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ARTICLE

Increased Levels of Circulating Interleukin 6, Interleukin 8, C-Reactive Protein, and Risk of Lung Cancer

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Background Previous studies that were based primarily on small numbers of patients suggested that certain circulating proinflammatory cytokines may be associated with lung cancer; however, large independent studies are lacking.

Methods Associations between serum interleukin 6 (IL-6) and interleukin 8 (IL-8) levels and lung cancer were analyzed among 270 case patients and 296 control subjects participating in the National Cancer Institute-Maryland (NCI-MD) case–control study. Results were validated in 532 case patients and 595 control subjects in a nested case–control study within the prospective Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Association with C-reactive protein (CRP), a systemic inflammation biomarker, was also analyzed. Associations between biomarkers and lung cancer were estimated using logistic regression models adjusted for smoking, stage, histology, age, and sex. The 10-year standardized absolute risks of lung cancer were estimated using a weighted Cox regression model.

Results Serum IL-6 and IL-8 levels in the highest quartile were associated with lung cancer in the NCI-MD study (IL-6, odds ratio [OR] = 3.29, 95% confidence interval [CI] = 1.88 to 5.77; IL-8, OR = 2.06, 95% CI = 1.19 to 3.57) and with lung cancer risk in the PLCO study (IL-6, OR = 1.48, 95% CI = 1.04 to 2.10; IL-8, OR = 1.57, 95% CI = 1.10 to 2.24), compared with the lowest quartile. In the PLCO study, increased IL-6 levels were only associated with lung cancer diagnosed within 2 years of blood collection, whereas increased IL-8 levels were associated with lung cancer diagnosed more than 2 years after blood collection (OR = 1.57, 95% CI = 1.15 to 2.13). The 10-year standardized absolute risks of lung cancer in the PLCO study were highest among current smokers with high IL-8 and CRP levels (absolute risk = 8.01%, 95% CI = 5.77% to 11.05%).

Conclusions Although increased levels of both serum IL-6 and IL-8 are associated with lung cancer, only IL-8 levels are associated with lung cancer risk several years before diagnosis. Combination of IL-8 and CRP are more robust biomarkers than either marker alone in predicting subsequent lung cancer.

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Lung cancer is the most common cause of cancer-related deaths worldwide (1,2). The average 5-year lung cancer survival rate in the United States remains at 15%, but for patients diagnosed with localized disease, up to 50% survive beyond 5 years (1). Thus, early detection of lung cancer may improve survival. To date, screening methods such as chest radiograph alone or in combination with sputum cytology have failed to reduce mortality rates (3). If more refined risk stratification methods can be developed to identify the higher-risk individuals among those already at a high risk of lung cancer (eg, heavy smokers), then further screening might prove beneficial to these higher-risk individuals.

Chronic inflammation is associated with lung carcinogenesis. In previous studies, we and others have shown that C-reactive protein (CRP), a systemic marker of chronic inflammation, is associated with increased lung cancer risk (4–9). Furthermore, it is suggested that circulating proinflammatory cytokines may be associated with lung cancer; interleukin 6 (IL-6) and interleukin 8 (IL-8) are of particular interest because they are expressed in premalignant epithelial cells, and their expression is associated with a poor prognosis in lung cancer patients (10,11). High circulating levels of IL-6 and IL-8 were reported to be associated with lung cancer (12–15), but there were too few case patients to examine subgroups. None of the reports included multivariable analyses to adjust for potential confounding such as smoking. Adjustment for smoking is particularly relevant because IL-8 expression is higher in small-airway epithelial cells in smokers, and serum IL-6 and IL-8 levels are higher in smokers (16–18).

In this study, our goal was to determine whether circulating IL-6 and IL-8 levels were associated with lung cancer, and if these biomarkers accurately predicted subsequent diagnosis of lung

cancer. For this, we performed two independent case–control studies using participants from the National Cancer Institute-Maryland (NCI-MD) study and from the prospective Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. We then examined if a combination of circulating IL-6 and IL-8 levels with circulating CRP levels could further stratify patients.

Subjects and Methods

Study Population

NCI-MD Study. Participants were prospectively recruited as part of an ongoing NCI-MD study from the greater Baltimore, Maryland region from May 18, 1998, to November 10, 2003, as described previously (19,20). Briefly, eligible participants were free of known diagnosis of HIV infection, hepatitis B, and hepatitis C and born in the United States. White (of European descent) case patients (N = 346) had histologically confirmed non–small cell lung cancer (NSCLC), did not have any other cancer at the time of enrollment, and were enrolled within 24 months after diagnosis (median time = 2.8 months). Case patients resided in Metropolitan Baltimore or the Maryland Eastern Shore and were recruited from seven hospitals in Baltimore, after obtaining physician's consent. White hospital-based control subjects (N = 180) were frequency matched to case patients by sex, ethnicity, age, smoking history, and hospital. Hospital-based control subjects were cancer-free patients recruited from the same hospitals as lung cancer case patients and recruited from internal medicine clinics, primary care, pulmonology, and cardiology clinics. White population-based control subjects (N = 195) were identified from the Maryland Department of Motor Vehicles lists and frequency matched to case patients by age, sex, and race. Race was self-described. All participants completed a questionnaire in the presence of an interviewer. Blood specimens were processed immediately after collection for isolation of serum and stored at −80°C.

Among the enrolled whites, 304 (88%) of 346 case patients and 345 (92%) of 375 control subjects provided serum samples. Because of depletion of samples during previous studies (21), 270 case patients and 296 control subjects were analyzed in this study. Participants with cytokine measurements were representative of all those enrolled with respect to demographics assessed (Supplementary Table 1, available online).

PLCO Study. Participants were selected as part of a nested case–control study within the screening arm of the PLCO Cancer Screening Trial, as previously detailed (5). Briefly, the PLCO study was a randomized trial aimed at evaluating the efficacy of screening in reducing cancer mortality, which recruited approximately 155 000 men and women aged 55–74 years from 1992 to 2001, from 10 recruiting centers throughout the United States (22). Participants in the screening group provided blood samples annually for the first 6 years of the study. Baseline blood samples were used. Lung cancers were ascertained through annual questionnaires mailed to the participants, and positive reports were reviewed by abstracting hospital medical records or death certificates from the National Death Index.

