Sputum Cytological Atypia as a Predictor of Incident Lung Cancer in a Cohort of Heavy Smokers with Airflow Obstruction[1]

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Abstract

Individuals with cytological atypia in sputum may be at increased risk for lung cancer. We conducted a longitudinal analysis of the association between lung cancer incidence and cytological atypia in sputum samples collected prospectively from an ongoing cohort of adults at high risk for lung cancer. Cohort members had a smoking history of ≥30 pack-years and chronic obstructive pulmonary disease documented by pulmonary airflow testing. Sputum samples collected at baseline and periodically thereafter were examined by standard cytological methods. From the cohort of 2006 people, there were 83 incident lung cancers over 4469 person-years of observation. At baseline, the association between personal and behavioral characteristics, and sputum cytological atypia was assessed by multiple logistic regression. The association between sputum cytological atypia and incident lung cancer was then assessed by hazard ratios using proportional hazards regression analysis, adjusting for potential confounding factors. Cytological atypia graded as moderate or worse was associated with continuing cigarette smoking (adjusted odds ratio, 2.5; 95% confidence interval, 1.5–4.1), and with lower levels of intake of fruits and vegetables (*P* for trend = 0.04). Atypia was not associated with several other factors, including the degree of airflow obstruction, the use of vitamin supplements, nonsteroidal anti-inflammatory drugs, or metered-dose steroid inhalers. Incident lung cancer was increased among those with moderate or worse cytological atypia (adjusted hazards ratio, 2.8; 95% confidence interval, 1.4–5.5). This association was not confounded by other risk factors. We conclude that in this high-risk cohort, cytological atypia is associated with continuing smoking and low intake of fruits and vegetables, but that independent of these and other factors, the risk of incident lung cancer is increased among those with moderate or worse grades of cytological atypia in their sputum.

Introduction

Mortality from lung cancer is greater than the mortality of the next three most common cancers combined (breast, prostate, and colon cancers; Ref. 1). The overall 5-year survival rate for lung cancer is <15%, attributable largely to the late stage at which most patients present and the lack of effective treatments for systemic disease. Earlier diagnosis of lung cancer using helical, low-dose CT[3] radiological imaging is now under intensive investigation (2, 3). CT imaging is likely more sensitive for lesions in the peripheral lung fields than for lesions in the central airways, so biomarkers for lung cancer risk that might be identifiable in the sputum could complement radiological imaging. Identifying people at high lung cancer risk based on sputum findings could then indicate bronchoscopic evaluation for the identification of preneoplasia to lead to more intensive follow-up, enrollment into chemoprevention trials, or finding early invasive lung cancers, which can be effectively treated with surgical resection (4).

The idea that the cytological appearance of bronchial epithelial cells exfoliated into the sputum might predict lung cancer risk comes from the concept that bronchial epithelial cytology changes as cells progress along the multistep pathway from inflammation to lung cancer (4). Findings in support of this idea include the frequent finding of bronchial squamous metaplasia and atypia of exfoliated cells in association with invasive lung cancer, and the observations from several case series that patients with a diagnosis of moderate atypia or worse on sputum cytology may be at high risk for future development of lung cancer (5–7). In the Johns Hopkins Lung Project, 14% (86 of 626) of the participants with a diagnosis of moderate sputum cytological atypia or worse later progressed to lung cancer as compared with only 3% (147 of 4600) of those without aytpia (7). However, the clinical utility of sputum cytological atypia is uncertain, as previous randomized, controlled trials focused on the identification of cancer cells in sputum failed to demonstrate reduced lung cancer mortality (8).

In the current era, in which both CT imaging and fluorescence bronchoscopy hold promise for early lung cancer detection (2, 9), and new chemoprevention agents are being developed (10), it is reasonable to again consider whether biomarkers

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[3] The abbreviations used are: CT, computed tomography; FEV1, forced expiratory volume in 1 s; FEVC, forced expiratory vital capacity NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; CI, confidence interval.

of lung cancer risk identifiable in the sputum might be helpful to identify people who could benefit from more intensive diagnostic examination and/or who could be enrolled into lung cancer chemoprevention trials. The University of Colorado Specialized Program of Research Excellence in Lung Cancer initiated a cohort study to assess whether sputum cytology or other molecular biomarkers in sputum might be early predictors of lung cancer risk. The cohort was defined as adults at high lung cancer risk because of tobacco smoking history and the presence of chronic obstructive pulmonary disease (11–16). This is a report of the findings of analyses conducted within this cohort to assess the factors that are associated with cytological atypia, as well as to assess the association between atypia and lung cancer incidence over the first 7 years of the cohort study.

