index is not a biomarker of risk for either disease. However, the current results decrease our enthusiasm for Ki-67 index as a lung cancer or COPD risk biomarker. This study does not address the utility of Ki-67 as an intermediate end point in trials of chemoprevention agents, although again, we would be more enthusiastic about its use if there had been a strong association between Ki-67 and disease.

Conclusions

Ki-67 index is increased in current smokers, compared with ex- or never smokers and is higher in men than women. Ki-67 index seems to decrease quickly after smoking cessation. Ki-67 index increases with increasing histologic dysplasia. Ki-67 index is not elevated in individuals with lung cancer or COPD, after adjustment for smoking, age, and gender. The current study does not support Ki-67 index as a useful biomarker of lung cancer or COPD risk and casts doubt on its utility as a surrogate end point in chemoprevention trials.

References

Bronchial Epithelial Ki-67 Index Is Related to Histology, Smoking, and Gender, but Not Lung Cancer or Chronic Obstructive Pulmonary Disease

York E. Miller, Patrick Blatchford, Dae Sung Hyun, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/16/11/2425

Cited articles
This article cites 29 articles, 12 of which you can access for free at:
http://cebp.aacrjournals.org/content/16/11/2425.full.html#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
/content/16/11/2425.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.