As of December 31, 2004, 898 lung cancers were diagnosed among the 77 464 participants in the screening group. Patients were excluded because of missing baseline questionnaire, history of any cancer, diagnosis of multiple cancers during follow-up, missing smoking information at baseline, missing consent for utilization of biological specimens for etiologic studies, or unavailability of serum specimens. A total of 592 lung cancer patients were available for this case–control study. Race was self-described. Serum cytokine levels can vary across ethnicities (23); and because there were too few nonwhite case patients to perform well-powered analyses stratified by race, we excluded such subjects. Thus, 532 white case patients were included in the analysis. Among the case patients in the screening arm, 210 were detected through screening, and the remaining 322 case patients had clinically diagnosed lung cancer either before screening started or after at least one screen was completed.

Control subjects were free of cancer at the time of a case patient's lung cancer diagnosis and were matched to the 532 case patients by age, sex, year of random assignment, follow-up time since enrollment, and smoking status at enrollment (never, former, or current smoker). Current and former smokers were matched on

CONTEXT AND CAVEATS

Prior knowledge

High levels of serum proinflammatory cytokines such as interleukin 6 (IL-6) and interleukin 8 (IL-8) could be associated with lung cancer risk. Serum levels of C-reactive protein (CRP), a systemic biomarker of chronic inflammation, are also associated with lung cancer risk.

Study design

Case patients and control subjects from the National Cancer Institute-Maryland (NCI-MD) case–control study were included to analyze the associations between IL-6 and IL-8 and lung cancer. Results were validated in a nested case–control study within the prospective Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Associations with CRP individually as well as in combination with IL-6 and IL-8 were also investigated. Analyses were adjusted for smoking, stage, histology, age, and sex.

Contribution

High levels of IL-6 and IL-8 were associated with increased lung cancer in the NCI-MD study and lung cancer risk in the PLCO study. High IL-8 levels were noted up to 5 years before lung cancer diagnosis, whereas high IL-6 levels were only noted within 2 years before lung cancer diagnosis. The 10-year absolute risks of lung cancer were highest in current smokers with high IL-8 and high CRP levels.

Implication

Serum testing of IL-6, IL-8, and CRP, after further assay development, has the potential to identify people at increased risk of lung cancer.

Limitations

The specificity of the serum biomarkers exclusively for lung cancer is not known because other cancers were not included in this study. More effective methods to standardize the biomarker measurements are necessary.

From the Editors

cumulative amount of smoking (0–29, 30–39, 40–49, and ≥50 pack-years) and time since quitting (≤15 and >15 years) for former smokers. We matched never-smoking control subjects to lung cancer case patients using a 3 : 1 ratio to enhance statistical power, whereas former- and current-smoking control subjects were matched to case patients using a 1 : 1 ratio.

Institutional review board approval was obtained from all participating institutions and the NCI. Informed consent was obtained from all participants.

Cytokine Measurements

Blood was collected at the time of enrollment and allowed to clot. Serum was isolated by centrifugation and stored at −80°C. After thawing, 25 μL of serum (566 and 1127 samples from the NCI-MD and PLCO studies, respectively) were measured for IL-6 and IL-8 concentrations using two sets of electrochemiluminescence immunoassay (ECLIA) plates (Mesoscale Discovery, Gaithersburg, MD). For the NCI-MD study, the ultrasensitive 10-plex ECLIA plates were custom-designed, as described earlier (24) and for the PLCO study, 4-plex (MS6000 Human ProInflammatory-4 II Ultra-Sensitive Kit, Mesoscale Discovery, Gaithersburg, MD) ECLIA plates were used. Both sets of ECLIA plates were analyzed on the MesoScale Discovery 6000 instrument, following the manufacturer's assay and analysis protocols. PLCO samples were assayed approximately 2 years after the NCI-MD study. All samples were blinded and randomly distributed. For the PLCO study, duplicates were performed on 100% of the samples and results were recorded as the average of the duplicates. As an added quality control measure of accuracy within and across plates, an additional 12% of samples were duplicated and randomly distributed across the plates (6% intraplate and 6% interplate duplicates for NCI-MD, and 5% intraplate and 7% interplate duplicates for PLCO). Reproducibility of blinded duplicates was evaluated using the Spearman correlation coefficient (25). The coefficient of variation was estimated for control samples included as laboratory standards on each plate. Samples with cytokine values lower than the detection limit were assigned a value of one-half of the detection limit. The detection limit for each plate was determined based on linearity of the standard curve following the manufacturer's instructions. Quality control results are summarized in Supplementary Table 2 (available online).

CRP Measurements

Serum samples from case patients and control subjects in the nested case–control PLCO study were measured for CRP exactly as reported earlier (5). Briefly, 10 μL of serum from 1127 samples were measured for CRP using the Immulite 1000 High Sensitivity CRP chemiluminescent immunometric assay and instrumentation (Siemens Medical Solutions Diagnostics, Los Angeles, CA), following the manufacturer's protocol. Samples were blinded and randomly distributed. The limit of detection was 0.2 μg/mL, and samples below the detection limit were assigned a level of 0.2 μg/mL.

Statistical Analysis

All analyses were performed using Stata software, version 11 (StataCorp LP, College Station, TX), except where indicated. All reported *P* values were two-sided, and all *P* values less than or equal to .05 were considered statistically significant. Univariate comparison of characteristics between case patients and control subjects was performed for categorical variables using the χ^2 test and continuous variables using either the Student *t* test or the Kruskal–Wallis test of normally or nonnormally distributed data, respectively. Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of lung cancer (NCI-MD study) or lung cancer risk (PLCO study) with IL-6 and IL-8, categorized by median or quartile values based on the control subjects in each study (25th, 50th, and 75th percentile quartiles: NCI-MD study, IL-6 quartiles, 1.4, 2.1, 3.8 pg/mL, respectively and IL-8 quartiles, 7.0, 10.8, 28.5 pg/mL, respectively; PLCO study, IL-6 quartiles, 2.7, 4.0, 6.6 pg/mL, respectively and IL-8 quartiles 13.1, 17.4, 23.3 pg/mL, respectively) and were adjusted for age, sex, and smoking status. Subgroup analyses were performed by stratifying on each of the following factors: smoking (described below), tumor stage (stage I, II, III, and IV), histology (squamous cell carcinoma [SSC], adenocarcinoma [AC], NSCLC not otherwise specified [NSCLC NOS], and small cell lung cancer [SCLC]), age (≤65 and > 65 years), sex, education (high school or less and more than high school), regular use of aspirin or ibuprofen (yes and no), body mass index (BMI) (≤26.5 and >26.5 kg/m^2), history of emphysema or bronchitis (yes and no), and family history of lung cancer (yes and no). PLCO case patients were stratified into patients who were diagnosed within 2 years vs patients diagnosed more than 2 years after the blood was collected. PLCO case patients were further stratified by patients whose lung cancers were screen detected vs clinically detected. Among control subjects, predictors of increased IL-6 and IL-8 levels were assessed using linear regression. Deviation from multiplicative statistical interactions was assessed through product terms in the logistic regression models. Correlation between cytokine levels (eg, IL-6 vs IL-8 levels) was performed on log-transformed serum cytokine levels, using the Spearman correlation coefficient.

