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Methylation-derived neutrophil-to-lymphocyte ratio and lung cancer risk in heavy smokers

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Abstract

The neutrophil-to-lymphocyte ratio (NLR) is a biomarker that indicates systemic inflammation and can be estimated using array-based DNA methylation data as methylation-derived NLR (mdNLR). We assessed the relationship between pre-diagnosis mdNLR and lung cancer risk in a nested case-control study in the β-Carotene and Retinol Efficacy Trial (CARET) of individuals at high risk for lung cancer due to heavy smoking or substantial occupational asbestos exposure. We matched 319 incident lung cancer cases to controls based on age at blood draw, smoking, sex, race, asbestos, enrollment year, and time at risk. We computed mdNLR using the ratio of predicted granulocyte and lymphocyte proportions derived from DNA methylation signatures in whole blood collected prior to diagnosis (median 4.4 years in cases). Mean mdNLR was higher in cases than controls (2.06 vs 1.86, p=0.03). Conditional logistic regression models adjusted for potential confounders revealed a 21% increased risk of lung cancer per unit increase in mdNLR (Odds Ratio (OR) 1.21, 95% Confidence Interval (CI) 1.01–1.45). A 30% increased risk of non-small cell lung cancer (NSCLC) was observed for each unit increase in mdNLR (n=240 pairs; OR 1.30, 95% CI 1.03–1.63), and there was no statistically significant association between mdNLR and small cell

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lung cancer risk. The mdNLR-NSCLC association was most pronounced in those with asbestos exposure (n=42 male pairs; OR 3.39, 95% CI 1.32–8.67). A better understanding of the role of mdNLR in lung cancer etiology may improve prevention and detection of lung cancer.

Introduction

Inflammation is an established characteristic and hallmark of cancer development and progression (1). White blood cell distributions, such as increased neutrophil counts and decreased lymphocyte counts, are markers of systemic inflammation that are associated with morbidity and mortality in cancer patients (2,3). The neutrophil-to-lymphocyte ratio (NLR) is computed as the ratio of the respective whole-blood proportions of neutrophils and lymphocytes, and therefore reflects the balance between the innate immune response and adaptive immune response (4). Elevated pre-treatment NLR is an emerging biomarker of cancer prognosis, as it has been associated with worse outcomes for various tumor types including lung cancer (2,3,5–13). Investigations of NLR and cancer risk are limited (14–16), and only one study to date has evaluated the association between NLR and lung cancer risk (15).

White blood cell distributions are easily determined from peripheral blood in routine clinical care; however, the assessment of these parameters becomes unstable in stored blood after as little as 24 hours (17), which makes their examination in biorepository collections challenging. Blood cells originate from hematopoietic stem cells, and the lineage of each blood cell is dependent on epigenetic differentiation signatures including methylation (18). Obtaining blood cell distribution estimates from methylation data measured in archival blood using deconvolution algorithms has been described and validated (19–22). Recent work has highlighted the utility of these methods for estimating NLR, denoted methylation-derived NLR (mdNLR), and in utilizing these estimates to examine associations with cancer risk and outcomes (16,21,23). The lifetime probability that a smoker will develop lung cancer is estimated to be <15% (24). Thus, there is a great need for biomarkers to differentiate between smokers who will and will not develop lung cancer, especially in the context of lung cancer screening, which generates a large proportion of false positives. We evaluated whether increased levels of inflammation as indicated by higher mdNLR values are associated with lung cancer risk in a case-control study nested in the β-Carotene and Retinol Efficacy Trial (CARET) of heavy smokers at high risk for lung cancer. The CARET population is primarily composed of individuals who would have been eligible for lung cancer screening at blood draw based on the current guidelines from various organizations including the U.S. Preventive Task Force (e.g., ages 55–80, 30+ pack year smoking history, current smokers or former smokers who quit less than 15 years ago) (25).

Materials and methods

Study population

Participants in this study were drawn from the multicenter β-Carotene and Retinol Efficacy Trial (CARET) (26). CARET was a randomized, double-blinded, placebo-controlled chemoprevention trial designed to assess the safety and efficacy of daily supplementation

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with β-carotene and retinyl palmitate among individuals at high risk of developing lung cancer (27–29). Men and women ages 50–69 years were eligible for the trial if they were current or former smokers (quit at most six years prior to enrollment) with a heavy cigarette smoking history of 20 pack-years (n=14,254). Additionally, men ages 45–69 years with occupational asbestos exposure who were current or former heavy smokers (quit at most fifteen years prior to enrollment) were included (n=4,060). Extensive information about smoking history and other risk factors was collected through annual questionnaires. Whole blood was collected after enrollment at participant visits between 1994 and 1997. Due to higher lung cancer incidence and overall mortality rates in the intervention versus placebo arm, the intervention was stopped in 1996.

