Relationship between Circulating Inflammation Proteins and Lung Cancer Diagnosis in the National Lung Screening Trial

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Abstract

Background: Previously, we observed a strong relationship between circulating serum inflammation proteins in relation to lung cancer diagnosis and risk, both in case–control and prospective cohorts. Low-dose computed tomography (LDCT) screening has a high prevalence of false-positive nodules, thus companion noninvasive biomarkers that can distinguish between benign and malignant nodules could have clinical utility and positive impact on patient outcomes.

Methods: We conducted a nested case–control study within the National Lung Screening Trial. Concentrations of 30 inflammation proteins were measured on plasma samples of 262 cases and 528 controls using a highly sensitive and analytically validated electrochemiluminescence V-PLEX immunoassay.

Results: Comparing the fourth quartile with the first quartile, we found increased IFNγ and IL12/IL23p40 associated with increased odds of a lung cancer diagnosis [OR 1.89, 95%

confidence intervals (CI), 1.16–3.09; OR 2.49, 95% CI, 1.46–4.23, respectively]. Confirming our previous observations, we also detected a relationship between increased IL6, IL8, and C-reactive protein (CRP) with lung cancer diagnosis. These relationships were significant after adjustment for age, gender, race, smoking, body mass index (BMI), family history of lung cancer, and previous diagnoses of inflammatory conditions. However, none of these proteins could distinguish between a benign and malignant lung nodule (IL6: OR 1.25, 95% CI, 0.59–2.64; IL8: OR 1.40, 95% CI, 0.70–2.81; CRP: OR 0.98, 95% CI, 0.45–2.12).

Conclusions: We have discovered new associations for IFNγ and IL12/IL23p40 with lung cancer but have no evidence that these proteins can distinguish between benign and malignant lung nodules.

Impact: Circulating inflammation proteins are unlikely to have utility as companion LDCT biomarkers.

Introduction

Lung cancer is the leading cause of cancer-related mortality in the United States and worldwide (1). For all stages of lung cancer combined, the 5-year survival rates are still approximately 15%. One of the major contributing factors to this dismal survival rate is the stage at which the majority of lung cancers are diagnosed. Unfortunately, greater than 50% of lung cancer is diagnosed at stage 3 or 4, a time when both local and systemic treatments are unlikely to be curative. The results of the National Lung Screening Trial (NLST) mark a new gold standard for lung cancer screening

based on low-dose computed tomography (LDCT) imaging (2, 3). However, the enhanced sensitivity of LDCT is tempered by its low specificity and the simultaneous detection of benign indolent nodules. Up to 97% of suspicious lesions detected with LDCT are benign at time of surgery, and the number of false positives diagnosed by LDCT significantly increases the cost of screening and patient morbidity (3, 4). Biomarkers that can differentiate between benign and malignant tumors could reduce the number of surgeries and invasive procedures for patients and ultimately improve lung cancer outcomes.

The role of circulating immune proteins as cancer biomarkers is of interest given the documented relationship between smoking and inflammation with this disease (5–9). A chronic inflammatory state can cause a high rate of cell turnover and an increase in oxidative and nitrosative stress (10). These reactive oxygen species can then bind to DNA, leading to damage and mutations, possibly also exacerbating the DNA damage caused by tobacco smoke (5, 11–14). Several chronic inflammatory and infectious conditions are associated with an increased risk of lung cancer (8, 15). Furthermore, cytokine concentrations are altered when inhaled smoke particulates and chemical irritants induce an immune response (16–18), and research has shown that microenvironmental, lung cancer and premalignant epithelial cells can also secrete cytokines (19–21).

Modulation of the inflammatory milieu within the tissue microenvironment can be evident systemically (21–23). Indeed, we and others have previously demonstrated an elevation of certain inflammation proteins at the time of lung cancer diagnosis

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(22, 24–26). In addition, some inflammation proteins were also associated with lung cancer risk (22, 25, 27), suggesting that they may be useful for risk stratification. Although molecular epidemiology studies do not directly imply causation, they do suggest that inflammation is related to the etiology of lung cancer. This suggestion is supported by epidemiologic studies that show a relationship between inflammatory conditions and risk of developing lung cancer (8, 28), and mouse models that define a causal relationship between inflammation and lung cancer (11).

Considering the relationship between inflammation with lung cancer risk and diagnosis, and the inflammatory signals that egress from malignant tumors, we sought to investigate if circulating inflammation proteins could be a companion diagnostic tool for lung cancer screening and potentially discriminate between malignant and benign nodules.

