

Circulating Markers of Interstitial Lung Disease and Subsequent Risk of Lung Cancer

Meredith S. Shiels¹, Anil K. Chaturvedi¹, Hormuzd A. Katki¹, Bernadette R. Gochoico², Neil E. Caporaso¹, and Eric A. Engels¹

Abstract

Background: Inflammation and pulmonary diseases, including interstitial lung diseases, are associated with increased lung cancer risk. Circulating levels of surfactant protein-D (SP-D) and Krebs von Lungren-6 (KL-6) are elevated in interstitial lung disease patients and may be useful markers of processes contributing to lung cancer.

Methods: We conducted a nested case-control study, including 532 lung cancer cases, 582 matched controls, and 150 additional controls with chest X-ray (CXR) evidence of pulmonary scarring, in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Serum SP-D and KL-6 levels were measured using enzyme immunoassay. Logistic regression was used to estimate the associations of SP-D and KL-6 with lung cancer and CXR scarring.

Results: Cases had higher levels than controls for SP-D (median 118.7 vs. 105.4 ng/mL, $P = 0.008$) and KL-6 (372.0 vs. 325.8 $\mu\text{g/mL}$, $P = 0.001$). Lung cancer risk increased with SP-D ($P_{\text{trend}} = 0.0003$) and KL-6 levels ($P_{\text{trend}} = 0.005$). Compared with the lowest quartile, lung cancer risk was elevated among those with the highest quartiles of SP-D (OR = 1.87, 95% CI: 1.32–2.64) or KL-6 (OR = 1.58, 95% CI: 1.11–2.25). Among controls, participants with CXR scarring were more likely than those without scarring to have elevated levels of SP-D (quartile 4 vs. quartile 1: OR = 1.67, 95% CI: 1.04–2.70, $P_{\text{trend}} = 0.05$) but not of KL-6 (OR = 1.04, 95% CI: 0.64–1.68, $P_{\text{trend}} = 0.99$).

Conclusion: Circulating levels of SP-D and KL-6 are associated with subsequent lung cancer risk.

Impact: Our findings support a potential role for interstitial lung disease in lung cancer etiology or early detection, but additional research is needed. *Cancer Epidemiol Biomarkers Prev*; 20(10); 2262–72. ©2011 AACR.

Introduction

Lung cancer is the most common malignancy and causes the greatest number of cancer-related deaths worldwide, with 1.61 million new cases and 1.38 million deaths in 2008 (1). Tobacco has been well established as the major cause of lung cancer for more than 50 years (2), increasing lung cancer risk 15- to 30-fold (3). More recently, studies have examined the role of inflammation in the etiology of lung cancer (4). Both infectious and noninfectious lung diseases that cause extensive and

prolonged pulmonary inflammation are associated with an increased risk of lung cancer. Pulmonary tuberculosis, *Chlamydia pneumoniae* infection, chronic bronchitis, and emphysema have each been associated with increased lung cancer risk (5–10). Furthermore, increased lung cancer risk is observed in people with asymptomatic pulmonary scarring (11) and with interstitial lung diseases, such as idiopathic pulmonary fibrosis (12–14).

Idiopathic pulmonary fibrosis and other interstitial lung diseases are associated with measurably elevated serum levels of 2 glycoproteins: surfactant protein-D (SP-D) and Krebs von Lungren-6 (KL-6; refs. 15, 16). SP-D is a component of pulmonary surfactant, plays a role in innate immunity, and modulates inflammation in the lung (16). SP-D levels are also elevated in patients with chronic obstructive pulmonary disease (COPD), particularly during COPD exacerbations (17, 18). KL-6 is a glycoprotein classified as a MUC1 mucin (15). It has been used as a diagnostic marker for interstitial lung diseases (19) and is expressed by a number of adenocarcinomas, including lung adenocarcinoma (15). SP-D and KL-6 are both produced by pulmonary alveolar type II cells, which proliferate during lung injury (20). In addition, SP-D is expressed by Clara cells, and KL-6 is expressed by

Authors' Affiliations: ¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville; and ²Medical Genetics Branch, National Human Genome Research Institute, Bethesda, Maryland

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Corresponding Author: Meredith S. Shiels, Infections and Immunoepidemiology Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, EPS 7059, Rockville, MD 20852. Phone: 301-402-5374; Fax: 301-402-0817; E-mail: shielsms@mail.nih.gov

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respiratory bronchiolar epithelial cells and serous cells of the bronchial glands (15, 16). Although increased serum levels of SP-D and KL-6 have been evident for specific lung diseases (15–18, 21–23), little is known about their utility in detecting more limited lung damage among relatively healthy people.

Although lung cancer risk is elevated in people with clinically evident lung diseases, it is unknown whether subclinical levels of lung damage and disease are associated with development of lung cancer. In this study, we measured serum levels of SP-D and KL-6 in lung cancer cases and controls from the screening arm of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Our goal was to evaluate a largely healthy population, to assess whether low levels of lung damage or disease increase subsequent lung cancer risk. We carefully adjusted for cigarette smoking to assess the independent associations of SP-D and KL-6 with lung cancer risk.

Methods

Study population and laboratory methods

Study participants were enrolled in the PLCO trial, which recruited approximately 155,000 people from the general population at 10 U.S. screening centers from 1992 to 2001 (24). Participants aged 50 to 74 years were randomized to receive either screening for prostate, lung, colorectal, and ovarian cancers or routine health care. Participants were excluded if they were undergoing treatment for cancer or had a history of prostate, lung, colorectal, or ovarian cancer. Lung cancer screening included a chest X-ray (CXR) at baseline and yearly follow-up intervals. In addition, baseline blood samples were obtained and demographic, behavioral, and dietary information was collected via questionnaire. Lung cancer cases were ascertained through periodic self-report and death certificate review and confirmed by medical chart abstraction.

