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Circulating markers of cellular immune activation in pre-diagnostic human serum in relation to lung cancer risk in the Lung Cancer Cohort Consortium (LC3)

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Abstract

Cell-mediated immunity may play an important role in lung carcinogenesis. We investigated the associations for circulating levels of tryptophan, kynurenine, kynurenine:tryptophan ratio (KTR), kynurenine metabolite—quinolinic acid (QA), and neopterin as markers of interferon-gamma-induced cellular immune activation with lung cancer risk in 5,364 cases and 5,364 individually matched control subjects from 20 prospective cohorts included the international Lung Cancer Cohort Consortium (LC3). Tryptophan, kynurenine, QA, and neopterin were quantified by mass spectrometry-based methods in serum/plasma samples collected on average 6 years before lung cancer diagnosis. Odds ratios (ORs) and 95% confidence intervals (CIs) for lung cancer associated with different levels of these metabolites and KTR were calculated using conditional logistic regression with adjustment for matched smoking variables and circulating cotinine. Overall, the highest quintiles of circulating kynurenine, KTR, QA and neopterin were associated with a 20–30% higher risk of lung cancer, and tryptophan with a 15% lower risk compared with the lowest quintile (all P_{trend} <0.05). The strongest associations were seen for current smokers, where the adjusted ORs (95% CIs) of lung cancer for the highest quintile of KTR, QA and neopterin were 1.42 (1.15–1.75), 1.42 (1.14–1.76) and 1.45 (1.13–1.86), respectively, compared with the lowest quintile. The associations with KTR and QA were strongest for lung squamous cell carcinoma followed by adenocarcinoma and for lung cancer diagnosed within the first 2 years after blood draw. Strongest associations among current smokers suggest a key role for cell-mediated immunity in smoking-related lung carcinogenesis.

Keywords

lung cancer; kynurenine; tryptophan; neopterin; quinolinic acid

INTRODUCTION

Lung cancer is one of the most common cancers accounting for 2.09 million incident cases and 1.76 million deaths worldwide in 2018 [1]. The 5-year survival rate for lung cancer cases

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is only 17.7% in the United States (US) [2], and is even worse globally [3]. This underscores the importance of improving prevention and treatment to reduce lung cancer morbidity and mortality. Whilst the role of the immune system in the development of lung cancer has been increasingly recognized, the mechanisms by which immune mediators influence risk are only partially understood [4, 5].

Previous epidemiological studies that focused on the associations between circulating cytokines and risk of lung cancer have provided inconsistent results. For example, interleukin-6 and interleukin-8 were associated with increased risk of lung cancer in two prospective studies in the US [6] and Europe [7], but these same markers were not associated with lung cancer risk in a second US cohort [8] that evaluated the associations between 77 inflammatory markers and lung cancer risk, perhaps due to low statistical power. Furthermore, these previous studies were not well powered to study risk in important sub-groups, such as never smokers. In addition, concentrations of cytokines are generally low in the circulation of healthy individuals who have no active infection or malignancy [9]. Thus investigations of alternative biomarkers associated with inflammation, immune response, and lung cancer risk is warranted.

Interferon gamma (IFN-gamma) is a cytokine produced predominantly by cells involved in cell-mediated immunity such as natural killer cells, natural killer T cells, and CD4 and CD8 cytotoxic T cells [10]. Animal studies have shown that IFN-gamma-induced cellular immunity is important for the inhibition of lung cancer development [11, 12]. However, the association between IFN-gamma and lung cancer risk in human epidemiology is understudied. The half-life of IFN-gamma in the circulation is short [13] and it is therefore not readily measurable in large-scale epidemiological studies. IFN-gamma activates indoleamine 2,3-dioxygenase (IDO), and upregulates metabolism through the kynurenine pathway of tryptophan metabolism [14]. As such, the kynurenine:tryptophan ratio (KTR) can be used as a surrogate of IDO activity and IFN-gamma-mediated cellular immune activation [15]. IFN-gamma also stimulates the production of neopterin, a metabolite of guanosine triphosphate, by macrophages [16]. One previous epidemiological study observed an association between KTR and higher risk of lung cancer [17]. A second prospective study showed associations between KTR or neopterin and risk of cancer overall, but no association was observed for risk of lung cancer specifically, possibly due to lack of statistical power [18]. Thus replication studies are needed to confirm these earlier findings. In addition, the downstream metabolites of kynurenine pathway (Figure 1) may also play a role in the initiation and progression of lung cancer. For example, quinolinic acid (QA), a downstream metabolite of kynurenine, has immuno-regulatory effects [19, 20] and may be important in lung cancer risk, but has not been studied in large epidemiological studies.

The purpose of the current study conducted among 20 prospective cohorts from Asia, Australia, Europe and the US is to comprehensively investigate the associations between circulating concentrations of KTR, QA and neopterin as markers of IFN-gamma-induced cellular immune activation and lung cancer risk. Our large sample size (5364 case-control pairs) allows us to further investigate these associations by smoking status, histology, and time from blood draw to diagnosis.

MATERIALS AND METHODS

Study population

The design of the Lung Cancer Cohort Consortium (LC3) including cohort design and follow-up procedures has been reported previously [21]. The current investigation included case-control studies of incident lung cancer cases and individually matched controls nested within 20 prospective cohorts from the US, Europe, Australia, and Asia. At recruitment into each cohort, participants signed informed consent forms, completed questionnaires, had blood sample drawn and anthropometric measurements taken. The LC3 was approved by the Institutional Review Board of each contributing cohort.

