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Circulating Inflammation Markers, Risk of Lung Cancer, and Utility for Risk Stratification

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Abstract

Background: We conducted two independent nested case-control studies to identify circulating inflammation markers reproducibly associated with lung cancer risk and to investigate the utility of replicated markers for lung cancer risk stratification.

Methods: Nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, the previously published discovery study included 526 lung cancer patients and 592 control subjects and the replication study included 526 lung cancer case patients and 625 control subjects. Control subjects were matched by sex, age, smoking, study visit, and years of blood draw and exit. Serum levels of 51 inflammation markers were measured. Odds ratios (ORs) were estimated with conditional logistic regression. All statistical tests were two-sided.

Results: Of 11 markers identified in the discovery study, C-reactive protein (CRP) (odds ratio [OR] [highest vs. lowest category] = 1.77, 95% confidence interval [CI] = 1.23 to 2.54), serum amyloid A (SAA) (OR = 1.88, 95% CI = 1.28 to 2.76), soluble tumor necrosis factor receptor-2 (sTNFRII) (OR = 1.70, 95% CI = 1.18 to 2.45), and monokine induced by gamma interferon (CXCL9/MIG) (OR = 2.09, 95% CI = 1.41 to 3.00) were associated with lung cancer risk in the replication study ($P_{trend} < .01$). In pooled analyses, CRP, SAA, and CXCL9/MIG remained associated with lung cancer more than six years before diagnosis ($P_{trend} < .05$). The incorporation of an inflammation score combining these four markers did not improve the sensitivity (77.6% vs 75.8%, $P = .33$) or specificity (56.1% vs 56.1%, $P = .98$) of risk-based lung cancer models.

Conclusions: Circulating levels of CRP, SAA, sTNFRII, and CXCL9/MIG were reproducibly associated with lung cancer risk in two independent studies within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, underscoring an etiologic role for inflammation in lung carcinogenesis, though replication is needed in other populations. Markers did not improve lung cancer risk stratification beyond standard demographic and behavioral characteristics.

Inflammation plays a key role in the immune response, eliminating pathogens and repairing tissue damage. However, inflammation can become chronic and processes that play a pivotal role in immunity, such as the generation of reactive oxygen and nitrogen species, promotion of angiogenesis and proliferation of cells, may play a key role in carcinogenesis (1,2).

There is a growing body of epidemiological evidence implicating chronic inflammation in lung cancer etiology (3–9). Recently, in a broad investigation of circulating inflammation markers and lung cancer, we identified 11 markers that were prospectively associated with lung cancer risk (10). These markers represent several components of the inflammation

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process, including acute-phase proteins (C-reactive protein [CRP], serum amyloid A [SAA]), pro-inflammatory cytokines (soluble tumor necrosis factor receptor-2 [sTNFRII]), anti-inflammatory cytokines (IL-1RA), lymphoid differentiation cytokines (IL-7), growth factors (TGF-A), and chemokines (CXCL5/ENA 78, monokine induced by gamma interferon [CXCL9/MIG], CXCL13/BCA-1, CCL17/TARC, CCL22/MDC) (10).

Our prior work also showed that an inflammation score derived from four markers (CRP, IL-1RA, CXCL13/BCA-1, and CCL22/MDC) provided good separation in cumulative risks, with current smokers in the highest quartile, having a 7.9% 10-year cumulative risk of lung cancer compared with 2.3% in the lowest quartile (10). This observation could have important clinical implications. In 2013, the US Preventive Services Task Force (USPSTF) issued a grade B recommendation for annual screening with low-dose computed tomography (LDCT) for people age 55 to 80 years with 30 or more pack-years of smoking and less than 15 years since smoking cessation (11). Given the costs of LDCT screening and the potential harms, such as false-positive screen results, there is growing consensus to target screening to smokers at highest lung cancer risk (12–14). It is unclear whether inflammation markers can aid in improved lung cancer risk stratification beyond currently available demographic and behavioral predictors.

Here, we report results from an independent case-control study of 51 circulating markers of inflammation and lung cancer risk also nested within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Additionally, pooling data from the discovery and replication studies, we investigated the utility of replicated markers in lung cancer risk stratification.

Methods

Study Population

The PLCO trial recruited approximately 155 000 men and women age 55 to 74 years from the general population between 1992 and 2001 (15). Our study was nested within the screening arm. Lung cancer screening included a chest x-ray at baseline, followed by two (never smokers) or three (smokers) annual screens (16). Blood samples were obtained at baseline and five subsequent annual visits, and participants provided demographic, behavioral, and dietary information at baseline. Lung cancers were ascertained through annual questionnaires and confirmed by medical chart abstraction, death certificate review, or clinical follow-up after a positive screening (15,16). PLCO was approved by the institutional review boards at each screening center and the National Cancer Institute. All participants gave informed consent.