Materials and Methods

Study population

This study utilized data and biospecimens from the NLST, a randomized screening trial that recruited subjects at high risk for lung cancer (2, 3). The NLST compared the use of low-dose CT with chest radiography. It was a collaborative effort of the Lung Screening Study (LSS), administered by the NCI Division of Cancer Prevention, and the American College of Radiology Imaging Network (ACRIN), sponsored by the NCI Division of Cancer Treatment and Diagnosis, Cancer Imaging Program. The NLST population selection and eligibility criteria, methods, and results have been previously described (2, 3). Briefly, all subjects were aged between 55 and 74 years old with at least a 30 pack-year history of cigarette smoking. Former smokers were defined as those who had quit smoking less than 15 years before entry into the study. Participants were randomly assigned into two study arms. Participants in the first arm were randomized to receive three annual low-dose CT assessments, with the second arm scheduled to receive three annual single-view posterior–anterior chest radiographs. Both arms were then followed for 5 years.

Positive low-dose CT scans were defined as one or more noncalcified nodules at least 4 mm in the longest diameter (2, 3). Subjects within the low-dose CT arm with a positive scan were further evaluated for lung cancer diagnoses by follow-up with their physician. Participants were given a questionnaire upon entry into the study that included questions related to their past medical history, smoking habits, and family history of cancer. In total, 53,454 persons at high-risk for lung cancer were enrolled in the NLST. There were 649 lung cancers, for which diagnostic information was complete, diagnosed in the LDCT-arm.

Study design

Biospecimens were collected from a subset of ACRIN centers ($n = 15$). Of the 649 lung cancers diagnosed with LDCT, plasma was collected and available for 265. Of the 265, analysis was successful on 262 (note, plasma for three individuals was not included due to insufficient volume). Of these 262 cases, 61 were diagnosed at T0, 62 were diagnosed at T1, and 139 were diagnosed at T2. Further, of these 262 cases, 134 were diagnosed at the time of their screen, 122 had a sample drawn before diagnosis, and 6 were drawn after diagnosis. A breakdown of these collections, stratified by time of cancer diagnosis, is presented in Supplementary Table S1. Controls were then selected based on incidence-density matching of each case to two random controls. The samples were two to one incidence density matched based on timepoint sample taken (T0, T1, T2), number of screens, age at diagnosis (± 5 years), smoking status (current/former), gender (male/female), cigarettes per day (± 1), and duration of smoking (± 3 years). These criteria were possible for 187 cases. The cigarettes/day criterion was relaxed to ± 5 for 42 individuals. There was matching based only on time on study, number of screens, age, and gender for 73 individuals, matching based on time on study and number of screens for 12 individuals and matching only by time on study for 100 individuals. We performed two nested case–control studies. The first sought to validate and confirm our previous observations within the NLST samples using a traditional case–control subset. The second sought to compare inflammation profiles between benign and malignant nodules. Matching was not conducted with consideration for the positive or negative status of the scan. A summary of the participant characteristics is outlined in Table 1.

Inflammation protein measurement

Concentrations of 30 inflammation proteins were measured on serum samples of 265 cases and 528 controls using a highly sensitive and analytically validated electrochemiluminescence V-PLEX immunoassay (MSD, Rockville), following the manufacturer's instructions. Plasma samples from all participants were randomly distributed across the plates. Control samples to assess interplate variability, and analyte-specific standards to generate standard curves, were also included with each plate. Briefly, 25 μL of patient sera were assayed for circulating levels of Chemokine Panel 1 Kit (Eotaxin, Eotaxin-3, IL8, IP10, MCP1, MCP4, MDC, MIP1α, MIP1β, TARC), Proinflammatory Panel 1 Kit (IFNγ, IL10, IL12p70, IL13, IL1β, IL2, IL4, IL6, TNFα), Cytokine Panel 1 Kit (GMCSF, IL1α, IL5, IL7, IL12/IL23p40, IL15, IL16, IL17A, TNFβ, and VEGFA), and C-reactive protein (CRP). All signal results were extrapolated into concentrations (pg/mL) from the standard curves. Five control samples (random plasma samples from healthy volunteers) were also included on each plate to assess intraplate variance (see Supplementary Table S2, for coefficients of variance). To ensure quality data for further analyses and interpretation, detection level criteria were applied to the measurements obtained for each protein (Supplementary Tables S3 and S4) based on fit curve ranges defined by the plate-specific-standard curves generated for each analyte using standard dilutions (computations were conducted using Workbench 4.0; MSD, Rockville). If an analyte had more than 50% of samples that fell outside of the detection range, the protein was excluded from further analysis (this criterion applied to high-sensitivity IL8, MIP1α, GMCSF, IL1α, IL5, IL1β, IL12p70, IL13, and IL4). Median and quartile levels are chosen based on the respective control populations (i.e., negative scan or positive scan).