Smokers were categorized as never, former quit 15 years or less, former quit for more than 15 years, and current. A never-smoker was defined as a person who smoked less than 100 cigarettes in his lifetime, a former smoker was defined as a person who had quit smoking more than 1 year before the interview or baseline questionnaire, and a current smoker was defined as a smoker who smoked within 1 year of the interview or baseline questionnaire. Age was categorized as less than 65 years vs 65 years or older. Education was categorized as completed high school or less vs some college or more. BMI was dichotomized by the median value of control subjects in the PLCO study (≤26.5 vs >26.5 kg/m^2). Tumor staging was based on the American Joint Committee on Cancer (AJCC) manual (26) for both studies.

Standardized 10-year lung cancer absolute risks and risk differences for IL-8 and CRP were calculated using a weighted Cox regression model using the R package NestedCohort (http://dceg.cancer.gov/tools/analysis/nested-cohort), as described in previous studies (5,27,28). Briefly, for each white participant of the PLCO trial's screening arm cohort, who was eligible for selection into the serum component of the nested case–control study (N = 51 989), we calculated their probability of being selected into the case–control study based on combinations of age strata, strata of smoking duration and intensity, sex, and year of randomization.

The inverse of these selection probabilities was used as the sampling weights in a weighted Cox regression model to estimate absolute risks of lung cancer (27,28). We standardized the 10-year absolute risks of lung cancer to the screening arm cohort's joint distribution of age, sex, year of randomization, and pack-years of smoking for former and current smokers or time since quitting for former smokers. There was no material difference between the hazard ratios for IL-8 and CRP from this weighted Cox model and their corresponding odds ratios from the logistic regressions (data not shown). There was also no material difference between the estimated cohort survival curve from the weighted Cox model and the crude Kaplan–Meier survival curve (data not shown).

Several health conditions that may be associated with systemic inflammation were recorded as part of the questionnaires for all NCI-MD study participants, including chronic bronchitis, emphysema, adult asthma, tuberculosis, asbestosis, pneumonia, lupus, and arthritis. For the PLCO study participants, only emphysema and chronic bronchitis were recorded.

Results

Characteristics of Participants From the NCI-MD and PLCO Studies

The demographic and clinicopathologic features of participants are presented in Table 1. In both NCI-MD and PLCO studies, sex and age were similar between case patients and control subjects. Also in both studies, fewer case patients had college education or higher and more had a family history of lung cancer than control subjects, and the most common histological type was adenocarcinoma, followed by squamous cell carcinoma. There were more current smokers and greater number of smoking pack-years among case patients compared with control subjects in NCI-MD study. Because never-smoking control subjects were matched with lung cancer case patients at a ratio of 3 : 1, there were more never-smokers among the control subjects in the PLCO study. In the NCI-MD study, case patients were less likely to use aspirin or ibuprofen and had a lower BMI than control subjects. In the PLCO study, more case patients had a family history of lung cancer than control subjects. In contrast to the PLCO case patients, the majority of the NCI-MD case patients had stage I tumors, which could reflect a possible bias for recruiting surgical case patients, who primarily have stage I tumors, in the NCI-MD study.

Association Between Serum IL-6 and IL-8 Concentrations and Lung Cancer

Serum IL-6 and IL-8 Levels. In both NCI-MD and PLCO participants, the median levels of IL-6 and IL-8 were statistically significantly higher among the case patients compared with the control subjects (NCI-MD: IL-6, case patients vs control subjects, median = 3.7 pg/mL, interquartile range [IQR] = 2.3–7.2 pg/mL vs median = 2.1 pg/mL, IQR = 1.4—3.8 pg/mL, $P = 1 \times 10^{-4}$; IL-8, case patients vs control subjects, median = 15.9 pg/mL, IQR = 9.5—40.5 pg/mL vs median = 10.8 pg/mL, IQR 7.0–28.5 pg/mL, $P = 1 \times 10^{-4}$ and PLCO: IL-6, case patients vs control subjects, median = 4.4 pg/mL, IQR = 2.9–7.2 pg/mL vs median = 4.0 pg/mL, IQR = 2.7–6.6 pg/mL, $P = .02$; IL-8, case patients vs control subjects, median = 19.0 pg/mL, IQR = 14.2 to 25.1 pg/mL vs median = 17.4 pg/mL, IQR = 13.1 to 23.3 pg/mL, $P = 7 \times 10^{-4}$) (Table 1). Because both studies had statistically significantly higher proportions of never-smokers among the control subjects compared with case patients, and serum cytokine levels may be influenced by smoking, analyses were also performed after excluding never-smokers. Median IL-6 and IL-8 levels remained statistically significantly higher among case patients compared with control subjects after exclusion of never-smokers (NCI-MD: IL-6, $P = 1 \times 10^{-4}$ and IL-8, $P = .05$; PLCO: IL-6, $P = .02$, and IL-8, $P = .005$) (Table 1).

Association of Serum IL-6 and IL-8 Levels With Lung Cancer and Risk of Lung Cancer. In multivariable unconditional logistic regression analyses among NCI-MD participants, IL-6 and IL-8 levels in the highest quartiles were statistically significantly associated with lung cancer compared with the lowest quartiles (IL-6: odds ratio = 3.29, 95% CI =1.88 to 5.77, $P_{trend} = 3.6 \times 10^{-7}$; IL-8: OR = 2.06, 95% CI = 1.19 to 3.57, $P_{trend} = .003$) (Table 2). Results were similar when NCI-MD control subjects were grouped into population- and hospital-based control subjects (Supplementary Table 3, available online). In the PLCO study, participants with serum IL-6 and IL-8 levels within the highest quartiles showed a statistically significantly increased risk of lung cancer compared with those within the lowest quartiles (IL-6: OR = 1.48, 95% CI = 1.04 to 2.10, $P_{trend} = .02$; and IL-8: OR = 1.57, 95% CI = 1.10 to 2.24, $P_{trend} = .003$), although the differences were smaller in magnitude than the NCI-MD study participants. The reduced magnitude of the odds ratio in the PLCO study compared with the NCI-MD study could reflect the fact that all control subjects in the PLCO study were matched by smoking history, which is a confounder. The magnitude of the odds ratios increased in the third and fourth quartiles compared with the first and second quartiles; thus, we also performed analyses on dichotomized cytokine levels. IL-6 and IL-8 serum levels higher than the median values were statistically significantly associated with lung cancer in the NCI-MD study and with lung cancer risk in the PLCO study (NCI-MD, IL-6, OR = 2.82, 95% CI = 1.90 to 4.20, $P = 3.0 \times 10^{-7}$; PLCO, IL-6, OR = 1.26, 95% CI = 1.00 to 1.61, $P = .05$; NCI-MD, IL-8, OR = 1.86, 95% CI = 1.29 to 2.68, $P = .001$; PLCO, IL-8, OR = 1.47, 95% CI = 1.15 to 1.88, $P = .002$) (Table 2).