Our prior study of circulating inflammation markers (ie, discovery study) has been described in detail (526 lung cancer case patients diagnosed through 2004 and 592 matched control subjects) (10), and was similarly designed to the replication study noted herein. The replication study included 526 of 1776 lung cancers that were not included in the discovery study and occurred through 2012 (10). Additional exclusion criteria were: missing/incomplete smoking information, history of cancer, multiple cancers during follow-up, unavailable serum, or missing consent. Case patients were selected to have the same distribution of time from sample collection to diagnosis as the discovery study. Six hundred twenty-five control subjects were matched to case patients (1:1 for ever smokers, 3:1 for never smokers) on age at random assignment (55–59, 60–64, 65–69, 70–74 years), sex, study year of blood draw, calendar year of blood draw, study year of exit, smoking status (current, former, never), cumulative smoking at baseline for ever smokers (0–29, 30–39, 40–49, 50+ pack-years), and time since quitting for former smokers (<15, 15+ years).

Laboratory Methods

Circulating levels of 51 markers were measured in serum specimens collected at baseline (15%) or at one of the annual follow-up visits (visit 1: 14%, 2: 31%, 4: 29%, 5: 10%) (processed at 2400–3000 rpm for 15 minutes, frozen ≤2 hours of collection, stored at -80°C). Analytes that performed poorly in the discovery study were not included (10). Markers were measured using a Luminex bead-based assay (EMD Millipore Inc., Billerica, MA). Concentrations were calculated using either a four- or five-parameter standard curve. Serum samples were assayed in duplicate and averaged to calculate concentrations. Case patients and matched control subjects were included on the same analytical batch in adjacent wells. We assayed one pair of blinded duplicates within each batch and a pooled serum sample across batches to evaluate assay reproducibility and drift across batches. We calculated coefficients of variation (CVs) and intraclass correlation coefficients (ICCs). Two markers with more than 90% of values below the lowest limit of quantification (LLOQ) and two markers with 0% detectability in the blinded duplicates were excluded from all analyses, resulting in 47 evaluable markers. ICCs were greater than 0.8 in all but one of these markers (Supplementary Table 1, available online).

Statistical Analyses

We assessed independent replication of our prior results by estimating associations in the replication study for those markers identified in the discovery study (ie, CRP, SAA, sTNFRII, IL-1RA, IL-7, TGF-A, CCL5/ENA-78, CXCL9/MIG, CXCL13/BCA-1, CCL17/TARC, and CCL22/MDC). Because of lot-to-lot variability, which is inherent to immunoassays that are utilized for research purposes (17), absolute marker levels differed notably between the two studies (Supplementary Table 2, available online).We believe these differences led to monotonic distributional shifts, as evidenced by similar log-variance values between the two studies (Supplementary Table 2, available online), which do not change the rank order of analyte concentrations. Thus, we do not anticipate a systematic bias in our results. Importantly, the ICCs were similar between the two studies (Supplementary Table 2, available online), underscoring similar statistical power.

Study-specific cutpoints were used to generate categories based on the proportion of individuals with measurements under the LLOQ as follows: markers with less than 25% below the LLOQ (n = 36) were categorized into quartiles (based on the distribution among control subjects); markers with 25% to 50% below the LLOQ (n = 5) were categorized as less than the LLOQ, and tertiles of detectable measurements; markers with 50% to 75% below the LLOQ (n = 2) were categorized as less than the LLOQ, and below and above the median; and markers with 75% to 90% below the LLOQ (n = 4) were categorized as less than the LLOQ and equal to or greater than LLOQ.

Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of each marker with lung cancer risk. In addition to the matching variables, we adjusted all models for history of chronic bronchitis/emphysema, coronary heart disease or heart attack, family history of lung cancer, regular use of aspirin/ibuprofen,

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body mass index, race, and education. P_{trend} values were calculated by treating marker categories as ordinal variables.

To increase power, we pooled results from the discovery and replication studies (retaining study-specific marker categories) and carried out stratified analyses by latency (ie, time from serum collection to diagnosis in two-year categories), histology (squamous cell carcinoma and adenocarcinoma), smoking status (never, former, current), and sex for replicated markers. Multiplicative statistical interactions with latency, smoking, and sex and heterogeneity across histologies were evaluated using the Wald test.

For replicated markers, we estimated an inflammation score through five-fold cross-validation separately for the discovery and replication studies, as previously described (18). The association between study-specific quartiles of the inflammation score and lung cancer was assessed with conditional logistic regression.

We then formally investigated whether the incorporation of CRP (marker commonly used for cardiovascular disease risk stratification) or the inflammation score (based on replicated markers) provided improvements in lung cancer risk prediction. Specifically, pooling data from both studies, we compared the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the identification of lung cancers over five years of follow-up among ever smokers across four incremental risk stratification approaches: 1) current USPSTF guidelines (≥30 pack-years smoked, ≤15 years since smoking cessation), 2) risk-based models based on age, sex, pack-years, and time since quitting, 3) risk-based models plus CRP, and 4) risk-based models plus the inflammation score. We first estimated the number of screening arm participants that met the USPSTF criteria for LDCT screening (n = 15 076), and calculated sensitivity, specificity, PPV, and NPV for the occurrence of lung cancer during five years of follow-up. We then selected 15 076 individuals with the highest predicted five-year probability of lung cancer in each of the latter three approaches and calculated sensitivity, specificity, PPV, and NPV in each approach. These predicted five-year probabilities of lung cancer were estimated using weighted Cox regression models (see the Supplementary Statistical Appendix, available online).