Data processing and normalization

Discovery Workbench (MesoScale Diagnostics LLC) was used to fit analyte- and plate-specific signal versus concentration standard curves based on seven calibrator dilutions. Fitted curves were applied to convert measured signals to concentrations on biological samples. In the process of calibration, samples with signal intensities outside the standard curve range were assigned out-of-range (OOR) concentrations. Concentrations were $\log_2$-transformed. The subsequent analysis was separately carried out for unknown and control samples. For each analyte,

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Table 1. Characteristics of the lung cancer cases and controls

| | | Controls | | | |
| | | Negative scan | Positive scan | | |
Demographics	Cases (*n* = 262)	*N* = 418	*N* = 110	*P*[a]	*P*[b]
Age					
Median (IQR) in years	64 (60–68)	64 (59–68)	64.5 (61–68)	0.50	0.57
Gender					
Male	159	263	59	0.56	0.21
Female	103	155	51		
Race					
White	245	384	104		
Black or African American	14	19	3		
Asian	1	6	0	0.36	0.27
American Indian or Alaskan Native	1	1	0		
Native Hawaiian or other Pacific Islander	0	3	0		
More than one race	0	4	1		
Missing	1	1	2		
Smoking status at randomization					
Former	124	182	49	0.33	0.62
Current	138	236	61		
Pack-years of smoking					
Median (IQR) in years	57 (43–76.5)	51 (43–76)	50 (42.4–69)	0.22	0.05
BMI					
Median (IQR) in years	26.4 (24.0–29.2)	26.6 (24.4–30.1)	26.2 (22.9–30.13)	0.06	0.77
Family history of lung cancer					
No	192	304	82	0.71	0.61
Yes	57	84	21		
Missing	13	30	7		
Tumor stage					
IA	160				
IB	25				
IIA	5				
IIIA	15				
IIIB	23				
IV	27				
Unable to assess	7				
Tumor histology					
Large cell carcinoma, NOS	6				
Large cell neuroendocrine carcinoma	2				
Tumorlet, NOS	14				
Oat cell carcinoma	1				
Small cell carcinoma	3				
Non–small cell carcinoma	21				
Squamous cell carcinoma	52				
Squamous cell carcinoma, keratinizing, NOS	6				
Squamous cell carcinoma, large cell, nonkeratinizing	1				
Adenocarcinoma	87				
Carcinoid	2				
Bronchioloalveolar adenocarcinoma	40				
Bronchioloalveolar adenocarcinoma, nonmucinous	3				
Bronchioloalveolar adenocarcinoma, mixed	5				
Adenocarcinoma with mixed subtypes	3				
Papillary adenoma	6				
Signet ring cell carcinoma	2				
Acinar cell adenoma	3				
Mixed squamous cell and glandular papilloma	4				
Adenocarcinoma with squamous metaplasia	1				

Abbreviations: BMI, body mass index; IQR, interquartile range; NOS, not otherwise specified.
[a]*P* denotes differences between controls with a negative LDCT scan and patients with lung cancer.
[b]*P* denotes differences between controls with a positive LDCT scan and patients with lung cancer.

the fraction of samples with concentrations labeled as OOR was calculated; analytes with OOR concentrations on >15% of the samples were removed from the subsequent analysis. Analytes that passed this sensitivity-based quality control filter were grouped by plex (CRP, chemokine, cytokine, and proinflammatory; ref. 29).

Each of these groups was separately normalized across plates using the R package MDimNormn, which minimizes plate effects based on a multidimensional MA (multi-MA) mean centering normalization approach (27). For each analyte, one-way ANOVA analysis was performed to verify that any significant plate effects (*F*-value < 0.05) were indeed removed after multi-MA normalization. For each protein, the difference between each sample and the median across samples in units of mean absolute deviation (MAD) was used as a robust statistic of deviation. Outlier samples were defined as samples that had absolute deviation >3 for >15%

Table 2. Relationship between inflammation proteins with lung cancer diagnosis among individuals with a negative LDCT screen

