Progression of Airway Dysplasia and C-Reactive Protein in Smokers at High Risk of Lung Cancer

Don D. Sin, S. F. Paul Man, Annette McWilliams, and Stephen Lam

Division of Respirology, Department of Medicine, University of British Columbia; James Hogg iCAPTURE Center for Cardiovascular and Pulmonary Research; and the Lung Tumor Group, British Columbia Cancer Agency, Vancouver, British Columbia, Canada

Rationale: **Chronic inflammation has been implicated in the development of airway dysplasia and lung cancer. It is unclear whether circulating biomarkers of inflammation could be used to predict progression of airway dysplasia.**

Objective: **We determined whether circulating levels of C-reactive protein (CRP) or other inflammatory biomarkers could predict progression of bronchial dysplasia in smokers over 6 mo.**

Methods: **The plasma levels of CRP, interleukins 6 and 8, and monocyte chemoattractant protein 1 were measured at baseline in 65 ex- and current smokers who had at least one site of bronchial dysplasia > 1.2 mm in size. Additional bronchial biopsies were taken after 6 mo from the same sites where dysplastic lesions were discovered at baseline. Progressive dysplastic lesions were defined as worsening of the dysplastic lesion by at least two grades or development of new dysplastic lesions.**

Results: **Half of the participants developed progressive dysplastic lesions after 6 mo. The baseline CRP levels in these participants were 64% higher than those without progressive disease ($p = 0.027$). Only one of eight (13%) participants with CRP ≤ 0.5 mg/L developed progressive disease, whereas 31 of 57 (54%) participants with CRP > 0.5 mg/L developed progressive disease ($p = 0.011$). The odds of developing progressive disease were 9.6-fold higher in the latter than in the former group.**

Conclusion: **Plasma CRP, in concert with lung function and pack-years of smoking, appears to have excellent predictive powers in identifying participants with bronchial dyplastic lesions whose lesions progress to more advanced stages of dysplasia.**

Keywords: airway dysplasia; C-reactive protein; lung inflammation

Lung cancer is a worldwide epidemic. More than 1 million people die of this disease annually (1). In the United States alone, 170,000 new cases of lung cancer are reported each year (2). Most of these are non–small cell lung cancer (NSCLC) and the overall prognosis once diagnosed is dismal (2). The only reasonable chance of cure for NSCLC is surgical resection for early-stage tumors (3). However, most patients with early lung cancer are often asymptomatic (4). Symptoms usually develop when the tumors become invasive or disseminated and curative resection is infeasible. In response, there has been a growing effort to discover novel (non- or semiinvasive) methods to identify individuals harboring precancerous lesions and to use a chemopreventive agent to prevent progression of these lesions to invasive carcinomas (5).

In animal models, chronic inflammation has been demonstrated to be an important risk factor for tumor genesis (6), and several susceptibility loci in mice for lung neoplasia also contain susceptibility genes for lung injury and inflammation. Several of these genes are homologous to the human asthma loci (7). Intriguingly, it has been known for decades that obstructive and fibrosing lung conditions associated with chronic inflammation, such as pulmonary fibrosis, sarcoidosis, and chronic obstructive pulmonary disease, also increase the risk of lung cancer (8–10). More recently, it has been shown that these conditions also present with systemic inflammation (11–13). The relationship between systemic inflammation and NSCLC is less clear. However, a recent study by McKeown and coworkers showed that, compared with healthy control subjects, individuals with NSCLC have significantly higher circulating levels of inflammatory markers, such as C-reactive protein (CRP) and interleukin 6 (IL-6) (14), and that the levels of these molecules correlate with prognosis (15). Whether CRP and other inflammatory cytokines are also elevated in asymptomatic individuals with precancerous bronchial lesions and whether these blood biomarkers can predict future progression of bronchial dysplasia are unknown. If these relationships exist, these data would be useful in designing future chemoprevention and early detection trials because blood biomarkers are relatively noninvasive and easily obtainable in a clinical setting. The primary purpose of the present study was to determine whether inflammatory biomarkers at baseline could predict progression of bronchial dysplasia over 6 mo.

METHODS

See online supplement for further details on methods used.

Subjects

We used data collected from a chemoprevention trial that evaluated the effects of budesonide in individuals at high risk of developing lung cancer (16). Briefly, individuals were 40 yr or older, had a smoking history of 30 pack-years or longer, had no comorbidities, and demonstrated sputum atypia. Bronchoscopies were performed and samples were taken from areas with abnormal fluorescence that were larger than 1.2 mm. Only subjects with dysplastic lesions larger than 1.2 mm were then invited to participate in the study. Half of the subjects were randomized to budesonide at a dose of 800 μg twice daily by inhalation or placebo for 6 mo. After 6 mo, all participants underwent a second fluorescence bronchoscopy and biopsies were obtained from the same sites biopsied at baseline plus any new sites suspicious of dysplasia or cancer. All study personnel were blinded to the study codes.