Materials and Methods

The University of Colorado Cancer Center Sputum Screening Cohort Study is an ongoing prospective study initiated in 1993 to determine whether exfoliated sputum cytological atypia or other molecular biomarkers identifiable within sputum might predict future lung cancer development. The study methodology has been described previously (17). Briefly, subjects have been recruited from community and academic pulmonary clinics primarily in the Denver, Colorado metropolitan area. At the time of enrollment all of the subjects were ages 25 years or older, with a cigarette smoking history of 30 or more pack-years, and with pulmonary air flow obstruction documented by a spirometry finding of FEV1 of $\leq$75% than predicted for age and an FEV1/FEVC ratio of $\leq$0.75. Excluded were those who had a diagnosis of cancer within 5 years before the time of recruitment (excluding nonmelanoma skin cancer), a current acute respiratory infection, or who were judged by their physician to have a life expectancy of $<$5 years.

Patient demographic information, tobacco history, and pulmonary function test results were obtained by direct interview and medical record review at the time of enrollment. From 1993 through 1997, the risk factors measured at baseline were limited to selected personal medical conditions, pulmonary function, and tobacco history. Beginning in July, 1997, a self-administered questionnaire ascertained additional information on smoking status, occupational history, current use of medications including vitamin supplements, inhaled corticosteroids, and NSAIDs, and the frequency of fruit and vegetable intake using a validated six-item questionnaire (18). That questionnaire asks about the frequency of intake during the past year of fruit juices such as orange, grapefruit, or tomato; fruit, not counting juice; green salad; potatoes, not including french fries, fried potatoes, or potato chips; carrots; and vegetables, not counting potatoes, carrots, or green salads. The frequency options were never or less than once per month; 1–3 times/month; once per week; 2–4 times/week; 5–6 times/week; once per day; twice per day; and 3 or more times per day. All of the newly enrolled subjects after July 1997 and previously enrolled subjects who returned for a routine annual follow-up sputum sample completed this expanded questionnaire.

There were 2550 patients enrolled into the cohort as of July 1, 2001, but 537 of those enrollees (21%) did not submit at least one sputum specimen for analysis, and 7 (0.3%) had lung cancer diagnosed at the time of the baseline examination. Therefore, this analysis is limited to those 2006 cohort members who were enrolled in the cohort between 1993 and July 1, 2001, who submitted at least one sputum sample for cytological analysis, and were not found to have lung cancer at the time of enrollment. The study protocol was approved by the Colorado Multi-Institutional Review Board.

Sputum Collection, Processing, and Interpretation. Participants were provided with two containers filled with a fixative solution of 2% carbowax and 50% alcohol, and instructed to collect an early morning, spontaneous cough sputum specimen for 6 consecutive days: 3 days into the first container and 3 into the second. The second 3-day pooled sputum samples were those examined in this study (19). Specimens were blended, then centrifuged at 1500 rpm for 15 min. Four slide smears were then prepared from the resuspended cell pellets and were air-dried, fixed with 95% alcohol, and stained using the Papanicolaou technique.

Slides were independently screened by trained cytotechnologists and cytopathologists as not adequate for diagnosis, normal, squamous metaplasia, mild atypia, moderate atypia, severe atypia, or carcinoma (17). When interpretation discrepancies occurred, the cytotechnologist and cytopathologist met to achieve a consensus opinion on specimen adequacy, level of inflammation, and diagnosis. To assess inter-reader reliability of cytology diagnosis among cytopathologists, a total of 86 samples representative of each diagnostic category underwent an initial and a second blinded quality control review with an overall categorical agreement of 85% (κ statistic 0.80; $P < 0.001$; Ref. 17).