	Control/case	Univariable participants with a negative LDCT scan		Multivariable participants with a negative LDCT scan	
		OR (95% CI)	P	OR (95% CI)	P[a]
IL6					
<25th percentile	103/42	Reference		Reference	
25th–50th percentile	103/58	1.38 (0.85–2.24)	0.19	1.48 (0.87–2.53)	0.15
50th–75th percentile	103/73	1.74 (1.09–2.77)	0.02	1.88 (1.11–3.21)	0.02
>75th percentile	103/83	2.00 (1.26–3.16)	0.003	2.82 (1.64–4.86)	<0.001
Trend		1.40 (1.18–1.66)	0.002	1.40 (1.18–1.66)	<0.001
<Median	206/100	Reference		Reference	
>Median	205/156	1.57 (1.14–2.15)	0.005	1.84 (1.26–2.68)	0.001
IL8					
<25th percentile	105/50	Reference		Reference	
25th–50th percentile	104/69	1.40 (0.88–2.19)	0.15	1.57 (0.95–2.60)	0.08
50th–75th percentile	105/52	1.04 (0.65–1.67)	0.87	1.08 (0.63–1.86)	0.77
>75th percentile	104/91	1.84 (1.18–2.85)	0.007	2.25 (1.37–3.71)	0.001
Trend		1.18 (1.02–1.35)	0.02	1.25 (1.06–1.46)	0.006
<Median	209/119	Reference		Reference	
>Median	209/143	1.20 (0.88–1.63)	0.25	1.30 (0.90–1.86)	0.16
CRP					
<25th percentile	105/50	Reference		Reference	
25th–50th percentile	104/69	1.57 (0.98–2.52)	0.06	1.65 (0.96–2.83)	0.07
50th–75th percentile	105/52	1.81 (1.14–2.87)	0.01	2.18 (1.28–3.71)	0.004
>75th percentile	104/91	1.76 (1.11–2.80)	0.02	2.46 (1.43–4.23)	0.001
Trend		1.19 (1.03–1.37)	0.02	1.33 (1.13–1.58)	0.001
<Median	209/119	Reference		Reference	
>Median	209/143	1.39 (1.02–1.90)	0.04	1.73 (1.20–2.49)	0.003
IFNγ					
<25th percentile	104/50	Reference		Reference	
25th–50th percentile	103/57	1.15 (0.72–1.84)	0.56	1.11 (0.65–1.87)	0.72
50th–75th percentile	104/64	1.28 (0.81–2.03)	0.29	1.28 (0.76–2.13)	0.35
>75th percentile	103/91	1.84 (1.18–2.85)	0.007	1.89 (1.16–3.09)	0.01
Trend		1.22 (1.06–1.40)	0.005	1.24 (1.06–1.45)	0.007
<Median	207/107	Reference		Reference	
>Median	207/155	1.45 (1.06–1.98)	0.02	1.50 (1.06–2.14)	0.02
IL12/IL23p40					
<25th percentile	105/50	Reference		Reference	
25th–50th percentile	104/65	1.31 (0.83–2.07)	0.24	1.25 (0.73–2.12)	0.42
50th–75th percentile	105/62	1.24 (0.78–1.97)	0.36	1.59 (0.93–2.72)	0.09
>75th percentile	104/85	1.72 (1.10–2.67)	0.02	2.49 (1.46–4.23)	0.001
Trend		1.17 (1.02–1.35)	0.03	1.36 (1.15–1.61)	0.001
<Median	209/115	Reference		Reference	
>Median	209/147	1.27 (0.94–1.74)	0.12	1.77 (1.22–2.57)	0.002

[a]P adjusted for age, gender, smoking status, pack-years of smoking, race, BMI, history of COPD, childhood asthma, adulthood asthma, asbestosis, chronic bronchitis, bronchiectasis, emphysema, fibrosis of the lung, pneumonia, sarcoidosis, silicosis, tuberculosis, diabetes, and family history of lung cancer.

of analytes (and the same sign in all of the deviates above the absolute deviation threshold).

Statistical analysis

Demographic and smoking status variables were described for cases and controls. To evaluate differences between cases and controls, chi-squared tests were used for categorical variables and Student t tests for continuous variables. Tumor characteristics were further described for cases. Univariable and multivariable logistic regression was utilized to estimate the magnitude of association between inflammation protein expression and case/control status. Multivariable analyses were used to control for possible confounding variables: age, gender, race, smoking status at randomization, and pack-years of smoking. Final adjusted models included the above variables with body mass index (BMI), family history of cancer and for previous diagnoses of inflamma-tory conditions including chronic obstructive pulmonary disease (COPD), emphysema, bronchiectasis, sarcoidosis, tuberculosis, or chronic bronchitis. ORs and 95% confidence intervals (CI) were derived. A Cox proportional hazards model was used to estimate HRs and 95% CIs for time from randomization to status at last follow up among cases. Models were adjusted for age, gender, smoking status, pack-years of smoking, tumor stage, histologic subtype, and treatment status. A comparison of ROC and AUC statistics was conducted using the roccomp command. All statistical analyses were performed using STATA (version 14) and two-sided significance levels were set at $P < 0.05$.

There were 40 tumors diagnosed as bronchioalveolar carcinoma (BAC) in this dataset. Since the completion of the NLST, BAC has undergone a reclassification as adenocarcinoma *in situ*, minimally invasive adenocarcinoma (MIA), lepidic predominant adenocarcinoma (LPA), predominantly invasive