Selection of cases and controls

Lung cancer cases were defined on the basis of the International Classification of Diseases for Oncology, Second Edition (ICD-O-2), and included all invasive cancers coded as C34.0 to C34.9. Altogether, 11,399 incident lung cancer cases with pre-diagnostic serum or plasma samples among members of the US National Cancer Institute Cohort Consortium in 2009 were eligible for participation. From this, the LC3 selected a total of 5,545 lung cancer cases, and to optimize the statistical power in smoking stratified analyses, never and former smoking cases were oversampled. For each case, one control was randomly selected within the same cohort among all eligible participants who were alive and free of cancer (except non-melanoma skin cancer) at the same length of time from enrollment as was the index case at diagnosis. Matching criteria were race (US only), sex, date of blood collection (± 1 month, relaxed to ± 3 months for sets without available controls), and date of birth (± 1 year, relaxed to ± 3 years), as well as smoking status in 5 categories (never smokers, short and long-term quitters among former smokers with <10 years or ≥10 years since quitting, and light and heavy current smokers (<15 or ≥15 cigarettes per day). After excluding cases who were not able to be correctly matched on smoking status in 5 categories defined above (n=126 cases), had insufficient serum/plasma samples (n=42), or had a revised date of diagnosis prior to blood draw (n=13), a total of 5364 lung cancer case-control pairs remained eligible for the current analysis.

Biochemical analyses

Serum or plasma samples from all LC3 study participants were sent on dry ice to the Bevital A/S laboratory (http://www.bevital.no) in Bergen, Norway, and were kept at −80°C until later analysis. Concentrations of tryptophan, kynurenine [22], quinolinic acid (QA), neopterin and cotinine [23] – a biomarker of recent tobacco exposure were determined by mass spectrometry based methods (LC-MS/MS, GC-MS/MS). Biochemical analysis was performed in 96-well plates, each containing 86 study samples, 6 calibration samples, 3 quality control samples, and 1 blank sample. Samples of the index case and the matched control subjects were put next to each other in a random order and always analyzed together in the same batch. The laboratory personnel were blind to the case/control status of the test samples.

Statistical Analysis

The KTR ratio was calculated as kynurenine concentration divided by tryptophan multiplied by 1000. We logarithmically transformed (base e) original values of all biomarker concentrations and KTR to normalize their skewed distributions. The pair-wise correlations between biomarkers were assessed using Spearman correlation coefficients. The relationships between circulating concentrations of biomarkers and socio-demographic, lifestyle and clinical factors, including age at blood draw, body mass index (BMI), estimated glomerular filtration rate (eGFR; a measurement of kidney function that influences the circulating levels of kynurenine and its metabolites), and geographical region were evaluated using Analysis of Covariance (ANCOVA). The eGFR was calculated based on participant's age, gender, and creatinine concentration in plasma or serum according to the previously published method [24].

Study participants were divided into quintiles based on the distributions of biomarker concentrations among controls within a specific cohort. Odds ratios (ORs) of lung cancer for quintiles of biomarker concentrations were calculated relative to the first quintile using conditional logistic regression [25]. Ordinal values (e.g., 1, 2, 3, 4, and 5) for individual biomarkers were used for testing linear trends across quintiles in the biomarker-lung cancer risk associations.

In addition to matching on cohort, race (US only), sex, date of blood draw, date of birth, and the combination of smoking status with years of quitting and number (for former smokers) and number of cigarettes per day (for current smokers), the multivariable conditional logistic regression models included the following reported risk factors for lung cancer and determinants of kynurenine metabolites as potential confounders: cotinine concentration (continuous, a biomarker of recent nicotine intake) [26], educational attainment (six categories), body mass index (BMI) in kg/m^2 (<18.5, 18.5– <25, 25– <30, ≥30), and eGFR.

Fully adjusted regression models were used in analyses stratified by smoking status (current, former, never smokers), histological subtypes of lung cancer (adenocarcinoma, large-cell carcinoma, small-cell carcinoma, and squamous cell carcinoma), time between blood draw and lung cancer diagnosis (<2, 2–<5, and ≥5 years), and geographical region (US, Europe/Australia, Asia). Potential effect modification of associations between biomarkers and lung cancer risk by demographic, lifestyle, or other factors were examined by including their product term in the multivariate regression models.

Statistical analyses were carried out using SAS software version 9.3 (SAS Institute, Cary, NC). All *P* values reported are two-sided, and those that were <0.05 were considered to be statistically significant.

RESULTS

Baseline characteristics of cases and controls

The current study sample included 5,364 incident lung cancer cases and 5,364 individually matched controls (Table 1). Overall, slightly more participants were male (54.2%). Participants from Europe/Australia (EU/AU) and Asia were also predominantly male (57.9%

and 69.2%, respectively) whereas participants from the US were predominantly female (58.7%). Current smokers accounted for nearly half the overall study participants (47%, 2,519 case-control pairs), with former (28.3%, 1,518 case-control pairs) and never smokers (24.7%, 1,327 case-control pairs) contributing approximately one-quarter each. Cases and controls had, on average, similar characteristics including BMI and age at recruitment (60 years).

Median age at lung cancer diagnosis was 69.8 (range 53.6 – 82.0) with little variation across geographic regions. The median time between blood draw and lung cancer diagnosis was 5.2 years for the US, 5.8 years for Asian, and 10 years for cohorts in Australia and Europe. Histologically, the majority of lung cancer cases were adenocarcinoma, followed by squamous cell, small cell and large cell carcinoma. Due to a larger overall sample size, the US cohorts contributed the majority of all adenocarcinoma cases (50.3%), small cell carcinoma cases (49.8%), and large cell carcinoma cases (64.4%). The proportion of squamous cell cases did not differ substantially by region, with each region contributing approximately one-third of cases.