Our case-control study was nested within the screening arm of PLCO (Supplementary Fig. S1). Of the 898 lung cancer cases that occurred during PLCO follow-up through December 31, 2004, we included 532 in our analysis. Cases were excluded if they had a missing baseline questionnaire ($n = 17$), a history of cancer at baseline ($n = 57$), multiple cancers during follow-up ($n = 88$), incomplete tobacco use information ($n = 11$), and if they did not give consent for their samples to be used ($n = 31$) or did not have an available serum specimen ($n = 162$). Controls (i.e., "matched controls;" $n = 582$) were matched to cases on the basis of following criteria: age at randomization (55–59, 60–64, 65–69, and 70–74 years), sex, year of randomization (1993–1995, 1996–1997, 1998–1999, and 2000–2001), length of follow-up during the study (1-year interval), smoking status (current, former, and never), cumulative smoking at baseline for current and former smokers (0–29, 30–39, 40–49, and 50+ pack-years), and time since quitting for former smokers (<15

and 15+ years). We selected 1 control for each case who was a current or former smoker, and 3 controls for each case who was a never-smoker.

As part of the PLCO trial, study radiologists blinded to participant characteristics determined whether radiological findings interpreted from CXRs were suggestive of lung cancer (11). Furthermore, radiologists recorded other CXR abnormalities on trial evaluation forms, including the presence and location of "scarring/pulmonary fibrosis/honeycombing." Although the matched controls described above included some subjects with evidence of pulmonary scarring on baseline CXRs ($n = 53$), we selected 150 additional such controls (i.e., "scarred controls," including 50 controls with right lung scarring, 50 controls with left lung scarring, and 50 controls with bilateral scarring). These scarred controls were matched to the original "matched controls" by age, sex, and smoking status.

Enzyme immunoassays were used to measure levels of SP-D (BioVendor, LLC), KL-6 (SankoJanyaku Co., Ltd), and a third marker, e-selectin (Bender MedSystems, Inc.), in serum samples collected from participants at PLCO baseline. We also included 10 serum samples collected from patients with biopsy-confirmed idiopathic pulmonary fibrosis as positive controls. Average SP-D levels in positive controls were 318.5 ng/mL compared with 46.3 ng/mL in healthy blood donors, and average KL-6 levels in positive controls were 1,465 µg/mL compared with 276 µg/mL in healthy blood donors. Because e-selectin levels were not found to be elevated in patients with idiopathic pulmonary fibrosis (not shown), we did not assess this biomarker further. Mean coefficients of variation within each plate calculated from 50 quality control samples tested in pairs on the same plate were 17.3% for SP-D and 18.3% for KL-6. KL-6 was tested in single runs and SP-D was tested in duplicate. Duplicate SP-D measurements for each participant were averaged for analysis. In a previous study (25), high-sensitivity C-reactive protein (CRP) was measured in baseline serum samples using a chemiluminescent immunoassay (Diagnostic Products Corporation).

The study was approved by Institutional Review Boards at the National Cancer Institute and the National Human Genome Research Institute, and all participants provided written informed consent.

Statistical analysis

Participant characteristics were compared between cases and matched controls using the χ^2 test and tests of medians (26). Geometric mean levels of SP-D and KL-6 were also compared across participant characteristics among matched controls. With data from the cases and matched controls, unconditional logistic regression was used to measure the associations of SP-D and KL-6 with lung cancer risk, adjusting for the matching factors. SP-D and KL-6 levels were categorized into quartiles based on the distribution of these markers in controls. In an additional logistic regression model, we also included

Table 1. Characteristics of study subjects from the PLCO Cancer Prevention Trial

	Lung cancer cases, n (%)	Matched controls, n (%)	P
Total	532 (100)	582 (100)	
Age at randomization, y			
55–59	96 (18)	104 (18)	0.68
60–64	145 (27)	163 (28)	
65–69	182 (34)	182 (31)	
70–74	109 (20)	133 (23)	
Sex			
Female	169 (32)	205 (35)	0.22
Male	363 (68)	377 (65)	
Randomization year			
1993–1995	220 (41)	246 (42)	0.99
1996–1997	164 (31)	175 (30)	
1998–1999	109 (20)	120 (21)	
2000–2001	39 (7)	41 (7)	
Race			
White	470 (88)	525 (90)	0.06
Black	42 (8)	28 (5)	
Asian	9 (2)	6 (1)	
Pacific Islander	9 (2)	21 (4)	
American Indian	2 (0.4)	2 (0.3)	
Smoking status			
Never	33 (6)	109 (19)	<0.001 ^c
Current	208 (39)	206 (35)	
Former	291 (55)	267 (46)	
Cumulative smoking, pack-years ^a			
1–30	126 (25)	119 (25)	0.95
30–40	136 (27)	126 (27)	
40–50	42 (8)	45 (10)	
50–230	195 (39)	183 (39)	
Years since quitting ^b			
0.5–15	185 (64)	167 (63)	0.80
15–53	106 (36)	100 (37)	
BMI			
14.0–18.5	6 (1)	3 (0.5)	0.54
18.6–25.0	184 (35)	189 (33)	
25.1–30.0	231 (44)	269 (47)	
30.1–52.5	104 (20)	112 (20)	
Regular aspirin use			
No	235 (44)	270 (46)	0.51
Yes	294 (56)	312 (54)	
Regular ibuprofen use			
No	393 (74)	401 (69)	0.06
Yes	138 (26)	181 (31)	
History of chronic bronchitis			
No	472 (89)	539 (93)	0.02
Yes	59 (11)	42 (7)	
History of emphysema			
No	470 (89)	550 (95)	<0.001
Yes	61 (11)	31 (5)	
Scarring on baseline chest X-ray			
No	461 (87)	529 (91)	0.02
Yes	71 (13)	53 (9)	

^aAnalysis was limited to former and current smokers only.