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A

B

C
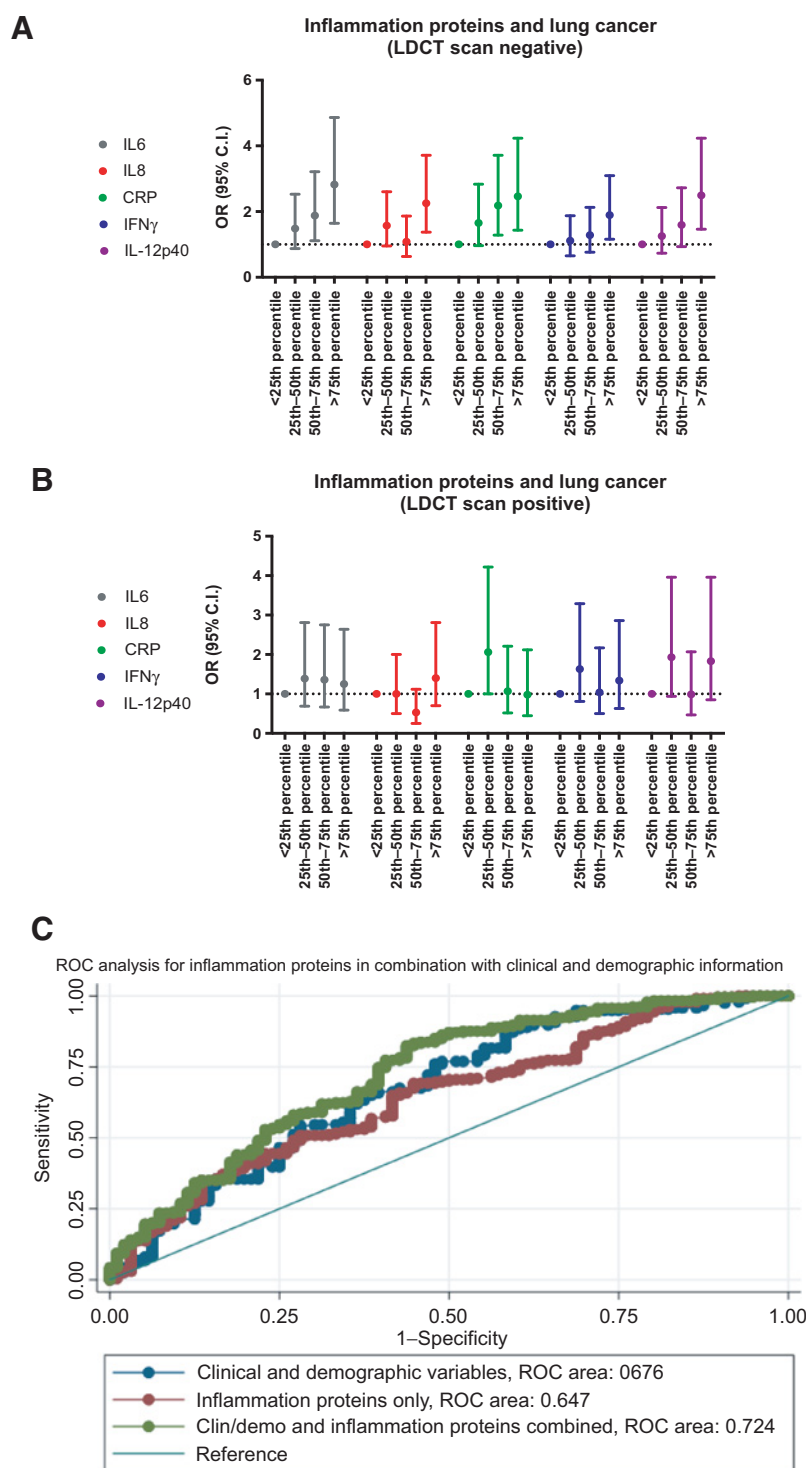
Figure 1.
A graphical representation of ORs for inflammation proteins associated with lung cancer diagnosis among screen negative controls (**A**) and screen positive controls (**B**). **C,** An ROC analysis for clinical (Clin) and demographic (demo) variables and inflammation proteins among lung cancer patients and controls with positive LDCT scans.

adenocarcinoma with some nonmucinous lepidic component and invasive mucinous adenocarcinoma. The previous classification included a heterogenous spectrum of subtypes that usually has a better prognosis (in some cases 5-year survival was 100% at 5 years; ref. 30), therefore for the survival analysis of this paper we also conducted a nested analysis with removal of the BAC subtype.

Results

Study population characteristics

Descriptive characteristics of controls and lung cancer cases are presented in Table 1. The population was well matched, with no differences by age, gender, BMI, race, or smoking status (Table 1). As this was an NLST population, the pack-year distribution was high for both cases and controls. As expected, the majority of the cases were

Table 3. Relationship between inflammation proteins with lung cancer diagnosis among individuals with a positive LDCT scan