The present study includes individuals selected for a nested case-control study based on endpoint information collected from 1985 to 2005, during active participant follow-up, as described previously (26). Briefly, all participants who provided a blood specimen and who were free of lung cancer at the time of blood collection were eligible to be matched in a 2:1 ratio to 793 lung cancer cases. Matching factors included age at enrollment (±4 years), sex, race/ethnicity, enrollment year (±2 years), smoking status at enrollment (current or former), history of occupational asbestos exposure, and length of follow-up. For our study, we selected those with known histotype to the extent possible (240 NSCLC, 68 SCLC, 11 unknown). We matched controls to the 319 cases in a 1:1 ratio using age at blood draw (±5 years), sex, race/ethnicity, enrollment year (±2 years), smoking status at blood draw (current or former), history of occupational asbestos exposure, and length of follow-up. The Institutional Review Boards for each of the participating CARET institutions approved all study protocols, which were in accordance with recognized ethical guidelines, and written informed consent was obtained from all participants.

Methylation array, Quality Control, Preprocessing and Normalization of Methylation Data

We assessed DNA methylation in whole blood using the Illumina HumanMethylation850 BeadArray platform at the University of Southern California Epigenomics Core Facility, following standardized protocols described by the manufacturer (Illumina, Inc). Quality control, preprocessing, and normalization of Illumina HumanMethylation850 BeadArray methylation data was performed using both *minfi* and *wateRmelon* Bioconductor packages (30,31). To ensure high-quality methylation data, we utilized the within-array normalization combination of Noob+β-mixture quantile (BMIQ), as this processing combination has been previously demonstrated to result in improved signal intensity compared to competing approaches (32). Within-array correction for background fluorescence and dye biases were performed using the Noob methodology via the function “preprocessNoob” in the *minfi* Bioconductor package (30). Following application of the Noob, we identified and removed poor quality samples and probes. Samples were excluded if >20% of their probes had detection p-values $>1\times10^{-5}$ or if they exhibited irregularities in the distribution of their control probes (33). In addition, probes with a median detection p-value >0.05 were removed and excluded from subsequent statistical analyses. After sample- and probe-level quality control, we corrected the type II probe bias to make the methylation distribution of type II features comparable to the distribution of type I features using the BMIQ dilation methodology (34), implemented using the function “BMIQ” in the *wateRmelon*

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Bioconductor package. For each sample, BMIQ involves fitting a three-class β-mixture model (class 1=un-methylated, class 2=hemi-methylated, and class 3=methylated probes) to the type I and type II probes separately. Probes are assigned to the class with the highest posterior predictive probability (i.e., probability of class membership conditional on the observed data). β-values for the type II features are then normalized by class to the distributions for the same class estimated in type I features. Following the Noob+BMIQ processing combination, the presence of batch-effects and/or other technical sources of variation were inspected by first performing a principal components analysis (PCA) on the normalized methylation data, followed by an examination of the association between the top K principal components (K determined using random matrix theory (35) and plate, beadchip, and chip position). We observed only mild associations between these technical features and the top K principal components, and concluded that a formal batch-effect correction was not required for these data.

Estimation of the methylation-derived neutrophil-to-lymphocyte ratio (mdNLR)

We used a recently developed method by Koestler et al. to estimate the mdNLR for each of the study samples from blood-derived DNA methylation data (16). We first applied reference-based cell-mixture deconvolution to estimate the proportion of leukocyte subtypes (i.e., CD4T, CD8T, natural killer cells, B cells, monocytes, and granulocytes) for each sample using the "estimateCellCounts" function in the minfi Bioconductor package. In total, 600 leukocyte discriminating cytosine-phosphate-guanine (CpG) sites were used as the basis for cell composition estimation (36). The mdNLR was computed for each sample by taking the ratio of its predicted granulocyte and lymphocyte fractions (the sum of the predicted fraction of lymphocyte subtypes) (16).