Bronchial Biopsies

The biopsy samples were fixed in buffered formalin, embedded in paraffin, and serially sectioned. Hematoxylin and eosin–stained sections were systematically reviewed by two pathologists who were blinded to intervention assignments (17). Two pathologists resolved minor (i.e., one grade) differences in sample classification by telephone consultation. If the histopathology diagnosis differed by two or more grades, both pathologists reviewed the slides again and reached a consensus diagnosis after verbal communication by phone or e-mail. For analytic purposes,

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Correspondence and requests for reprints should be addressed to Stephen Lam, M.D., British Columbia Cancer Agency, Cancer Imaging Department, 675 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3. E-mail: slam@bccancer.bc.ca

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progressive disease was defined as worsening of the dysplastic lesion present at baseline by two or more grades (e.g., mild dysplasia to severe dysplasia or worse) or development of new lesions that were mild dysplasia or worse. Everything else was classified as stable disease.

Plasma Measurements

The levels of cytokines and CRP in the plasma samples were determined using commercially available solid-phase sandwich enzyme-linked immunosorbent assay kits. All samples were measured in duplicate except CRP, which was measured in triplicate.

Statistical Analysis

The primary endpoint was the relationship between baseline CRP and progression of airway dysplasia; analyses involving all other inflammatory proteins were secondary. Plasma biomarkers were nonnormally distributed and were log-transformed to achieve normality. Multiple regression modeling was used to determine the independent relationship between these biomarkers and progression of disease. In this model, we adjusted for age, pack-years of smoking, FEV_1 as percent predicted, current smoking status, body mass index (BMI), treatment effect (budesonide vs. placebo), and sex. To construct a parsimonious model, we used a stepwise selection method in which variables were considered for further evaluation when their p value was 0.20 or less. From the logistic regression models, we determined the area under the receiver operating characteristic curve (also known as a C-statistic) to evaluate the predictive ability of each of the baseline variable in predicting progression of the dysplastic lesions over the 6-mo period (18). The C-statistic can range from 0.5 (prediction no better than chance) to 1.0 (perfect prediction); p values less than 0.05 (two-tailed) were considered significant. Continuous variables are presented as mean ± SD, unless otherwise indicated. The study was approved by the institutional review board at the University of British Columbia, and informed consent was obtained from participants.

RESULTS

Subjects

Of the 105 subjects who had completed the 6-mo study, plasma samples were available in 65 and were included in this analysis. The remaining subjects were excluded because we did not have sufficient volume of plasma in these subjects to perform the necessary biomarker measurements. There were no significant differences in the baseline characteristics or in the risk of progressive dysplastic lesions between those who were and were not included in the present study (Table 1). The mean age of the participants included in the analysis was 57 ± 8 yr. Of these, 49 (75%) were men and 48 (74%) were active smokers at the time of enrollment. On average, the participants had 52 ± 17 pack-years of smoking. The mean FEV_1 was 2.86 ± 0.81 L (83 ± 20% of predicted). Of the total participants, 27 (42%) had an FEV_1 to FVC ratio of less than 70% (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stages 1 or more [19]); 16 (25%) had an FEV_1 of less than 80% of predicted (GOLD stage 2 or more); and only three (5%) had an FEV_1 of less than 50% (GOLD stage 3 or more). Of these individuals, 32 (49%) had progressive dysplastic lesions in the bronchial biopsy after 6 mo, whereas 33 (50%) had stable lesions or lesions that regressed. The baseline characteristics of those with and without progressive lesions are summarized in Table 2. At baseline, those who developed progressive disease were more likely to be active smokers and had lower FEV_1 compared with those whose lesions did not progress.