	Control/case	Participants with a positive LDCT scan OR (95% CI)	P^a
IL6			
<25th percentile		Reference	
25th–50th percentile	28/57	1.39 (0.69–2.81)	0.35
50th–75th percentile	27/66	1.36 (0.67–2.75)	0.40
>75th percentile	28/78	1.25 (0.59–2.64)	0.56
Trend	27/55	1.07 (0.84–1.36)	0.57
<Median	88/123	Reference	
>Median	55/133	1.10 (0.66–1.86)	0.71
IL8			
<25th percentile		Reference	
25th–50th percentile	28/69	1.00 (0.50–2.00)	0.99
50th–75th percentile	27/63	0.53 (0.25–1.12)	0.10
>75th percentile	28/39	1.40 (0.70–2.81)	0.34
Trend	27/91	1.06 (0.86–1.32)	0.57
<Median	55/132	Reference	
>Median	55/130	0.96 (0.57–1.61)	0.87
CRP			
<25th percentile		Reference	
25th–50th percentile	28/50	2.06 (1.00–4.22)	0.05
50th–75th percentile	27/104	1.07 (0.52–2.21)	0.86
>75th percentile	28/62	0.98 (0.45–2.12)	0.96
Trend	28/46	0.91 (0.71–1.17)	0.47
<Median	55/154	Reference	
>Median	55/108	0.68 (0.41–1.13)	0.14
IFNγ			
<25th percentile		Reference	
25th–50th percentile	27/56	1.63 (0.81–3.29)	0.17
50th–75th percentile	27/85	1.04 (0.50–2.17)	0.92
>75th percentile	27/56	1.34 (0.63–2.86)	0.45
Trend	27/65	1.04 (0.82–1.32)	0.75
<Median	54/141	Reference	
>Median	54/121	0.89 (0.53–1.51)	0.68
IL12/IL23p40			
<25th percentile		Reference	
25th–50th percentile	28/66	1.93 (0.94–3.96)	0.07
50th–75th percentile	27/89	0.99 (0.47–2.07)	0.98
>75th percentile	28/51	1.83 (0.85–3.96)	0.12
Trend	27/66	1.12 (0.88–1.43)	0.37
<Median	55/145	Reference	
>Median	55/117	0.93 (0.55–1.59)	0.80

[a]P adjusted for age, gender, smoking status, pack-years of smoking, race, BMI, history of COPD, childhood asthma, adulthood asthma, asbestosis, chronic bronchitis, bronchiectasis, emphysema, fibrosis of the lung, pneumonia, sarcoidosis, silicosis, tuberculosis, diabetes, and family history of lung cancer.

diagnosed with stage I disease ($n = 160$ stage IA and $n = 25$ stage IB, combined stage 1 = 71%). Although the histologic subtype distribution was broad, adenocarcinoma was the most prevalent (33%), followed by squamous cell carcinoma (20%) and BAC (19%).

Association between plasma inflammation proteins and lung cancer diagnosis

As shown in Supplementary Table S5, most inflammation proteins had a similar distribution between cases and controls. However, several proteins, including IL8, CRP, and IFNγ, were significantly higher among cases.

We initially attempted to confirm our previous observations with regard to CRP, IL6, and IL8 in the NLST population and conducted a nested case–control study based on cases with a positive screen and controls with a negative screen. Comparing

quartile 4 with quartile 1, IL6, IL8, and CRP were associated with increased odds of a lung cancer diagnosis (OR 2.82, 95% CI, 1.64–4.86; OR 2.25, 95% CI, 1.37–3.71; OR 2.46, 95% CI, 1.43–4.23, respectively). These observations confirmed our previous findings (22). We also found increased IFNγ and IL12/IL23p40 associated with increased odds of a lung cancer diagnosis (OR 1.89, 95% CI, 1.16–3.09; OR 2.49, 95% CI, 1.46–4.23, respectively; Table 2; Fig. 1A). These relationships were significant after adjustment for age, gender, race, smoking status, pack-years of smoking, BMI, family history of lung cancer, and previous diagnoses of inflammatory conditions. The results for all inflammation proteins included in this study are shown in Supplementary Table S6.

One hundred and twenty-two patients had their blood taken before the lung cancer diagnosis. As the period between the first and final screen was 2 years, it is not possible to compare the relationship between these proteins with long-term lung cancer risk. However, we conducted a sensitivity analysis to filter out the samples taken at the time of a positive screen/lung cancer diagnosis (Supplementary Table S7). We then compared the levels of each inflammation protein in these cases to control participants with a negative LDCT scan ($n = 418$). Increased levels of IL6 (OR 1.25, 95% CI, 1.00–1.57), IFNγ (OR 1.27, 95% CI, 1.04–1.56) and IL12/IL23p40 (OR 1.32, 95% CI, 1.06–1.65) were each associated with increased likelihood of a lung cancer diagnosis in samples taken 1 to 2 years before a positive scan (Supplementary Table S7).

Given our repeated observations of elevated inflammation proteins at the time of lung cancer diagnosis among lung cancer patients, compared with population controls, we then conducted a second nested case–control study looking at levels of circulating proteins in malignant compared with benign nodules detected by LDCT. As shown in Table 3 and Fig. 1B, none of the circulating inflammatory proteins measured in this study could distinguish a benign from malignant lung nodule (IL6: OR 1.25, 95% CI, 0.59–2.64; IL8: OR 1.40, 95% CI, 0.70–2.81; CRP: OR 0.98, 95% CI, 0.45–2.12; IFNγ: OR 1.34, 95% CI, 0.63–2.86; IL12/IL23p40: OR 1.83; 95% CI, 0.85–3.96). We also conducted an ROC-analysis looking at covariables alone (AUC = 0.676), the five inflammation proteins alone (AUC = 0.647) and covariables and inflammation proteins together. The combined AUC value was 0.724 (Fig. 1C).