^bAnalysis was limited to former smokers only.

^cBy design, an excess of controls who were never smokers were included.

baseline CXR scarring and levels of both SP-D and KL-6. We evaluated models separately according to lung cancer histology (squamous cell carcinoma, adenocarcinoma, small-cell carcinoma, and large-cell carcinoma). Further analyses were carried out stratified by time from baseline blood collection to lung cancer/control selection or by smoking status. As controls were matched to cases on follow-up time, time from baseline was defined among controls as time from baseline blood collection to the date of cancer diagnosis in the matched case. We also utilized linear regression to assess the association between time from baseline and levels of SP-D and KL-6 among cases.

In a control-only analysis (both matched and scarred controls), we assessed the associations of SP-D and KL-6 levels with CXR scarring using unconditional logistic regression, adjusted for age, sex, smoking status, time since quitting, cumulative smoking, and randomization year. Associations with any scarring, unilateral scarring, and bilateral scarring were examined in separate models.

Results

Table 1 presents the characteristics of 532 lung cancers and 582 matched controls in our study. Lung cancers were predominantly adenocarcinomas ($n = 189$) and squamous cell carcinomas ($n = 114$) but also included 68 small-cell carcinomas and 30 large-cell carcinomas. No significant differences were observed for the matching characteristics, with the exception of smoking status, where by design we included an excess of controls who were never-smokers. As reported previously (11), cases were more likely to have CXR evidence of scarring than controls (71 cases vs. 53 controls, $P = 0.02$). Cases were also more likely than controls to have a history of emphysema or chronic bronchitis at baseline ($P \leq 0.001$ and 0.02 , respectively).

Table 2 describes levels of SP-D and KL-6 among matched controls. Age was not significantly associated with levels of SP-D or KL-6, but men had significantly higher levels of both SP-D and KL-6 compared with women. SP-D levels were also higher in whites than other racial groups. Significant differences in SP-D were observed by smoking status, with the highest levels observed in current smokers, but no significant trends in SP-D were observed with cigarettes smoked per day, pack-years smoked, or years since smoking cessation among former smokers. SP-D levels decreased with increasing body mass index (BMI) and with regular ibuprofen use. KL-6 was positively associated with increasing CRP levels, and there was a borderline positive association for SP-D. KL-6 levels did not vary significantly by measures of smoking, and neither marker differed according to history of chronic bronchitis or emphysema. KL-6 and SP-D were correlated with each other among controls ($r = 0.30$, $P < 0.0001$).

Cases had higher serum levels than controls for both SP-D (median 118.7 vs. 105.4 ng/mL, $P = 0.008$) and KL-6

Table 2. Levels of SP-D and KL-6 among matched controls in the PLCO Cancer Screening Trial

	SP-D (ng/mL)			KL-6 (µg/mL)		
	Geometric mean	95% CI	P ^c	Geometric mean	95% CI	P ^c
Age at randomization, y						
≤59	94.6	85.2–105.0	0.08	344.6	314.9–377.2	0.26
60–64	105.3	96.9–114.5		337.1	313.7–362.4	
65–69	101.0	93.3–109.3		342.1	319.6–366.3	
70+	110.6	100.8–121.3		366.4	338.2–396.8	
Gender						
Male	108.1	102.3–114.2	0.004	361.4	344.8–378.9	0.003
Female	94.5	87.7–101.8		320.8	300.9–341.9	
Race						
White	108.6	103.8–113.5	<0.0001	347.7	334.0–362.0	0.38
Black	74.2	61.1–90.0		372.4	312.9–443.2	
Asian	57.2	37.7–86.9		297.8	204.5–433.8	
Pacific Islander	56.6	45.3–70.7		316.1	258.6–386.5	
American Indian	41.6	20.2–85.7		214.3	111.8–411.0	
Smoking status						
Never	92.9	83.9–102.9	0.03	334.2	306.0–365.0	0.41
Current	110.3	102.4–118.8		341.0	319.8–363.6	
Former	102.1	95.7–109.0		356.1	336.6–376.8	
Cigarettes smoked per day ^a						
10–20	101.3	95.4–107.5	0.91	348.1	330.7–366.4	0.57
21–30	106.1	96.6–116.6		345.3	318.3–374.5	
31–40	104.3	91.9–118.4		342.2	306.9–381.5	
41–100	105.4	91.2–121.8		346.2	305.7–392.1	
Cumulative smoking, pack-years ^a						
1–30	93.3	84.6–102.9	0.06	360.6	331.2–392.7	0.66
30–40	113.0	102.8–124.3		335.4	308.8–364.4	
40–50	104.0	88.7–121.9		387.9	337.7–445.5	
50–230	109.7	101.4–118.7		343.3	320.5–367.6	
Years since quitting ^b						
0.5–15	104.0	95.8–112.9	0.47	357.4	332.0–384.8	0.87
15–53	99.0	89.1–110.1		354.0	321.8–389.4	
BMI						
14.0–25.0	111.5	103.2–120.4	0.03	353.5	330.7–378.0	0.38
25.1–30.0	99.4	93.2–106.1		345.9	326.9–366.0	
30.1–52.5	98.0	88.6–108.4		336.7	308.5–367.4	
History of chronic bronchitis						
No	102.6	97.9–107.4	0.41	346.1	332.6–360.1	0.73
Yes	110.2	93.4–130.0		355.2	308.1–409.5	
History of emphysema						
No	102.5	97.9–107.3	0.33	346.9	333.6–360.8	0.73
Yes	113.1	93.3–137.1		336.9	285.4–397.5	
Regular aspirin use						
No	101.3	94.9–108.2	0.48	348.7	329.7–368.8	0.77
Yes	104.7	98.5–111.2		344.7	327.2–363.2	
Regular ibuprofen use						
No	106.2	100.7–112.0	0.05	350.6	334.8–367.1	0.38
Yes	96.5	89.2–104.5		337.8	315.4–361.7	
CRP, mg/L						
0.30–0.99	97.5	88.6–107.2	0.11	321.2	296.0–348.4	0.04
1.0–2.4	102.7	94.4–111.8		351.0	326.3–377.5	
2.5–5.3	102.8	94.1–112.2		345.3	320.2–372.3	
5.4–147.0	109.0	99.8–119.1		366.4	339.7–395.3	

