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Circulating markers of cellular immune activation in prediagnostic blood sample and lung cancer risk in the Lung Cancer Cohort Consortium (LC3)

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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

Additional Supporting Information may be found in the online version of this article.

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Cell-mediated immune suppression may play an important role in lung carcinogenesis. We investigated the associations for circulating levels of tryptophan, kynurenine, kynurenine:tryptophan ratio (KTR), quinolinic acid (QA) and neopterin as markers of immune regulation and inflammation with lung cancer risk in 5,364 smoking-matched case–control pairs from 20 prospective cohorts included in the international Lung Cancer Cohort Consortium. All biomarkers 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 individual biomarkers were calculated using conditional logistic regression with adjustment for circulating cotinine. Compared to the lowest quintile, the highest quintiles of kynurenine, KTR, QA and neopterin were associated with a 20–30% higher risk, and tryptophan with a 15% lower risk of lung cancer (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. A stronger association was also seen for KTR and QA with risk of lung squamous cell carcinoma followed by adenocarcinoma, and for lung cancer diagnosed within the first 2 years after blood draw. This study demonstrated that components of the tryptophan–kynurenine pathway with immunomodulatory effects are associated with risk of lung cancer overall, especially for current smokers. Further research is needed to evaluate the role of these biomarkers in lung carcinogenesis and progression.

What's new?

The kynurenine pathway, by which tryptophan is degraded and NAD+ is synthesized, plays a role in inflammation and immune response. It may also be involved in cancer development and progression. Here, the authors investigated the relationship between the metabolites of the kynurenine pathway and risk of lung cancer. They found that lower levels of tryptophan and higher levels of kynurenine and quinolinic acid were associated with increased risk of lung cancer, especially in smokers. These biomarkers may be signs that immune suppression in the tumor microenvironment may boost cancer progression.

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 for lung cancer cases is only 17.7% in the United States (US),[2] and is even lower globally.[3] This underscores the importance of improving prevention and treatment to reduce lung cancer morbidity and mortality. While 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 partly 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. Also, these previous studies were not well powered to study risk in important subgroups, 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 for immune regulation and response with the risk of lung cancer are warranted.

Among the pathways involved in cancer innate and adaptive immune tolerance, the catabolism of tryptophan has increasingly been recognized as playing a fundamental role.[10] Interferon gamma (IFN-γ)-inducible indoleamine 2,3-dioxygenase (IDO) catalyzes the first rate-limiting reaction that converts tryptophan to kynurenine, which in turn leads to the depletion of local tryptophan and accumulation of kynurenine and their derivatives (Fig. 1). This results in a highly tolerogenic microenvironment characterized by reduced T effector lymphocytes and natural killer cells and an increased number of functionally active T regulatory cells and myeloid-derived suppressor cells.[11] The ratio between circulating kynurenine and tryptophan (KTR) can therefore be used as a surrogate of IDO activity and IFN-γ-mediated immune regulation in tumor microenvironment.[12] IFN-gamma also stimulates the production of neopterin, a metabolite of guanosine triphosphate, by macrophages.[13] One previous epidemiological study observed an association between KTR and higher risk of lung cancer.[14] 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.[15] In addition, the downstream metabolites of the kynurenine pathway such as quinolinic acid (QA) have immuno-regulatory effects[16,17] and may contribute to the development and progression of lung cancer, but has not been investigated in large epidemiological studies.

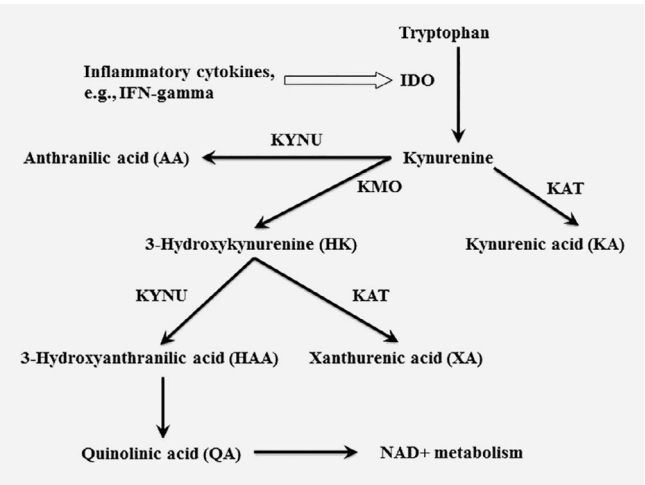

Figure 1. The kynurenine pathway of tryptophan metabolism.

The purpose of the current study conducted using 20 prospective cohorts from Asia, Australia, Europe and the US was to comprehensively investigate the associations for circulating concentrations of the tryptophan–kynurenine pathway metabolites and neopterin as markers of IFN-γ-induced immune regulation with the risk of developing lung cancer. Our large sample size (5,364 case–control pairs) allowed 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.[18] 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 and those of participating registries as required.