All tests were two-sided; *P* values of less than .05 were considered statistically significant.

Results

Characteristics of Lung Cancer Case Subjects and Control Subjects

Demographic, behavioral, and health history characteristics were largely similar between the discovery and replication studies (Table 1). In both studies, compared with control subjects, case patients were more likely to have a personal history of emphysema or chronic bronchitis and have a family history of lung cancer. Compared with control subjects, case patients in the discovery study were less likely to have more than 12 years of education and case patients in the replication study were more likely to be black.

Associations Between Inflammation Markers and Lung Cancer

Of the 11 circulating inflammation markers that were associated with lung cancer risk in our discovery study (P_{trend} < .05) (10), four markers remained statistically significantly associated with lung cancer in the replication study (Table 2): CRP (quartile 4 vs 1: OR = 1.77, 95% CI = 1.23 to 2.54, P_{trend} < .001), SAA (OR = 1.88, 95% CI = 1.28 to 2.76, P_{trend} = .003), sTNFRII (OR = 1.70, 95% CI = 1.18 to 2.45, P_{trend} = .006), and CXCL9/MIG (OR = 2.09, 95% CI = 1.41 to 3.08, P_{trend} < .001). When each of these four markers was included in one model, odds ratios for CRP (P_{trend} = .03) and CXCL9/MIG (P_{trend} = .002) remained statistically significant. An additional seven markers were statistically significantly associated with lung cancer in the replication study alone (ie, CXCL11/I-TAC, CXCL1/GRO, CXCL10/IP-10, CCL15/MIP-1D, sEGFR, sILRII, sVEGFR3; P_{trend} < .05) (Supplementary Table 3, available online).

Pooling the data from the discovery and replication studies gave us more power to assess associations stratified by smoking status, histology, latency, and sex. CRP, SAA, and CXCL9/MIG were each associated with lung cancer risk among both former and current smokers, while sTNFRII was only associated with lung cancer among former smokers (Supplementary Figure 1, available online). None of the four markers was associated with lung cancer risk among never smokers (all P_{trend} > .05). Further, none of the nine markers that were statistically significantly associated with lung cancer risk among never smokers in the discovery study replicated. Each marker was associated with risk of both adenocarcinomas and squamous cell carcinomas (all P_{trend} < .05) (Supplementary Figure 2, available online). Statistically significant interactions ($P_{interaction}$ [between marker levels and sex] < .05) between the markers and sex were only present for CRP and SAA, where odds ratios were stronger among men than women (data not shown).

CRP, SAA, and CXCL9/MIG were consistently associated with lung cancer across categories of latency, with associations present six or more years prior to diagnosis (Table 3). Though a statistically significant interaction was not present between any of the markers and latency (all $P_{interaction}$ [between marker levels and latency] > .05), the association between sTNFRII and lung cancer risk appeared to attenuate with increasing time.

The inflammation score, which combined the four replicated markers (CRP, SAA, CXCL9, and sTNFRII), was similarly associated with lung cancer in each study ($P_{interaction}$ = .81) (Supplementary Table 4, available online). In pooled analysis, the highest quartile of the inflammation score was associated with an increase in lung cancer risk (OR = 2.43, 95% CI = 1.86 to 3.18, P_{trend} < .001). An inflammation score that excluded CRP was similarly associated with lung cancer (OR = 2.18, 95% CI = 1.67 to 2.84, P_{trend} < .001).

Utility of Inflammation Markers in Lung Cancer Risk Stratification

Of the 916 lung cancer patients estimated to have occurred within five years of follow-up among smokers in the screening arm of the PLCO, 624 would have been included among the 15 076 screening-eligible individuals, according to USPSTF guidelines (sensitivity = 68.1%, specificity = 55.8%, PPV = 4.1% and NPV = 98.4%) (Figure 1). Sensitivity (75.2%) statistically significantly increased (*P* = .006) when a risk-based approach that incorporated age, sex, and smoking characteristics was utilized to select the same number of individuals, while no differences were noted for specificity (56.1%), PPV (4.6%), or NPV (98.8%). Importantly, further inclusion of CRP or the inflammation score into the risk-based model did not statistically significantly improve sensitivity (75.8% and 77.6%, respectively), specificity (56.1% and 56.1%), PPV (4.6% and 4.7%), or NPV (98.8% and 98.9%) for the identification of