Analysis of Potential Confounding. We next examined if the associations between IL-6 and IL-8 serum levels and lung cancer in the NCI-MD study, or risk of lung cancer in the PLCO study, were independent of factors that could potentially influence the associations. Results remained statistically significant after adjustment for education, BMI, regular use of aspirin and/or ibuprofen, family history of lung cancer, and history of heart disease, in both studies (Supplementary Table 4, available online). Furthermore, IL-6 and IL-8 remained associated with lung cancer after adjusting for conditions associated with systemic inflammation in the NCI-MD study (chronic bronchitis, emphysema, adult asthma, tuberculosis, asbestosis, pneumonia, lupus, or arthritis) and remained statistically significantly associated with increased risk of lung cancer in the PLCO study (emphysema and chronic bronchitis) (Supplementary Table 4, available online). These data suggest that the associations between serum IL-6 and IL-8 levels, and lung

Table 1. Characteristics and clinical data of lung cancer and control participants in the NCI-MD and PLCO studies*

	NCI-MD study			PLCO study		
	Case patients (N = 270)	Control subjects (N = 296)		Case patients (N = 532)	Control subjects (N = 595)	
Characteristic	**No. (%)**	**No. (%)**	***P***	**No. (%)**	**No. (%)**	***P***
Age, y Mean (SD)	66.6 (10.0)	65.2 (10.4)	.10†	64.7 (5.1)	64.5 (5.3)	.52†
Sex						
Men	142 (52.6)	148 (50.0)	.38‡	359 (67.5)	380 (63.9)	.20‡
Women	128 (47.4)	148 (50.0)		173 (32.5)	215 (36.1)	
Smoking status						
Never	22 (8.2)	86 (29.1)		37 (7.0)	106 (17.8)	
Former quit ≤15 y	57 (21.2)	68 (23.1)		186 (35.0)	184 (30.9)	
Former quit >15 y	64 (23.4)	83 (27.8)		105 (19.7)	102 (17.2)	
Current	127 (47.2)	59 (20.0)	4.6×10^{-14}‡	204 (38.3)	203 (34.1)	1.4×10^{-6}‡,§
Pack-years, mean (SD)||	47.8 (26.4)	39.7 (31.3)	.001†	47.4 (29.8)	45.1 (29.9)	.20†
Education¶						
High school or less	146 (60.1)	127 (49.8)	.02‡	200 (37.6)	190 (31.9)	.05‡
College or higher	97 (39.9)	128 (50.2)		332 (62.4)	405 (68.1)	
Regular aspirin or ibuprofen use¶						
No	174 (64.7)	145 (49.0)	1.7×10^{-4}‡	182 (34.3)	202 (34.0)	.91‡
Yes	95 (35.3)	151 (51.0)		349 (65.7)	393 (66.0)	
BMI, kg/m^2¶						
≤26.5	74 (27.4)	52 (17.6)	.005‡	267 (50.6)	291 (49.8)	.81‡
>26.5	196 (72.6)	244 (82.4)		261 (49.4)	293 (50.2)	
History of heart disease¶						
No	204 (76.1)	217 (73.3)	.44‡	423 (83.1)	498 (85.1)	.36‡
Yes	64 (23.9)	79 (26.7)		86 (16.9)	87 (14.9)	
History of emphysema or bronchitis¶						
No	204 (75.8)	225 (76.0)	.96‡	411 (78.0)	521 (88.9)	3.9×10^{-5}‡
Yes	65 (24.2)	71 (24.0)		103 (20.0)	65 (11.1)	
Family history of lung cancer¶						
No	221 (82.2)	253 (85.5)	.28‡	408 (81.6)	502 (88.4)	.002‡
Yes	48 (17.8)	43 (14.5)		92 (18.4)	66 (11.6)	
Histology¶						
AC	105 (42.9)	–		226 (43.2)	–	
SCC	53 (21.6)	–		119 (22.8)	–	
SCLC	0	–		68 (13.0)	–	
NSCLC, NOS	59 (24.1)	–		35 (6.7)	–	
Other	28 (11.4)	–		77 (14.3)	–	
Tumor stage¶						
I	104 (71.2)	–		165 (31.3)	–	
II–IV	42 (28.8)	–		362 (68.7)	–	
IL-6, pg/mL, median (interquartile range)	3.7 (2.3 to 7.2)	2.1 (1.4 to 3.8)	1×10^{-4}#	4.4 (2.9 to 7.2)	4.0 (2.7 to 6.6)	.02#
IL-8, pg/mL, median (interquartile range)	15.9 (9.5 to 40.5)	10.8 (7.0 to 28.5)	1×10^{-4}#	19.0 (14.2 to 25.1)	17.4 (13.1 to 23.3)	7.0×10^{-4}#
IL-6, pg/mL, median (interquartile range)||	3.7 (3.3 to 4.2)	2.3 (2.1 to 2.7)	1×10^{-4}#	4.4 (4.1 to 4.8)	4.0 (3.7 to 4.4)	.02#
IL-8, pg/mL, median (interquartile range)||	15.7 (13.2 to 18.2)	11.9 (9.9 to 14.9)	.05#	19.1 (18.4 to 20.0)	17.6 (16.6 to 18.5)	.005#

* AC = adenocarcinoma; BMI = body mass index categorized by median value of controls; IL-6 = interleukin 6; IL-8 = interleukin 8; NCI-MD = National Cancer Institute-Maryland; NOS = not otherwise specified; NSCLC = non–small cell lung cancer; PLCO = Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCC = squamous cell carcinoma; SCLC = small cell lung cancer; – = Not applicable.

† *P* values were calculated using a two-sided Student *t* test.

‡ *P* values were calculated using a two-sided χ^2 test.

§ The statistically significant difference between case patients and controls subjects in the PLCO study is because never-smoking control subjects were matched to lung cancer case patients using a 3 : 1 ratio.

|| Excludes individuals who had never smoked.

¶ Numbers do not add to 100% of total because of missing information. Tumor staging was based on the American Joint Committee on Cancer (AJCC) manual.

P values were calculated using a two-sided Kruskal–Wallis test.