For data analysis, the sputum cytology diagnoses were first categorized into five groups: (*a*) unreadable specimen (containing no informative bronchial epithelial cells); (*b*) normal or squamous metaplasia; (*c*) mild atypia; (*d*) moderate atypia; or (*e*) worse than moderate atypia (severe atypia or carcinoma). We used moderate or worse cytological atypia as the principal exposure to be comparable with previous studies and to define a single cut-point for screening abnormality.

Cohort Follow-Up. Cohort members who had provided a sputum sample in the previous year were mailed a postcard once a year to ask for continued participation by providing another sputum sample. Other cohort members were followed by active methods such as mail, and by passive methods such as the matching to the Colorado Department of Public Health and Environment Vital Statistics records (through June 1, 2001) and the National Death Index (searched through December 31, 1999). The Colorado Central Cancer Registry was used to identify incident cases among those subjects who had signed an appropriate consent form permitting cancer registry linkage (January, 1, 1993 to July, 1, 2001). Among the 2006 cohort members in this analysis there were 721 documented deaths and 83 documented incident lung cancers. The date of diagnosis was imputed for the 45 cases ascertained only by death certificate, based on the median 6-month time between diagnosis and death observed in the remainder of the cases in the cohort.

Data Analysis. The association between behavioral risk factors and atypia was assessed among the approximately half of the analytic cohort subjects ($n = 1005$) who had completed at least one full behavioral risk factor questionnaire and had a sputum sample adequate for interpretation collected within 24 months of questionnaire completion. Former smokers were defined as those individuals who had quit smoking 1 year or more at the time of questionnaire completion. Pack-years of cigarette smoking at enrollment was defined as the average number of packs smoked per day multiplied by the number of years of smoking. To assess fruit and vegetable intake an index variable based on the number of servings per month was created from the six-question food frequency instrument. Categorical tables were first examined, with χ^2 testing of associations.

Table 1 Characteristics of 2006 cohort members and the association between selected factors and cytological finding from the baseline sputum sample, University of Colorado Lung Cancer Specialized Program of Research Excellence cohort, 1993–2001[a]

Cohort characteristics			Baseline sputum cytological reading											
			Unreadable		Normal or squamous metaplasia		Mild atypia		Moderate atypia		Worse than moderate atypia		Moderate atypia or worse	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Total cohort	2006	100	234	11.7	438	21.8	980	48.8	322	16.1	32	1.6	354	17.7
Gender														
Female	597	29.7	82	13.7	138	23.1	290	48.6	82	13.7	5	0.8	87	14.6
Male	1409	70.3	152	10.8	300	21.3	690	49.0	240	17.0	27	1.9	267	19.0
Age														
30–59 years	424	21.1	36	8.5	86	20.3	221	52.1	74	17.5	7	1.7	81	19.1
60–69 years	793	39.4	88	11.1	181	22.8	390	49.2	122	15.4	12	1.5	134	16.9
70+ years	789	39.3	110	13.9	171	21.7	369	46.8	126	16.0	13	1.7	139	17.6
Race/ethnicity														
Caucasian	1867	93.1	215	11.5	418	22.4	910	48.7	297	15.9	27	1.5	324	17.4
Hispanic	61	3.0	8	13.1	6	9.8	31	50.8	15	24.6	1	1.6	16	26.2
African American	53	2.6	7	13.2	9	17.0	23	43.4	10	18.9	4	7.6	14	26.4
Asian, Native American, Other	25	1.3	4	16.0	5	20.0	16	64.0	0	0.0	0	0.0	0	0.0
Lifetime smoking														
Quartile 1 < 43 pack-years	448	22.3	47	10.5	99	22.1	235	52.5	61	13.6	6	1.3	67	15.0
Quartile 2 43–54 pack-years	474	23.6	60	12.7	102	21.5	214	45.2	88	18.6	10	2.1	98	20.7
Quartile 3 55–80 pack-years	591	29.5	70	11.8	132	22.3	289	48.9	95	16.1	5	0.9	100	16.9
Quartile 4 ≥ 81 pack-years	493	24.6	57	11.6	105	21.3	242	49.1	78	15.8	11	2.2	89	18.1
Smoking status														
Current smoker	819	40.8	70	8.6	154	18.8	428	52.3	150	18.3	17	2.1	167	20.4
Former smoker	1187	59.2	164	13.8	284	23.9	552	46.5	172	14.5	15	1.3	187	15.8

Next, logistic regression models were examined to assess the association between moderate atypia or worse and other risk factors. First, univariate models were examined, then multivariate models adjusting for the most important covariates. Associations were expressed as ORs and their corresponding 95% CI.