Table 1. Characteristics of lung cancer case patients and control subjects

Characteristic	Replication study		Discovery study	
	Control subjects, % (n = 625)	Lung cancer case patients, % (n = 526)	Control subjects, % (n = 592)	Lung cancer case patients, % (n = 526)
Age at randomization, y*				
≤59	21.1	21.9	17.6	18.3
60–64	27.8	28.9	28.2	27.8
65–69	29.3	29.9	32.4	33.7
70+	21.8	19.4	21.8	20.3
Sex*				
Female	46.2	43.4	35.8	31.9
Male	53.8	56.7	64.2	68.1
Smoking status*				
Never	24.0	9.5	16.7	6.3
Former	40.8	48.7	49.7	55.9
Current	35.2	41.8	33.6	37.8
Pack-years smoked among ever smokers*				
<30	20.8	20.8	25.2	25.3
30–40	9.9	9.9	26.0	26.0
40–50	18.5	18.7	8.5	8.3
50+	50.7	50.6	40.4	40.4
Time since quitting smoking among former smokers*, y				
<15	57.3	57.4	62.6	62.6
15+	42.8	42.6	36.4	36.4
Race†				
White	89.9	89.0	90.9	88.8
Black	4.3	7.2	4.7	7.6
Other	5.8	3.8	4.4	3.6
Education‡				
≤12 y/completed high school	33.3	37.6	33.6	40.1
>12 y	66.7	62.4	66.4	59.9
Body mass index, kg/m^2				
<25	32.3	38.8	33.5	37.1
25–29.9	44.8	41.1	45.6	42.6
30+	21.1	19.0	19.3	19.2
Missing	1.8	1.1	1.7	1.1
History of emphysema or chronic bronchitis§				
No	89.6	94.8	89.9	81.4
Yes	10.4	15.2	10.1	18.6
History of coronary heart disease or heart attack				
No	89.1	89.0	87.7	84.6
Yes	10.9	11.0	12.3	15.4
Family history of lung cancer§				
No	85.6	79.1	85.8	75.7
Yes	13.3	21.0	13.0	23.4
Missing	1.1	0	1.2	1.0
Regularly uses aspirin/ibuprofen				
No	38.1	35.9	36.8	35.6
Yes	61.9	64.1	63.2	64.5
Lung cancer histology				
Squamous cell carcinoma	—	18.1	—	21.9
Adenocarcinoma	—	41.1	—	45.6
Other	—	40.8	—	32.5
Lung cancer stage‖				
I	—	25.7	—	28.8
II	—	4.9	—	6.8
III	—	21.3	—	25.1
IV	—	31.6	—	24.1
Missing	—	16.5	—	15.2
Years from blood collection to diagnosis, median (IQR)	—	3.65 (2.46–5.92)	—	2.93 (1.15–5.07)
Year of diagnosis, median (IQR)	—	2004 (2002–2006)	—	2000 (1998–2002)

* Matching variable. The distribution of case subjects and control subjects appears different because of matching three control subjects per case for never-smokers and one control per case for former and current smokers. IQR = interquartile range.

† Statistically significant difference between case patients and control subjects in the replication study only.

‡ Statistically significant difference between case patients and control subjects in discovery study only.

§ Statistically significant difference between case patients and control subjects in both the discovery and replication study.

‖ Staging based on the American Joint Committee on Cancer staging system.

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Table 2. Association between 11 circulating markers of inflammation and lung cancer risk in the replication study, in the discovery study, and in pooled data from the two studies combined