As the case–control analysis included disease ranging from stage I to stage IV, it was possible that our case–control results reflected an elevation of inflammation proteins in late stage disease, which would explain the inability of the inflammation proteins to detect early stage disease. To test this possibility, we also conducted an analysis restricted to stage I disease (Supplementary Table S8). As shown, each of the five proteins were strongly associated with stage 1 disease.

Relationship between circulating inflammation proteins and lung cancer survival in a screened population

We previously found evidence that IL6, IL8, CRP, and IL17A were associated with lung cancer survival (31, 32). These studies included lung cancer cases diagnosed based on clinical symptoms. We therefore asked whether any of the circulating proteins measured in this study were associated with survival in the NLST patient cohort. As shown in Fig. 2, when considering levels of CRP only among cases where the samples was taken at the time of diagnosis, increased levels of CRP were associated with poor outcome (Table 4; HR 2.28, 95% CI, 1.02–5.07, $P = 0.04$).
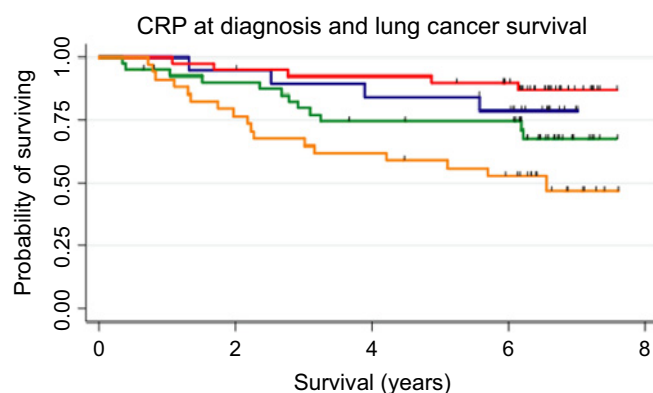
Figure 2.
A Kaplan–Meier curve depicting the relationship between circulating levels of CRP with survival among patients with lung cancer diagnosed with LDCT in the NLST.

Discussion

We and others previously demonstrated that circulating proteins are altered among lung cancer cases compared with controls in both a case–control and prospective study setting (22, 24–26). Herein, we replicated these previous observations for IL6, CRP, and IL8. Our previous studies focused on a limited number of inflammation proteins ($n = 10$), therefore we increased that number to 30 in this study and identified two additional proteins associated with lung cancer diagnosis, i.e., IFNγ and IL12/IL23p40. To our knowledge, this is the first time that circulating levels of these two proteins have been linked with lung cancer diagnosis and/or risk. The participants in this study were high-risk, heavy smokers, with a smoking profile that was higher that other studies. This is a potential reason why our study was the first to report these observations. Of note, our findings were observed in plasma, whereas previous studies focused on serum (22, 25–27), which could also explain why we found an association. Our validation of previous findings for IL6, CRP, and IL8 in a different type of biospecimen further strengthens those observations.

A secondary aim of this study was to assess whether the utility of circulating inflammation proteins could be extended to use as companion biomarkers in the setting of LDCT screening for lung cancer. Previously, levels of CRP, IL6, IL8 proteins were found to be elevated in clinically-detected lung cancer patients compared with population controls. Further, in this analysis when only considering screen negative patients, levels were also elevated, including for IFNγ and IL12/IL23p40. Surprisingly, however, these proteins were not elevated in lung cancer cases with a positive screen, compared with individuals with a suspicious lesion but no confirmed cancer diagnosis. The power in this analysis was lower, but the results appeared to be clear. We conducted an ROC analysis looking at whether the inflammation proteins added information to a discrimination model. Although the AUC value increased when these proteins were added, the value itself, 0.724, was still low, suggesting again that these proteins have little discrimination ability in this setting.

As nodules detected by LDCT screening are small, we tested whether the relationship between inflammation proteins and lung cancer diagnosis in the case–control analysis was influenced by the detection of large, late-stage tumors—the study cohort included tumors from stage I to stage IV. Therefore, we conducted a sensitivity analysis restricted to stage I tumors only. However, our data show that the inflammation proteins were associated with diagnosis of stage I disease. These data suggest that these proteins are elevated among patients with tumors 30 mm or less (size cut-off for stage I disease). It is possible that these inflammation proteins are not useful for detection of smaller lesions, that is, <30 mm, but our findings also suggest that even inflammation proteins associated with lung cancer risk, and lung cancer diagnosis, may not be useful biomarkers for benign and malignant lung nodule discrimination. One reason for this could be

Table 4. Relationship between CRP with lung cancer survival in the NLST[a]