Plasma Markers of Inflammation and Bronchial Dysplasia

Of the cytokines examined, only the baseline CRP differed between those who did and did not develop progression in the bronchial lesions (Table 3). On average, CRP levels were 64% higher in the group that had progressive disease ($p = 0.027$). Only one of eight (13%) individuals with CRP of 0.5 mg/L or less developed progressive disease, whereas 31 of 57 (54%) subjects with CRP of greater than 0.5 mg/L developed progressive disease. The odds of developing progressive disease were 9.6-fold higher in the group that had a CRP greater than 0.5 mg/L compared with the group with CRP levels less than this threshold ($p = 0.011$). At higher threshold (e.g., 1 mg/L), the relationships were much weaker, which may have been in part related to small sample sizes in these categories (Table 4). Other factors associated with progressive disease included having more pack-years of smoking and lower FEV_1 (Table 5). Interestingly, age, current smoking status, treatment status, and sex were not significantly associated with progressive disease. Baseline CRP, pack-years of smoking, and FEV_1, (as percent predicted) in concert had a C-statistic of 0.83, indicating that three variables had excellent predictive power in estimating progression of disease (18). In this model, baseline CRP ($p = 0.044$), %predicted FEV_1 ($p = 0.004$), and pack-years of smoking ($p = 0.033$) were all independently related to progression.

Among those with progressive disease, 17 (53%) subjects had worsening of dysplastic lesions present at baseline by two or more grades and 15 (47%) subjects developed new dysplastic lesions at follow-up. Log-CRP levels were significantly higher in those who had worsening of dysplastic lesions compared with those without progressive lesions (1.011 ± 0.174 vs. 0.348 ± 0.178 mg/L; $p = 0.011$). The log-CRP levels were slightly (but not significantly) higher in those with new dysplastic lesions compared with those without any dysplasia (0.652 ± 0.177 vs. 0.348 ± 0.178 mg/L; $p = 0.235$; *see* Table E1 of the online supplement).

TABLE 1. BASELINE CHARACTERISTICS OF SUBJECTS WHO WERE AND WERE NOT INCLUDED IN THE ANALYSIS

	Included	Not Included	p Value
No. subjects	65	40	
Age, yr	57 ± 8	58 ± 8	0.925
Men, %	49 (75)	27 (68)	0.380
Current smoker, %	48 (74)	33 (83)	0.305
Pack-years of smoking	52 ± 17	47 ± 17	0.172
FEV_1, L	2.86 ± 0.81	2.84 ± 0.78	0.879
FEV_1, % predicted	83 ± 20	85 ± 14	0.490
FEV_1/FVC, %	71 ± 10	72 ± 7	0.572
Budesonide, %	32 (49)	20 (50)	0.939
Progressive disease over 6 mo	32 (49)	18 (45)	0.673

Continuous data are presented as mean ± SD and dichotomous data are presented as number (% of column totals).

TABLE 2. BASELINE CHARACTERISTICS OF SUBJECTS ACCORDING TO WHETHER THEY HAD PROGRESSIVE OR NONPROGRESSIVE EPITHELIAL LESIONS

	Nonprogressive	Progressive	p Value
No. Subjects	33	32	
Age, yr	56 ± 7	59 ± 8	0.042
Men, %	23 (70)	26 (81)	0.280
BMI, kg/m^2	27.9 ± 5.6	28.1 ± 4.9	0.843
Current smoker, %	22 (67)	26 (81)	0.021
Pack-years of smoking	48 ± 14	57 ± 26	0.657
FEV_1, L	3.16 ± 0.71	2.61 ± 1.11	0.006
FEV_1, % predicted	92 ± 22	74 ± 26	0.001
FEV_1/FVC, %	73 ± 7	68 ± 12	0.014
Budesonide, %	18 (55)	14 (44)	0.384

Definition of abbreviation: BMI = body mass index.

Continuous data are presented as mean ± SD and dichotomous data are presented as number (% of column totals).

TABLE 3. BASELINE LEVELS OF PLASMA CYTOKINES AND C-REACTIVE PROTEIN IN INDIVIDUALS WHO DID AND DID NOT HAVE PROGRESSIVE LESIONS

	Nonprogressive Disease	Progressive Disease	Difference*	p Value
CRP, mg/L	0.348 ± 0.178 (1.42)	0.843 ± 0.127 (2.32)	0.500 ± 0.219 (↑64%)	0.027
IL-6, pg/ml	0.335 ± 0.852 (1.40)	0.630 ± 0.123 (1.88)	0.295 ± 0.193 (↑34%)	0.132
IL-8, pg/ml	2.39 ± 0.169 (10.91)	2.67 ± 0.167 (14.44)	0.286 ± 0.238 (↑33%)	0.234
MCP-1, pg/ml	3.84 ± 0.171 (46.53)	3.94 ± 1.44 (51.42)	0.103 ± 0.224 (↑11%)	0.647

Definition of abbreviations: CRP = C-reactive protein; IL = interleukin; MCP-1 = monocyte chemoattractant protein 1.

The data are presented as logarithmic mean ± SE, unless otherwise specified. The number in parentheses in the second and third columns represents the geometric mean of the plasma biomarker value.