Table 2. Association of IL-6 and IL-8 serum levels with lung cancer in the NCI-MD study, and lung cancer risk in the PLCO study*

	NCI-MD Study			PLCO Study		
Cytokine level	**No. of case patients/No. of control subjects**	**OR (95% CI)†**	**Adjusted OR (95% CI)‡**	**No. of case patients/No. of control subjects**	**OR (95% CI)†**	**Adjusted OR (95% CI)‡**
IL-6						
First quartile§	27/73	1.00 (referent)	1.00 (referent)	107/149	1.00 (referent)	1.00 (referent)
Second quartile	30/75	1.08 (0.59 to 1.99)	0.98 (0.51 to 1.86)	112/138	1.22 (0.87 to 1.72)	1.14 (0.79 to 1.65)
Third quartile	83/74	3.03 (1.76 to 5.21)	2.28 (1.29 to 4.06)	147/158	1.36 (0.96 to 1.93)	1.25 (0.88 to 1.78)
Fourth quartile	130/74	4.75 (2.81 to 8.03)	3.29 (1.88 to 5.77)	166/150	1.55 (1.10 to 2.19)	1.48 (1.04 to 2.10)
P_{trend}		3.5×10^{-12}	3.6×10^{-7}		.01	.02
Low IL-6¶	57/148	1.00 (referent)	1.00 (referent)	237/304	1.00 (referent)	1.00 (referent)
High IL-6	213/148	3.74 (2.58 to 5.41)	2.82 (1.90 to 4.20)	295/291	1.30 (1.03 to 1.64)	1.26 (1.00 to 1.61)
P		3.0×10^{-7}||	3.0×10^{-7}||		.03||	.05||
IL-8						
First quartile§	34/74	1.00 (referent)	1.00 (referent)	102/147	1.00 (referent)	1.00 (referent)
Second quartile	57/74	1.68 (0.98 to 2.86)	1.48 (0.84 to 2.63)	114/153	1.07 (0.76 to 1.52)	1.03 (0.72 to 1.48)
Third quartile	96/74	2.82 (1.70 to 4.69)	2.62 (1.52 to 4.51)	148/147	1.43 (1.01 to 2.01)	1.41 (0.99 to 2.01)
Fourth quartile	83/74	2.44 (1.46 to 4.08)	2.06 (1.19 to 3.57)	169/147	1.66 (1.18 to 2.32)	1.57 (1.10 to 2.24)
P		2.0×10^{-4}||	.003||		.001||	.003||
Low IL-8¶	91/148	1.00 (referent)	1.00 (referent)	219/306	1.00 (referent)	1.00 (referent)
High IL-8	179/148	1.97 (1.40 to 2.76)	1.86 (1.29 to 2.68)	323/300	1.49 (1.17 to 1.88)	1.47 (1.15 to 1.88)
P		9.6×10^{-5}||	.001||		.001||	.002||

* CI = confidence interval; IL-6 = interleukin 6; IL-8 = interleukin 8; NCI-MD = National Cancer Institute-Maryland study; PLCO = Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; OR = odds ratio.

† Univariate analysis; analysis was done by unconditional logistic regression.

‡ Multivariable unconditional logistic regression analysis adjusted for age (continuous), sex, smoking pack-years (continuous), smoking status (never, former quit ≤15 years, former quit >15 years, and current), PLCO study adjusted additionally for year of randomization and number of years in the study.

§ Quartiles were based on serum IL-6 and IL-8 cutoff levels among controls in NCI-MD study (25th, 50th, and 75th percentile: IL-6, 1.4, 2.1, 3.8 pg/mL, respectively; IL-8, 7.0, 10.8 and 28.5 pg/mL, respectively) and PLCO study (IL-6, 2.7, 4.0, 6.6 pg/mL, respectively; IL-8, 13.1, 17.4, 23.3 pg/mL, respectively).

¶ Serum IL-6 and IL-8 levels were dichotomized (≤median vs >median) among control subjects in the NCI-MD (IL-6 2.1 pg/mL; IL-8 10.8 pg/mL) and PLCO (IL-6, 4.0 pg/mL; IL-8, 17.4 pg/mL) studies.

|| *P* values were calculated using a two-sided Wald χ^2 statistic.

cancer and lung cancer risk, were independent of these tested potential confounding factors.

When multiplicative interactions were assessed through product terms within the logistic regression model, there was no statistically significant interaction between IL-6 or IL-8 with lung cancer in the NCI-MD study, or lung cancer risk in the PLCO study, and the variables used in the multivariable models or listed in Table 1 (data available upon request). In addition, in subgroup analyses stratified by tumor histology and stage, there was a considerable overlap of the 95% confidence intervals (Supplementary Figure 1, available online), suggesting that there was no heterogeneity of lung cancer in the NCI-MD study, or lung cancer risk in the PLCO study, among these subgroups, although there was lower power within subgroup analyses.

In the NCI-MD study, some of the lung cancer case patients (42 of 270 patients) were treated with radiation and/or chemotherapy before blood collection, and because this could potentially alter the circulating cytokine levels and confound the results, we performed the analyses among participants who had not received previous lung cancer therapy. There was no statistically significant difference in median serum IL-6 levels between case patients who received previous therapy vs patients who did not (median = 3.7 pg/mL, IQR = 2.3–7.3 pg/mL vs median = 4.2 pg/mL, IQR = 2.2 –7.3 pg/mL, respectively, Kruskal–Wallis $P = .73$). However, serum IL-8 levels were statistically significantly lower among case patients who received previous therapy vs patients who did not (median = 9.9 pg/mL, IQR = 8.0–16.4 pg/mL vs median = 17.4 pg/mL, IQR = 9.8–48.9 pg/mL, respectively, Kruskal–Wallis $P = 1 \times 10^{-4}$). Serum IL-6 and IL-8 levels higher than the median value were statistically significantly associated with lung cancer after exclusion of treated patients (IL-6, OR = 2.93, 95% CI = 1.93 to 4.45, $P = 4.7 \times 10^{-7}$; IL-8, OR = 2.22, 95% CI = 1.51 to 3.28, $P = 5.7 \times 10^{-5}$). Because serum cytokine levels might be affected by the time between diagnosis and sample collection, we stratified analyses by those who had their blood drawn within 3 months or more than 3 months after diagnosis. The time between diagnosis and blood collection did not affect the associations between IL-6 (0–3 months after diagnosis, OR = 3.14, 95% CI = 1.84 to 5.34 and >3 months after diagnosis, OR = 2.51, 95% CI = 1.55 to 4.08) or IL-8 (0–3 months after diagnosis, OR = 1.80, 95% CI = 1.14 to 2.85 and >3 months after diagnosis, OR = 1.83, 95% CI = 1.17 to 2.85), with lung cancer. Thus, the associations between IL-6 and IL-8 and lung cancer were independent of previous treatment and time after diagnosis that blood was collected.