Each potential risk factor for lung cancer incidence was examined first by univariate categorical analysis, then by proportional hazards regression analysis (20) and Kaplan-Meier plots. Proportional hazards regression analyses were conducted in three stages: first using crude models for the association between sputum atypia and lung cancer risk, then using multivariate models, adjusting for the potential confounding factors of age, sex, and year of enrollment into the cohort, then in full models that also added smoking status and pack-years of smoking history at enrollment. Cohort members were included in the analysis from the age at the first sputum sample, then censored at the time of their death, lung cancer incidence, or the last date of contact if lost to follow-up on December 31, 2001 (the close of the cohort follow-up period for this analysis). Sensitivity analyses were conducted in which time of censoring was alternatively assumed to be on December 31, 1999 (the last day covered by the National Death Index search) for all of the members lost to follow-up. Findings from that analysis were unchanged from the analysis shown here, indicating that the assumption of date of censoring for those lost to follow-up did not influence the findings. Two sets of regression models were examined, one in which we considered only the first sputum sample collected at baseline, and one in which we used information from both that baseline sample and up to five subsequent sputum samples (the maximum number obtained from any case), using a time-varying covariate analysis of the repeated measures of sputum cytology (20). All of the analyses were carried out using Statistical Analysis Software (SAS, version 8.1; SAS Institute, Inc., Cary, NC).

Results

The cohort was predominantly older (79% age 60 or older), Caucasians (93%), males (70%), and former smokers (59%); and cohort members had a substantial history of tobacco use, with 54% reporting >55 pack-years of tobacco use (Table 1). A finding of moderate atypia or worse in the population was apparent among 17.7% of the population at the time of the baseline sputum sample.

The association between atypia and other risk factors was assessed in detail among the 1005 cohort members who had filled out a full risk factor questionnaire (Table 2). Moderate atypia or worse was found more common among continuing smokers (OR, 2.5; 95% CI, 1.5–4.1), but there was no association with lifetime cigarette pack-years or with severity of airflow obstruction in the multivariate models (Table 2). There was also no relationship between moderate atypia or worse and the use of NSAIDs (OR, 1.2, 95% CI, 0.7–1.9) or metered-dose steroid inhalers (OR, 1.2; 95% CI, 0.7–1.8); but fruit and vegetable intake was found to be inversely associated with atypia (P for trend = 0.04).

There were 83 cases of incident lung cancer in the analytic cohort over the period of follow-up (Table 3). The incidence rate of lung cancer was 1.86 per 100 person-years in the entire analytic cohort (95% CI, 1.46–2.26). Among those 537 cohort members who were excluded from this analysis because they did not submit a sputum sample, there were 21 incident lung cancers, for a rate similar to that of the cohort members who did contribute sputum samples, included in this analysis. Lung cancer incidence per 100 per-years was higher among males

Table 2 The association between selected risk factors and sputum cytology in the baseline sample from the 1010 subjects who completed a full risk factor questionnaire, University of Colorado Lung Cancer Specialized Program of Research Excellence cohort, 1993–2001.

