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# Differential Serum Cytokine Levels and Risk of Lung Cancer between African and European Americans

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## Abstract

**Background—**African Americans have a higher risk of developing lung cancer than European Americans. Previous studies suggested that certain circulating cytokines were associated with lung cancer. We hypothesized that variations in serum cytokine levels exist between African Americans and European Americans, and increased circulating cytokine levels contribute to lung cancer differently in the two races.

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**Methods—**Differences in ten serum cytokine levels, interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, granulocyte macrophage colony-stimulating factor (GMCSF), interferon (IFN)-γ and tumor necrosis factor (TNF)-α between 170 African-American and 296 European-American controls from the National Cancer Institute-Maryland (NCI-MD) case-control study were assessed. Associations of the serum cytokine levels with lung cancer were analyzed. Statistically significant results were replicated in the prospective Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial and the Wayne State University (WSU) Karmanos Cancer Institute case-control study.

**Results—**Six cytokines: IL-4, IL-5, IL-8, IL-10, IFNγ, and TNFα, were significantly higher among European-American as compared to African-American controls. Elevated IL-6 and IL-8 levels were associated with lung cancer among both races in all three studies. Elevated IL-1β, IL-10 and TNFα levels were associated with lung cancer only among African Americans. The association between elevated TNFα levels and lung cancer among European Americans was significant after adjustment for additional factors.

**Conclusions—**Serum cytokine levels vary by race and might contribute to lung cancer differently between African Americans and European Americans.

**Impact—**Future work examining risk prediction models of lung cancer can measure circulating cytokines to accurately characterize risk within racial groups.

### Keywords

inflammation; cytokines; lung cancer; racial disparities

## Introduction

Lung cancer is the leading cause of cancer deaths in the United States. The poor 16%, 5-year lung cancer survival rate is partly attributed to late stage at diagnosis for most patients (1). Identifying biomarkers of lung cancer may improve early detection, and ultimately lung cancer survival. African Americans have higher lung cancer incidence and mortality rates as compared to European Americans, which may be due to differences in genetics, environment, or modalities of care (2, 3). Identification of biomarkers that uniquely distinguish African Americans at a high risk of lung cancer may help bridge the gap in lung cancer racial health disparities.

Insurmountable evidence demonstrates that chronic inflammation is involved in the development and progression of lung cancer (4, 5). An inflammatory state, which is partly mediated by cytokines, causes a high rate of cell turnover and an increase in oxidative and nitrosative stress, leading to increased DNA damage and mutations. Furthermore, cytokine concentrations are altered when inhaled smoke particulates and chemical irritants induce an immune response (6, 7). Human lung cancer and pre-malignant epithelial cells can also secrete cytokines (8, 9). Thus, circulating cytokines are attractive potential biomarkers for early detection of lung cancer.

There are substantial racial differences in inflammation between African Americans and European Americans. For example, African Americans have higher incidence rates of

several autoimmune diseases including systemic lupus erythematosus (SLE) and multiple sclerosis (MS), as well as infectious diseases such as tuberculosis, septicemia, and HIV/AIDs (10). African Americans have higher levels of circulating C-reactive protein, a non-specific marker of inflammation (11, 12), higher levels of IL-6, and reduced levels of TNFα as compared to European Americans (12, 13). African Americans and European Americans also have significantly different frequencies of single nucleotide polymorphisms (SNPs) in cytokine genes that functionally alter serum cytokine concentrations (11, 14–16). Given the racial differences in some inflammatory markers, susceptibility to autoimmune diseases and allele frequencies in inflammatory gene SNPs, we hypothesized that there are marked variations in serum cytokine levels between African Americans and European Americans and these cytokines are differentially associated with lung cancer risk in these two groups.

Most previous studies comparing racial differences in cytokine levels and their associations with cancer investigated only a few cytokines. The lack of sufficient numbers of African Americans in population-based case-control studies has been a limiting factor in determining racial differences in circulating cytokines and race-specific associations between cytokines and lung cancer. We previously reported that, among European Americans, increased serum levels of IL-6 and IL-8 were associated with lung cancer, and IL-8 serum levels were associated with increased risk of subsequently developing lung cancer (17). We report here an investigation of the association of five additional pro-inflammatory (IL-1β, IL-12, GMSCF, IFN-γ TNF-α, and three anti-inflammatory (IL-4, IL-5, and IL-10) cytokines with lung cancer among African-American and European-American participants from the National Cancer Institute-Maryland (NCI-MD) study. Significant associations were evaluated by replication in European Americans from the prospective Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, and African Americans enrolled in the Wayne State University (WSU), Karmanos Cancer Institute study.

## Materials and Methods

### Study Population

Institutional review board approval was obtained from all participating institutions, and informed consent was obtained from all participants.

**NCI-Maryland (NCI-MD) Study—**Participants were recruited during an ongoing case-control study from the greater Baltimore, Maryland region from May 18, 1998, to November 10, 2003, as described in detail previously (17–19). Cases had histologically confirmed non–small cell lung cancer (NSCLC), were enrolled within 24 months after diagnosis, and did not have any other cancer at the time of enrollment. Cases lived in Metropolitan Baltimore or the Maryland Eastern Shore and were recruited from a total of seven hospitals after obtaining physician's consent. Hospital-based controls were cancer-free patients recruited from internal medicine, primary care, pulmonology, or cardiology clinics, and were frequency matched to cases by age, sex, race, smoking history, and hospital. Population-based controls were identified from lists obtained from the Maryland Department of Motor Vehicles and were frequency matched to cases by age, sex, and race.

Serum was collected from 988 of the 1,110 (89%) study participants enrolled during the study period. Ample quantities of serum for this study were available for 913 participants. Due to cost limitations, the cytokine concentrations were assayed on a subset of the samples from controls; however, all lung cancer cases were included (N = 821). Controls were selected based on the availability of genotyping data for other analyses not described in this manuscript.

**Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) Screening Trial Study—**Participants within the screening arm of the PLCO Cancer Screening Trial were selected for this nested case–control study, as described previously (17, 20). The PLCO study recruited 155,000 men and women aged 55–74 years from 1992 to 2001, from 10 centers throughout the United States (21). Participants in the screening group provided blood samples annually for 6 years. Baseline blood samples were used in this study. Lung cancer cases were identified through annual questionnaires that were mailed to the participants. All positive reports were confirmed by examination of hospital medical records or death certificates from the National Death Index. At the time of the December 31, 2004 sample selection cut-off date, 898 lung cancers had been diagnosed among the 77,464 participants in the screening group. Five hundred thirty-two serum samples from the European-American and 44 serum samples from the African-American lung cancer patients were available.