^aAnalysis was limited to former and current smokers only.

^bAnalysis was limited to former smokers only.

^cP value is for overall difference or trend.

Table 3. Associations of serum levels of SP-D and KL-6 with subsequent risk of lung cancer

	Lung cancer cases, <i>n</i> (%)	Matched controls, <i>n</i> (%)	Adjusted OR ^a (95% CI)	Mutually adjusted OR ^b (95% CI)
SP-D, ng/mL				
16.5–71.7	97 (18)	146 (25)	1.00	1.00
71.8–105.3	120 (23)	145 (25)	1.25 (0.87–1.79)	1.19 (0.83–1.72)
105.4–143.8	122 (23)	146 (25)	1.17 (0.81–1.68)	1.13 (0.79–1.63)
143.9–615.5	193 (36)	145 (25)	1.87 (1.32–2.64)	1.77 (1.24–2.51)
			<i>P</i> _{trend} = 0.0003	<i>P</i> _{trend} = 0.001
KL-6, µg/mL				
178.8–231.0	98 (18)	145 (25)	1.00	1.00
231.1–325.7	113 (21)	146 (25)	1.11 (0.77–1.59)	1.08 (0.75–1.56)
325.8–465.7	160 (30)	145 (25)	1.66 (1.17–2.35)	1.60 (1.13–2.28)
465.8–4,225.9	161 (30)	146 (25)	1.58 (1.11–2.25)	1.43 (1.00–2.05)
			<i>P</i> _{trend} = 0.005	<i>P</i> _{trend} = 0.03

^aSeparate models were used for each marker. Estimates were adjusted for age, sex, smoking status, time since quitting, cumulative smoking, and randomization year.

^bA single model included quartiles of both markers. Estimates were adjusted for age, sex, smoking status, time since quitting, cumulative smoking, randomization year, and scarring on baseline chest X-ray.

(372.0 vs. 325.8 µg/mL, *P* = 0.001). As shown in Table 3, lung cancer risk increased with SP-D level (*P*_{trend} = 0.0003), and participants with levels in the fourth quartile of SP-D had nearly twice the risk of lung cancer compared with the first quartile (OR = 1.87, 95% CI: 1.32–2.64). Likewise, lung cancer risk increased with KL-6 levels (*P*_{trend} = 0.005, Table 3), and participants with KL-6 levels in the highest quartile had 60% higher risk than participants in the lowest quartile (OR = 1.58, 95% CI: 1.11–2.25). Further adjustment for history of emphysema or chronic bronchitis did not alter the associations between the markers and lung cancer (results not presented). In models that additionally included quartiles of CRP, we observed independent associations with lung cancer for SP-D (*P*_{trend} = 0.0006) and CRP (*P*_{trend} = 0.003) and for KL-6 (*P*_{trend} = 0.01) and CRP (*P*_{trend} = 0.003). In a model that included SP-D, KL-6, and CXR scarring, SP-D and KL-6 each was independently associated with lung cancer risk (*P*_{trend} = 0.001 and 0.03, respectively, Table 3). In addition, we dichotomized SP-D and KL-6 at the median and examined joint categories. Compared with those below the median for both SP-D and KL-6, those with higher levels of both markers had double the risk of lung cancer (OR = 2.01, 95% CI: 1.43–2.83). No significant interactions were observed between SP-D and KL-6 (*P*_{interaction} = 0.24), SP-D and CXR scarring (*P*_{interaction} = 0.91), or KL-6 and CXR scarring (*P*_{interaction} = 0.87).