Biomarker distribution in study population

The geometric means of biomarkers were significantly different among groups by smoking status in control subjects. The mean concentration of tryptophan was highest for current smokers and lowest for never smokers whereas the levels of kynurenine, KTR, QA, and neopterin were the highest for former smokers (Supplementary Table 1). Concentrations [median (20^{th} −80^{th} percentile)] of circulating biomarkers did not differ substantially across cohorts within geographic region, with few exceptions. For US cohorts, circulating tryptophan concentrations were 20 μmol/L higher in the American Cancer Society Cancer Prevention Study-II (CPS-II) Nutrition cohort compared to the Women's Health Initiative (WHI) cohort (Supplementary Table 2). In addition, circulating neopterin concentrations were different among different cohorts within a region; the highest levels were observed in the Multiethnic Cohort (MEC) among the US cohorts and in the Singapore Chinese Health Study among Asian cohorts. Overall kynurenine, KTR, QA, and neopterin concentrations were positively correlated with each other (Spearman correlation coefficient [r] = 0.34–0.66) whereas tryptophan was positively correlated with kynurenine (r = 0.45) and QA (r = 0.13), but inversely correlated with KTR (r = −0.43) and was not correlated with neopterin (r = −0.01) (Supplementary Table 3).

Overall and stratified associations of circulating biomarkers and lung cancer risk

The highest quintiles of circulating kynurenine, KTR, QA, and neopterin were associated with a 22–31% higher risk of lung cancer as compared with the lowest quintiles after controlling for smoking status, duration and intensity, circulating levels of cotinine, and other potential confounders (Table 2). In contrast, the highest quintile of tryptophan was associated with a 15% lower risk of lung cancer compared with the lowest quintile (Table 2).

Table 3 shows the odds ratios for lung cancer associated with higher quintiles of biomarkers in current, former, and never smokers separately. Among current smokers, ORs (95% CIs) for lung cancer in the highest quintiles of KTR, QA and neopterin were 1.42 (1.15–1.75),

1.42 (1.14–1.75), and 1.45 (1.13–1.86), respectively (all $P_{trend} \leq 0.005$). The corresponding ORs (95% CIs) in former smokers were 1.32 (1.00–1.74), 1.20 (0.90–1.59), and 1.43 (0.97–1.86) (all P_{trend} were borderline significant). There was no association between these biomarkers and lung cancer risk among never smokers (all $P_{trend} > 0.16$). However, no interaction between any biomarker and smoking status for lung cancer risk was detected all *P's* for multiplicative interaction > 0.05) (data not shown).

When data were analyzed by histological subtype of lung cancer, associations for KTR and QA with risk of lung squamous cell carcinoma, and for QA with adenocarcinoma were seen (Table 4). The associations for other biomarkers with risk of adenocarcinoma or squamous cell carcinoma, and for all biomarkers with large cell and small cell carcinomas did not reach statistical significance.

In the sensitivity analysis, the associations for circulating levels of kynurenine, KTR and QA were observed for the risk of lung cancer diagnosed within 2 years after blood draw (Table 5). Higher levels of neopterin were associated with higher risk of lung cancer diagnosed within 2 to <5 years after blood draw. The association between QA and lung cancer risk remained, albeit weakened, even 5 or more years after blood collection.

DISCUSSION

Principal findings

The current study is the largest prospective epidemiological study to investigate the novel associations of KTR and neopterin, proinflammatory immune biomarkers with risk of lung cancer. The study demonstrated significant associations between increasing levels of these cellular immunity biomarkers and higher risk of lung cancer in current smokers, and to a lesser extent, in former smokers. These positive associations were strongest for lung squamous cell cancer and for lung cancer cases diagnosed within the first two years after blood draw.

Higher circulating KTR concentrations and risk of lung cancer

The observed associations between KTR and lung cancer risk in the current study have plausible biological explanations. Tryptophan is an essential amino acid for immune cell proliferation, and depletion of tryptophan results in T cell apoptosis [27]. IDO catalyzes the initial step of the kynurenine pathway, the conversion of tryptophan to formylkyurenine, which is rapidly converted to kynurenine. Thus when IDO is high, the KTR would be expected to be higher. IDO can be induced by a series of inflammatory cytokines, especially IFN-gamma. In addition, IDO is over-expressed in several cancers, including lung cancer [28]. Clinical studies conducted in lung cancer patients showed that mRNA expression of IDO was higher in lung cancer tissues than adjacent non-malignant lung tissues [29]. IDO-expressing dendritic cells were found in tumor tissues and tumor-draining lymph nodes in patients with lung cancers [30]. Serum KTR was higher in lung cancer patients than in healthy controls [31]. The stronger associations between kynurenine or KTR and risk of lung cancer in individuals within <2 years of blood draw may reflect potential reverse causation. A prior epidemiological study in the European Prospective Investigation into Cancer and Nutrition

(EPIC)[17] reported associations for lower circulating levels of tryptophan and higher KTR with higher risk of lung cancer, similar to those found in the present study which included a much larger sample size and diverse populations.