Controls that were cancer-free at the time of a case's lung cancer diagnosis were matched to cases 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 additionally matched on smoking amount (0–29, 30–39, 40–49, and ≥50 pack-years) and time since quitting (≤15 and >15 years) for former smokers. To improve statistical power among the never-smokers, the never-smoking controls were matched to lung cancer cases using a 3:1 ratio.

**Wayne State University (WSU) study—**Cases were identified through the population-based Metropolitan Detroit Cancer Surveillance System, an NCI-funded Surveillance, Epidemiology, and End Results Program (SEER) registry, as part of the Exploring Health, Ancestry, and Lung Epidemiology (EXHALE) study, as previously described (22). Rapid case ascertainment was used to identify histologically-confirmed lung cancer patients within several months after diagnosis. African Americans diagnosed with a first primary lung cancer from November 1, 2005 through June 30, 2010, who were also residents of the three county metropolitan Detroit area (Wayne, Oakland and Macomb counties), were recruited. Controls were recruited through community-based methods and were frequency matched on age (±5 years), sex, and race. Serum was successfully obtained from 73.9% of the cases and 83.0% of the controls.

Blood specimens were processed immediately and isolated sera were stored at −80°C until needed, for all three studies. Histology and staging procedures were described (17, 22).

## Cytokine Assays

Laboratory personnel were blinded to each participant's case-control status for all three studies. Cytokine concentrations were measured at the Frederick National Laboratory for

Cancer Research, from 25 μl of serum using Mesoscale ultrasensitive electrochemiluminescence immunoassays on the Meso Scale Discovery 6000 instrument, following the manufacturer's instructions (Meso Scale Discovery, Gaithersburg, MD). For the NCI-MD study participants, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, GMCSF, IFNγ, and TNFα were measured on custom-designed 10-plex plates. For validation of European Americans and African Americans in the PLCO and WSU studies, IL-1β, IL-6, IL-8 and TNFα were measured on 4-plex ultrasensitive plates (MS6000, Human ProInflammatory-4 II Ultra-Sensitive Kit, Mesoscale Discovery). WSU participants were additionally assayed for IL-10 on single-plex ultrasensitive plates (K151AOC, Human IL-10 Ultra-Sensitive Kit, Mesoscale). For the PLCO and WSU studies, 100% of the samples were assayed in duplicates and results are shown as the average of the duplicates. The PLCO study samples were assayed approximately 2 years after the NCI-MD study samples were measured for cytokine concentrations, and the WSU study samples were assayed approximately 1 year after the PLCO study. Serum samples from all participants were randomly distributed across the plates, and controls for standard curves were included with each plate. As an added quality control, 12% of the samples within each of the three studies were blindly duplicated and evenly distributed inter- and intra-plate.

## Statistical analyses

All analyses were performed using Stata software, version 12 (StataCorp LP, College Station, TX). Reported p-values were two-sided and the significance threshold level was specified as $p = 0.05$. For all three studies, a never smoker was defined as a person who had never smoked more than 100 cigarettes in his/her lifetime, and a former smoker was defined as a person who had quit smoking more than one year prior to the interview. Race was self-reported. Participants with at least one family member with lung cancer were defined as having a family history of lung cancer. Differences in serum cytokine concentrations between cases and controls, and between African-American and European-American controls, were calculated using a Wilcoxon rank sum test. Serum cytokine levels below the detection limit were recorded as half of the detection limit. For IL-1β, IL-4, IL-5, GMCSF, and IFNγ, cytokine concentrations were below the assay detection limit for greater than 10% of study participants. Because the data was skewed and a large number of samples were below the detection limit, data is presented comparing the medians.

Univariate comparisons of characteristics between cases and controls were performed for continuous variables using the Student's t test or the Kruskal-Wallis test of normally or non-normally distributed data, respectively. Comparisons for categorical variables were performed using the $\chi^2$ test. Unconditional logistic regression models were constructed to assess the relationship among lung cancer risk and serum cytokine concentrations, and were adjusted for age, sex and smoking status. Analyses for PLCO study participants were additionally adjusted for number of years in the study and the year of randomization. Consistent with our previous study (17), cytokine concentrations were divided into quartiles based on serum cytokine levels in controls a priori to provide easily interpretable comparisons between the studies. The adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by using the lowest quartile as the referent group.

Correlations between cytokine levels and lung cancer stage were performed by Kruskal Wallis tests. Quality control results are summarized in Supplementary Table S1.

## Results

### Characteristics of African-American and European-American Participants from the NCI-MD, PLCO, and WSU Studies

The demographic and clinical features of participants are shown in Table 1. There were more current smokers among cases as compared with controls in all studies. European-American and African-American cases in the NCI-MD and WSU studies, respectively, had higher pack-years of smoking as compared to controls. In the NCI-MD study, European-American cases were less likely to use aspirin or ibuprofen than controls. Cases from both races had a lower BMI as compared to controls in the NCI-MD, but not PLCO, study, possibly reflecting the retrospective design of the NCI-MD study. African-American cases in the WSU study were more likely than controls to have a history of emphysema or bronchitis and family history of lung cancer. The most common histological type was adenocarcinoma, followed by squamous cell carcinoma in all three studies, with the exception of African Americans in the NCI-MD study, in which most patients had unspecified NSCLC. In contrast to the PLCO and WSU cases, the majority of the NCI-MD cases had stage I tumors, which could reflect a possible bias for recruiting surgical cases, who primarily have stage I tumors.

### Comparison of Serum Cytokine Concentrations between African Americans and European Americans

In the NCI-MD study, serum cytokine concentrations were similar among the hospital- and population-based control groups stratified by race, except for IL-6 among the European Americans, where the hospital controls had a significantly higher median value than the population controls (median = 2.5 pg/mL, interquartile range (IQR) = 1.4 to 4.0 pg/mL vs median = 2.0 pg/mL, IQR = 1.4 to 3.6 pg/mL, $P = 0.0002$) (Supplementary Table S2). Therefore, all analyses were performed with the two control groups combined, unless otherwise noted.

We observed statistically significant differences in six of the ten measured serum cytokine concentrations between African-American and European-American controls in the NCI-MD study. European-American controls had significantly higher median values of IL-4, IL-5, IL-8, IL-10, IFNγ, and TNFα as compared with African-American controls (Table 2). There were no statistically significant differences in serum IL-6 concentrations between African Americans and European Americans within the hospital- and population-based control groups (P>0.05, data not shown).

