Progression of Airway Dysplasia And C-reactive Protein In Smokers At High Risk of Lung Cancer

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METHODS

Subjects

The primary purpose of the present study was to determine whether inflammatory biomarkers at baseline could predict progression of bronchial dysplasia over 6 months. We used data collected from a chemoprevention trial that evaluated the effects of budesonide in individuals at high risk of developing lung cancer. The details of subject recruitment have been previously reported (E1). In brief, potential subjects were recruited using television programs, radio broadcasts, and local newspapers between June 1, 2000, and November 1, 2001. To be invited for the study, individuals had to be 40 years of age or older, had a smoking history of at least 30 pack-years, and no co-morbidities. Individuals who met these criteria underwent sputum induction using simultaneous high-frequency chest wall oscillation with a ThAIRapy Vest (Advanced Respiratory, Inc., St. Paul, MN) and inhalation of 3% hypertonic saline from an ultrasonic nebulizer for 12 minutes. Individuals who demonstrated sputum atypia, defined as the presence of more than or equal to five cells having a DNA index > 1.2, subsequently underwent bronchoscopy to localize areas of dysplasia using the LIFE-Lung device (Xillix Technologies Corp., Richmond, British Columbia, Canada). Biopsy samples were taken from areas with abnormal fluorescence that were at least 1.2 mm in size, as well as from at least two control areas of normal fluorescence. Only subjects with dysplastic lesions > 1.2 mm were then invited to participate in the study. Half of the invited subjects were then randomized to budesonide (Pulmicort Turbuhaler; AstraZeneca, Lund, Sweden) at a dose of 800 μg twice daily by inhalation or placebo for 6 months. After the 6 months, all participants underwent a second fluorescence bronchoscopy and biopsies were obtained

from the same sites biopsied at baseline. Biopsy samples were also taken from new areas that displayed abnormal fluorescence. The bronchoscopist was blinded to the intervention assignment. All study personnel were blinded to the study codes, as was confirmed by an independent review.

Bronchial Biopsies

The median number of biopsy samples obtained per subject was 6 (range, 4–14 samples). The biopsy samples were fixed in buffered formalin, embedded in paraffin, and serially sectioned. H&E-stained sections were systematically reviewed by two pathologists who were blinded to intervention assignments. All biopsy samples were classified into one of the following seven groups (normal, basal cell hyperplasia, metaplasia, mild/moderate/severe dysplasia, or carcinoma *in situ*) according to World Health Organization criteria (E2). Because individual biopsies frequently contained more than one histologic cell type, the diagnosis was based on the most advanced histology present. 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, if necessary, reached a consensus diagnosis after verbal communication by phone or e-mail. For analytic purposes, 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

Plasma samples were taken from the subjects in the morning after overnight fasting using standard venipuncture techniques. Plasma samples were immediately separated from the rest of the blood components and were stored in -70^{O}C conditions. The samples were thawed once for measurement of plasma cytokines. The levels of cytokines and CRP in the plasma samples were determined by using commercially-available solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kits (Alpha Diagnostics, San Antonio, Tx for CRP and R and D Systems Inc, Minneapolis, MN for the other cytokines). All samples were measured in duplicate except CRP, which was measured in triplicate. The median interassay coefficient of variation was 5.2% for CRP; 7.8% for interleukin (IL)-6; 12.8% for IL-8; and 11.2% for monocyte chemoattractant protein (MCP)-1.

Statistical Analysis

The baseline characteristics of subjects who did and did not have progressive disease were compared using a Student t test for continuous variables and a chi-square test for dichotomous variables. Plasma CRP and cytokines were non-normally distributed and for analytic purposes, were log-transformed to achieve normality. Multiple regression modeling was used to determine the independent relationship between these cytokines and progression (or non-progression) of disease. In this model, we adjusted for age, pack-years of smoking, forced expiratory volume in one second (FEV_1) as percent predicted, current smoking status, body mass index (BMI), treatment effect (budesonide versus placebo) and gender. 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 (a generous threshold) to ensure that predictive variables were not excluded

prematurely in the analysis. In the final model, we included variables only if the p-value was less than 0.10. The candidate variables were age (in quintiles), gender, current smoking status (current versus former smoker), BMI, treatment effect, pack-years of smoking (in quintiles), FEV_1 % predicted (in quintiles), and plasma cytokines (CRP, IL-6, IL-8, MCP-1). From the logistic regression models, we determined the area under the receiver operating characteristic curve (also known as a C statistic) in order to evaluate the predictive ability of each of the baseline variable in predicting progression of the dysplastic lesions over the 6 month period (E3). The C statistic can range from 0.5 (prediction no better than chance) to 1.0 (perfect prediction). We also evaluated the C statistic in a multi-variate analysis using the variables retained in the stepwise regression method described previously. P-values less than 0.05 (two-tailed) were considered significant. Continuous variables are presented as mean±SD, unless otherwise indicated.

REFERENCES

E1. Lam S, leRiche JC, McWilliams A, Macaulay C, Dyachkova Y, Szabo E, Mayo J, Schellenberg R, Coldman A, Hawk E, Gazdar A. A randomized phase IIb trial of pulmicort turbuhaler (budesonide) in people with dysplasia of the bronchial epithelium. *Clin Cancer Res* 2004;10:6502-6511.

E2. Brambilla E, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. *Eur Respir J* 2001;18:1059-1068.

E3. Hosmer DW, Lemeshow W. Applied logistic regression. 2nd ed. New York, NY: John Wiley & Sons; 2000.