		Replication study			Discovery study	Pooled estimate
Marker	Category	Lung cancer case patients	Control subjects	ORs (95% CIs)*	ORs (95% CIs)*	ORs (95% CIs)*
CRP	1	112	156	1.0	1.0	1.0
	2	99	156	0.94 (0.63 to 1.38)	1.58 (1.06 to 2.35)	1.21 (0.92 to 1.59)
	3	121	156	1.09 (0.75 to 1.59)	1.69 (1.14 to 2.51)	1.34 (1.03 to 1.75)
	4	192	156	1.77 (1.23 to 2.54)	2.27 (1.51 to 3.41)	1.99 (1.52 to 2.61)
P_{trend}†				<.001	<.001	<.001
SAA	1	95	156	1.0	1.0	1.0
	2	138	156	1.84 (1.27 to 2.66)	1.21 (0.83 to 1.77)	1.51 (1.16 to 1.96)
	3	147	156	1.90 (1.31 to 2.75)	1.59 (1.08 to 2.33)	1.72 (1.32 to 2.25)
	4	144	156	1.88 (1.28 to 2.76)	2.18 (1.48 to 3.22)	2.03 (1.55 to 2.66)
P_{trend}†				.003	<.001	<.001
sTNFRII	1	102	156	1.0	1.0	1.0
	2	132	156	1.29 (0.90 to 1.85)	1.27 (0.89 to 1.81)	1.29 (1.00 to 1.65)
	3	130	156	1.35 (0.93 to 1.96)	1.35 (0.93 to 1.97)	1.35 (1.04 to 1.76)
	4	160	156	1.70 (1.18 to 2.45)	1.50 (1.01 to 2.21)	1.62 (1.25 to 2.11)
P_{trend}†				.006	.05	<.001
IL-1RA	1	374	462	1.0	1.0	1.0
	2	79	81	1.10 (0.77 to 1.59)	0.71 (0.51 to 1.00)	0.92 (0.74 to 1.14)
	3	72	82	1.09 (0.75 to 1.58)	‡	‡
P_{trend}†/*P*				.57	.05	.44
IL-7	1	223	258	1.0	1.0	1.0
	2	89	122	0.98 (0.69 to 1.39)	1.47 (1.05 to 2.06)	1.17 (0.95 to 1.44)
	3	102	123	1.00 (0.70 to 1.42)	‡	‡
	4	111	122	1.14 (0.80 to 1.63)	‡	‡
P_{trend}†/*P*				.52	.02	.15
TGF-A	1	118	158	1.0	1.0	1.0
	2	106	154	1.05 (0.73 to 1.52)	1.26 (0.86 to 1.84)	1.14 (0.88 to 1.48)
	3	173	157	1.55 (1.09 to 2.22)	1.40 (0.96 to 2.05)	1.47 (1.14 to 1.90)
	4	128	156	1.12 (0.77 to 1.63)	1.56 (1.07 to 2.27)	1.31 (1.01 to 1.71)
P_{trend}†				.22	.02	.01
CXCL5/ENA 78	1	118	156	1.0	1.0	1.0
	2	129	156	0.99 (0.69 to 1.41)	1.43 (1.01 to 2.03)	1.21 (0.94 to 1.54)
	3	142	157	1.20 (0.83 to 1.72)	1.13 (0.79 to 1.63)	1.17 (0.91 to 1.51)
	4	136	156	0.96 (0.96 to 1.43)	1.68 (1.15 to 2.45)	1.29 (0.98 to 1.68)
P_{trend}†				.87	.03	.10
CXCL9/MIG	1	97	156	1.0	1.0	1.0
	2	108	156	1.14 (0.78 to 1.67)	0.92 (0.63 to 1.34)	1.02 (0.79 to 1.33)
	3	135	157	1.44 (0.98 to 2.11)	1.38 (0.95 to 2.00)	1.40 (1.08 to 1.83)
	4	185	156	2.09 (1.41 to 3.08)	1.63 (1.12 to 2.36)	1.82 (1.40 to 2.37)
P_{trend}†				<.001	.003	<.001
CXCL13/BCA-1	1	115	151	1.0	1.0	1.0
	2	116	152	0.91 (0.63 to 1.31)	0.97 (0.67 to 1.40)	0.95 (0.73 to 1.23)
	3	133	152	1.08 (0.76 to 1.53)	0.99 (0.68 to 1.45)	1.06 (0.82 to 1.37)
	4	145	152	1.21 (0.85 to 1.74)	1.59 (1.10 to 2.29)	1.40 (1.08 to 1.80)
P_{trend}†				.20	.01	.005
CCL17/TARC	1	120	156	1.0	1.0	1.0
	2	102	156	0.73 (0.50 to 1.07)	1.16 (0.80 to 1.67)	0.93 (0.72 to 1.21)
	3	113	157	0.76 (0.53 to 1.11)	1.09 (0.76 to 1.57)	0.91 (0.71 to 1.18)
	4	190	156	1.26 (0.89 to 1.78)	1.50 (1.06 to 2.13)	1.38 (1.08 to 1.76)
P_{trend}†				.13	.03	.01
CCL22/MDC	1	143	156	1.0	1.0	1.0
	2	110	156	0.80 (0.56 to 1.14)	1.13 (0.78 to 1.65)	0.93 (0.72 to 1.19)
	3	98	157	0.68 (0.48 to 0.97)	0.96 (0.66 to 1.41)	0.80 (0.62 to 1.04)
	4	174	156	1.13 (0.79 to 1.60)	1.72 (1.18 to 2.50)	1.38 (1.07 to 1.78)
P_{trend}†				.70	.009	.03

* Odds ratios and 95% confidence intervals were estimated in conditional logistic regression models. Models were adjusted for matching criteria, personal history of bronchitis/emphysema, history of coronary heart disease or heart attack, family history of lung cancer, use of aspirin/ibuprofen, body mass index, race, and education. BCA = B-cell attracting chemokine; CI = confidence interval; CXCL9/MIG = monokine induced by gamma interferon; CRP = C-reactive protein; ENA = epithelial-derived neutrophil-activating peptide; IL = interleukin; MDC = macrophage-derived chemokine; OR = odds ratio; SAA = serum amyloid A; sTNFRII = soluble tumor necrosis factor receptor-2; TARC = thymus- and activation-regulated chemokine; TGF = transforming growth factor-alpha.

† Two-sided P_{trend} values across marker categories were assessed with the Wald test using marker levels as an ordinal variable with 1 degree of freedom.

‡ For IL-7 and IL-1Ra, the fraction of values above the lowest limit of quantification (LLOQ) was greater in the replication study than in the discovery study. Therefore, more categories were created for the replication study, while these markers were classified as above or below the LLOQ in the discovery study and the pooled analysis.