Of the 600 CpGs used to estimate cell composition (36), 96 CpGs overlapped with the 18,760 false discovery rate-significant CpGs identified in the 2016 Joehanes et al. meta-analysis of differential DNA methylation between current and never smokers (37). We performed permutation testing to assess whether there was significant enrichment of smoking-associated CpGs among those used to deconvolute cell composition. We also compared cell type proportion estimates, and the resultant mdNLR, obtained before (600 CpGs) and after removing the 96 smoking-associated CpGs (504 CpGs) in our population (n=638) by computing the Pearson correlations and root mean squared error (RMSE).

Statistical analysis

Given the nested case-control study design with 1:1 case-control matching, a series of conditional logistic regression models were used to examine the association between continuous mdNLR and lung cancer risk. Models were fit using the "clogit" function in the survival R package with the following matching factors: age at blood draw (±5 years), sex, race/ethnicity, enrollment year, smoking status at blood draw, history of occupational asbestos exposure, and length of follow-up, and were adjusted for potential confounding factors, including: age (continuous), pack years of smoking, cigarettes per day, and body mass index (BMI), all at blood draw. Adjustment for continuous age and smoking variables help to control for any existing residual confounding in the corresponding matching factors. We selected potential confounders based on biologic plausibility and the impact of addition

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to crude conditional logistic regression models, i.e. met or exceeded a 10% change in the mdNLR coefficient.

Conditional logistic regression models were fit to estimate the relationship between mdNLR and lung cancer risk overall, as well as the association between mdNLR and risk by lung cancer histotype (NSCLC, SCLC), race (white, non-white), sex (male, female), study (asbestos exposure, heavy smoking only), smoking status at blood draw (yes, no), and intervention arm (placebo, active). NSCLC cases were defined as those with a diagnosis of lung adenocarcinoma, squamous cell carcinoma, and not otherwise specified (NOS) NSCLC. Because the time between blood draw and diagnosis varied considerably across cases (0.1 years to 10.1 years, median=4.4 years), conditional logistic regression models stratified by the median length of time between blood draw and diagnosis (<4.4 years and 4.4 years) were fit as a means to gain insight into whether the association between mdNLR and risk varies by the proximity to the time of diagnosis.

Results

Characteristics of the cases (all, NSCLC, and SCLC) and controls are presented in Table 1. Briefly, both cases and controls were approximately 64.0 years of age at blood draw and the majority were white (97%), male (66%), and current smokers at blood draw (65%). Cases were generally diagnosed at late stage, with 73% presenting with stage III or IV lung cancer. Compared to controls, mean mdNLR was higher for cases (mean, standard deviation (SD) for controls versus cases: 1.86 (0.90) versus 2.06 (1.31), p=0.03) and was higher for NSCLC than SCLC (mean, SD: 2.07, 1.31 and 1.88, 1.21, respectively).

Permutation-based testing indicated statistically significant enrichment of smoking-associated CpGs (37) among the 600 CpGs used to estimate white blood cell proportions (permutation p<0.0001) (36). However, cell type estimates for our population (n=638) obtained with and without the removal of the 96 smoking-associated CpGs were highly correlated (Pearson *r* 0.99 across all cell types). The average difference between estimates obtained with and without the removal of smoking-associated CpGs was less than 2% in absolute percentage points (e.g., RMSE 2 for all cell types) and less than 1% in 4 out of the 6 cell types. Importantly, mdNLR estimates were highly consistent (Pearson *r*=1.00, RMSE=0.11).

Higher mdNLR values were associated with risk of lung cancer, especially NSCLC, in our study. After controlling for age, pack years, cigarettes per day, and BMI, all at blood draw, each unit increase in mdNLR was associated with a 21% increased risk of lung cancer (95% CI: 1.01–1.44; Table 2). For NSCLC, we observed a 30% increased risk of lung cancer (95% CI: 1.03–1.63) for each unit increase in mdNLR. Associations with mdNLR were similar for adenocarcinoma and squamous cell carcinoma. We did not observe statistically significant associations between mdNLR and SCLC, which may have been due to a smaller sample size (68 pairs; Table 2).