Risk factor	Total sample n^a (%)	Sputum cytology[b]			Odds ratios for moderate atypia or worse, compared to normal cytology	
		Normal *n* (%)	Squamous metaplasia and mild atypia *n* (%)	Moderate atypia or worse *n* (%)	Crude odds ratio (95% confidence interval)	Multivariate-adjusted odds ratio[c] (95% confidence interval)
All study subjects	1005 (100)	261 (26.0)	602 (59.9)	142 (14.1)		
Gender						
Female	284 (28.3)	77 (27.1)	171 (60.2)	36 (12.7)	1.0	1.0
Male	721 (71.7)	184 (25.5)	431 (59.8)	106 (14.7)	1.23 (0.78–1.96)	1.15 (0.69–1.91)
Age						
30–59 years	213 (21.2)	41 (19.2)	138 (64.8)	34 (16.0)	1.0	1.0
60–69 years	378 (37.4)	102 (27.1)	231 (56.7)	61 (16.2)	0.72 (0.41–1.26)	0.79 (0.44–1.44)
70+ years	416 (41.4)	118 (28.4)	251 (60.3)	47 (11.3)	0.48 (0.27–0.85)	0.65 (0.35–1.22)
					$p_{trend} = 0.008$	$p_{trend} = 0.27$
Smoking status						
Former smoker	701 (70.1)	212 (30.3)	406 (57.9)	83 (11.8)	1.0	1.0
Current smoker	299 (29.9)	49 (16.4)	191 (63.9)	59 (19.7)	3.08 (1.95–4.85)	2.50 (1.52–4.13)
Use of vitamin supplements						
No	372 (37.0)	88 (23.6)	222 (59.7)	62 (16.7)	1.0	1.0
Yes	633 (63.0)	173 (27.3)	380 (60.0)	80 (12.7)	0.65 (0.43–0.998)	1.01 (0.63–1.61)
Use of NSAIDs						
No	285 (28.4)	86 (30.2)	157 (55.1)	42 (14.7)	1.0	1.0
Yes	720 (71.6)	175 (24.3)	445 (61.8)	100 (13.9)	1.17 (0.75–1.82)	1.15 (0.70–1.89)
Use of steroid inhalers						
No	468 (46.6)	129 (27.6)	263 (56.2)	76 (16.2)	1.0	1.0
Yes	537 (53.4)	132 (24.6)	339 (63.1)	66 (12.3)	0.85 (0.56–1.28)	1.16 (0.73–1.84)
Fruit and vegetable servings per month						
Quartile 1 (<47 servings)	254 (25.6)	57 (22.5)	153 (60.2)	44 (17.3)	1.0	1.0
Quartile 2 (47–74 servings)	248 (25.1)	62 (25.0)	151 (60.9)	35 (14.1)	0.73 (0.41–1.30)	1.02 (0.55–1.89)
Quartile 3 (75–110 servings)	245 (24.8)	68 (27.7)	144 (58.8)	33 (13.5)	0.63 (0.36–1.11)	0.84 (0.45–1.57)
Quartile 4 (≥111 servings)	242 (24.5)	70 (28.9)	147 (60.8)	25 (10.3)	0.46 (0.25–0.85)	0.60 (0.31–1.16)
					$p_{trend} = 0.01$	$p_{trend} = 0.04$

[a] In some cases, total number is <1005 because of missing data.
[b] All $P > 0.01$ by χ^2 across all categories except for age ($P = 0.03$) and smoking status ($P < 0.0001$).
[c] Adjusted for age (continuous), race, sex, current smoking status, enrollment date (continuous), FEVI (continuous), and fruit and vegetable servings (continuous).

(1.98; 95% CI, 1.49–2.47), continuing smokers (2.20; 95% CI, 1.52–2.88) and in those with the highest quartile of pack-year smoking histories (2.65; 95% CI, 1.72–3.59). Lung cancer incidence was also associated with sputum atypia. There was little association between mild atypia and lung cancer risk, but a moderately elevated risk was observed among those with moderate cytologic atypia (incidence rate ratio, 1.7), and substantially elevated risk among those with greater than moderate atypia (incidence rate ratio, 18.1).

The independence of the association between sputum atypia and lung cancer risk was then assessed in the two sets of multivariate models (Table 4). Those models showed that the association between sputum cytology and lung cancer risk is largely independent of age, sex, time of enrollment, and tobacco use. Analyses using up to six sputum samples per person from the repeated measures of sputum cytology indicate somewhat stronger associations with lung cancer risk than are seen in the analyses using only the baseline sputum samples. When a single cut point is used to define an abnormal sputum screening test (moderate atypia or worse), the adjusted hazard ratio for lung cancer risk is 3.18 (95% CI, 1.60–6.31). Additional models limited to the 1005 cohort members who had completed the full risk factor questionnaire showed that the association between cytological atypia and lung cancer incidence was also not confounded by fruit and vegetable intake, vitamin supplement use, NSAIDS, or steroid inhalers (data not shown).