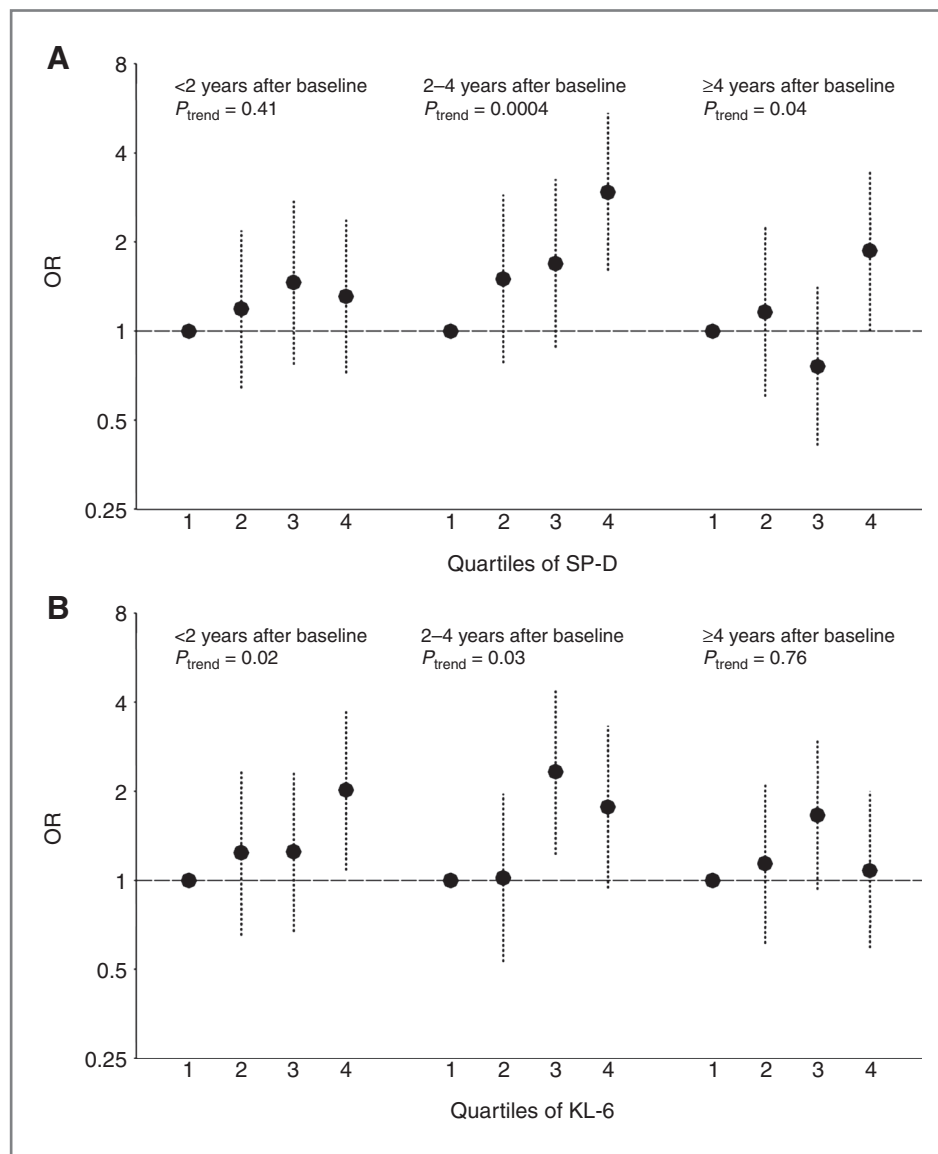
In addition, we examined the association of SP-D and KL-6 with lung cancer stratified by the time following the baseline blood collection (Fig. 1A and B). For SP-D, no association with lung cancer risk was observed within the 2 years after blood collection (quartile 4 vs. quartile 1: OR = 1.31, *P*_{trend} = 0.41). However, significant associations were observed after blood collection in the 2.0- to 4.0-year

period (quartile 4 vs. quartile 1: OR = 2.95, *P*_{trend} = 0.0004) and 4.1- to 10.6-year period (OR = 1.87, *P*_{trend} = 0.04). In contrast, associations between KL-6 and lung cancer risk were observed in the time periods 0.03 to 1.9 years (quartile 4 vs. quartile 1: OR = 2.02, *P*_{trend} = 0.02) and 2.0 to 4.0 years (OR = 1.77, *P*_{trend} = 0.03) following blood collection, but no association was observed 4.1 years or more after blood collection (OR = 1.08, *P*_{trend} = 0.76). Tests for interaction with time from baseline were not significant for SP-D (*P*_{interaction} = 0.16) or KL-6 (*P*_{interaction} = 0.15). Similarly, when the analysis was limited to lung cancer cases, KL-6 levels were significantly higher (*P* = 0.0003) in cases arising 0.03 to 1.9 years after blood collection compared with 4.1 years or more after blood collection. In contrast, SP-D levels tended to be lower (*P* = 0.10) in cases 0.03 to 1.9 versus 4.1 years or more after blood collection.

Associations between the serum markers and specific histologic types of lung cancer were statistically significant for adenocarcinoma (quartile 4 vs. quartile 1: SP-D: OR = 1.65, *P*_{trend} = 0.008 and KL-6: OR = 1.74, *P*_{trend} = 0.008) and squamous cell carcinoma (quartile 4 vs. quartile 1: SP-D: OR = 4.22, *P*_{trend} = 0.0002 and KL-6: OR = 1.82, *P*_{trend} = 0.01, Fig. 2A and B). No significant trends were observed for small-cell (quartile 4 vs. quartile 1: SP-D: OR = 1.72, *P*_{trend} = 0.21 and KL-6: OR = 1.29, *P*_{trend} = 0.77) or large-cell carcinomas (quartile 4 vs. quartile 1: SP-D: OR = 1.13, *P*_{trend} = 0.42 and KL-6: OR = 2.36, *P*_{trend} = 0.85).

When stratified by smoking status, associations were observed between SP-D levels and lung cancer among current smokers (quartile 4 vs. quartile 1: OR = 2.05, *P*_{trend} = 0.01) and former smokers (OR = 2.00, *P*_{trend} = 0.004), but not among never-smokers (OR =

Figure 1. ORs and 95% CIs for lung cancer across quartiles of SP-D (A) and KL-6 (B), stratified by time since baseline blood collection (<2.0, 2.0–4.0, and 4.1+ years) are presented. P values for trend across quartiles are also presented.



0.73, $P_{\text{trend}} = 0.75$, Fig. 3A and B). The association between KL-6 and lung cancer was limited to current smokers (quartile 4 vs. quartile 1: OR = 2.17, $P_{\text{trend}} = 0.005$), with no associations observed among former (OR = 1.24, $P_{\text{trend}} = 0.26$) or never-smokers (OR = 1.44, $P_{\text{trend}} = 0.53$). Nonetheless, tests of interaction with smoking status were not significant for SP-D ($P_{\text{interaction}} = 0.81$) or KL-6 ($P_{\text{interaction}} = 0.79$).