Subgroup analyses revealed that lung cancer risk associations were most notable in males (OR, 95% CI: 1.30, 1.04–1.63), those with a history of asbestos exposure (OR, 95% CI:

2.13, 1.12–4.05), and those assigned to the trial's active intervention arm (OR, 95% CI: 1.70, 1.12–2.58). As in the overall analyses, associations were larger when restricted to NSCLC pairs for males (OR, 95% CI: 1.34, 1.02–1.75) and asbestos exposure (OR, 95% CI: 3.39, 1.32–8.67), but the NSCLC association for the active intervention arm was not statistically significant (OR, 95% CI: 1.53, 0.89–2.62). We observed associations of similar magnitude for early (I/II) and late (III/IV) stage lung cancers, although the late stage odds ratios were statistically significant for overall and NSCLC (Table 2). Associations were similar though no longer statistically significant in strata of smoking status or time at risk.

Discussion

We observed that elevated pre-diagnosis mdNLR is associated with an increased risk of lung cancer in a population of heavy smokers at high risk for lung cancer. Our findings are compelling, as blood was collected prior to diagnosis (median 4.4 years) and lung cancer cases and controls share a history of heavy smoking. Smoking has been reported to be associated with blood methylation signatures (38–40) and immune profiles (41). We observed that the residual effects due to smoking on the 600 CpGs used for white blood cell deconvolution have a minimal effect on cell composition estimates and therefore on mdNLR estimation. Nevertheless, smokers tend to have higher NLR values than non-smokers (42) and both smoking and nicotine are reportedly immunosuppressive (43). Observing an association between mdNLR and lung cancer risk after rigorous control for smoking history through matching and model adjustment implies that there are other factors, intrinsic or extrinsic, that are likely contributing to the relationship between mdNLR and lung cancer risk. Identifying a biomarker for lung cancer risk among those at highest risk is a salient issue, as <25% of smokers will develop lung cancer during their lifetime assuming they survive competing causes of death, or <15% without conditioning on competing causes of death (24). Successfully distinguishing between those who ultimately will develop lung cancer among those eligible for screening could improve lung cancer screening by limiting false-positives and overdiagnosis, two of the major concerns about lung cancer screening (44).

The strongest subgroup association between mdNLR and lung cancer risk was observed in those with occupational asbestos exposure. Asbestos dysregulates the immune response both alone (45,46) and in combination with cigarette smoke (47,48). In our study, cases and controls were matched on asbestos and smoking exposure prior to lung cancer diagnosis; even after controlling for these well-recognized risk factors for lung cancer, we still observed that mdNLR was associated with lung cancer risk. We note that all asbestos exposed workers included in our study were male, and that NSCLC associations for male (OR, 95% CI: 1.15, 0.88–1.51) and female (OR, 95% CI: 1.22, 0.75–1.98) participants without asbestos exposure were similar. Therefore, we attribute the strengthened association among men to the subset of men with occupational asbestos exposure. We postulate that elevated pre-diagnosis mdNLR in asbestos-exposed lung cancer cases may be indicative of an altered inflammatory profile induced by asbestos exposure that is permissive to the development of lung cancer.

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Previous work has established the relationship between systemic inflammation and cancer, which includes tumor development, angiogenesis, and inhibition of apoptosis (49). Thus it is plausible that the high inflammatory profile reflected by elevated NLR (i.e., high neutrophil counts and/or low lymphocyte counts) may be more favorable for cancer development, tumor growth, tumor initiation, or early tumor progression. Specifically, lymphocytes act as tumor defense by inducing cytotoxic cell death and inhibiting proliferation and migration (49), while neutrophils have been shown to inhibit the activity of lymphocytes such as T-cells (4). Our findings parallel research in circulating immune markers and lung cancer risk (43,46) that have helped to establish roles for inflammation in the initiation and promotion of lung cancer (50).

Research on the possible relationship between elevated NLR or mdNLR and cancer risk is limited, and the only other lung cancer study reported a null association between NLR and lung cancer risk (15). The Sanchez-Salcedo study (15) included 135 participants from an LCDT lung cancer screening cohort, 32 of whom developed lung cancer during 4 to 10 years of follow-up, with the majority (72%) of cases diagnosed at early stage (I or II). This is in contrast to our study, where the majority (73%) of participants were diagnosed with late stage (III or IV) lung cancers and were diagnosed prior to use of lung cancer screening. In addition, the enrollment criteria for the Sanchez-Salcedo study (15) was relaxed in comparison to the U.S. Preventive Task Force guidelines for lung cancer screening eligibility (25), as participants were enrolled and screened as young as 40 years with a minimum smoking history of 10 pack years. Therefore, participants screened in the Sanchez-Salcedo study would not have been eligible for lung cancer screening in the U.S. We note that the majority (76%) of CARET case-control pairs included in this study meet the eligibility criteria for screening in the U.S., but were not screened since enrollment occurred long before lung cancer screening was implemented in the U.S. (44). Regarding other cancer types, a study of NLR and breast cancer risk observed that elevated NLR was associated with increased risk (14), which was confirmed in a study of mdNLR (16). In that study, elevated mdNLR was also observed to be associated with risk of head and neck squamous cell carcinoma and ovarian cancer, but there was no association with bladder cancer (16).