Association Between Serum IL-6 and IL-8 Levels and Subsequent Diagnosis of Lung Cancer. We next examined if increased IL-6 and IL-8 serum levels were associated with subsequent diagnosis of lung cancer. Because subclinical malignancies may be responsible for increased circulating IL-6 and IL-8 levels, we excluded case patients diagnosed within 2 years after blood collection (baseline) in the PLCO study, a time period in which clinically undetected tumors are likely to be present. When excluding these case patients, there was no evidence of association of IL-6 levels higher than the median (4.0 pg/mL) with lung cancer risk (OR = 1.01, 95% CI = 0.76 to 1.32, $P = .97$), despite a greater than 95% power to detect a statistically significant difference for an odds ratio greater than 1.5. In contrast, when limiting to case patients diagnosed within 2 years after blood collection, there was a statistically significant association between IL-6 levels above the median (4.0 pg/mL) and lung cancer risk (OR = 1.99, 95% CI = 1.39 to 2.84, $P = 1.5 \times 10^{-5}$) (Table 3).

IL-8 was associated with an increased risk of lung cancer when case patients diagnosed more than 2 years after baseline were excluded (OR = 1.53, 95% CI = 1.09 to 2.17, $P = .02$), as well as when case patients diagnosed within 2 years were excluded (OR = 1.57, 95% CI = 1.15 to 2.13, $P = .004$) (Table 3). Moreover, high IL-8 serum levels were associated with lung cancer risk even when case patients diagnosed within 5 years after blood collection were excluded from the analysis (OR = 1.46, 95% CI = 1.01 to 2.10, $P = .04$). These results cannot be attributed to serum storage time because there was no association between IL-6 or IL-8 levels and storage time in control subjects (Kruskal–Wallis $P = .77$ and .75, respectively), and control subjects were matched with case patients

Table 3. Association of high IL-6 and IL-8 serum levels with lung cancer risk in the PLCO study by time before diagnosis*

		Lung cancers diagnosed within 2 y†			Lung cancers diagnosed within 2 y excluded†		
Cytokinelevel	Control subjects (N = 595), No. (%)	Case patients (N = 185), No. (%)	OR (95% CI)‡	P§	Case patients (N = 347), No. (%)	OR (95% CI)‡	P§
IL-6||							
Low	304 (51.1)	63 (34.1)	1.00 (referent)		174 (50.1)	1.00 (referent)	
High	291 (48.9)	122 (65.9)	1.99 (1.39 to 2.84)	1.5×10^{-5}	173 (49.9)	1.01 (0.76 to 1.32)	.97
IL-8||							
Low	300 (50.4)	72 (38.9)	1.00 (referent)		144 (41.5)	1.00 (referent)	
High	295 (49.6)	113 (61.1)	1.53 (1.09 to 2.17)	.02	203 (58.5)	1.57 (1.15 to 2.13)	.004

* CI = confidence ratio; IL-6 = interleukin 6; IL-8 = interleukin 8; OR = odds ratio; PLCO = Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial.

† Case patients were grouped into those who were diagnosed within 2 years after baseline blood collection and those who were diagnosed more than 2 years after baseline.

‡ Multivariable unconditional logistic regression adjusted for age (continuous), sex, smoking pack-years (continuous), smoking status (never, former quit ≤15 years, former quit >15 years, and current), year of randomization, and number of years in the study.

§ *P* values were calculated using a two-sided Wald χ^2 statistic.

|| Serum IL-6 and IL-8 levels were dichotomized (≤median vs >median) among control subjects (IL-6 4.0 pg/mL; IL-8 17.4 pg/mL).

on storage time. Therefore, high IL-8 serum levels were associated with both current and subsequent diagnosis of lung cancer, whereas IL-6 level was increased only at lung cancer diagnosis and possibly during subclinical disease.

Association Between Serum IL-6 and IL-8 Levels and Lung Cancer Risk Among Screen-Diagnosed vs Clinically Diagnosed Case Patients. We next assessed if there was an association between IL-6 and IL-8 levels and lung cancer risk among case patients who had screen-detected or clinically detected lung cancers. By design of the nested PLCO case–control study, case patients were selected from the screening arm. Among the 532 case patients in the screening arm, 210 were detected through screening, and the remaining 322 case patients had clinically diagnosed lung cancer either before the screening started or after at least one screen was completed. IL-6 levels above the median were not statistically significantly associated with subsequent screen-diagnosed (OR = 1.34, 95% CI = 0.94 to 1.93, $P = .11$) or clinically diagnosed (OR = 1.24, 95% CI = 0.92 to 1.65, $P = .16$) lung cancer. The lack of statistical significance likely reflected a loss of power in subgroup analyses. In contrast, IL-8 levels above the median were associated with subsequent screen-diagnosed (OR = 1.51, 95% CI = 1.06 to 2.16, $P = .02$) and clinically diagnosed (OR = 1.45, 95% CI = 1.08 to 1.96, $P = .01$) lung cancer. Thus the association between IL-6 and IL-8 with lung cancer risk appeared to be independent of whether the case patients were screen or clinically diagnosed.

Predictors of High Serum IL-6 and IL-8 Levels

We assessed predictors of increased IL-6 and IL-8 levels among control subjects (Supplementary Table 5, available online). Median IL-6 levels were statistically significantly higher among current smokers (NCI-MD, $P = .006$; PLCO, $P = .006$) and those with a higher BMI (≥26.5 kg/m^2) (NCI-MD, $P = .04$; PLCO, $P = .02$). Median IL-6 levels were statistically significantly higher among those with less education (high school or less vs some college or higher) in the PLCO study ($P = .03$), and there was a similar trend in NCI-MD, though not statistically significant ($P = .09$). Median IL-8 levels were statistically significantly higher among smokers ($P = .005$) and those with heart disease ($P = 2 \times 10^{-4}$) in the NCI-MD study, and there was a similar trend, though not statistically significant in the PLCO study (smokers, $P = .30$; heart disease, $P = .07$). Median IL-8 levels were higher in control subjects with less education (high school or lower) ($P = .05$) and lower BMI (≤26.5 kg/m^2) ($P = .001$) in the PLCO study, and there was a similar trend in the NCI-MD study, though not statistically significant (education, $P = .12$; BMI, $P = .41$). The association between higher median cytokine levels and education was possibly mediated by an increased percentage of current smokers with less education in both the NCI-MD and PLCO studies ($P = .001$ and .03, respectively).