Among controls, those with CXR scarring had marginally higher SP-D levels than those without scarring (median 105.0 vs. 113.9 ng/mL, $P = 0.12$), and as shown in Table 4, participants with the highest levels were more likely to have CXR scarring (quartile 4 vs. quartile 1: OR = 1.67, 95% CI: 1.04–2.70, $P_{\text{trend}} = 0.05$). Also, higher SP-D levels were strongly associated with an increased frequency of bilateral CXR scarring ($P_{\text{trend}} = 0.005$), but not

unilateral lung scarring ($P_{\text{trend}} = 0.68$). In contrast, KL-6 levels were similar in participants with and without scarring (median 321.6 vs. 325.5 $\mu\text{g/mL}$, $P = 0.56$), and no association was observed with bilateral or unilateral scarring (Table 4).

Discussion

We observed significant associations between 2 circulating markers of interstitial lung disease and subsequent lung cancer risk in a large cohort study, the PLCO Cancer Screening Trial. After adjustment for cigarette smoking, lung cancer risk was increased 1.9-fold during follow-up for those in the highest quartile of SP-D, and 1.6-fold for those in the highest quartile of KL-6, compared with people with the lowest levels of these markers.

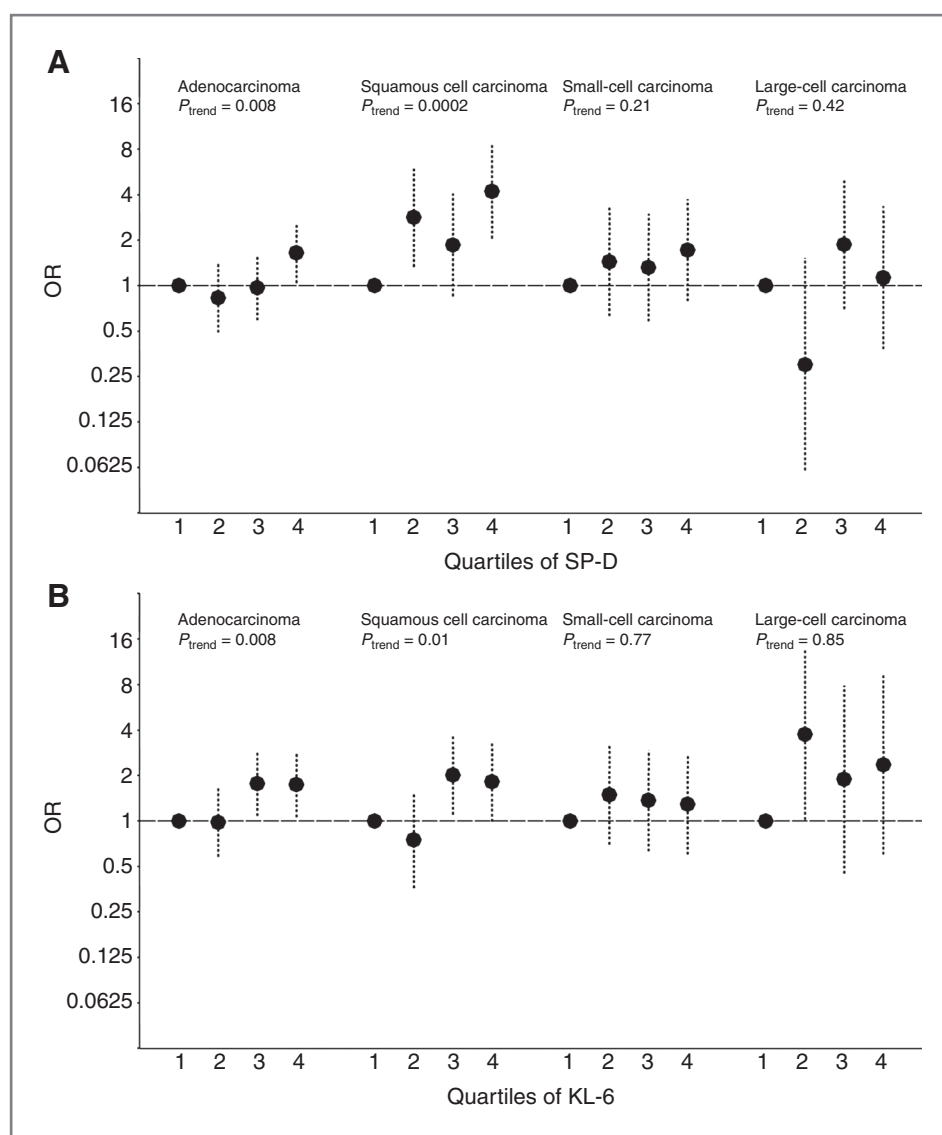


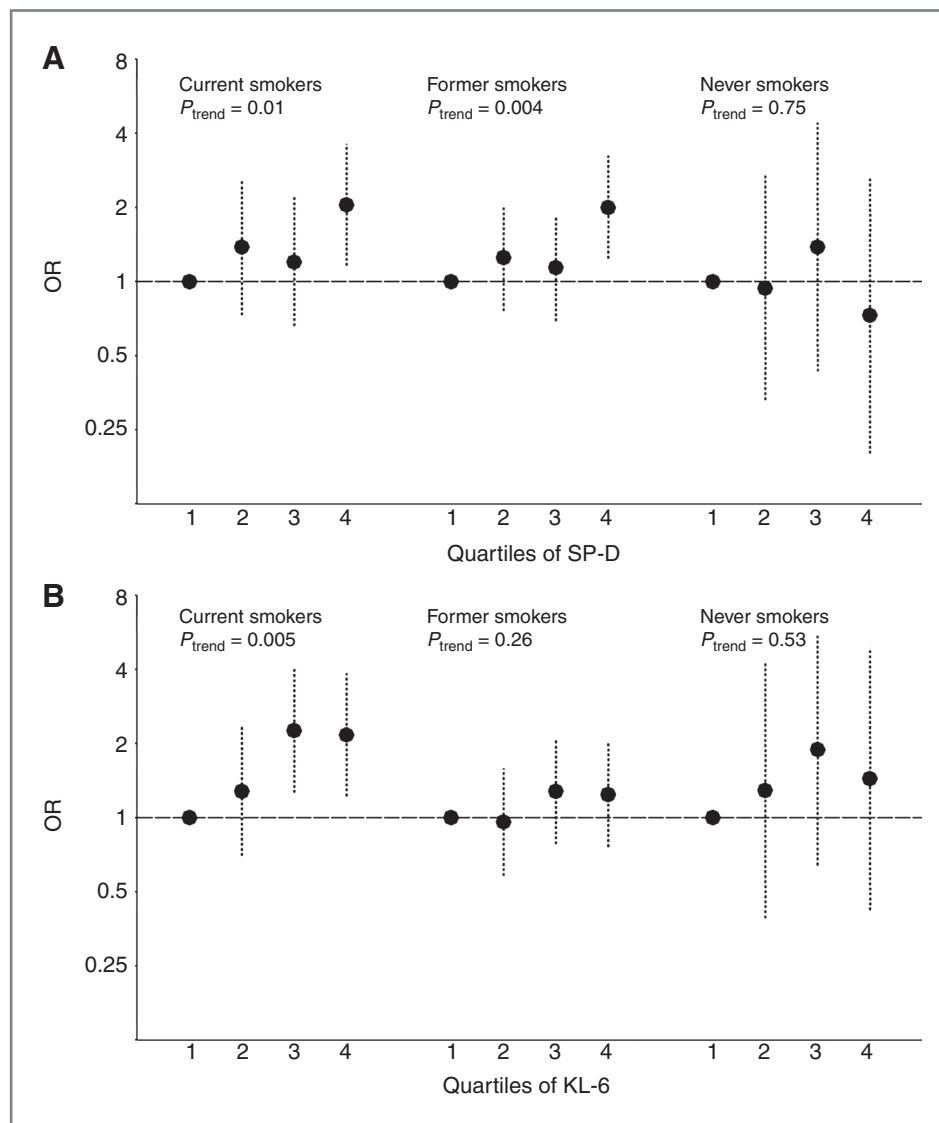
Figure 2. ORs and 95% CIs for lung cancer across quartiles of SP-D (A) and KL-6 (B), stratified by tumor histology (adenocarcinoma, squamous cell carcinoma, small-cell carcinoma, and large-cell carcinoma) are presented. P values for trend across quartiles are also presented.

These associations with lung cancer risk are biologically plausible because elevated circulating SP-D and KL-6 levels have been detected in patients with a number of pulmonary diseases, including idiopathic pulmonary fibrosis (15, 21), idiopathic pulmonary alveolar proteinosis (22), and acute respiratory distress syndrome (16, 23). Individuals with COPD also have elevated SP-D levels (17, 18). Both glycoproteins are produced by type II alveolar cells, which proliferate and replace type I alveolar cells during lung injury (27). Subsequently, the reparative type II cells transform into type I cells or undergo epithelial-to-mesenchymal transition (28). However, a disproportionately large number of type II alveolar cells persist in the presence of interstitial fibrosis and secrete high SP-D and KL-6 levels detectable in serum or plasma (27). Idiopathic pulmonary fibrosis has been associated with increased lung cancer risk and mortality