Table 3. Association between CRP, SAA, sTNFRII, and CXCL9/MIG and lung cancer risk, stratified by time from blood collection to lung cancer diagnosis using data from both the discovery and replication studies

		<2 y (257 case patients, 299 control subjects)	2–4 y (468 case patients, 397 control subjects)	4–6 y (198 case patients, 180 control subjects)	6+ y (252 case patients, 219 control subjects)
Marker	Category	ORs (95% CIs)*	ORs (95% CIs)*	ORs (95% CIs)*	ORs (95% CIs)*
CRP	1	1.0	1.0	1.0	1.0
	2	1.18 (0.67 to 2.08)	1.12 (0.71 to 1.76)	1.37 (0.69 to 2.70)	1.46 (0.77 to 2.79)
	3	1.33 (0.76 to 2.33)	1.42 (0.92 to 2.19)	1.11 (0.56 to 2.19)	1.61 (0.86 to 3.04)
	4	2.20 (1.23 to 3.95)	1.77 (1.16 to 2.70)	2.26 (1.17 to 4.36)	2.25 (1.17 to 4.33)
P_{trend}†		.005	.003	.03	.02
$P_{interaction}$ = .34					
SAA	1	1.0	1.0	1.0	1.0
	2	1.47 (0.83 to 2.57)	1.54 (1.00 to 2.39)	1.92 (0.97 to 3.81)	1.58 (0.88 to 2.83)
	3	1.87 (1.06 to 3.30)	1.40 (0.92 to 2.13)	3.29 (1.49 to 7.26)	1.84 (1.02 to 3.33)
	4	2.29 (1.32 to 3.99)	1.54 (0.99 to 2.38)	3.34 (1.60 to 6.98)	3.00 (1.53 to 5.87)
P_{trend}†		.003	.09	<.001	.002
$P_{interaction}$ = .99					
sTNFRII	1	1.0	1.0	1.0	1.0
	2	2.33 (1.36 to 3.98)	1.26 (0.82 to 1.93)	1.28 (0.67 to 2.47)	0.71 (0.41 to 1.25)
	3	2.26 (1.29 to 3.96)	1.19 (0.78 to 1.82)	1.60 (0.78 to 3.26)	0.90 (0.49 to 1.63)
	4	2.00 (1.13 to 3.53)	1.68 (1.11 to 2.56)	1.62 (0.83 to 3.16)	1.24 (0.65 to 2.37)
P_{trend}†		.02	.03	.15	.43
$P_{interaction}$ = .26					
CXCL9/MIG	1	1.0	1.0	1.0	1.0
	2	1.75 (1.01 to 3.06)	0.72 (0.46 to 1.12)	0.93 (0.47 to 1.84)	1.09 (0.60 to 1.99)
	3	3.07 (1.69 to 5.58)	0.98 (0.63 to 1.51)	0.99 (0.52 to 1.88)	1.54 (0.84 to 2.83)
	4	2.46 (1.41 to 4.32)	1.39 (0.89 to 2.16)	1.80 (0.95 to 3.39)	2.08 (1.12 to 3.85)
P_{trend}†		<.001	.05	.06	.01
$P_{interaction}$ = .63					

* Odds ratios and 95% confidence intervals were estimated in conditional logistic regression models. Models were adjusted for matching criteria, personal history of bronchitis/emphysema, history of coronary heart disease or heart attack, family history of lung cancer, use of aspirin/ibuprofen, body mass index, race, and education. CI = confidence interval; CXCL9/MIG = monokine induced by gamma interferon; CRP = C-reactive protein; OR = odds ratio; SAA = serum amyloid A; sTNFRII = soluble tumor necrosis factor receptor-2.

† Two-sided P_{trend} values across marker categories were assessed with the Wald test using marker levels as an ordinal variable with 1 degree of freedom.

lung cancers (all $P > .05$). The lack of improvement in lung cancer risk prediction arose from the relatively modest net movement of lung cancer patients into the high-risk group of 15 076 smokers (Figure 2). For example, the incorporation of CRP into a risk-based model resulted in a net increase of six lung cancers in the high-risk group, a nonsignificant improvement relative to the total number of lung cancer patients.

Discussion

In the largest and most comprehensive efforts to date to discover and replicate the associations of circulating inflammation markers and lung cancer, our key observation was that levels of CRP, SAA, CXCL9/MIG, and sTNFRII were consistently associated with increased lung cancer risk. These observations reinforce the role of inflammation in lung carcinogenesis. Importantly, we show that these four markers do not provide meaningful improvements in lung cancer risk stratification beyond age, sex, and smoking characteristics.

CRP, SAA, sTNFRII, and CXCL9/MIG each play an important role in the inflammatory response and have functions that may contribute to lung carcinogenesis. CRP and SAA are both acute-phase proteins, which are primarily stimulated by IL-6 in response to infection or tissue damage, resulting in concentrations that increase up to 1000-fold (19). CRP activates the complement pathway and plays a role in opsonization and phagocytosis, while CRP and SAA both induce cytokine production and chemotaxis (19–21). Tumor necrosis factor, a pro-inflammatory cytokine, plays a key role in the inflammatory response and binds to TNFRI and TNFRII. Unlike TNFRI, which is related to apoptosis, TNFRII promotes cell survival through the activation of NF-κB, potentially resulting in cell proliferation, a hallmark of cancer development (22). CXCL9/MIG is a glutamic acid-leucine-arginine (ELR)–negative chemokine that is induced by interferon-gamma, a key component of the inflammatory response, and primarily attracts T-cells and natural killer cells (23). CXCL9/MIG is anti-angiogenic; thus, perhaps increased levels observed prior to lung cancer diagnosis reflect compensatory efforts by the immune system to prevent ongoing pro-angiogenic changes in the tissue microenvironment.