Despite our careful control of confounders in our study, this work is not without limitations. Our study sample consisted solely of heavy smokers who elected to participate in the CARET clinical trial, and therefore our results may have limited generalizability. Since heavy smokers are at the highest risk for lung cancer, and because the majority of smokers will not develop lung cancer during their lifetimes (24), this is an ideal population in which to investigate features that can differentiate between smokers who go on to develop lung cancer from those who will not. Based on our results, it is possible that mdNLR may be able to differentiate lung cancer risk in individuals at high enough risk to be eligible for screening. Additional work is needed to clarify whether mdNLR in screening-eligible populations can be leveraged to identify lung cancer at earlier stages among those who are screened. Small sample sizes for subgroups may have limited our statistical power to detect associations. We did not have cytological NLR measurements for the participants in our study. Nevertheless, previous work has validated that mdNLR is highly correlated with cytological NLR (R^2=0.99) (16), and our estimated mdNLR aligns with the mean cytological NLR of 2.15 reported for a nationally representative sample of over 9,000

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individuals from the National Health and Nutrition Examination Survey (NHANES) (42). Finally, our results have not yet been replicated in an external validation data set.

Our results demonstrate the utility of DNA methylation profiling for NLR estimation (e.g., mdNLR) for prospective analyses using archival samples. NLR determined from complete blood count measures assessed for standard clinical care are inexpensive and reproducible (6), and mdNLR is a validated proxy for NLR in archival blood samples (16). Thus, there is broad potential for mdNLR approaches to be used to improve biomarker discovery for risk prediction in other prospective studies of cancer.

We posit that NLR may convey information about both lung cancer risk and etiology among heavy smokers, especially those with a history of asbestos exposure. A deeper understanding of the mechanisms and pathways reflected by elevated NLR in lung cancer development will inform whether this potential marker has clinical utility in prevention and/or early detection, especially in high-risk populations who are actively undergoing (or have undergone) screening for lung cancer.

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Abbreviations:

BMI	body mass index
BMIQ	β-mixture quantile
CARET	β-Carotene and Retinol Efficacy Trial
CI	confidence interval
CpG	cytosine-phosphate-guanine
mdNLR	methylation-derived neutrophil-to-lymphocyte ratio
NLR	neutrophil to lymphocyte ratio
NOS	not otherwise specified
NSCLC	non-small cell lung cancer
OR	odds ratio
RMSE	root mean squared error
SCLC	small cell lung cancer

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Table 1.

Participant characteristics

	Controls (n=319)	Cases		
		All[a] lung cancers (n=319)	Non-small cell lung cancer[b] (n=240)	Small cell lung cancer (n=68)
Matching variables				
Age at blood draw, mean years (SD)	63.5 (5.7)	64.5 (5.6)	64.5 (5.5)	64.5 (5.8)
45–54, n (%)	21 (7)	21 (7)	16 (7)	4 (6)
55–59, n (%)	77 (24)	42 (13)	31 (13)	9 (13)
60–64, n (%)	86 (27)	101 (32)	77 (32)	23 (34)
65–74, n (%)	135 (42)	155 (49)	116 (48)	32 (47)
Sex, n (%) female	109 (34)	109 (34)	78 (33)	28 (41)
Enrollment year, n (%)				
1985–1986	13 (4)	15 (5)	13 (5)	2 (3)
1987–1988	13 (4)	14 (4)	9 (4)	4 (6)
1989–1990	79 (25)	78 (24)	57 (24)	16 (24)
1991–1992	146 (46)	144 (45)	107 (45)	33 (49)
1993–1994	68 (21)	68 (21)	54 (22)	13 (19)
Asbestos exposure, n (%)	53 (17)	52 (16)	42 (18)	9 (13)
Current smoker at blood draw, n (%)	205 (64)	206 (65)	155 (65)	41 (60)
Other characteristics at blood draw				
Stage III/IV[c], n (%)	-	197 (73)	143 (67)	53 (95)
Cigarettes per day, lifetime mean (SD)	14.0 (13.3)	15.5 (15.0)	15.0 (14.7)	15.7 (15.7)
Pack-years, mean (SD)	53.8 (23.7)	59.3 (22.5)	58.5 (22.2)	60.1 (22.9)
Years since quit smoking, mean (SD)	2.4 (5.0)	2.3 (4.3)	2.4 (4.3)	2.5 (4.3)
BMI (kg/m^2), mean (SD)	28.1 (5.6)	27.7 (4.9)	27.6 (4.8)	28.1 (5.4)
<18.5 (%)	4 (1)	2 (1)	2 (1)	--
18.5–24.9 (%)	85 (27)	92 (29)	69 (29)	19 (28)
25.0–29.9 (%)	130 (41)	136 (43)	101 (42)	30 (44)
30.0 (%)	100 (31)	89 (28)	68 (28)	19 (28)
Intervention arm, n (%) assigned to active	168 (53)	166 (52)	123 (51)	39 (57)
mdNLR at blood draw				
Mean (SD)	1.86 (0.90)	2.06 (1.31)	2.07 (1.31)	1.88 (1.21)