### Association between Serum Cytokine Concentrations and Lung Cancer among African Americans and European Americans

**Serum cytokine levels—**Concentrations of several circulating cytokines were higher in lung cancer cases than controls for both races in the NCI-MD study. Cases had higher concentrations of IL-6, IL-8, and TNFα among both African-American and European-

American controls (Table 2). There were also racial differences in levels of several serum cytokines between cases and controls. African-American cases had statistically significantly higher levels of IL-1β and IL-10 than controls, which was not observed among European Americans (Table 2). Among cases, serum cytokine levels were not associated with increasing lung cancer stage (data not shown).

**Association between serum cytokine levels and lung cancer—**Similar to what was observed among European Americans in our previous study (17), African Americans within the highest quartile IL-6 and IL-8 serum concentrations had a statistically significantly increased risk of lung cancer as compared to those in the lowest quartile (Table 3). Surprisingly, increased levels of three additional cytokines were associated with lung cancer among African Americans but not European Americans. Compared to the lowest quartile, African Americans within the highest quartile of serum cytokine concentrations of IL-1β, IL-10 and TNFα had an increased risk of lung cancer compared to those in the lowest quartile (Table 3).

To further assess whether increased IL-6 and IL-8 serum concentrations are associated with lung cancer among both African Americans and European Americans, and the associations of elevated IL-1β, IL-10 and TNFα with lung cancer are present in African Americans, we set out to replicate the data in independent case-control studies.. Among European Americans in the PLCO study, serum concentrations of IL-6 and IL-8 (17), but not IL-1β or TNFα, in the highest quartiles were associated with increased risk of lung cancer as compared to individuals in the lowest quartiles, (Table 4). To be more comparable to timing of sample collection from the NCI-MD case-control study, we restricted the analyses to PLCO participants diagnosed with lung cancer within two years after blood collection, and results were similar (Supplementary Table S3). Furthermore, among African Americans in the WSU study, serum concentrations of all five cytokines, IL-1β, IL-6, IL-8, IL-10, and TNFα, in the highest quartiles were associated with an increased risk of lung cancer as compared to individuals in the lowest quartiles (Table 4).

There were too few African-American participants in the PLCO study to perform association analyses, but when we combined African-American cases and controls from the NCI-MD, PLCO and WSU studies, and adjusted for study, participants with the highest quartile levels of IL-1β, IL-6, IL-8, IL-10 and TNF-α had an increased risk of lung cancer compared to those with the lowest quartile concentrations (Table 5).

**Analysis of potential confounding—**We next assessed whether potential demographic or clinico-pathologic factors contributed to the observed racial differences. We additionally adjusted the models for education level, BMI, regular use of aspirin and/or ibuprofen, family history of lung cancer, systemic inflammation, and history of heart disease. The results remained statistically significant (Supplementary Tables S4, S5). Additionally, among European Americans in the NCI-MD study, elevated levels of TNFα were significantly associated with lung cancer after adjustment for the additional factors (Supplementary Table S4). Because many of the clinical-pathological factors were not recorded in the WSU study, we only adjusted those analyses for education level and COPD, and the results were similar

(Supplementary Table S5). Thus, no consistent trend across studies was observed to suggest that any of these variables confounded the associations.

**Effects of smoking—**We assessed whether racial differences in the associations between serum cytokine levels and lung cancer were modulated by exposure to tobacco smoke. We examined the associations between serum cytokine concentrations and lung cancer among all the African-American participants combined from the three studies. The magnitudes of the odds ratios were similar across smoking status subgroups for IL1β, IL-6, IL-8, IL-10 and TNFα (Table 5). Furthermore, there was substantial overlap of the 95% confidence intervals, indicating there was no evidence of interaction between smoking and any of these serum cytokine levels with lung cancer risk in the African-American participants. We also examined if smoking status could modify the risk of lung cancer among European-American participants. There were no changes in trends for associations of IL-6 or IL-8 with lung cancer. In addition, no significant associations emerge between IL-1β, IL-10 or TNFα and lung cancer risk among European Americans, with the exception that there was an association between elevated TNFα levels and lung cancer among former smokers in the PLCO study (Supplementary Table S6). These data in total suggest that the observations of racial differences of serum cytokine levels of IL1-β, and IL10 and lung cancer were not due to differences in smoking history.

## Discussion

African Americans have a higher incidence of lung cancer as compared to European Americans (2, 3). Because inflammation is a risk factor for numerous cancers, and there are marked differences in inflammation between the two races (4, 5, 10–13), we investigated the role of circulating cytokine concentrations as a risk factor for lung cancer among African Americans and European Americans. In this study, serum concentrations of six out of ten cytokines were significantly higher among European-American as compared to African-American controls. We and others previously reported that higher concentrations of IL-6 and/or IL-8 were associated with an increased risk of lung cancer among Caucasians and Asians (17, 23, 24). Our report here strengthens the observations of these earlier studies and extends them to include African Americans. We also report that additional cytokines, IL-1β, IL-10, and TNFα, were associated with lung cancer among African Americans but not European Americans in two independent studies, although the association between TNFα and lung cancer in the NCI-MD study emerged in European Americans after adjusting for additional factors.

The reason for differences in circulating IL-4, IL-5, IL-8, IL-10, IFNγ and TNFα levels between African Americans and European Americans could be partly explained by functional polymorphisms in several cytokine genes that were reported to modulate cytokine concentrations (25–28). Moreover, in several studies, the distributions of polymorphisms in cytokine genes were different between African-American and Caucasian or European American populations (14, 25, 27, 29–31). For example, in IL-10, several SNPs and their haplotypes are associated with differential IL-10 expression (15, 16) and the homozygous AA genotype of -1082A>G within IL-10 is related to lower IL-10 expression and a stronger inflammatory response (32). African Americans have a significantly higher frequency of the

1082AA genotype (33). Racial differences in serum cytokine levels could be due to other factors, such as other genetic differences, infection, or different biological responses to tobacco smoke exposure. Given the higher incidence and mortality of lung cancer among African Americans, as well as differences in frequency of auto-immune disorders (34), these observations may provide useful avenues for future study.

The results of the present study suggest that high serum concentrations of IL-6 and IL-8 are potential universal biomarkers of lung cancer, but IL-1β, IL-10 and TNFα are potential biomarkers for lung cancer among African Americans. The similar results for IL-6 and IL-8 in both African Americans and Caucasians suggest these cytokines are not a direct cause of the racial disparity in lung cancer rates, whereas IL-1β, IL-10 and TNFα may represent differing mechanisms underlying lung cancer development in African Americans. This represents a promising line of inquiry to explore regarding lung cancer health disparities.