IDO in tumor cells and infiltrating inflammatory cells, such as dendritic cells and regulatory B cells, serves as an immunosuppressive enzyme that limits T cell responses against tumors via depletion of tryptophan and by producing immuno-regulatory kynurenine metabolites [30, 32]. Inhibition of IDO by 1-methyltryptophan significantly delayed the tumor outgrowth in a mouse model of Lewis Lung carcinoma [28]. Kynurenine can promote the proliferation of regulatory T cells, which suppress the antitumor immune response, contributing to cancer immune escape [33]. Another possible mechanism linking the kynurenine pathway and lung cancer development is through the interaction between kynurenine metabolites and aryl hydrocarbon receptor, a protein that regulates the metabolic pathways of exogenous chemicals. Kynurenine is a ligand for aryl hydrocarbon receptor, which activates the carcinogenesis pathway of polycyclic aromatic hydrocarbons, in particular benzo[a]pyrene, a strong lung carcinogen derived from tobacco smoke [34, 35]. Lung squamous cell carcinoma is more strongly associated with polycyclic aromatic hydrocarbons present in tobacco smoke [36] whereas adenocarcinoma is more strongly associated with tobacco-specific nitrosamines such as 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) in animal models [37]. This may explain why our observed associations between KTR and lung cancer risk were confined to current smokers, and stronger for squamous cell carcinoma than adenocarcinoma of the lung.

Higher circulating quinolinic acid concentrations and risk of lung cancer

The current study is the first to evaluate the association between QA and lung cancer risk. We found that a higher concentration of QA in pre-diagnostic blood samples was associated with higher risk of lung cancer. QA, a downstream metabolite of kynurenine, has long been known as a neurotoxin via stimulation of the presynaptic receptor which induces oxidative stress, and enhances the production of pro-inflammatory cytokines in the brain [38]. Increased levels of QA were found in cerebrospinal fluid of patients with neuro-degenerative disorders, such as Huntington’s disease, Alzheimer’s disease, and AIDS dementia complex [20]. In gerbils, QA level increased following acute systemic immune stimulation via upregulation of enzymes in the kynurenine pathway [39]. In the current study, circulating QA concentrations were moderately correlated with the inflammatory markers KTR (r=0.57) and neopterin (r=0.40), which is consistent with the fact that QA concentrations are correlated with IDO expression [20]. Previous studies showed that during inflammation, QA synthesis occurs mainly in immune cells [20]. Given that QA is a precursor of nicotinamide adenine dinucleotide, a coenzyme for redox reactions, accumulation of QA within immune cells could provide substrates for nicotinamide adenine dinucleotide synthesis to meet the enhanced requirements during an immune response [20]. Taken together, the observed association between QA and increased risk of lung cancer could reflect immune response against cancer prior to its clinical presentation. In addition, recent evidence showed that QA can inhibit the proliferation of cancer-killing T and natural killer cells [40]. Therefore, the higher concentrations of QA may promote tumor growth via its role in immune suppression. The association for QA with risk of lung cancer within <2 years of blood draw is stronger

than those with longer time intervals, which suggests that this marker may be related to the progression of lung cancer and could be developed as biomarker for early detection of lung cancer.

Strengths and limitations

The strengths of the study include a prospective design using pre-diagnostic plasma/serum samples, and a large sample size with sufficient power to conduct analysis stratified by smoking status, and histology of lung cancer. We measured circulating metabolites of the kynurenine pathway, including the novel use of QA as an inflammatory marker. In addition to matching on smoking status, intensity and duration in the study design, the analysis for the association between biomarkers of cellular immune activation and lung cancer risk was adjusted for circulating cotinine, a biomarker of recent tobacco exposure [41], and eGFR, a renal function measurement that is a strong determinant for circulating concentrations of kynurenine and its metabolites [42]. The present study measured novel biomarkers for cellular immune activation, such as KTR and neopterin with a high intra-individual correlation in our previously published work on the development of the methodology, in which the intraclass correlation coefficients (ICCs) for KTR and neopterin in 4 different sampling visits over 3.5 years were 0.74 and 0.67, respectively [43], indicating that a single time point measurement is a relatively reliable biomarker for long-term exposure, and these biomarkers may be better than traditional cytokine biomarkers such as IFN-gamma and interleukins with lower ICCs [44]. As any observational study, the observed association between serum biomarkers and lung cancer risk could be confounded by other factors such as smoking. For example, smoking is an established risk factor for lung cancer. Smokers in the present study had significantly higher concentration of tryptophan and lower levels of KTR and QA, as well as neopterin. Furthermore, lung cancer risk was only significantly inversely associated with tryptophan and positively associated with KTR and QA, as well as neopterin, among current smokers. Although smoking status, density and duration were matched for cases and controls in the present study and circulating cotinine was additionally adjusted for in the statistical analysis, the residual confounding of smoking on the observed biomarker-lung cancer risk associations cannot completely be ruled out.

CONCLUSION

The current study demonstrates that markers of cellular immunity, KTR and QA, as well as neopterin, were associated with increased risk of lung cancer for current smokers. Findings from experimental studies also support a role for inflammation and immunity in the development of lung cancer. Taken together, our results suggest that biomarkers for early detection of smoking related lung cancer could be found in the kynurenine pathway. Our novel observation of a positive association between QA and lung cancer risk warrants mechanistic studies on the role of QA in lung carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ANCOVA Analysis of Covariance

BMI	body mass index
CI	confidence interval
eGFR	estimated glomerular filtration rate
IDO	Indoleamine 2,3-dioxygenase
IFN-gamma	interferon-gamma
KTR	kynurenine to tryptophan ratio
LC3	Lung Cancer Cohort Consortium
OR	odds ratio
QA	quinolinic acid