Though these inflammation markers may play an etiologic role in lung cancer development, associations may also be driven by inflammatory exposures that are risk factors for lung cancer. Smoking has notable effects on immunity and causes nearly all lung squamous cell carcinomas and 70% to 80% of adenocarcinomas (Freedman ND, Abnet CC, Caporaso NE, et al., unpublished data). However, we carefully matched both studies on smoking behaviors, and smoking has been associated with only one of these four markers (ie, CRP), indicating that these associations are not driven by residual confounding by smoking (24). Though the associations observed in our studies were limited to smokers, our study was underpowered to assess associations among never smokers (n = 83 total lung cancer case patients) and additional well-powered studies of never smokers are needed. Of

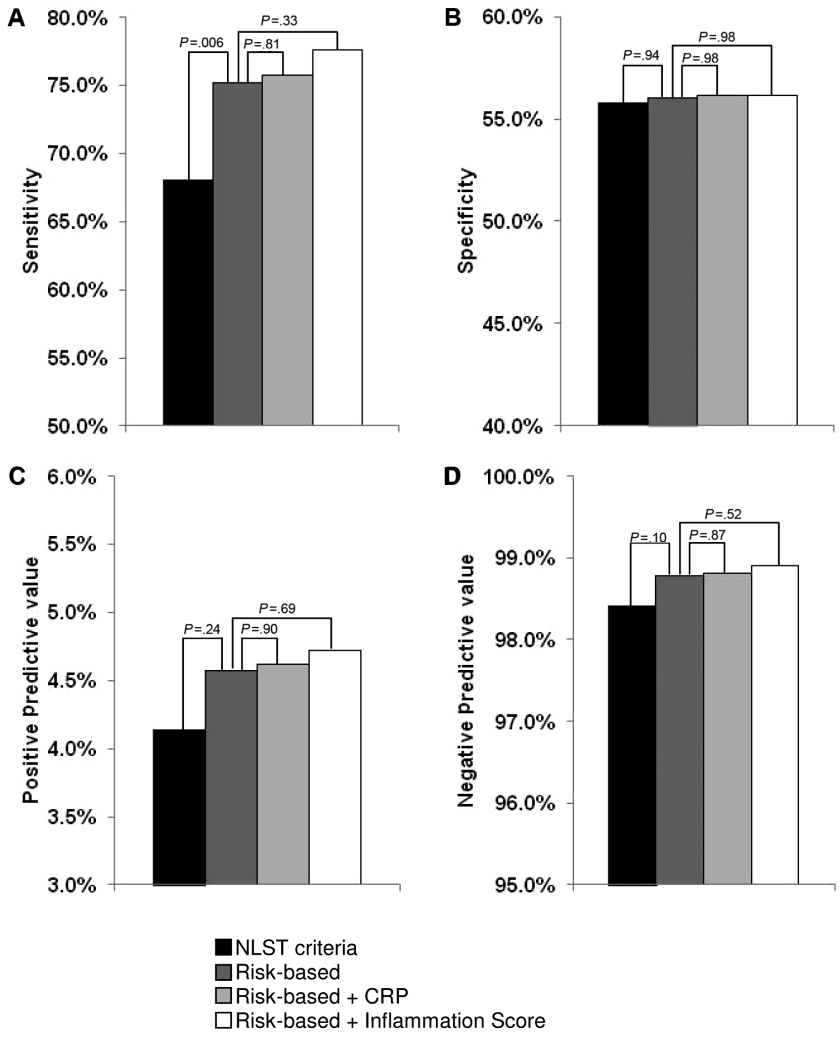

Figure 1. Sensitivity, specificity, positive predictive value, and negative predictive value of low-dose computed tomography (LDCT) screening eligibility criteria, risk-based models; risk-based models plus C-reactive protein (CRP) levels and risk-based models plus an inflammation score. **Bars** indicate the **(A)** sensitivity, **(B)** specificity, **(C)** positive predictive value, and **(D)** negative predictive value of LDCT screening eligibility criteria, based on US Preventive Services Task Force guidelines (**black bars**); risk-based models (**dark gray bars**); risk-based models plus CRP levels (**light gray bars**) and risk-based models plus an inflammation score based on levels of CRP, serum amyloid A, monokine induced by gamma interferon, and soluble tumor necrosis factor receptor-2 (**white bars**). A two-sided Wald Chi-Square test was used to calculate *P* values. *P* values compare **dark gray bars** to **black bars**, **light gray bars** to **dark gray bars,** and **white bars** to **dark gray bars**. CRP = C-reactive protein.

note, despite adjustment for self-reported chronic bronchitis and emphysema, residual confounding may remain, as these markers have been associated with chronic obstructive pulmonary disease, as well as other inflammatory lung conditions in the literature (25–28). Further, we were unable to adjust for other potential confounders such as asthma, allergies, and certain medications. Despite the potential for residual confounding, we believe that the associations observed in this study may reflect the role of individual variability in immune response in lung cancer risk.