The role of inflammation and immunity in tumor biology is complex. When the immune response is functioning normally, inflammation is self-limiting. The production of pro-inflammatory or Th-1 cytokines is followed by anti-inflammatory or Th-2 cytokines (35, 36). During chronic inflammation, the balance between Th-1 and Th-2 cytokines is disrupted and increased inflammation results in increased oxygen and/or nitrogen radicals, which are associated with cancer development. Th-2 dominant cytokine profiles have been correlated with enhanced tumor promotion and progression (36) and tumor cells which produce immunosuppressive (Th-2) cytokines may escape host tumor response (37). Both IL-6 and IL-8 are considered Th-2 cytokines (38), even though IL-6 was reported to produce both Th-1 and Th-2 responses (36). Our laboratory previously reported that elevated IL-6 and IL-8 mRNA in normal tissue was associated with lymph node metastasis, while higher IL-8 mRNA in tumor tissue, and elevated circulating IL-6 and IL-8 levels were associated with worsened lung cancer prognosis (39, 40). The data presented here suggests that an increase in these Th-2-associated cytokines is a more central mechanism in lung cancer development that spans across races. However, the fact that IL-1β and TNFα, both Th-1 cytokines and IL-10, a Th-2 cytokine, were associated with lung cancer only among African Americans in our study underscores the complexities of the disease in differences among racial groups.

A major strength of the present study is that it is one of the largest studies that have begun to explore the differences in circulating cytokines between African Americans and European Americans. We thus not only provided the initial investigation of these cytokines and lung cancer specifically among African Americans but also provided a comparison between the two races. In addition, by adjusting for cigarette smoking exposure, our data suggests that the association of cytokines with lung cancer was not solely due to differences in smoking history.

This study also had several potential limitations. Cytokines were measured only once and may be influenced by illnesses (other than lung cancer) or anti-inflammatory medications. However, with the exception of IL-6, cytokine concentrations were similar between hospital- and population-based controls; and furthermore, the association between IL-6 and lung cancer was observed when compared with population (likely healthier/less illness) or

hospital (likely more illness) controls. The observations were also not affected by regular use of anti-inflammatory medications, suggesting that circulating cytokine levels might be independently associated with lung cancer even among patients with chronic inflammatory conditions. Another limitation is that due to the limited availability of information on tumor histology we were unable to examine specific histological subtypes rigorously. Last, cytokine concentrations were measured after lung cancer diagnosis in the NCI-MD and WSU studies. However, the samples from the PLCO study were collected prior to lung cancer diagnosis. Prospective studies with multiple serial measures on participants from both races are needed.

The National Lung Screening Trial has shown that low-dose computed tomography (CT) screening can detect lung tumors at the millimeter range and reduce overall lung cancer mortality (41). Many screening facilities across the United States now utilize low-dose CT scans for individuals at high risk of lung cancer. However, the high rate of false-positive results instigates concern about whether exposure to x-rays, cost, and patient anxiety outweigh the benefits. Several recommendations have been put in place regarding nodule management (42), although improved modeling is needed to interpret nodule size and other imaging characteristics to maximize the ability to detect a “positive” tumor. Our study suggests that circulating serum cytokine levels are promising candidate biomarkers for early detection of lung cancer and that certain cytokines may be better predictors in certain races. Prospective trials are necessary to determine if serial serum testing of cytokines can improve the positive predictive value of the current screening modalities and improve lung cancer survival.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Table 1**

Characteristics and clinical data for lung cancer cases and matched controls in the NCI-MD, PLCO and WSU studies

| | African Americans | | | European Americans | | |
|---|---|---|---|---|---|---|
| | **Cases** | **Controls** | ***P*** | **Cases** | **Controls** | ***P*** |
| **NCI-MD Study** | | | | | | |
| Number of participants | 85 | 170 | | 270 | 296 | |
| Age, years, mean ± SD[a] | 62.9 ± 9.9 | 64.3 ± 12.4 | 0.37 | 66.6 ± 10.0 | 65.2 ± 10.4 | 0.10 |
| Gender, *N* (%)[b] | | | | | | |
| Male | 39 (45.9) | 88 (51.8) | 0.38 | 142 (52.6) | 148 (50.0) | 0.54 |
| Female | 46 (54.1) | 82 (48.2) | | 128 (47.4) | 148 (50.0) | |
| Smoking status, *N* (%)[b] | | | | | | |
| Never | 7 (8.2) | 67 (39.6) | | 22 (8.2) | 86 (29.1) | |
| Former quit ≤15 years | 22 (25.9) | 25 (14.8) | | 57 (21.2) | 68 (23.1) | |
| Former quit >15 years | 9 (10.6) | 50 (29.6) | | 64 (23.4) | 82 (27.8) | |
| Current | 47 (55.3) | 27 (16.0) | <0.001 | 127 (47.2) | 59 (20.0) | <0.001 |
| Pack-years, mean ± SD[a,c] | 47.4 ± 29.8 | 45.1 ± 29.9 | 0.20 | 47.8 ± 26.4 | 39.7 ± 31.3 | 0.001 |
| Education, *N* (%)[b] | | | | | | |
| High school or less | 60 (76.0) | 78 (52.7) | 0.001 | 146 (60.1) | 127 (49.8) | 0.02 |
| College or higher | 19 (24.0) | 70 (47.3) | | 97 (39.9) | 128 (50.2) | |
| Regular aspirin/ibuprofen use, *N* (%)[b,d] | | | | | | |
| No | 52 (61.9) | 93 (54.7) | 0.28 | 174 (64.7) | 145 (49.0) | <0.001 |
| Yes | 32 (38.1) | 77 (45.3) | | 95 (35.3) | 151 (51.0) | |
| BMI, *N* (%)[b,d] | | | | | | |
| <26.5 | 267 (50.6) | 18 (10.6) | <0.001 | 74 (27.4) | 52 (17.6) | 0.005 |
| ≥26.5 | 261 (49.4) | 152 (89.4) | | 196 (72.6) | 244 (82.4) | |
| History of heart disease, *N* (%)[b,d] | | | | | | |
| No | 67 (79.8) | 136 (80.0) | 0.96 | 204 (76.1) | 217 (73.3) | 0.44 |
| Yes | 17 (20.2) | 34 (20.0) | | 64 (23.9) | 79 (26.7) | |
| History of emphysema/bronchitis, *N* (%)[b,d] | | | | | | |
| No | 62 (73.8) | 137 (80.6) | 0.22 | 175 (65.1) | 197 (66.6) | 0.71 |