* These data are interpreted as difference in mean ± SE between those who developed progressive disease and those whose lesions did not progress, and the number in parenthesis indicate the percent increase in biomarker values in those who developed progressive disease compared with those who did not.

Over the 6-mo period, the plasma levels of cytokines and CRP did not change significantly (*see* Figure E1). For CRP, the R^2 value was 58% ($p < 0.001$); for IL-6, it was 52% ($p < 0.001$); for IL-8, it was 66% ($p < 0.001$); and for monocyte chemoattractant protein 1 (MCP-1), it was 71% ($p < 0.001$). These data suggest that baseline cytokine measurements are robust and stable over 6 mo and as such the baseline levels may be used for prediction purposes.

DISCUSSION

The present study found a significant relationship between CRP levels at baseline and progression of dysplastic lesions in former and current smokers who were at a high risk of developing NSCLC. On average, CRP levels were 64% higher in those who developed progressive dysplastic lesions in bronchial biopsies compared with those who did not. The odds of developing progressive lesions were almost 10-fold higher in those with CRP levels exceeding 0.50 mg/L compared with those with levels lower than this threshold. Importantly, over a 6-mo period, CRP levels were remarkably stable in the study population, indicating that a single measurement is a reasonable surrogate of the CRP "burden" in these subjects. Another important observation in our study was that baseline CRP, coupled with age and pack-years of smoking, was associated with a C-statistic of 0.83, suggesting that knowing these three values may allow investigators to predict with a reasonable degree of confidence which subjects will experience progressive disease and which subjects will not. These data suggest that CRP levels, in conjunction with other simple clinical data, may be helpful in predicting which subjects with sputum atypia and bronchial dyplastic lesions will and will not progress to more advanced stages of dysplasia. However, before CRP levels can be routinely advocated for clinical settings, additional studies are needed to confirm these early findings and to better define the exact thresholds at which CRP levels can optimally risk-stratify patients.

It is not entirely clear whether CRP is intrinsically involved in the pathogenesis of airway dysplasia or whether it is an epiphenomenon of a generalized inflammatory state of subjects. To date, there is a marked paucity of data on this topic. In advanced stages of NSCLC, CRP rises, probably as an inflammatory reaction to the tumor (20). Whether similar reactions occur with bronchial dysplastic lesions is uncertain. There are murine models, which suggest that inflammation may be a predisposing factor for lung tumorigenesis, and antiinflammatory agents, such as glucocorticoids, may be effective in certain histologic subtypes of NSCLC (21, 22). Whether inflammation plays a material role in human NSCLC is much more controversial.

Interestingly, the present analysis also showed that reduced FEV_1 (as percent predicted) and pack-years of smoking were both independently associated with progression of disease. Cigarette smoking is a well-established risk factor for airway dysplasia and progression (23). The FEV_1 findings, on the other hand, are less well known but are consistent with previous reports. Our previous study showed that for every 1-L decrease in FEV_1, the odds of high-grade preinvasive lesion increased by approximately 50% (odds ratio, 1.49) (24). Papi and colleagues showed that the odds of squamous cell carcinoma increased by over fourfold when patients developed COPD (25). The mechanisms linking reduced FEV_1 with airway dysplasia and squamous cell carcinoma are uncertain. However, it is plausible that reduced

TABLE 4. SENSITIVITY AND SPECIFICITY OF VARIOUS THRESHOLD OF PLASMA C-REACTIVE PROTEIN LEVELS IN PREDICTING PROGRESSION OF DYSPLASTIC LESIONS

CRP threshold (*mg/L*)	Sensitivity	1-Specificity	p Value
> 0.50	96.8	78.8	0.011
> 0.75	93.8	70.0	0.024
> 1.00	84.4	66.6	0.110
> 1.25	84.4	57.6	0.038
> 1.50	75.0	51.5	0.055
> 2.00	56.3	48.5	0.641

Definition of abbreviation: CRP = C-reactive protein.

The p values have been adjusted for FEV_1 and sex.