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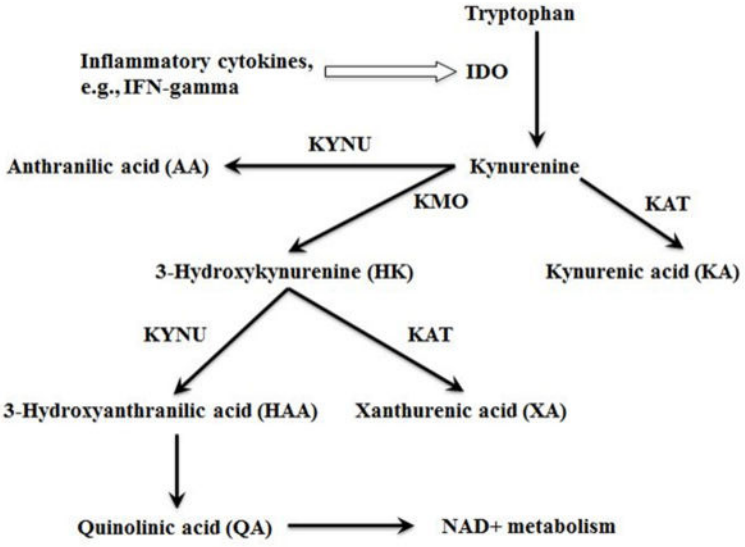

Figure 1:
The kynurenine pathway of tryptophan metabolism

Table 1.

Baseline and clinical characteristics of study participants overall and by continent, the Lung Cancer Cohort Consortium (LC3) Study

Baseline and Clinical Characteristics	US cohorts No.(%) of participants in group		EU/AU cohorts No.(%) of participants in group		Asian cohorts No.(%) of participants in group		Overall No.(%) of participants in group	
	Cases (n=2400)	Matched controls (n=2400)	Cases (n=1189)	Matched controls (n=1189)	Cases (n=1775)	Matched controls (n=1775)	Cases (n=5364)	Matched controls (n=5364)
Sex								
Men	991 (41.3%)	991 (41.3%)	688 (57.9%)	688 (57.9%)	1229 (69.2%)	1229 (69.2%)	2908 (54.2%)	2908 (54.2%)
Women	1409 (58.7%)	1409 (58.7%)	501 (42.1%)	501 (42.1%)	546 (30.8%)	546 (30.8%)	2456 (45.8%)	2456 (45.8%)
Smoking status								
Never	569 (23.7%)	569 (23.7%)	156 (13.1%)	156 (13.1%)	602 (33.9%)	602 (33.9%)	1327 (24.7%)	1327 (24.7%)
Former	1007 (42%)	1007 (42%)	335 (28.2%)	335 (28.2%)	176 (9.9%)	176 (9.9%)	1518 (28.3%)	1518 (28.3%)
Current	824 (34.3%)	824 (34.3%)	698 (58.7%)	698 (58.7%)	997 (56.2%)	997 (56.2%)	2519 (47%)	2519 (47%)
Education								
less than high school	237 (9.9%)	215 (9%)	662(55.6%)	598(50.2%)	898 (50.6%)	883 (49.7%)	1797 (33.5%)	1696 (31.6%)
completed high school	357 (14.9%)	374 (15.6%)	159 (13.4%)	180 (15.2%)	243 (13.7%)	230 (13%)	759 (14.1%)	784 (14.6%)
vocational school	422 (17.6%)	435 (18.1%)	180 (15.2%)	200 (16.8%)	289 (16.3%)	279 (15.7%)	891 (16.6%)	914 (17%)
some college	402 (16.8%)	393 (16.4%)	107 (9%)	129 (10.9%)	171 (9.6%)	196 (11%)	680 (12.7%)	718 (13.4%)
college graduate	357 (14.9%)	319 (13.3%)	63 (5.3%)	64 (5.4%)	104 (5.9%)	113 (6.4%)	524 (9.8%)	496 (9.2%)
graduate studies	574 (23.9%)	637 (26.5%)	10 (0.8%)	8 (0.7%)	62 (3.5%)	65 (3.7%)	646 (12%)	710 (13.2%)
Unknown	51 (2.1%)	27 (1.1%)	8 (0.7%)	10 (0.8%)	8 (0.5%)	9 (0.5%)	67 (1.2%)	46 (0.9%)
Body Mass index[a]								
<18.5	30 (1.3%)	31 (1.3%)	14 (1.2%)	10(0.8%)	157 (8.8%)	113 (6.4%)	201 (3.7%)	154 (2.9%)
18.5– <25	1088 (45.3%)	1020 (42.5%)	521 (43.8%)	435 (36.6%)	1203 (67.8%)	1192 (67.2%)	2812 (52.4%)	2647 (49.3%)
25– <30	841 (35%)	858 (35.8%)	468 (39.4%)	536 (45.1%)	369 (20.8%)	424 (23.9%)	1678 (31.3%)	1818 (33.9%)
≥30	378 (15.8%)	430 (17.9%)	185 (15.6%)	206(17.4%)	46 (2.6%)	46 (2.6%)	609 (11.4%)	682 (12.7%)
Unknown	63 (2.6%)	61 (2.5%)	1 (0.1%)	2 (0.2%)	0 (0%)	0 (0%)	64 (1.2%)	63 (1.2%)

Continuous variables, median (5th-95th percentile)