Abbreviations: SD = Standard Deviation; BMI = Body Mass Index.

[a] “All lung cancers” includes adenocarcinoma, squamous cell carcinoma, and small cell lung cancer as well as cases for whom histotype was not otherwise specified non-small cell lung cancer (NOS; n=9) or missing (n=11).

[b] The “Non-small cell lung cancer” category includes adenocarcinoma, squamous cell carcinoma, and not otherwise specified non-small cell lung cancer (NOS; n=9).

[c] Stage information was not available for 50 lung cancer cases (28 NSCLC, 12 SCLC, and 10 missing histotype).

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Table 2.

Continuous methylation-derived neutrophil-to-lymphocyte ratio and lung cancer risk overall and by sex, asbestos exposure, smoking status, intervention arm, and median time at risk.

	All[a] lung cancers		Non-small cell lung cancer		Small cell lung cancer	
	OR[b]	(95% CI)	OR[b]	(95% CI)	OR[b]	(95% CI)
Continuous mdNLR	**1.21**	**(1.01, 1.44)**	**1.30**	**(1.03, 1.63)**	1.06	(0.77, 1.47)
Sex						
Male	**1.30**	**(1.04, 1.63)**	**1.34**	**(1.02, 1.75)**	1.24	(0.79, 1.93)
Female	1.05	(0.76, 1.45)	1.22	(0.75, 1.98)	0.94	(0.53, 1.66)
Asbestos exposure						
Yes	**2.13**	**(1.12, 4.05)**	**3.39**	**(1.32, 8.67)**	1.27	(0.54, 2.95)
No	1.09	(0.90, 1.32)	1.15	(0.92, 1.45)	0.96	(0.61, 1.52)
Smoking status						
Never/former	1.26	(0.98, 1.63)	1.24	(0.92, 1.68)	1.52	(0.80, 2.90)
Current	1.16	(0.91, 1.47)	1.40	(0.98, 2.00)	0.76	(0.44, 1.34)
Stage						
Early (I/II)	1.30	(0.79, 2.13)	1.29	(0.79, 2.12)	--	--
Late (III/IV)	**1.35**	**(1.08, 1.70)**	**1.44**	**(1.08, 1.92)**	1.19	(0.81, 1.74)
Intervention arm						
Placebo	1.11	(0.78, 1.59)	1.32	(0.86, 2.02)	0.70	(0.26, 1.87)
Active	**1.70**	**(1.12, 2.58)**	1.53	(0.89, 2.62)	2.47	(0.79, 7.73)
Time at risk (median in cases = 4.4 years)						
<4.4 years	1.25	(0.99, 1.58)	1.34	(0.99, 1.80)	1.20	(0.81, 1.76)
4.4 years	1.18	(0.89, 1.57)	1.27	(0.88, 1.84)	0.80	(0.37, 1.71)

Abbreviations: OR = odds ratio; CI = confidence interval; mdNLR = methylation-derived neutrophil-to-lymphocyte ratio; BMI = body mass index.

[a] "All lung cancers" includes non-small cell lung cancer pairs (N=240) and small cell lung cancer pairs (N=68), as well as pairs for whom case histotype was missing (N=11).

[b] Conditional logistic regression models adjusted for age, pack years, cigarettes per day, and BMI at blood draw.

Bold OR and CI indicate statistical significance.