Studies have shown that tumor cells can produce SAA, sTNFRII, and CXCL9/MIG (29–32). Thus, it is possible that these associations are induced by inflammation markers produced by preclinical lung tumors. However, CRP, SAA, and CXCL9/MIG were associated with lung cancer risk six years or more prior to diagnosis, reducing the likelihood of reverse causality. Further, if associations were induced by markers produced by the tumors, stronger associations should be present in larger tumors. However, each marker was similarly associated with stage I/II and stage III/IV lung cancers (data not shown). Future studies with longer latencies from blood collection to cancer diagnosis should be explored.

The utility of risk-based models for the identification of high-risk smokers to maximize the benefits of LDCT, while reducing the harms, has been shown previously (33,34). Consistent with these findings, we found that a risk-based approach aids in the identification of a larger fraction of lung cancers (ie, sensitivity) when compared with USPSTF guidelines. However, the further inclusion of CRP levels alone or an inflammation score based on CRP, SAA, sTNFRII, and CXCL9/MIG did not notably improve these sensitivity, specificity, PPV, or NPV. The lack of improvement of these parameters at the population level may have been driven by the modest effect sizes of the associations between inflammation markers and lung cancer as well as modest attributable fractions for lung cancer, resulting in little net movement of lung cancer patients into the high-risk group. Therefore, these markers may not be suitable candidates for inclusion in lung cancer risk prediction models.

The main strength of our study was the observations of these associations in two independent case-control sets, which allowed for independent replication of our results. Additionally, our studies were nested within a large, well-established,

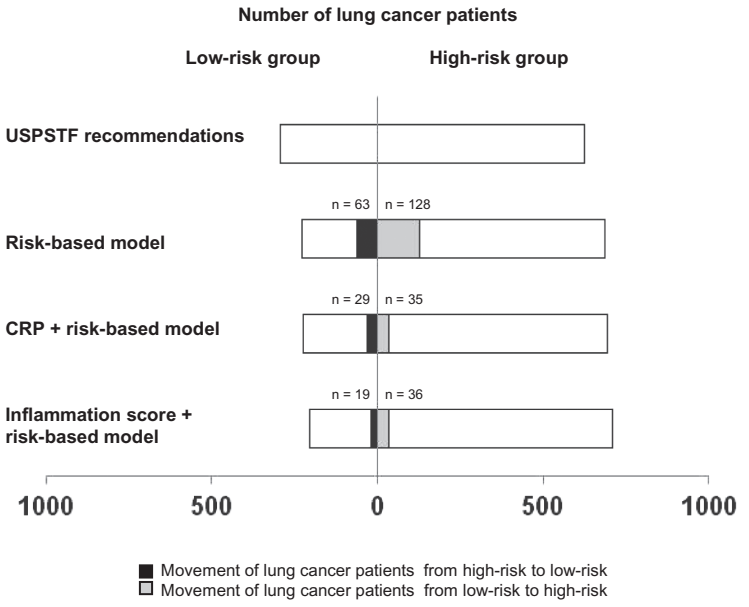

Figure 2. Movement of lung cancer patients between high- and low-risk groups defined by US Preventive Services Task Force (USPSTF) guidelines, risk-based models, risk-based models plus C-reactive protein (CRP) levels, and risk-based models plus an inflammation score. **Bars** indicate the number of lung cancer patients occurring in the high- and low-risk groups based on USPSTF guidelines, risk-based models, risk-based models plus CRP levels and risk-based models plus an inflammation score based on levels of CRP, serum amyloid A, monokine induced by gamma interferon, and soluble tumor necrosis factor receptor-2. The **gray bars** represent the number of patients that moved from the low-risk to the high-risk group, and the **black bars** represent the number of patients that moved from the high-risk to the low-risk group as you move from one scenario to the next (ie, comparing risk-based models to USPSTF guidelines, comparing risk-based + CRP models to risk-based models, and comparing risk-based + inflammation score models to risk-based + CRP models). CRP = C-reactive protein; USPSTF = US Preventive Services Task Force.

population-based cohort with uniform specimen collection procedures and measured a large number of key markers of inflammation with high ICCs in a relatively low specimen volume.

Our study is not without limitations. Our observations need further replication in additional studies carried out in completely unique populations that utilize different technologies for marker measurement, particularly given the notable lot-to-lot variability in absolute levels. We note that there are many more markers of immune response that were not included in this study but could play an important role in lung cancer etiology. Additionally, future studies should examine changes in marker levels over time in relation to lung cancer risk and whether systemic markers of inflammation reflect local inflammation in the lung.

Circulating levels of CRP, SAA, sTNFRII, and CXCL9/MIG have been shown to be associated with prospective risk of lung cancer in two independent studies nested within the PLCO. Collectively, these studies show that inflammation likely plays an important role in the development of lung cancer and provide insight into potential inflammatory pathways associated with lung cancer etiology that should be explored in future studies. Though potentially important etiologic markers, inflammation markers may not be the most useful markers for risk stratification. Future studies should examine markers that have a higher specificity for lung cancer, such as biomarkers produced directly by lung cells and tumors.

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