Baseline and Clinical Characteristics	US cohorts No.(%) of participants in group		EU/AU cohorts No.(%) of participants in group		Asian cohorts No.(%) of participants in group		Overall No.(%) of participants in group	
	Cases (n=2400)	Matched controls (n=2400)	Cases (n=1189)	Matched controls (n=1189)	Cases (n=1775)	Matched controls (n=1775)	Cases (n=5364)	Matched controls (n=5364)
Age at recruitment (years)	60 (42–74)	60 (42–74)	60 (45–70)	60 (45–70)	60 (46–72)	60 (46–71	60 (44–72)	60 (44–72)
Circulating concentrations for biomakers								
Tryptophan, μmol/L	63.9 (41.3–89.1)	64.4 (43.7–90.5)	67.8 (48.9–92.7)	68.1 (50.1–91.1)	67.3 (48.6–91.2)	67.5 (49.1–90.1)	66.0 (44.9–90.8)	66.5 (46.2–90.7)
Kynurenine, μmol/L	1.51 (1.00–2.37)	1.53 (1.02–2.34)	1.52 (1.06–2.19)	1.52 (1.07–2.18)	1.49 (1.08–2.18)	1.48 (1.09–2.14)	1.50 (1.04–2.25)	1.51 (1.05–2.22)
Kynurinine:Tryptophan ratio (x 1000)	22.6 (16.6–38.6)	23.6 (16.6–37.0)	22.3 (16.4–33.8)	22.0 (16.5–32.1)	21.9 (15.9–34.1)	21.9 (16.0–32.1)	22.6 (16.2 −36.2)	22.6 (16.4–34.5)
Quinolinic acid, nmol/L	364 (200–789)	363 (207–741)	341 (201–633)	334 (202–605)	350 (207–651)	350 (216–605)	354 (203–708)	353 (208–685)
Neopterin, nmol/L	12.0 (5.74–25.0)	11.8 (5.66–25.5)	10.2 (4.78–20.9)	10.3 (4.38–19.5)	10.6 (5.29–24.0)	10.7 (5.28–24.6)	11.1 (5.31–24.0)	11.0 (5.14–24.6)
Clinical characteristics, case participants only								
Age at diagnosis, median (range), years	70 (55–83)		69 (54–80)		69 (52–80)		69.8 (53.6–82.0)	
Time from blood draw to diagnosis (years)	5.2 (1–15.5)		10.0 (1.5–16.0)		5.8 (0.7–16.5)		6.3 (1.0–16.0)	
Histology, No. (%)								
Large cell carcinoma	112 (4.6%)		46 (4%)		16 (1%)		174 (3.3%)	
Small cell carcinoma	245 (10.4%)		150 (12.5%)		99 (5.5%)		492 (9.2%)	
Squamous cell carcinoma	291 (11.9%)		231 (19.5%)		319 (17.9%)		836 (15.5%)	
Adenocarcinoma	1034 (42.7%)		419 (34.5%)		615 (34.6%)		2056 (38.4%)	
Missing / Unknown	735 (31.4%)		357 (29.5%)		726 (41%)		1806 (33.6%)	

[a]Body mass index is calculated as weight in kilograms divided by height in meters squared

Table 2:

Odds ratios of lung cancer incidence comparing higher quintiles with the lowest quintile of circulating biomarkers The Lung Cancer Cohort Consortium (LC3) Study (n = 5364 cases and 5364 controls)

	Odds Ratio (95% Confidence Interval) by quintiles of biomarker[a]					
Biomarkers	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**P trend**
Tryptophan (μmol/L)	1.00	0.86 (0.76–0.97)	0.87 (0.77–0.98)	0.86 (0.76–0.97)	0.85 (0.75–0.96)	0.019
Kynurenine (μmol/L)	1.00	1.05 (0.92–1.18)	1.02 (0.89–1.16)	1.01 (0.89–1.15)	1.22 (1.06–1.40)	0.033
KTR [b]	1.00	0.97 (0.86–1.10)	0.96 (0.84–1.09)	1.01 (0.89–1.15)	1.31 (1.14–1.50)	<0.001
Quinolinic acid (nmol/L)	1.00	0.95 (0.84–1.08)	0.94 (0.83–1.06)	1.09 (0.96–1.25)	1.31 (1.14–1.51)	<0.001
Neopterin (nmol/L)	1.00	1.12 (0.99–1.27)	1.09 (0.96–1.24)	1.12 (0.98–1.28)	1.31 (1.14–1.51)	0.001

[a] All models were adjusted for educational attainment (categorical), body mass index (kg/m^2) (categorical), serum cotinine concentrations (continuous), estimated glomerular filtration rate (continuous), and cohort.

[b] KTR, kynurenine to tryptophan ratio (x 1000),

Table 3:

Odds ratios of lung cancer incidence comparing higher quintiles with the lowest quintile of circulating biomarkers stratified by smoking status The Lung Cancer Cohort Consortium (LC3) Study (n = 5364 cases and 5364 controls)