The time to diagnosis of lung cancer for those with moderate or worse atypia is displayed in the Kaplan-Meier curves in Fig. 1, which show that the cumulative lung cancer incidence among those with moderate atypia or worse reaches 10% at ~3 years and 20% at ~6 years.

Discussion

This study provides evidence that the pattern of risk factors for sputum cytological atypia, particularly moderate atypia or worse, resembles that for behavioral factors thought to be risk factors for lung cancer; and that cytological atypia predicts higher subsequent risk for lung cancer incidence, independent of other risk factors. Our finding that current smokers had an increased risk for atypia is consistent with the relationship between smoking status and lung cancer. However, we did not see a relationship between lifetime pack-years of cigarette smoking, number of packs of cigarettes smoked per day, or years since quitting. It is quite likely that we could not see much of relationship in our study population because all of the subjects were heavy smokers, most with a cigarette history of over 55 pack-years. Another possibility is that perhaps after one acquires a certain degree of airflow obstruction, there is no additional increased risk from amount of tobacco consumption. A cohort analysis by Tockman *et al.* (12) is consistent with the latter explanation. In that analysis, the cumulative lifetime amount of cigarette consumption (pack-years) had little additional effect on lung cancer risk after adjustment was made

Table 3 Lung cancer incidence as related to selected factors among the 2006 members of the University of Colorado Lung Cancer Specialized Program of Research Excellence cohort, 1993–2001

	Person-years	Lung cancer cases	Lung cancer incidence rate per 100 person-years (95% confidence interval)	Incidence rate ratio (95% confidence interval)
Total cohort	4469.37	83	1.86 (1.46, 2.26)	
Gender				
Female	1236.98	19	1.54 (0.85, 2.23)	1.0 (reference)
Male	3232.25	64	1.98 (1.49, 2.47)	1.29 (0.77, 2.15)
Age				
30–59 years	887.01	14	1.58 (0.75, 2.40)	1.0 (reference)
60–69 years	1854.03	30	1.62 (1.04, 2.20)	1.03 (0.54, 1.93)
70+ years	1729.49	39	2.26 (1.55, 2.96)	1.43 (0.76, 2.63)
Race/ethnicity				
Caucasian	4198.88	79	1.88 (1.47, 2.30)	1.0 (reference)
Hispanic	119.38	1	0.84 (0, 2.48)	0.45 (0.06, 3.20)
African American	112.04	3	2.68 (0, 5.70)	1.42 (0.45, 4.51)
Asian, Native American, other	40.58	0	—	—
Lifetime smoking history				
Quartile 1 < 43 pack-years	877.63	11	1.25 (0.51, 1.99)	1.0 (reference)
Quartile 2 43–54 pack-years	1077.88	18	1.67 (0.90, 2.44)	1.33 (0.63, 2.82)
Quartile 3 55–80 pack-years	1345.71	23	1.71 (1.01, 2.41)	1.36 (0.66, 2.80)
Quartile 4 ≥ 81 pack-years	1168.90	31	2.65 (1.27, 3.59)	2.12 (1.06, 4.21)
Smoking status				
Current smoker	1818.99	40	2.20 (1.52, 2.88)	1.36 (0.88, 2.09)
Former smoker	2651.76	43	1.62 (1.14, 2.11)	1.0 (reference)
Baseline sputum cytology				
Unreadable	615.42	6	0.97 (0.19, 1.76)	0.76 (0.28, 2.06)
Normal	860.67	11	1.28 (0.52, 2.03)	1.0 (reference)
Mild atypia	2064.86	32	1.55 (1.01, 2.09)	1.21 (0.61, 2.41)
Moderate atypia	864.25	19	2.20 (1.21, 3.19)	1.72 (0.82, 3.61)
Worse than moderate atypia	64.96	15	23.09 (11.41, 34.78)	18.07 (8.30, 39.34)
Moderate atypia or worse	929.25	34	3.66 (2.43, 4.89)	2.86 (1.45, 5.65)

Table 4 Association between moderate or worse cytological atypia in sputum and lung cancer incidence among the 2006 members of the University of Colorado Lung Cancer Specialized Program of Research Excellence cohort, 1993–2001