TABLE 5. THE RELATIONSHIP BETWEEN PROGRESSION OF LESION AND VARIOUS BASELINE FACTORS

Variables	Odds Ratio (*95% CI*)	C-Statistic (*95% CI*)	p Value
CRP, mg/L	2.59 (1.21–5.52)	0.64 (0.51–0.78)	0.014
Pack-years*	2.34 (1.24–4.42)	0.62 (0.49–0.77)	0.009
FEV_1, % predicted*	0.40 (0.21–0.75)	0.69 (0.56–0.82)	0.005
Current smoker	2.94 (0.53–16.49)	0.57 (0.43–0.71)	0.218
Age, yr*	1.00 (0.54–1.88)	0.64 (0.51–0.78)	0.990
Men	1.22 (0.24–6.30)	0.56 (0.42–0.70)	0.812
BMI, kg/m^2	1.08 (0.92–1.10)	0.54 (0.39–0.68)	0.888
IL-6, pg/ml	1.23 (0.46–3.27)	0.67 (0.53–0.81)	0.686
IL-8, pg/ml	1.47 (0.68–3.18)	0.61 (0.47–0.75)	0.324
MCP-1, pg/ml	0.87 (0.40–1.89)	0.53 (0.39–0.68)	0.720

Definition of abbreviations: BMI = body mass index; CRP = C-reactive protein; IL = interleukin; MCP-1 = monocyte chemoattractant protein 1.

* Odds ratio reflects one quartile increase.

For biomarkers, the odds ratio reflects one unit increase in the logarithmic scale. For dichotomous variables, the odds ratio reflects the odds of developing progressive disease with the risk factor compared with those who did not have the risk factor.

FEV_1 may impair patients' ability to clear carcinogenic substances, leading to increased deposition of particulate matter in the larger bronchi, which may increase their risk for progression of airway dysplasia, regardless of other factors (26).

We also evaluated the potential relationship of bronchial dysplasia with other nonspecific markers of inflammation such as IL-6, IL-8, and MCP-1. We chose these markers because they have been implicated in the pathogenesis of smoking-induced lung diseases (27) and because they are readily measurable in plasma. However, none of these markers was significantly associated with progression of dypslastic lesions. It is possible that these inflammatory cytokines may not be involved in the progression of airway dysplasia. Alternatively, because these cytokines have shorter half-lives compared with CRP, and because the biochemical assays are less robust than those for CRP, the noise in the measurements may have precluded significant associations (28). Regardless of the exact cause, these biomarkers are likely to be less useful than CRP in predicting disease progression.

There are limitations to the study. First, we had limited plasma samples and as such could not evaluate other inflammatory cytokines. Second, the follow-up period was only 6 mo and as such our data cannot be generalized to longer time frames. Future studies are needed to determine whether baseline CRP levels have similar predictive powers over years of follow-up. Third, due to limited sample size, we could not adequately determine whether changes in CRP confer differential risks of disease progression. Studies addressing this question would require larger sample sizes and a longer period of follow-up. Fourth, our follow-up period was too short in determining the relationship between disease progression and the development of invasive carcinoma. A recent study by Breuer and colleagues found that 32% of individuals with severe airway dysplasia on bronchial biopsies went on to develop invasive squamous cell carcinoma or carcinoma *in situ* (CIS) over a median of 18 mo of follow-up (29). In contrast, only 9% of those with mild to moderate dysplasia developed squamous cell carcinoma or CIS. These data are consistent with the prevailing hypothesis that squamous cell carcinoma arises from preinvasive lesions in a stepwise fashion (i.e., sequential theory of cancer development) (30). This hypothesis is supported by animal experiments mimicking human carcinogenesis, demonstrating that squamous cell carcinoma develops along the pathway of squamous metaplasia, dysplasia (along various grades), CIS, and then to invasive carcinoma (31). Although imperfect, our definition of progressive disease in the context of the sequential theory of cancer development is likely relevant and useful for evaluating novel biomarkers of disease progression.

In summary, we found that CRP, FEV_1, and pack-years of smoking in concert have excellent predictive powers in identifying subjects with sputum atypia and bronchial dyplastic lesions who will and will not progress to a more advanced disease state. These data will be helpful in the design of future chemoprevention and early detection studies by identifying high-risk subjects for NSCLC. Future studies are needed to determine the precise role of CRP and other systemic biomarkers in the pathogenesis of NSCLC.

Conflict of Interest Statement: D.D.S. has received honoraria for speaking engagements from AstraZeneca (AZ) in 2003 ($4,000) and in 2004 ($3,000), and from GlaxoSmithKline (GSK) in 2003 ($4,000) and in 2004 ($8,000). He has also received unrestricted research funding as either the principal investigator or co-principal investigator from GSK in 2002 for $100,000, in 2003 for $80,000, and in 2004 for $1.5 million. He has also received $3,500 from GSK for consultancy work. S.F.P.M. has received as co-principal investigator a medical school grant from GSK ($140,000) and from Merck ($2.45 million) until 2003. A medical school grant has been approved by GSK (as co-principal investigator). Finally, he was invited to speak at an AZ-sponsored scientific meeting in April 2004. A.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.L. does not have a financial relationship with a commercial entity that has interest in the subject of this manuscript.

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