Smoking status and biomarker	Odds Ratio (95% Confidence Interval) by quintiles of biomarker[a]					
	Q1	Q2	Q3	Q4	Q5	P trend
Current smokers (2519 cases and 2519 controls)						
Tryptophan (μmol/L)	1.00	0.80 (0.66–0.98)	0.79 (0.65–0.96)	0.81 (0.66–0.98)	0.78 (0.63–0.95)	0.046
Kynurenine (μmol/L)	1.00	0.86 (0.72–1.04)	1.04 (0.86–1.26)	1.05 (0.86–1.28)	1.16 (0.93–1.46)	0.057
KTR [b]	1.00	0.94 (0.79–1.11)	0.95 (0.79–1.13)	1.04 (0.87–1.26)	1.42 (1.15–1.75)	0.005
Quinolinic acid (nmol/L)	1.00	0.97 (0.83–1.14)	1.04 (0.87–1.24)	1.26 (1.04–1.53)	1.42 (1.14–1.76)	<0.001
Neopterin (nmol/L)	1.00	1.10 (0.91–1.34)	1.21 (0.98–1.48)	1.28 (1.02–1.61)	1.45 (1.13–1.86)	0.003
Former smokers (1518 cases and 1518 controls)						
Tryptophan (μmol/L)	1.00	0.73 (0.58–0.92)	0.81 (0.64–1.03)	0.82 (0.65–1.04)	0.74 (0.58–0.94)	0.077
Kynurenine (μmol/L)	1.00	1.12 (0.84–1.49)	1.23 (0.95–1.6)	1.02 (0.78–1.34)	1.08 (0.82–1.41)	0.955
KTR [b]	1.00	1.06 (0.80–1.41)	1.18 (0.89–1.54)	1.13 (0.86–1.49)	1.32 (1.00–1.74)	0.035
Quinolinic acid (nmol/L)	1.00	1.00 (0.74–1.33)	0.82 (0.62–1.08)	1.17 (0.89–1.55)	1.20 (0.90–1.59)	0.037
Neopterin (nmol/L)	1.00	1.14 (0.86–1.50)	1.14 (0.85–1.54)	1.01 (0.74–1.37)	1.34 (0.97–1.86)	0.196
Never smokers (1327 cases and 1327 controls)						
Tryptophan (μmol/L)	1.00	1.00 (0.80–1.26)	1.04 (0.81–1.32)	1.16 (0.88–1.53)	0.87 (0.64–1.18)	0.911
Kynurenine (μmol/L)	1.00	1.06 (0.85–1.33)	1.12 (0.88–1.43)	1.05 (0.8–1.38)	1.17 (0.85–1.59)	0.406
KTR [b]	1.00	1.00 (0.79–1.27)	0.92 (0.72–1.19)	0.92 (0.71–1.19)	1.17 (0.88–1.54)	0.562
Quinolinic acid (nmol/L)	1.00	0.89 (0.69–1.13)	0.70 (0.54–0.9)	0.92 (0.71–1.2)	1.07 (0.79–1.44)	0.707
Neopterin (nmol/L)	1.00	1.09 (0.85–1.40)	1.18 (0.89–1.55)	1.30 (0.97–1.74)	1.19 (0.86–1.63)	0.168

[a] All models were adjusted for educational attainment (categorical), body mass index (kg/m^2) (categorical), serum cotinine concentrations (continuous), estimated glomerular filtration rate (continuous), and cohort.

[b] KTR, kynurenine to tryptophan ratio (x 1000).

Table 4:

Odds ratios of lung cancer incidence by histological subtype comparing higher quintiles with the lowest quintile of circulating biomarkers The Lung Cancer Cohort Consortium (LC3) Study (n = 3558 cases and 3558 controls)[a]

Histological subtype and biomarker	Odds Ratio (95% Confidence Interval) by quintiles of biomarker[b]					
	Q1	Q2	Q3	Q4	Q5	P trend
Large cell carcinoma (174 cases and 174 controls)						
Tryptophan (μmol/L)	1.00	1.88 (0.94–3.74)	1.94 (0.95–3.98)	1.97 (0.90–4.31)	1.96 (0.80–4.81)	0.142
Kynurenine (μmol/L)	1.00	1.62 (0.73–3.57)	1.25 (0.54–2.88)	2.05 (0.96–4.36)	2.28 (0.92–5.68)	0.048
KTR [b]	1.00	0.40 (0.17–0.90)	0.74 (0.34–1.61)	1.02 (0.47–2.18)	0.90 (0.40–2.04)	0.339
Quinolinic acid (nmol/L)	1.00	1.04 (0.49–2.19)	0.65 (0.31–1.37)	1.12 (0.50–2.48)	1.68 (0.68–4.12)	0.344
Neopterin (nmol/L)	1.00	1.33 (0.51–3.49)	1.70 (0.69–4.15)	1.11 (0.41–3.02)	1.97 (0.66–5.87)	0.390
Small cell carcinoma (492 cases and 492 controls)						
Tryptophan (μmol/L)	1.00	0.77 (0.51–1.17)	0.74 (0.48–1.13)	0.57 (0.37–0.88)	0.82 (0.53–1.25)	0.189
Kynurenine (μmol/L)	1.00	0.87 (0.58–1.32)	1.02 (0.67–1.55)	1.01 (0.64–1.59)	0.99 (0.62–1.57)	0.838
KTR [b]	1.00	0.65 (0.43–1.01)	1.08 (0.70–1.65)	0.74 (0.47–1.16)	1.13 (0.71–1.80)	0.447
Quinolinic acid (nmol/L)	1.00	0.83 (0.54–1.28)	0.79 (0.51–1.25)	1.37 (0.88–2.13)	1.32 (0.81–2.14)	0.071
Neopterin (nmol/L)	1.00	1.20 (0.75–1.92)	1.07 (0.64–1.80)	0.89 (0.53–1.50)	1.29 (0.71–2.36)	0.823
Squamous cell carcinoma (836 cases and 836 controls)						
Tryptophan (μmol/L)	1.00	0.67 (0.49–0.93)	0.68 (0.48–0.96)	0.72 (0.51–1.01)	0.76 (0.54–1.06)	0.304
Kynurenine (μmol/L)	1.00	0.71 (0.50–1.00)	1.21 (0.86–1.69)	1.04 (0.73–1.47)	1.22 (0.84–1.77)	0.066
KTR [c]	1.00	1.06 (0.77–1.46)	0.99 (0.71–1.38)	1.01 (0.72–1.41)	1.68 (1.17–2.43)	0.023
Quinolinic acid (nmol/L)	1.00	1.58 (1.15–2.16)	1.38 (0.99–1.93)	1.56 (1.11–2.20)	1.99 (1.35–2.91)	0.003
Neopterin (nmol/L)	1.00	1.61 (1.14–2.26)	1.21 (0.83–1.75)	1.36 (0.92–2.01)	1.34 (0.88–2.04)	0.468
Adenocarcinoma (2056 cases and 2056 controls)						
Tryptophan (μmol/L)	1.00	0.96 (0.79–1.17)	0.98 (0.80–1.20)	1.26 (1.01–1.56)	0.89 (0.70–1.12)	0.764
Kynurenine (μmol/L)	1.00	1.04 (0.85–1.27)	1.14 (0.93–1.39)	1.00 (0.80–1.24)	1.09 (0.86–1.39)	0.615
KTR [b]	1.00	1.02 (0.83–1.24)	1.00 (0.82–1.23)	1.00 (0.81–1.24)	1.12 (0.89–1.40)	0.426
Quinolinic acid (nmol/L)	1.00	0.85 (0.68–1.05)	0.85 (0.68–1.06)	1.01 (0.80–1.27)	1.36 (1.05–1.74)	0.009
Neopterin (nmol/L)	1.00	1.04 (0.84–1.29)	1.13 (0.90–1.43)	1.19 (0.92–1.52)	1.27 (0.97–1.66)	0.059