Figure Legend

Figure 1E. C-Reactive Protein (CRP) Levels At Baseline And At 6 Months Of Follow-Up In Subjects Who Did And Did Not Have Progressive Dysplastic Lesions On Bronchial Biopsies

Data are presented as mean±SE

*$p<0.05$ comparison of CRP levels between subjects with and without dysplastic lesions on bronchial biopsies at each time point.

Table E1. Baseline Levels Of Plasma Cytokines and C-Reactive Protein In Various Categories of Disease Progression

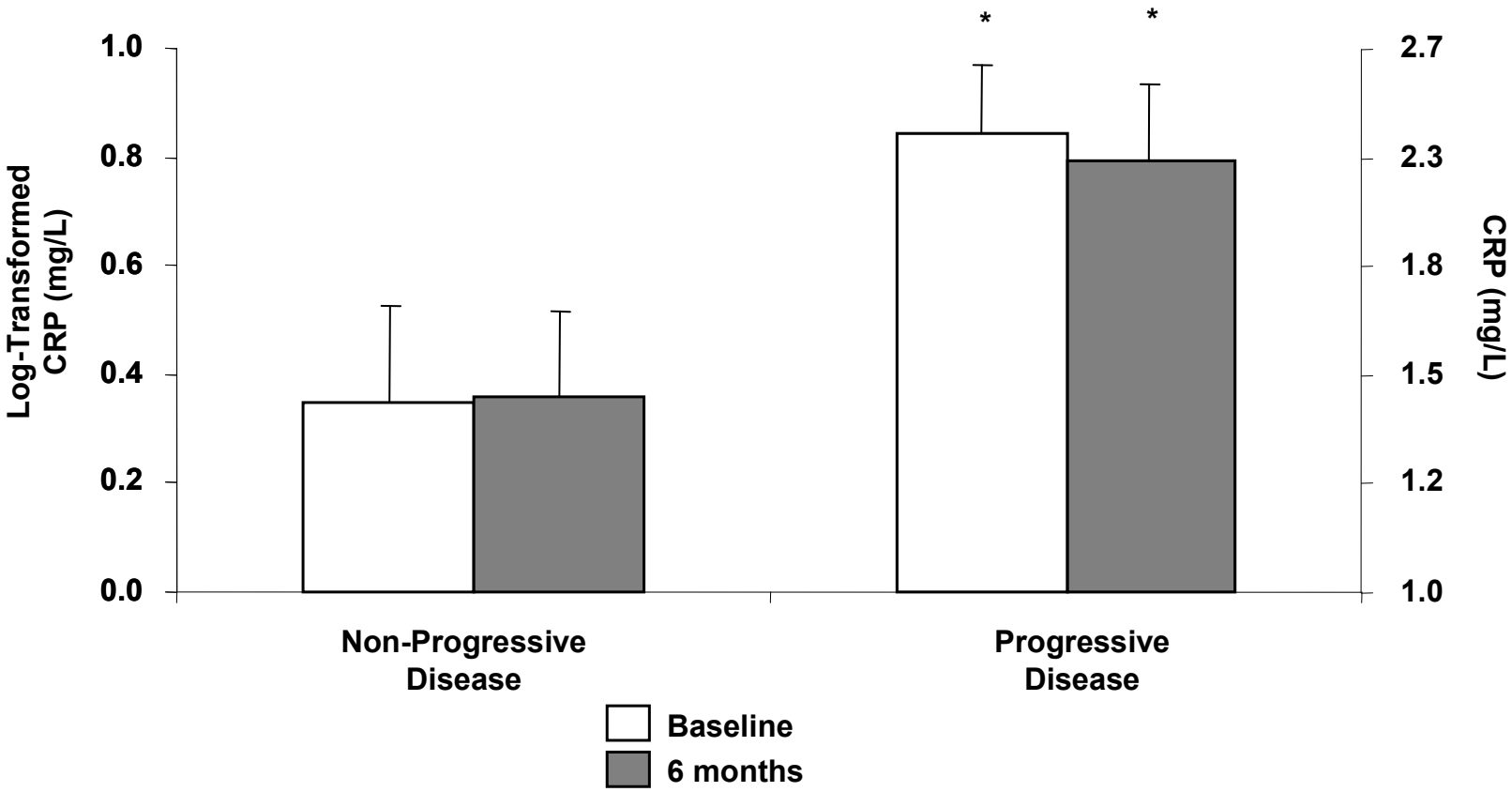
The data are presented as logarithmic mean±SE

	Non-Progressive Disease	Progression of Existing Lesion	Development of New Lesion
CRP, mg/L	0.348±0.178	**1.011±0.174***	0.652±0.177
Il-6, pg/mL	0.335±0.852	0.632±0.148	0.627±0.207
Il-8, pg/mL	2.39±0.169	2.53±0.235	2.83±0.238
MCP-1, pg/mL	3.84±0.171	3.84±0.171	3.80±0.165

*p=0.011 versus non-progressive disease; all other comparisons are non-significant.

Abbreviations: CRP, C-reactive protein; Il, interleukin; MCP, monocyte chemoattractant protein

Figure 1E.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*
1.0
0.8
0.6
0.4
0.2
0.0
Log-Transformed CRP (mg/L)
2.7
2.3
1.8
1.5
1.2
1.0
CRP (mg/L)
Non-Progressive Disease
Progressive Disease
Baseline
6 months