Addition of CRP and Smoking to the Risk Models

To better understand the interrelationship between cytokines and risk of lung cancer, we next correlated IL-6 and IL-8 levels to a nonspecific marker of inflammation, CRP. We found a positive correlation between IL-6 and CRP levels among case patients (Spearman's rank correlation coefficient [ρ] = 0.34, $P < .001$) and control subjects (ρ = 0.26, $P < .001$). In contrast, there was no correlation between IL-8 and CRP levels among case patients or control subjects (ρ = −0.04, $P = .41$; and ρ = −.01, $P = .77$, respectively). When IL-6, IL-8, and CRP levels were included as separate

Table 4. Association of circulating IL-6, IL-8, and CRP levels with lung cancer risk in the PLCO study*

Level of serum protein	Case patients (N = 532), No. (%)	Control subjects (N = 595), No. (%)	OR (95% CI)†	P‡
IL-6				
Low	237 (44.6)	304 (51.1)	1.00 (referent)	.33
High	295 (55.4)	291 (48.9)	1.13 (0.88 to 1.46)§	
IL-8				
Low	216 (40.6)	300 (50.4)	1.00 (referent)	.004
High	316 (59.4)	295 (49.6)	1.45 (1.13 to 1.86)||	
CRP				
Low	219 (41.2)	306 (51.4)	1.00 (referent)	.007
High	313 (58.8)	289 (48.6)	1.41 (1.09 to 1.81)¶	
IL-8 and CRP				
Low and low	91 (17.1)	154 (25.9)	1.00 (referent)	
Low and high or high and low	253 (47.6)	298 (50.1)	1.42 (1.03 to 1.95)	.03
High and high	188 (35.3)	143 (24.0)	2.11 (1.48 to 3.03)	3.6×10^{-5}

* CI = confidence interval; CRP = C-reactive protein; IL-6 = interleukin 6; IL-8 = interleukin 8; OR =odds ratio; PLCO = Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial.

† Multivariable unconditional logistic regression adjusted for age (continuous), sex, smoking pack-years (continuous), smoking status (never, former quit ≤15 years, former quit >15 years, and current), year of randomization, and number of years in the study, dichotomized by the median values among control subjects (IL-6 4.0 pg/mL; IL-8 17.4 pg/mL; CRP 2.7 μg/mL).

‡ *P* values were calculated using a two-sided Wald χ^2 statistic.

§ Circulating marker levels were additionally adjusted for serum CRP and IL-8 levels.

|| Circulating marker levels were additionally adjusted for serum IL-6 and CRP levels.

¶ Circulating marker levels were additionally adjusted for serum IL-6 and IL-8 levels.

variables in the multivariable models, IL-6 was not independently associated with lung cancer risk, whereas independent associations were observed for IL-8 (OR = 1.45, 95% CI = 1.13 to 1.86, P = .004) and CRP (OR = 1.41, 95% CI = 1.09 to 1.81, P = .007) (Table 4). There was no statistically significant interaction between IL-8 and CRP in their association with lung cancer risk ($P_{interaction}$ = .92). Levels of IL-8 and CRP were both dichotomized into low and high by the median levels among control subjects and combined in the multivariable analyses. Participants with high IL-8 and low CRP, or low IL-8 and high CRP, had a higher risk of lung cancer compared with participants with low IL-8 and low CRP levels (OR = 1.42, 95% CI = 1.03 to 1.95, P = .03). Participants with high IL-8 and high CRP showed an even higher risk of lung cancer (OR = 2.11, 95% CI = 1.48 to 3.03, $P = 3.6 \times 10^{-5}$) (Table 4). The model including both IL-8 and CRP performed better than models including only IL-8 or only CRP (Likelihood ratio test P = .001). Therefore, high levels of serum IL-8 and CRP were a better prediction classifier for lung cancer diagnosis than either marker alone.

Because smoking causes a chronic inflammatory state within the lungs, we assessed if the association between high IL-8 and CRP levels and lung cancer risk could be modulated by smoking. There was no detected interaction between high serum levels of both IL-8 and CRP and smoking status ($P_{interaction}$ = .18), but power to detect such an effect was limited because of the smaller number of never-smoking case patients (n = 37). High levels of both IL-8 and CRP were associated with an increased risk of lung cancer among current (OR = 3.33, 95% CI = 1.77 to 6.27, P_{trend} = .001) and former (OR = 1.76, 95% CI = 1.09 to 2.84, P_{trend} = .01) smokers (Figure 1). Although the magnitude of the odds ratio was similar to current and former smokers, the association of high levels of both IL-8 and CRP with lung cancer risk was not statistically significant among never-smokers (OR = 2.28, 95% CI = 0.66 to 7.87, P_{trend} = .19), which was not surprising given the small number of never-smokers. The associations among current and former smokers remained statistically significant after exclusion of case patients diagnosed within 2 years after blood collection (Figure 1), suggesting that the associations were not limited to those with current disease.

Standardized absolute risks of lung cancer over 10 years of follow-up among former and current smokers were calculated for those with IL-8 and CRP levels at or above median (categorized based on levels among control subjects) compared with those with IL-8 or CRP levels below the median. Never-smokers were excluded from analysis because of small numbers. Among former smokers, the 10-year absolute risk was statistically significantly higher among individuals with high IL-8 and high CRP levels (absolute risk = 3.18%, 95% CI = 2.15% to 4.68%) compared with individuals with low IL-8 and low CRP levels (absolute risk = 1.26%, 95% CI = 0.84% to 1.89%; risk difference = 1.92%, 95% CI of the difference = 0.57% to 3.27%). Among current smokers, the 10-year absolute risk was much higher among individuals with high IL-8 and high CRP levels (absolute risk = 8.01%, 95% CI = 5.77% to 11.05%) compared with individuals with low IL-8 and low CRP levels (absolute risk = 3.17%, 95% CI = 1.93% to 5.20%; risk difference = 4.83%; 95% CI of the difference = 1.84% to 7.83%) (Supplementary Table 6, available online).