[a] 1,806 cases were other or unknown histological types of cancer, thus they and their individually matched controls were excluded from this analysis.

[b] All models were adjusted for educational attainment (categorical), body mass index (kg/m^2) (categorical), serum cotinine concentrations (continuous), estimated glomerular filtration rate (continuous), and cohort.

[c] KTR, kynurenine to tryptophan ratio (x 1000).

Table 5:

Odds ratios of lung cancer incidence comparing higher quintiles with the lowest quintile of circulating biomarkers stratified by time from blood draw to cancer diagnosis, The Lung Cancer Cohort Consortium (LC3) Study[a]

Time from blood draw to Cancer diagnosis and biomarkers[a]	Odds Ratio (95% Confidence Interval) by quintiles of biomarker[b]					
	Q1	Q2	Q3	Q4	Q5	P trend
< 2 years (552 cases and 552 controls)						
Tryptophan (μmol/L)	1.00	0.56 (0.37–0.85)	0.65 (0.43–0.99)	0.73 (0.47–1.14)	0.72 (0.45–1.13)	0.542
Kynurenine (μmol/L)	1.00	1.21 (0.78–1.89)	1.22 (0.76–1.96)	1.15 (0.71–1.87)	1.86 (1.13–3.08)	0.032
KTR[c]	1.00	1.14 (0.72–1.80)	0.95 (0.60–1.52)	0.98 (0.61–1.57)	1.92 (1.17–3.14)	0.024
Quinolinic acid (nmol/L)	1.00	1.20 (0.76–1.90)	0.86 (0.53–1.38)	1.62 (1.00–2.61)	2.28 (1.38–3.77)	<0.001
Neopterin (nmol/L)	1.00	1.18 (0.73–1.89)	1.22 (0.74–2.01)	1.44 (0.82–2.51)	1.52 (0.85–2.72)	0.154
2–< 5 years (3093 cases and 3093 controls)						
Tryptophan (μmol/L)	1.00	1.02 (0.75–1.39)	1.03 (0.75–1.41)	0.97 (0.70–1.35)	0.83 (0.59–1.17)	0.279
Kynurenine (μmol/L)	1.00	1.02 (0.72–1.44)	0.97 (0.69–1.36)	0.95 (0.66–1.37)	1.13 (0.76–1.66)	0.689
KTR[c]	1.00	0.98 (0.71–1.35)	0.93 (0.67–1.29)	1.08 (0.78–1.51)	1.29 (0.90–1.84)	0.142
Quinolinic acid (nmol/L)	1.00	0.87 (0.62–1.21)	0.77 (0.55–1.07)	1.10 (0.79–1.54)	1.24 (0.85–1.79)	0.136
Neopterin (nmol/L)	1.00	0.98 (0.70–1.38)	1.05 (0.72–1.54)	1.76 (1.16–2.68)	1.72 (1.10–2.67)	0.003
≥ 5 years (467 cases and 467 controls)						
Tryptophan (μmol/L)	1.00	0.85 (0.70–1.03)	0.84 (0.70–1.02)	0.93 (0.77–1.13)	0.85 (0.69–1.04)	0.413
Kynurenine (μmol/L)	1.00	0.95 (0.79–1.13)	1.13 (0.94–1.35)	1.05 (0.87–1.28)	1.17 (0.94–1.45)	0.092
KTR[c]	1.00	0.99 (0.84–1.17)	1.08 (0.91–1.29)	1.09 (0.91–1.31)	1.21 (0.98–1.48)	0.058
Quinolinic acid (nmol/L)	1.00	0.96 (0.81–1.13)	0.96 (0.8–1.15)	1.21 (1.00–1.45)	1.28 (1.03–1.59)	0.005
Neopterin (nmol/L)	1.00	1.07 (0.89–1.28)	1.09 (0.89–1.33)	1.09 (0.87–1.35)	1.22 (0.96–1.56)	0.158

[a] 1,253 cases had missing information on the time interval from blood draw to cancer diagnosis, thus these cases and their individually matched controls were excluded from this analysis.

[b] All models were adjusted for educational attainment (categorical), body mass index (kg/m^2) (categorical), serum cotinine concentrations (continuous), estimated glomerular filtration rate (continuous), and cohort.

[c] KTR, kynurenine to tryptophan ratio (x 1000).