Selection of cases and controls

Lung cancer cases were defined according to the International Classification of Diseases for Oncology, Second Edition (ICD-O-2), and included all invasive cancers coded as C34.0–C34.9. Altogether, 11,399 incident lung cancer cases with prediagnostic serum or plasma samples in the members of the US National Cancer Institute Cohort Consortium in 2009 were eligible. From these, 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 from all eligible participants within the same cohort who were alive and free of cancer (except nonmelanoma 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 five categories: never

smokers, former smokers who had quit smoking for <10 or ≥10 years, and current smokers who smoked <15 or ≥15 cigarettes per day. After excluding cases who were not able to be correctly matched on smoking status in five 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 5,364 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 analysis. Concentrations of tryptophan, kynurenine,[19] quinolinic acid (QA), neopterin and cotinine[20]—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 from 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 personnelwas blind to the case/control status of the test samples. Between-batch coefficient of variation (CV) of quality-control samples for the five analyzed biomarkers was <6%.[21] Our previous studies also showed that tryptophan and kynurenine were stable between different types of blood tube, between serum and plasma, and over different processing lag time, and had high within-person reproducibility.[21,22]

Statistical analysis

The KTR ratio was calculated as the kynurenine concentration (nmol/l) divided by the tryptophan concentration (μmol/l). 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 difference in geometric means of biomarkers among three smoking groups (never, former and current smokers) was assessed using Analysis of Covariance (ANCOVA) in all control subjects with adjustment for cohort, age, sex and estimated glomerular filtration rate (eGFR; a measurement of kidney function that influences the circulating levels of kynurenine and its metabolites). The eGFR was calculated based on participant's age, gender and creatinine concentration in plasma or serum according to the previously published method.[23]

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.[24] 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 (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),[25] educational attainment (six categories), body mass index (BMI) in kg/m^2 (<18.5, 18.5 to <25, 25 to <30, ≥30) and eGFR.

Fully adjusted regression models were used in analyses stratified by smoking status (current, former and 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 to <5 and ≥5 years) and geographical region (US, Europe/Australia and 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.

Data availability

All data relevant to the present study are available upon request to the corresponding authors.

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

Table 1. Baseline and clinical characteristics of study participants overall and by continent (the Lung Cancer Cohort Consortium (LC3) study)

	US cohorts		EU/AU cohorts		Asian cohorts		Overall	
	No. (%) of participants in group		No. (%) of participants in group		No. (%) of participants in group		No. (%) of participants in group	
Baseline and clinical characteristics	Cases (n = 2,400)	Matched controls (n = 2,400)	Cases (n = 1,189)	Matched controls (n = 1,189)	Cases (n = 1,775)	Matched controls (n = 1,775)	Cases (n = 5,364)	Matched controls (n = 5,364)
Sex								
Men	991 (41.3%)	991 (41.3%)	688 (57.9%)	688 (57.9%)	1,229 (69.2%)	1,229 (69.2%)	2,908 (54.2%)	2,908 (54.2%)
Women	1,409 (58.7%)	1,409 (58.7%)	501 (42.1%)	501 (42.1%)	546 (30.8%)	546 (30.8%)	2,456 (45.8%)	2,456 (45.8%)
Smoking status								
Never	569 (23.7%)	569 (23.7%)	156 (13.1%)	156 (13.1%)	602 (33.9%)	602 (33.9%)	1,327 (24.7%)	1,327 (24.7%)
Former	1,007 (42.0%)	1,007 (42.0%)	335 (28.2%)	335 (28.2%)	176 (9.9%)	176 (9.9%)	1,518 (28.3%)	1,518 (28.3%)
Current	824 (34.3%)	824 (34.3%)	698 (58.7%)	698 (58.7%)	997 (56.2%)	997 (56.2%)	2,519 (47.0%)	2,519 (47.0%)
Education								
Less than high school	237 (9.9%)	215 (9%)	662 (55.6%)	598 (50.2%)	898 (50.6%)	883 (49.7%)	1,797 (33.5%)	1,696 (31.6%)
Completed high school	357 (14.9%)	374 (15.6%)	159 (13.4%)	180 (15.2%)	243 (13.7%)	230 (13.0%)	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 (17.0%)
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[1]								
<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%)
18.5–24.9	1,088 (45.3%)	1,020 (42.5%)	521 (43.8%)	435 (36.6%)	1,203 (67.8%)	1,192 (67.2%)	2,812 (52.4%)	2,647 (49.3%)
25.0–29.9	841 (35%)	858 (35.8%)	468 (39.4%)	536 (45.1%)	369 (20.8%)	424 (23.9%)	1,678 (31.3%)	1,818 (33.9%)
≥30.0	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 (0.0%)	64 (1.2%)	63 (1.2%)
Continuous variables, median (5th–95th percentile)								
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 biomarkers								
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 (nmol/μmol)	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)	