Discussion

We examined the association between circulating IL-6 and IL-8 levels and lung cancer in two independent studies. The first was a case–control study in which the participants resided in the Baltimore, Maryland region. The second was a nested case–control study within the prospective PLCO Cancer Screening Trial, recruited from 10 centers throughout the United States. Our findings demonstrated that increased serum IL-6 and IL-8 levels were associated with lung cancer in the NCI-MD study and lung cancer risk in the PLCO study. High serum IL-8 levels predated subsequent diagnosis of disease; increased IL-8 was present even 5 years before

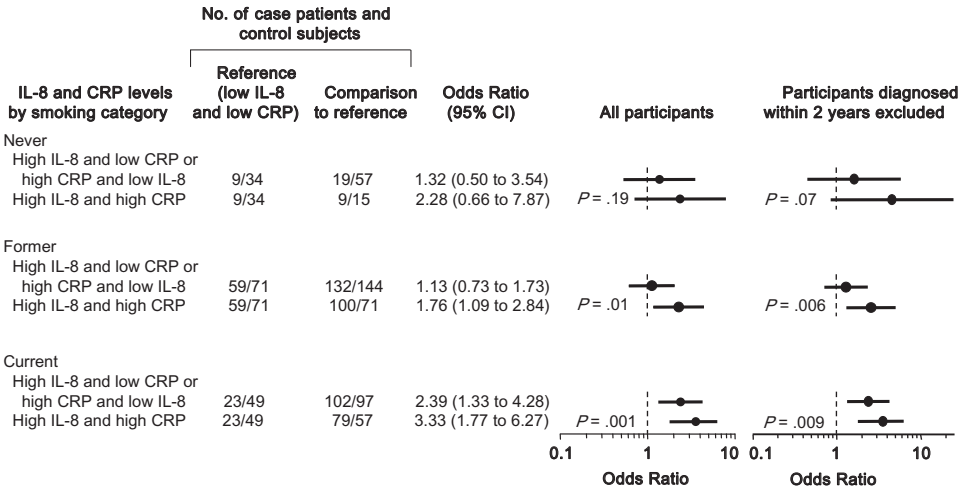

Figure 1. Associations of circulating interleukin 8 (IL-8) and C-reactive protein (CRP) levels with lung cancer risk. Associations across smoking status of Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial participants, and those participants diagnosed more than 2 years after baseline, are shown. High and low IL-8 and CRP levels were classified based on the median value among control subjects. Analyses were performed on 532 case patients and 595 control subjects. Odds ratios and 95% confidence intervals (CIs) are shown for all participants and were estimated by two-sided unconditional logistic regression analyses, adjusted for age, sex, smoking pack-years, smoking status (never, former quit ≤15 years, former quit >15 years, and current), year of random assignment, and number of years in the study. **Vertical dashed lines** represent an odds ratio of 1.0. **Solid black circles** represent the odds ratios and **solid horizontal bars** represent the 95% confidence intervals.

lung cancer diagnosis in the PLCO study. In contrast, IL-6 levels were increased only among those with diagnosed lung cancer (NCI-MD study) or those who soon developed lung cancer (<2 years in PLCO study), whereas no association was seen at longer intervals in the PLCO study. The associations were independent of smoking, age, sex, tumor histology, stage, systemic inflammation, and whether the case patients were screen or clinically diagnosed. In addition, IL-8 and CRP revealed substantial variation in 10-year absolute risk of lung cancer, both in current and former smokers, suggesting that these markers may identify smokers at a lower or higher risk of lung cancer.

Our findings are consistent with evidence that inflammatory mediators contribute to the pathogenesis of many human cancers, including lung cancer (29–31). For example, there is an increased risk of lung cancer associated with tuberculosis, adult asthma, and bacterial pneumonia (30). Under inflammatory stress, IL-6 and IL-8 participate in tumorigenesis by acting directly on lung epithelial cells via signaling through the nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1) pathway (30,32). Additionally, IL-6 and IL-8 are expressed by lung cancer cells and act in an autocrine and/or paracrine fashion to stimulate cancer cell proliferation (33,34), migration, and invasion (35).

It was surprising that IL-8, but not IL-6, was associated with subsequent development of disease, especially because IL-6 and IL-8 are secreted by premalignant and senescent cells surrounding premalignant lesions (10,36,37), which would likely be present up to several years before lung cancer diagnosis. The IL-6 data are consistent with a report of the prospective Health Aging and Body Composition study of 43 case patients, in which high IL-6 levels were not associated with development of lung cancer (7). Perhaps IL-8 plays a larger role in tumor initiation and promotion, whereas IL-6 participates primarily in tumor progression. This is supported by our previous report that increased circulating IL-6 levels are associated with lung cancer survival but IL-8 levels are not (24), and other reports that IL-6 participates in tumor progression in several cancer types (38-41). Serial examination of these markers, in addition to CRP, leading up to disease may provide additional crucial information about the relationship of these markers and disease status and progression.

Several factors should be considered in the interpretation of our findings. Strengths of this study include large sample sizes and inclusion of hospital- and population-based control subjects in the NCI-MD study. Although the associations were more robust among the population-based control subjects, there were statistically significant associations using both groups of control subjects. A major strength was replication of results in the PLCO study with a prospective design and broad representative group. An additional strength was the focus on a single race to avoid variability that could be introduced by a diverse population. However, a validation of the results among other ethnicities is needed, as well as an assessment of whether circulating cytokine levels contribute to racial health disparities in lung cancer.

This study has a few limitations. Patients with cancers other than lung cancer were excluded from the analyses, and therefore, we could not determine the specificity of the serum biomarkers for lung cancer among other cancer types. In addition, the absolute risk estimates did not account for competing mortality and thus, these values did not fully represent the observed proportion of individuals developing lung cancer. A minor limitation was the overlap of cytokine levels between case patients and control subjects. However, the relative risk assessment of IL-8 was considerably increased when used in combination with CRP levels, suggesting that development of a combination of biomarkers may yield even stronger predictive values. The cytokine concentrations measured in the NCI-MD and PLCO studies were different, making it difficult to use the same cutoffs for both populations. The reason for this difference is unknown, but may be because of differences in the populations, collection, and handling procedures for serum samples or analytical platforms. Therefore, more research is needed to standardize measurements of these biomarkers. Regardless, the concentrations were higher in case patients than in control subjects in both population groups, suggesting that these markers are targets for follow-up study.

Technologies such as low-dose spiral computed tomography may detect lung tumors at the millimeter range (42). However, the high rate of false-positive results instigates concern about whether exposure to x-rays, cost, and patient anxiety outweigh the benefits. Although the levels of IL-6, IL-8, and CRP were increased in clinically detected lung cancers, the levels of these biomarkers were increased in the PLCO study before lung cancer diagnosis, suggesting that they may be useful as biomarkers for lung cancer screening. Specifically, IL-6, IL-8, and CRP may be targets for further assay refinement, alone, or in combination with other non imaging screening targets under development. Furthermore, examination in prospective trials is necessary to determine if serial serum testing of IL-6, IL-8, and CRP can improve the positive predictive value of the current screening modalities, increase overall cost effectiveness, and potentially improve lung cancer survival.

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