| | African Americans | | | European Americans | | |
|---|---|---|---|---|---|---|
| | **Cases** | **Controls** | ***P*** | **Cases** | **Controls** | ***P*** |
| Yes | 22 (26.2) | 33 (19.4) | | 94 (34.9) | 99 (33.4) | |
| Family history of lung cancer, *N* (%)[b,d] | | | | | | |
| No | 70 (83.3) | 145 (85.3) | 0.68 | 221 (82.2) | 253 (85.5) | 0.28 |
| Yes | 14 (16.7) | 25 (14.7) | | 48 (17.8) | 43 (14.5) | |
| Histology, *N* (%)[d] | | | | | | |
| AC | 22 (26.8) | – | | 105 (42.9) | – | |
| SCC | 26 (31.7) | – | | 53 (21.6) | – | |
| SCLC | 0 | – | | 0 | – | |
| NSCLC, NOS | 28 (34.2) | – | | 59 (24.1) | – | |
| Other | 6 (7.3) | – | | 28 (11.4) | – | |
| Clinical stage, *N* (%)[d] | | | | | | |
| I | 25 (80.7) | – | | 104 (71.2) | – | |
| II–IV | 6 (19.4) | – | | 42 (28.8) | – | |
| **PLCO Study** | | | | | | |
| Number of participants | 44 | 29 | | 532 | 595 | |
| Age, years, mean ± SD[a] | 65.1 ± 5.1 | 65.7 ± 5.4 | 0.63 | 64.7 ± 5.1 | 64.5 ± 5.3 | 0.52 |
| Gender, *N* (%)[b] | | | | | | |
| Male | 34 (77.3) | 19 (65.5) | 0.27 | 359 (67.5) | 380 (63.9) | 0.20 |
| Female | 10 (22.7) | 10 (34.5) | | 173 (32.5) | 215 (36.1) | |
| Smoking status, *N* (%)[b] | | | | | | |
| Never | 0 (0) | 5 (17.2) | | 37 (7.0) | 106 (17.8) | |
| Former quit ≤15 years | 14 (31.8) | 8 (27.6) | | 186 (35.0) | 184 (30.9) | |
| Former quit >15 years | 2 (4.6) | 3 (10.3) | | 105 (19.6) | 102 (17.1) | |
| Current | 28 (63.6) | 13 (44.8) | 0.02 | 204 (38.4) | 203 (34.2) | <0.001 |
| Pack-years, mean ± SD[a,c] | 34.8 ± 23.4 | 34.1 ± 33.6 | 0.92 | 49.0 ± 30.2 | 45.6 ± 30.0 | 0.08 |
| Education, *N* (%)[b] | | | | | | |
| High school or less | 28 (63.6) | 12 (41.4) | 0.06 | 200 (37.6) | 190 (31.9) | 0.05 |
| College or higher | 16 (36.4) | 17 (58.6) | | 332 (62.4) | 405 (68.1) | |
| Regular aspirin/ibuprofen use, *N* (%)[b,d] | | | | | | |

| | African Americans | | | European Americans | | |
|---|---|---|---|---|---|---|
| | **Cases** | **Controls** | ***P*** | **Cases** | **Controls** | ***P*** |
| No | 20 (45.5) | 10 (34.5) | 0.35 | 182 (34.3) | 202 (34.0) | 0.91 |
| Yes | 24 (54.5) | 19 (65.5) | | 349 (65.7) | 393 (66.1) | |
| BMI, *N* (%)[b,d] | | | | | | |
| <26.5 | 19 (44.2) | 9 (31.0) | 0.26 | 267 (50.6) | 291 (49.8) | 0.81 |
| ≥26.5 | 24 (55.8) | 20 (69.0) | | 261 (49.3) | 293 (50.2) | |
| History of heart disease, *N* (%)[b,d] | | | | | | |
| No | 35 (92.1) | 23 (85.2) | 0.38 | 423 (83.1) | 498 (85.1) | 0.36 |
| Yes | 3 (7.9) | 4 (14.8) | | 86 (16.9) | 87 (14.7) | |
| History of emphysema/bronchitis, *N* (%)[b,d] | | | | | | |
| No | 32 (82.0) | 23 (85.2) | 0.74 | 411 (80.0) | 521 (88.9) | <0.001 |
| Yes | 7 (18.0) | 4 (14.8) | | 103 (20.0) | 65 (11.1) | |
| Family history of lung cancer, *N* (%)[b,d] | | | | | | |
| No | 22 (50) | 16 (55.2) | 0.34 | 408 (81.6) | 502 (88.4) | 0.002 |
| Yes | 22 (50) | 13 (44.8) | | 92 (18.4) | 66 (11.6) | |
| Histology, *N* (%)[d] | | | | | | |
| AC | 24 (55.8) | – | | 228 (43.1) | – | |
| SCC | 8 (18.6) | – | | 120 (22.7) | – | |
| SCLC | 7 (16.4) | – | | 68 (12.9) | – | |
| NSCLC, NOS | 2 (4.6) | – | | 38 (7.2) | – | |
| Other | 2 (4.6 | – | | 75 (14.2) | – | |
| Clinical stage, *N* (%) | | | | | | |
| I | 12 (27.9) | – | | 166 (31.3) | – | |
| II–IV | 31 (72.1) | – | | 366 (68.7) | – | |
| **WSU Study**[e] | | | | | | |
| Number of participants | 249 | 318 | | – | – | |
| Age, years, mean ± SD[a] | 61.9 ± 10.6 | 60.8 ± 9.2 | 0.19 | – | – | |
| Gender, *N* (%)[b] | | | | | | |
| Male | 116 (45.6) | 141 (44.3) | 0.59 | – | – | |
| Female | 133 (53.4) | 177 (55.7) | | – | – | |

| | African Americans | | | European Americans | | |
|---|---|---|---|---|---|---|
| | **Cases** | **Controls** | ***P*** | **Cases** | **Controls** | ***P*** |
| Smoking status, *N* (%)[b] | | | | | | |
| Never | 14 (5.6) | 100 (31.4) | | – | – | |
| Former quit ≤15 years | 36 (14.5) | 17 (5.4) | | – | – | |
| Former quit >15 years | 41 (16.5) | 59 (18.6) | | – | – | |
| Current | 158 (63.4) | 142 (44.6) | <0.001 | – | – | |
| Pack-years, mean ± SD[a,c] | 38.7 ± 31.4 | 18.0 ± 20.5 | <0.001 | – | – | |
| Education, *N* (%)[b] | | | | | | |
| High school or less | 148 (59.7) | 145 (45.6) | <0.001 | – | – | |
| College or higher | 100 (40.3) | 173 (54.4) | | – | – | |
| History of emphysema/bronchitis, *N* (%)[b,d] | | | | | | |
| No | 166 (66.7) | 268 (84.3) | <0.001 | – | – | |
| Yes | 83 (33.3) | 50 (15.7) | | – | – | |
| Family history of lung cancer, *N* (%)[b,d] | | | | | | |
| No | 190 (76.6) | 270 (84.9) | 0.01 | – | – | |
| Yes | 58 (23.4) | 48 (15.1) | | – | – | |
| Histology, *N* (%)[d] | | | | | | |
| AC | 105 (42.5) | – | | – | – | |
| SCC | 57 (23.0) | – | | – | – | |
| SCLC | 20 (8.1) | – | | – | – | |
| NSCLC, NOS | 40 (16.1) | – | | – | – | |
| Other/Not specified | 26 (10.5) | – | | – | – | |
| Clinical stage, *N* (%)[d] | | | | | | |
| Local | 64 (26.3) | – | | – | – | |
| Regional | 70 (28.8) | – | | – | – | |
| Distant | 109 (44.9) | – | | – | – | |

Abbreviations: NCI-MD, National Cancer Institute-Maryland; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; WSU, Wayne State University; BMI, body mass index categorized by median value of controls; AC, adenocarcinoma; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; SD, standard deviation; – Not applicable.