in a number of studies (12–14), and it may cause lung cancer through diffuse pulmonary inflammation (29). Chronic pulmonary inflammation could be an initiator or a promoter in the development of lung cancer, causing direct genotoxic injury due to a highly oxidative microenvironment, alteration of gene methylation, or increased cellular proliferation during tissue repair (4, 30).

In our study, current smoking status was associated with increased SP-D levels. However, we did not observe significant trends with cigarettes smoked per day, pack-years smoked, or years since quitting. A prior study also observed increased SP-D levels in smokers (17), whereas other studies have not (21, 31). We found no difference in KL-6 levels by smoking status, consistent with one prior study (31). The lack of a consistent association between these markers and cigarette smoking suggests SP-D and KL-6 reflect processes that have effects on lung cancer

Figure 3. ORs and 95% CIs for lung cancer across quartiles of SP-D (A) and KL-6 (B), stratified by smoking status (current, former, and never-smoker) are presented. P values for trend across quartiles are also presented.



that are independent of smoking and are not merely markers of smoking-related lung damage.

Participants in PLCO were drawn from the general population, so they were largely healthy and free of severe pulmonary conditions. Thus, it is not surprising that SP-D and KL-6 levels observed in our study were much lower than those observed in patients with interstitial lung disease. For example, in our study the median level of SP-D among matched controls was 105 ng/mL, much lower than the median of approximately 400 ng/mL in a prior study of patients with pulmonary fibrosis (21), and of 318.5 ng/mL observed in our positive control subjects with pulmonary fibrosis. These considerations highlight that, among our subjects, elevations in SP-D and KL-6 mostly reflect mild pulmonary disease or damage in the absence of clinical illness. CRP was positively correlated with SP-D and KL-6, indicating that levels of SP-D and KL-6 may increase in the setting of lung

inflammation. Nonetheless, our results showing independent associations with lung cancer risk suggest that SP-D, KL-6, and CRP each reflect somewhat independent processes that impact lung carcinogenesis.