	Crude model	Adjusted for age, sex, recruitment year	Adjusted for age, sex, recruitment year, pack-years, and smoking status
	Hazard ratio (95% CI)[a]	Hazard ratio (95% CI)[a]	Hazard ratio (95% CI)[a]
Initial baseline sputum			
Unreadable	0.76 (0.28, 2.06)	0.79 (0.29, 2.14)	0.81 (0.30, 2.20)
Normal	1.00 (reference)	1.00 (reference)	1.00 (reference)
Mild atypia	1.20 (0.61, 2.39)	1.20 (0.60, 2.40)	1.16 (0.58, 2.32)
Moderate atypia	1.64 (0.78, 3.48)	1.67 (0.79, 3.54)	1.66 (0.79, 3.53)
Worse than moderate atypia	18.4 (8.30, 40.6)	19.1 (8.53, 42.9)	17.7 (7.84, 39.8)
Moderate atypia or worse	2.77 (1.40, 5.50)	2.80 (1.41, 5.56)	2.75 (1.38, 5.47)
First six sputum samples			
Unreadable	0.61 (0.19, 1.93)	0.63 (0.20, 2.00)	0.65 (0.21, 2.06)
Normal	1.00 (reference)	1.00 (reference)	1.00 (reference)
Mild atypia	1.13 (0.57, 2.24)	1.13 (0.57, 2.26)	1.10 (0.55, 2.20)
Moderate atypia	1.67 (0.78, 3.57)	1.71 (0.80, 3.66)	1.68 (0.79, 3.60)
Worse than moderate atypia	33.2 (15.3, 71.7)	34.3 (15.7, 74.9)	31.4 (14.2, 69.4)
Moderate atypia or worse	3.27 (1.66, 6.46)	3.32 (1.68, 6.57)	3.18 (1.60, 6.31)

[a] Hazard ratio, as estimated from proportional hazards analysis; CI, confidence interval.

for impaired ventilation as measured by FEV1 among heavy smokers.

Our finding that a diet rich in fruits and vegetables is associated with a lower risk of sputum cytological atypia is in agreement with the inverse association between dietary fruit and vegetable intake and lung cancer risk (21). However, we observed no significant relationship between vitamin supplementation and atypia in multivariate models. We hypothesized that use of metered-dose steroid inhalers would reduce the risk of sputum cytological atypia, because suppression of inflammation could reduce lung cancer risk. Administration of aerosolized budesonide in rodents has been associated with a reduction in lung cancer incidence (22), but no observational studies have yet addressed the relationship between steroid administration and lung cancer or sputum cytological atypia. We did not find a relationship between current use of metered-dose steroid inhalers and moderate atypia or worse. Cyclooxygenase inhibitors such as NSAIDs have been shown in exper-

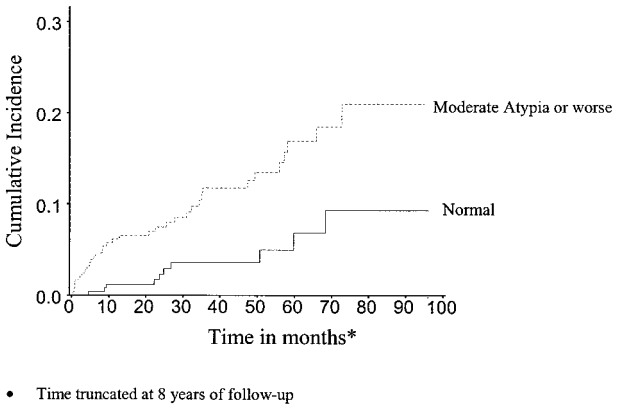

Fig. 1. Time to diagnosis of lung cancer by sputum cytology findings among 2006 members of the University of Colorado Lung Cancer Specialized Program of Research Excellence Cohort.

imental animal models to reduce lung cancer incidence (23). Limited observational data linking NSAID use to lung cancer incidence (24, 25) or mortality (26, 27) are inconsistent, as two studies found no relationship (24, 26) and two found a protective effect (25, 27). We did not find current use of NSAIDs to be associated with sputum cytology.