	N	HR (95% CI)	P[b]	N	HR (95% CI)	P[c]
CRP						
<25th percentile	19	Reference		12	Reference	
25th–50th percentile	40	0.65 (0.26–1.62)	0.35	32	0.87 (0.32–2.36)	0.78
50th–75th percentile	41	1.37 (0.60–3.12)	0.45	36	1.62 (0.64–4.09)	0.31
>75th percentile	34	2.28 (1.02–5.07)	0.04	33	2.77 (1.10–6.96)	0.03
Trend		1.52 (1.18–1.96)	0.001		1.57 (1.18–2.07)	0.002

[a]Denotes samples with a blood draw at the time of the screen/cancer diagnosis ($n = 134$).
[b]P adjusted for age, gender, smoking status, race, pack-years of smoking, treatment status, tumor stage, and tumor histology.
[c]P adjusted for age, gender, smoking status, race, pack-years of smoking, treatment status, tumor stage, and tumor histology (with BAC removed).

that patients with detected nodules or positive LDCT scans could have additional inflammatory conditions that elevate baseline levels. Senescent cells within the nodule microenvironment could acquire a senescent-associated secretory phenotype (SASP) or a premalignant nonprogressing lesion that secretes cytokines (20, 23). Although a null relationship was observed both before and after adjustment for many inflammatory conditions in our study, we cannot rule out this possibility. The lack of an association is also unlikely to be due to the specific smoking and age profile of the patients in the NLST, as our first analysis, which was case–control and where a significant relationship was observed, had a similar smoking profile to the nodule discrimination analysis.

Our data suggest that alternative strategies are needed to find biomarkers for nodule discrimination. Indeed, recent work by several groups have shown progress. For example, results of the PANOPTIC trial measuring LG3BP (33) and C163A, and the MILD trial, measuring microRNAs (34), suggest that some circulating biomarkers will have sufficient discriminatory power to be clinically useful. Other strategies, such as that of Spira and colleagues, harness the field effect theory of lung carcinogenesis measuring gene expression signatures in bronchial washings and nasal epithelia (35), whereas the field of radiomics is also rapidly evolving and poised to impact the discrimination of nodules (36–39).

The third aim of this study was to assess whether proteins previously associated with lung cancer survival among symptomatic patients would also be related to survival in a screen-detected population. Herein, we validated our observations for CRP. Because the NLST was completed, BAC has been reclassified. As BAC tends to have a better prognosis overall, we also conducted a sensitivity analysis with BAC tumors removed, however it did not change our findings.

Our study has several strengths and limitations. A key strength of the study was assessment of samples from the national lung screening trial and the ability to pull from a large population of controls. Further, simultaneously assessing these proteins both in the context of a case–control study and a screening study enabled us to extrapolate case control findings directly to the question of benign and malignant nodules within the same population. Another strength of the study was our ability to control for a broad range of inflammatory conditions (these data were extensively collected in the NLST), as these are potential confounders for the association between circulating inflammation proteins and lung cancer. One of the study's limitations was the inclusion of a smaller population of screen positive controls, but in this case the study was limited to what was available.

In summary, our data show that although inflammation is involved in the etiology of lung cancer, the detection of circulating inflammation proteins (at least those measured in this study) is unlikely to have clinical use in distinguishing between a benign and malignant lung nodule. The majority of this population were white and therefore this conclusion still needs to be made for other racial and ethnic groups. Although samples from screening studies are precious in nature, our data also highlight the need to possibly avoid extrapolation from case–control to prospective to screening studies for the purposes of identifying molecules that will discriminate between benign and malignant nodules. Additionally, studying the intended use population, possibly via agnostic means, could be the best approach, at least in the search for these specific kinds of biomarkers. This approach has been validated by Spira and others (34, 35). It is also worth noting that the issue of risk stratification for high-risk individuals is still a concern and biomarker work continues in this area as well (40). For example, although the general uptake of LDCT screening is still low (41), as its use broadens, there is not sufficient medical capacity to screen all eligible individuals, estimated at over 7 million (41, 42). Given previous data linking inflammation proteins to lung cancer risk (22, 25, 26), and the data from this study showing that IL6, IFNγ, and IL12/IL23p40 were also elevated among patients with lung cancer 1 to 2 years prior to a lung cancer diagnosis, it remains possible that there may be clinical utility for inflammation proteins in that setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D. Brown, B.M. Ryan
Development of methodology: D. Brown, L. Cao, B.M. Ryan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Brown, A. Zingone, Y. Yu, L. Cao, B.M. Ryan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Brown, A. Zingone, Y. Yu, B. Zhu, J. Candia, B.M. Ryan
Writing, review, and/or revision of the manuscript: D. Brown, A. Zingone, B. Zhu, J. Candia, L. Cao, B.M. Ryan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Brown, B.M. Ryan
Study supervision: L. Cao, B.M. Ryan

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