Along these lines, we found a relationship between SP-D levels and scarring on the baseline CXR, which was particularly strong for bilateral scarring, but KL-6 levels were not associated with CXR scarring. One possible explanation could be that KL-6 is a sensitive marker for the detection and monitoring of certain interstitial lung diseases, but it may not be as sensitive for the detection of much less severe or early pulmonary scarring. It is also important to recognize that a degree of measurement error exists in the detection of scarring by CXRs, as manifested by only fair agreement in the assessment of scarring across consecutive CXRs in PLCO (32). Thus, it is possible that some scarring diagnoses in our study were misclassified and that some of the CXR

Table 4. Associations of serum levels of SP-D and KL-6 with chest X-ray scarring among controls

	No scarring <i>n</i>	Any scarring		Unilateral scarring		Bilateral scarring	
		<i>n</i>	OR ^a (95% CI)	<i>n</i>	OR ^a (95% CI)	<i>n</i>	OR ^a (95% CI)
SP-D, ng/mL							
17.1–71.7	135	39	1.00	26	1.00	13	1.00
71.8–105.3	131	52	1.36 (0.84–2.22)	35	1.36 (0.77–2.40)	17	1.30 (0.60–2.80)
105.4–143.8	136	48	1.31 (0.80–2.15)	33	1.34 (0.75–2.39)	15	1.23 (0.56–2.73)
143.9–506.6	127	64	1.67 (1.04–2.70)	32	1.22 (0.68–2.19)	32	2.51 (1.24–5.09)
			<i>P</i> _{trend} = 0.05		<i>P</i> _{trend} = 0.68		<i>P</i> _{trend} = 0.005
KL-6, µg/mL							
178.8–231.0	135	47	1.00	29	1.00	18	1.00
231.1–325.7	135	56	1.14 (0.72–1.82)	35	1.16 (0.67–2.03)	21	1.13 (0.57–2.24)
325.8–465.7	132	53	1.16 (0.72–1.84)	34	1.20 (0.69–2.08)	19	1.09 (0.54–2.18)
465.8–4,225.9	127	47	1.04 (0.64–1.68)	28	1.00 (0.56–1.78)	19	1.07 (0.53–2.17)
			<i>P</i> _{trend} = 0.99		<i>P</i> _{trend} = 0.86		<i>P</i> _{trend} = 0.93

^aModels were adjusted for age, sex, smoking status, time since quitting, cumulative smoking, and randomization year.

findings may correspond to other conditions (e.g., congestive heart failure or atelectasis), which would have biased the associations of SP-D and KL-6 with CXR scarring toward the null.

Our results regarding the time intervals over which the associations between these markers and lung cancer risk were apparent are also informative (Fig. 1A and B). For SP-D, an association with lung cancer risk was observed after more than 2 years following measurement of serum levels, and no association was observed over a shorter interval. If lung damage promotes cancer over an extended period of time, then perhaps the associations observed at longer latencies represent prolonged exposure to lung damage, whereas associations observed proximal to cancer diagnosis do not. In contrast, KL-6 was predominantly associated with lung cancer over shorter intervals. The association with lung cancer during the 2 years immediately following measurement suggests that an alternative explanation for our findings for KL-6 may be reverse causality due to a disease effect, that is, that the presence of a cancer leads to production of KL-6. Indeed, KL-6 is secreted by some lung cancer tumors and has previously been considered as a possible diagnostic marker of lung adenocarcinoma (33, 34). Whether reverse causality could explain the associations between KL-6 and lung cancer observed over the longer interval of 2 to 4 years, or for squamous cell carcinoma, remains unclear.

Significant associations between each fibrosis marker and lung cancer were limited to adenocarcinomas and squamous cell carcinomas. Although we did not observe associations for small- and large-cell carcinomas, our analyses were limited by a small number of cases. Furthermore, significant trends in lung cancer risk with increasing levels of fibrosis markers were limited to current and former smokers for SP-D and current smok-

ers only for KL-6. However, the number of cases who were never-smokers was small, and because the interactions of SP-D and KL-6 with smoking status were not significant, we cannot conclude that the associations vary by smoking status.

The main strength of our study was the measurement of 2 circulating markers that are produced in the lung and are associated with both lung damage and severe pulmonary diseases. We utilized prediagnostic sera, so that levels of these markers could be evaluated as predictors of subsequent lung cancer risk. Furthermore, we were able to carefully control for potential confounding factors, including smoking, both in the study design and data analysis. A limitation was the potential measurement error of assessing pulmonary scarring with CXRs (11, 32). Furthermore, our statistical power was limited for certain subanalyses due to a smaller number of cases.

In conclusion, we observed positive associations between circulating levels of SP-D and KL-6 and subsequent risk of lung cancer. Our results add support to a model whereby pulmonary disease, inflammation, and scarring contribute to the etiology of lung cancer (4, 5, 7, 9–14, 35–38). Future research should further examine the role of interstitial lung disease and fibrosis in the etiology of lung cancer, and the measurement of serum levels of SP-D and KL-6, particularly in smokers. As KL-6 is secreted by some lung tumors, its use as an early detection marker should be further explored. The use of SP-D and KL-6 has been advocated for the clinical monitoring of interstitial lung diseases. These markers, along with other known predictors, may also offer promise to identify individuals at high risk of developing cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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BLOOD CANCER DISCOVERY

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Meredith S. Shiels, Anil K. Chaturvedi, Hormuzd A. Katki, et al.

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