This prospective study of individuals at high risk for lung cancer has shown that continuing cigarette smoking and lower levels of fruit and vegetable intake are associated with a higher grade of sputum cytological atypia, which, in turn, is associated with increased lung cancer risk. Clearly, sputum cytological findings of severe atypia or frank cancer cells may be markers of prevalent cancer, because the time to cancer diagnosis is very short for such individuals. However, also important is the observation that lower grades of atypia, particularly moderate atypia, are associated with an ~2-fold increase in risk for incident lung cancer. We suspect the process of field cancerization in the respiratory epithelium explains this observation (4). As respiratory epithelial cells become mutated from tobacco exposures, clonal selection can result in wide areas of respiratory epithelium carrying mutations that increase the risk of subsequent mutational changes leading to lung cancer. The observation that moderate atypia is associated with an increase in lung cancer risk may suggest that there are morphological changes in cytology that correlate with field carcinogenesis. Although some lung cancer risk factors such as continued smoking and fruit and vegetable intake are related to atypical sputum cytology in our cohort, it is important to note that those factors do not account for the relationship between cytological atypia and lung cancer risk that we observed in this study, as shown by the very small changes in hazard ratios in the multivariate as compared with the univariate models. Although other unmeasured or poorly measured risk factors for lung cancer could confound this relationship, their joint associations with cytology and lung cancer are likely weak enough to make such confounding unlikely.

Additional studies to examine the molecular correlates of cytological atypia and the molecular predictors in the sputum of lung cancer risk are needed. Techniques such as automated image cytometry may be helpful to improve and objectify cytopathology reading (28). Additionally, studies to extend this work to include molecular markers in sputum are promising (29). For example, we are trying to improve on the ability of cytology to predict lung cancer by examining patterns of methylation of genes important in the control of cell growth as detected in sputum (30).

Our findings of increased risk for incident lung cancer with sputum atypia are consistent with earlier observations from the National Cancer Institute Cooperative Early Lung Cancer Detection Program and others (31–35). The strength of this study is in its prospective design, because the sputum samples were all obtained before the diagnosis of lung cancer. Clearly, however, some of the incident lung cancers were present at the time of the initial sputum collection, and are, thus, best regarded as prevalent screen-detected cancers. The major weakness of this study is also the weakness of the entire field of cytology: the uncertainties inherent in pattern recognition and classification by cytopathologists (36, 37). The subjectivity of sputum cytological classifications has been a hindrance to this field of work. Importantly, however, because these readings were all done before diagnosis of lung cancer, the cytological readings were not biased relative to the diagnosis, and any misclassification in the readings likely biased these findings only toward the null. Because sputum cytology interpretation can be difficult and subject to misclassification, we are currently conducting a study to define the inter- and intracytopathologist reproducibility among various institutions. Other limitations of our study include the potential for misclassification of the risk factor status of an individual because the measurement of the behavioral risk factors was determined by self-report. Self-reports can lead to misclassification on factors such as nutritional supplements or medications, as well as diet.

In conclusion, this prospective study of individuals at high risk for lung cancer demonstrates that moderate or worse sputum cytological atypia predicts increased lung cancer risk. We suspect that these findings are attributable both to the exfoliation of cytologically abnormal cells associated with early lung cancer and by the exfoliation of cells with moderate atypia from wider areas of abnormal fields of respiratory epithelium involved in the field cancerization stages of the development of lung cancer. Additional studies of the relationships between both the cytological and molecular markers of lung cancer risk in sputum are warranted. If sputum cytology can identify those at substantially higher risk for lung cancer, with a cumulative incidence of 10% in 3 years and 20% in 6 years, this would constitute a group at substantively higher risk than those identified previously in other cohorts (38). Risk this high might justify more intense surveillance and/or chemoprevention that may be clinically useful. Conducting additional studies of the utility of sputum cytology and of other genetic and epigenetic changes in sputum in the context of CT screening trials now underway will be an excellent opportunity to better understand the basic biology of lung carcinogenesis. This research will better define the role, if any, of sputum screening as a complement to radiological imaging enhance the early detection of lung cancer.

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Sputum Cytological Atypia as a Predictor of Incident Lung Cancer in a Cohort of Heavy Smokers with Airflow Obstruction

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