[a] *P* values were calculated using a two-sided Student's *t* test.

[b] *P* values were calculated using a two-sided $x^2$ test.

[c] Excludes individuals who had never smoked.

[d] Numbers do not add to 100% of total due to missing information. Tumor staging was based on the AJCC manual.

[e] Intake of aspirin or ibuprofen, BMI, and history of heart disease were not recorded in the WSU study.

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**Table 2**

Serum levels of cytokines (pg/mL) of African American and European American participants in the NCI-MD study

| | African Americans | | | | | European Americans | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases (*N* = 85) | | Controls (*N* = 170) | | | Cases (*N* = 270) | | Controls (*N* = 296) | | | |
| **Cytokine** | **Median** | **IQR** | **Median** | **IQR** | ***P*[a]** | **Median** | **IQR** | **Median** | **IQR** | ***P*[a]** | ***P*[b]** |
| IL-1β | 0.5 | 0.2–1.1 | 0.4 | 0.2–0.7 | <0.01 | 0.5 | 0.2–0.9 | 0.4 | 0.2–0.9 | 0.44 | 0.12 |
| IL-4 | 1.2 | 0.6–2.5 | 0.9 | 0.3–1.8 | 0.07 | 1.4 | 0.7–2.2 | 1.4 | 0.7–2.8 | 0.72 | <0.01 |
| IL-5 | 0.7 | 0.4–1.6 | 0.7 | 0.3–1.3 | 0.33 | 0.7 | 0.4–1.3 | 0.8 | 0.5–1.4 | 0.47 | 0.04 |
| IL-6 | 5.0 | 3.2–8.5 | 2.3 | 1.4–3.8 | <0.01 | 3.7 | 2.3–7.1 | 2.1 | 1.4–3.7 | <0.01 | 0.75 |
| IL-8 | 17.0 | 9.3–35.5 | 8.3 | 5.7–13.5 | <0.01 | 15.8 | 9.5–39.0 | 10.4 | 7.0–28.1 | <0.01 | <0.01 |
| IL-10 | 13.0 | 8.4–22.8 | 8.4 | 4.9–17.5 | 0.01 | 12.7 | 7.6–25.1 | 11.0 | 7.6–22.1 | 0.09 | <0.01 |
| IL-12 | 6.3 | 3.0–13.1 | 7.6 | 3.1–15.4 | 0.80 | 5.3 | 3.0–13.1 | 7.2 | 3.6–14.6 | 0.05 | 0.52 |
| GMCSF | 0.9 | 0.5–2.7 | 0.9 | 0.3–2.0 | 0.58 | 1.1 | 0.6–3.0 | 1.0 | 0.6–2.6 | 0.45 | 0.25 |
| IFNγ | 1.6 | 1.0–3.6 | 1.5 | 0.7–3.0 | 0.14 | 1.8 | 1.1–3.6 | 1.9 | 1.2–4.4 | 0.51 | <0.01 |
| TNFα | 2.3 | 1.8–3.0 | 2.0 | 1.5–2.6 | 0.03 | 2.4 | 1.9–3.0 | 2.2 | 1.7–2.8 | <0.01 | 0.05 |

Abbreviations: NCI-MD, National Cancer Institute-Maryland; IQR, interquartile range; IL, interleukin; GMCSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; TNF, tumor necrosis factor.

[a] *P* values were calculated comparing cases and controls using non-parametric Wilcoxon rank sum test.

[b] *P* values were calculated comparing African-American controls to European-American controls using a non-parametric Wilcoxon rank sum test.

**Table 3**

Association between serum cytokine levels and lung cancer among participants in the NCI-MD study

| | | African Americans (*N* = 255) | | | European Americans (*N* = 566) | | |
|---|---|---|---|---|---|---|---|
| **Cytokine** | **Quartile**[a] | **Cases/Controls** | **OR (95% CI)**[b] | $P_{trend}$[c] | **Cases/Controls** | **OR (95% CI)**[b] | $P_{trend}$[c] |
| IL1β | <0.19 | 12/57 | 1.00 (reference) | | 61/59 | 1.00 (reference) | |
| | 0.19 – <0.37 | 20/35 | 2.28 (0.87–5.99) | | 52/77 | 0.69 (0.40–1.19) | |
| | 0.37 – <0.89 | 24/41 | 2.58 (1.02–6.50) | | 91/80 | 1.09 (0.66–1.81) | |
| | ≥0.89 | 29/37 | 3.61 (1.46–8.95) | 0.007 | 66/80 | 0.69 (0.41–1.17) | 0.47 |
| IL-4 | <0.58 | 19/58 | 1.00 (reference) | | 56/58 | 1.00 (reference) | |
| | 0.58 – <1.19 | 23/42 | 1.53 (0.64–3.63) | | 63/74 | 0.96 (0.56–1.64) | |
| | 1.19 – <2.36 | 21/42 | 1.31 (0.56–3.09) | | 84/76 | 1.02 (0.61–1.72) | |
| | ≥2.36 | 22/28 | 2.03 (0.83–4.94) | 0.17 | 67/88 | 0.79 (0.47–1.34) | 0.44 |
| IL-5 | <0.42 | 22/56 | 1.00 (reference) | | 69/60 | 1.00 (reference) | |
| | 0.42 – <0.74 | 21/34 | 1.41 (0.59–3.36) | | 51/69 | 0.54 (0.31–0.94) | |
| | 0.74 – <1.39 | 16/42 | 1.22 (0.50–2.94) | | 91/88 | 0.70 (0.43–1.16) | |
| | ≥1.39 | 26/38 | 1.72 (0.76–3.91) | 0.25 | 59/79 | 0.52 (0.30–0.88) | 0.05 |
| IL-6 | <1.36 | 6/42 | 1.00 (reference) | | 27/74 | 1.00 (reference) | |
| | 1.36 – <2.14 | 8/41 | 0.86 (0.23–3.19) | | 31/76 | 1.02 (0.55–1.93) | |
| | 2.14 – <3.79 | 19/46 | 2.02 (0.64–6.33) | | 80/71 | 2.39 (1.34–4.25) | |
| | ≥3.79 | 52/41 | 5.77 (1.99–16.78) | <0.001 | 132/75 | 3.36 (1.93–5.88) | <0.001[d] |
| IL-8 | <6.47 | 11/55 | 1.00 (reference) | | 26/62 | 1.00 (reference) | |
| | 6.47 – <9.31 | 10/44 | 1.07 (0.37–3.14) | | 38/72 | 1.21 (0.64–2.31) | |
| | 9.31 – <22.85 | 31/42 | 3.21 (1.26–8.18) | | 107/75 | 2.95 (1.65–5.27) | |
| | ≥22.85 | 33/29 | 6.53 (2.48–17.20) | <0.001 | 99/87 | 2.24 (1.26–4.00) | 0.001[d] |
| IL-10 | <6.12 | 16/55 | 1.00 (reference) | | 44/61 | 1.00 (reference) | |
| | 6.12 – <10.36 | 16/41 | 1.35 (0.53–3.40) | | 63/75 | 1.09 (0.63–1.91) | |
| | 10.36 – <20.63 | 31/38 | 4.11 (1.69–9.99) | | 78/80 | 1.21 (0.70–2.07) | |
| | ≥20.63 | 22/36 | 2.19 (0.88–5.24) | 0.02 | 85/80 | 1.36 (0.79–2.32) | 0.23 |
| IL-12 | <3.34 | 25/49 | 1.00 (reference) | | 76/67 | 1.00 (reference) | |
| | 3.34 – <7.31 | 23/36 | 1.01 (0.44–2.34) | | 82/81 | 1.01 (0.62–1.65) | |
| | 7.31 – <14.86 | 18/42 | 0.83 (0.35–1.97) | | 54/75 | 0.58 (0.35–0.98) | |

| | | African Americans (*N* = 255) | | | European Americans (*N* = 566) | | |
|---|---|---|---|---|---|---|---|
| **Cytokine** | **Quartile**[a] | **Cases/Controls** | **OR (95% CI)**[b] | $P_{trend}$[c] | **Cases/Controls** | **OR (95% CI)**[b] | $P_{trend}$[c] |
| | ≥14.86 | 19/43 | 0.98 (0.42–2.31) | 0.85 | 58/73 | 0.75 (0.45–1.25) | 0.08 |
| GMCSF | <0.50 | 21/55 | 1.00 (reference) | | 62/61 | 1.00 (reference) | |
| | 0.50 – <0.96 | 26/33 | 1.42 (0.61–3.33) | | 55/84 | 0.74 (0.44–1.26) | |
| | 0.96 – <2.51 | 16/45 | 0.77 (0.32–1.87) | | 79/72 | 1.06 (0.64–1.77) | |
| | ≥2.51 | 22/37 | 1.12 (0.47–2.64) | 0.88 | 74/79 | 0.95 (0.57–1.58) | 0.80 |
| IFNγ | <0.98 | 18/59 | 1.00 (reference) | | 54/57 | 1.00 (reference) | |
| | 0.98 – <1.70 | 27/39 | 2.58 (1.10–6.06) | | 73/78 | 0.97 (0.57–1.67) | |
| | 1.70 – <3.79 | 22/41 | 2.02 (0.83–4.92) | | 76/75 | 1.01 (0.59–1.74) | |
| | ≥3.79 | 18/31 | 2.07 (0.80–5.31) | 0.17 | 67/86 | 0.80 (0.47–1.37) | 0.43 |
| TNFα | <1.64 | 13/52 | 1.00 (reference) | | 38/63 | 1.00 (reference) | |
| | 1.64 – <2.10 | 23/40 | 2.54 (1.00–6.49) | | 65/75 | 1.48 (0.84–2.61) | |
| | 2.10 – <2.75 | 21/42 | 1.87 (0.74–4.79) | | 72/78 | 1.34 (0.77–2.35) | |
| | ≥2.75 | 28/36 | 3.09 (1.21–7.88) | 0.05 | 95/80 | 1.69 (0.97–2.92) | 0.11 |

Abbreviations: NCI-MD, National Cancer Institute-Maryland; OR, odds ratio; CI, confidence interval; IL, interleukin; GMCSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; TNF, tumor necrosis factor.

[a] Quartiles were based on serum cytokine cut-off levels among controls.

[b] Multivariate 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).

[c] *P* values were calculated using a two-sided Wald $\chi^2$ statistic.

[d] Data was previously reported (17).

**Table 4**

Association between serum cytokine levels and lung cancer among participants in the WSU and PLCO studies

| | | WSU African Americans (*N* = 567) | | | PLCO European Americans (*N* = 1127) | | |
|---|---|---|---|---|---|---|---|
| **Cytokine** | **Quartile**[a] | **Cases/controls *N*** | **OR (95% CI)**[b] | $P_{trend}$[c] | **Cases/controls *N*** | **OR (95% CI)**[b] | $P_{trend}$[c] |
| IL-1β | 1 | 40/75 | 1.00 (reference) | | 73/97 | 1.00 (reference) | |
| | 2 | 66/81 | 1.45 (0.84–2.52) | | 155/164 | 1.27 (0.84–1.91) | |
| | 3 | 55/82 | 1.24 (0.69–2.20) | | 170/177 | 1.37 (0.91–2.06) | |
| | 4 | 87/80 | 1.90 (1.09–3.31) | 0.05 | 134/157 | 1.15 (0.76–1.74) | 0.64 |
| IL-6 | 1 | 22/78 | 1.00 (reference) | | 96/138 | 1.00 (reference) | |
| | 2 | 37/81 | 1.12 (0.56–2.20) | | 131/158 | 1.17 (0.79–1.74) | |
| | 3 | 82/77 | 2.87 (1.54–5.35) | | 139/149 | 1.28 (0.86–1.89) | |
| | 4 | 107/82 | 3.57 (1.94–6.58) | <0.001 | 166/150 | 1.57 (1.07–2.30) | 0.02[d] |
| IL-8 | 1 | 43/79 | 1.00 (reference) | | 102/102 | 1.00 (reference) | |
| | 2 | 65/80 | 1.61 (0.92–2.82) | | 108/151 | 0.94 (0.64–1.40) | |
| | 3 | 52/79 | 1.15 (0.65–2.04) | | 148/149 | 1.37 (0.94–2.01) | |
| | 4 | 88/80 | 2.20 (1.28–3.79) | 0.02 | 174/153 | 1.55 (1.06–2.26) | 0.004[d] |
| IL-10 | 1 | 36/72 | 1.00 (reference) | | | N.D. | |
| | 2 | 55/77 | 1.44 (0.80–2.59) | | | | |
| | 3 | 47/77 | 1.28 (0.70–2.32) | | | | |
| | 4 | 98/77 | 2.40 (1.38–4.17) | 0.003 | | | |
| TNFα | 1 | 41/79 | 1.00 (reference) | | 114/131 | 1.00 (reference) | |
| | 2 | 41/80 | 0.98 (0.54–1.78) | | 123/160 | 0.94 (0.64–1.36) | |
| | 3 | 72/79 | 1.60 (0.91–2.79) | | 128/154 | 1.04 (0.71–1.52) | |
| | 4 | 94/80 | 2.31 (1.34–3.97) | <0.001 | 167/150 | 1.44 (1.02–1.64) | 0.07 |

Abbreviations: PLCO, Prostate, Lung, Colorectal, and Ovarian; WSU, Wayne State University; OR, odds ratio; CI, confidence interval; N.D., Not done

[a] Quartiles were based on serum cytokine levels among controls in the WSU study (25$^{th}$, 50$^{th}$, and 75$^{th}$ percentile: IL-1β, 0.9, 3.4, 10.4 pg/mL; IL-6, 3.1, 6.9, 19.6 pg/mL; IL-8, 27.1, 155.5, 1061.3 pg/mL; IL-10, 4.8, 7.1, 10.6 pg/mL; TNFα 6.8, 10.4, 20.5 pg/mL) and PLCO study (IL-1β, 0.4, 0.7, 1.4 pg/mL; TNF-α, 7.4, 9.1, 11.5 pg/mL).

[b] Unconditional multivariate logistic regression was adjusted for age (continuous), gender, smoking pack-years (continuous), smoking status (never, former quit ≥ 15 years, former quit <15 years, current), PLCO study adjusted additionally for year of randomization, number of years in the study.

[c] *P* values were calculated using a two-sided Wald $\chi 2$ statistic.

[d] Data was previously reported (17).

## Table 5

Association between serum cytokine levels and lung cancer among African Americans from all three studies, by smoking status

| | | OR (95% CI)[b] | | | |
|---|---|---|---|---|---|
| **Cytokine level[a]** | **Cases/controls *N*** | **Combined (*N* = 377/517)** | **Never smokers (*N* = 21/172)** | **Former smokers (*N* = 124/162)** | **Current smokers (*N* = 232/182)** |
| **IL-1β** | | | | | |
| First quartile | 55/137 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Second quartile | 102/123 | 1.82 (1.15–2.87) | 1.15 (0.27–4.97) | 1.56 (0.69–3.51) | 2.12 (1.13–3.97) |
| Third quartile | 91/132 | 1.48 (0.94–2.34) | 2.28 (0.62–8.41) | 2.15 (1.00–4.61) | 1.21 (0.64–2.31) |
| Fourth quartile | 129/125 | 2.32 (1.49–3.62) | 2.02 (0.53–7.73) | 3.15 (1.49–6.65) | 2.22 (1.19–4.15) |
| $P_{trend}$[c] | | 0.001 | 0.19 | 0.002 | 0.09 |
| **IL-6** | | | | | |
| First quartile | 33/126 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Second quartile | 54/128 | 1.03 (0.60–1.79) | 0.74 (0.06–8.50) | 1.96 (0.75–5.16) | 0.80 (0.38–1.69) |
| Third quartile | 116/134 | 2.26 (1.37–3.72) | 6.45 (1.24–33.52) | 2.30 (0.94–5.64) | 2.32 (1.17–4.60) |
| Fourth quartile | 174/129 | 3.82 (2.34–6.24) | 7.67 (1.58–37.29) | 5.89 (2.44–14.23) | 2.76 (1.42–5.37) |
| $P_{trend}$[c] | | <0.001 | 0.002 | <0.001 | <0.001 |
| **IL-8** | | | | | |
| First quartile | 63/140 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Second quartile | 85/132 | 1.25 (0.80–1.97) | 0.70 (0.12–4.10) | 1.04 (0.48–2.25) | 1.53 (0.81–2.87) |
| Third quartile | 90/128 | 1.28 (0.82–2.00) | 1.99 (0.49–8.17) | 1.78 (0.85–3.73) | 1.09 (0.58–2.05) |
| Fourth quartile | 139/117 | 2.36 (1.53–3.64) | 4.48 (1.25–16.04) | 2.71 (1.32–5.53) | 2.11 (1.14–3.90) |
| $P_{trend}$[c] | | <0.001 | 0.009 | 0.002 | 0.04 |
| **IL-10** | | | | | |
| First quartile | 52/127 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Second quartile | 71/118 | 1.42 (0.88–2.31) | 1.76 (0.46–6.74) | 1.41 (0.63–3.17) | 1.59 (0.80–3.16) |
| Third quartile | 78/115 | 1.76 (1.09–2.84) | 1.69 (0.44–6.45) | 2.57 (1.14–5.78) | 1.67 (0.85–3.30) |
| Fourth quartile | 120/113 | 2.33 (1.47–3.69) | 1.54 (0.38–6.24) | 2.13 (0.98–4.62) | 3.61 (1.85–7.03) |
| $P_{trend}$[c] | | <0.001 | 0.58 | 0.03 | <0.001 |
| **TNFα** | | | | | |
| First quartile | 12/57 | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |

| Cytokine level[a] | Cases/controls *N* | OR (95% CI)[b] | | | |
|---|---|---|---|---|---|
| | | Combined (*N* = 377/517) | Never smokers (*N* = 21/172) | Former smokers (*N* = 124/162) | Current smokers (*N* = 232/182) |
| Second quartile | 20/35 | 1.27 (0.80–2.01) | 0.76 (0.13–4.41) | 1.85 (0.83–4.15) | 1.35 (0.72–2.53) |
| Third quartile | 24/41 | 1.68 (1.08–2.61) | 2.72 (0.68–10.78) | 1.74 (0.80–3.77) | 1.81 (0.98–3.33) |
| Fourth quartile | 29/37 | 2.14 (1.38–3.30) | 4.06 (1.08–15.30) | 3.74 (1.74–8.00) | 1.45 (0.80–2.64) |
| $P_{trend}$[c] | | <0.001 | 0.02 | 0.001 | 0.18 |

Abbreviations: OR, odds ratio; CI, confidence interval; IL, interleukin; GMCSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; TNF, tumor necrosis factor.

[a] Quartiles were based on serum cytokine cut-off levels among European-American controls.

[b] Multivariate 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).

[c] *P* values were calculated using a two-sided Wald $\chi^2$ statistic.