(Continues)

Table 1. Baseline and clinical characteristics of study participants overall and by continent (the Lung Cancer Cohort Consortium (LC3) study) (Continued)

Baseline and clinical characteristics	US cohorts		EU/AU cohorts		Asian cohorts		Overall	
	No. (%) of participants in group		No. (%) of participants in group		No. (%) of participants in group		No. (%) of participants in group	
	Cases (n = 2,400)	Matched controls (n = 2,400)	Cases (n = 1,189)	Matched controls (n = 1,189)	Cases (n = 1,775)	Matched controls (n = 1,775)	Cases (n = 5,364)	Matched controls (n = 5,364)
Histology, *n* (%)								
Large cell carcinoma	112 (4.6%)		46 (4.0%)		16 (1.0%)		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	1,034 (42.7%)		419 (34.5%)		615 (34.6%)		2,056 (38.4%)	
Missing/Unknown	735 (31.4%)		357 (29.5%)		726 (41%)		1,806 (33.6%)	

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

did not differ substantially by region, with each region contributing approximately one-third of cases.

Biomarker distribution in study population

The geometric means of kynurenine, KTR, QA and neopterin were significantly higher in former smokers than never or current smokers, but no difference between current and never smokers among all control subjects after adjustment for age, sex, eGFR and cohort (Supporting Information Table S1). Never smokers had significantly higher concentrations of QA than current smokers (368.7 *vs.* 334.7 μmol/l, $p < 0.001$) and higher neopterin (10.8 *vs.* 10.3 μmol/l, $p = 0.002$). There was no difference in tryptophan concentrations among never, former and current smokers. Each of five analyzed biomarkers were significantly elevated in overweight and obese control subjects and the associations between these biomarkers and BMI were dose-dependent (Supporting Information Table S2). Median concentrations 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 (Supporting Information Table S3). 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 after adjustment for age and sex (partial 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; Supporting Information Table S4).

Overall and stratified associations of circulating biomarkers and lung cancer risk

Odds ratios for quintiles of each biomarker with overall lung cancer after controlling for smoking status, duration and intensity, circulating levels of cotinine and other potential confounders, are shown in Table 2. The OR for the top *vs.* bottom quartiles was 0.85 (0.75–0.96) for tryptophan, 1.22 (1.06–1.40) for kynurenine, 1.31 (1.14–1.50) for KTR, 1.31 (1.14–1.51) for quinolinic acid as well as for neopterin.

Table 3 shows the odds ratios for lung cancer associated with higher quintiles of biomarkers in current, former and never smokers separately (see numbers of cases and controls in Supporting Information Table S5). For current smokers, ORs (95% CIs) for lung cancer for 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) for 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 for never smokers (all $p_{trend} > 0.16$). However, no interaction between

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)

	Quintiles of biomarker[1]					
Biomarkers and risk estimates	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p_{trend}**
Tryptophan						
Cases/controls	1,199/1,073	1,020/1,073	1,057/1,073	1,095/1,073	993/1,072	
Adjusted odds ratio (95% CI)[1]	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						
Cases/controls	1,083/1,073	1,044/1,073	1,148/1,086	1,020/1,061	1,069/1,071	
Adjusted odds ratio (95% CI)[1]	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[3]						
Cases/controls	1,082/1,073	1,028/1,073	1,020/1,073	1,034/1,073	1,197/1,072	
Adjusted odds ratio (95% CI)[1]	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						
Cases/controls	1,158/1,073	1,033/1,074	902/1,072	1,123/1,073	1,148/1,072	
Adjusted odds ratio (95% CI)[1]	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						
Cases/controls[2]	1,022/1,072	1,065/1,071	1,087/1,070	1,051/1,069	1,128/1,071	
Adjusted odds ratio (95% CI)[1]	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**

[1]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. Bold figures indicate the 95% confidence intervals (CIs) of odds ratio did not include one or p values for trend were <0.05.
[2]Eleven case–control pairs were excluded due to missing value.
[3]KTR, kynurenine to tryptophan ratio.

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)

	Adjusted odds ratio (95% confidence interval) by quintiles of biomarker[1]					
Smoking status and biomarker	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	p_{trend}
Current smokers						
Tryptophan	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	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[2]	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	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	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						
Tryptophan	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	1.00	1.12 (0.84–1.49)	1.23 (0.95–1.60)	1.02 (0.78–1.34)	1.08 (0.82–1.41)	0.955
KTR[2]	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	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	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						
Tryptophan	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	1.00	1.06 (0.85–1.33)	1.12 (0.88–1.43)	1.05 (0.80–1.38)	1.17 (0.85–1.59)	0.406
KTR[2]	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	1.00	0.89 (0.69–1.13)	**0.70 (0.54–0.90)**	0.92 (0.71–1.20)	1.07 (0.79–1.44)	0.707
Neopterin	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

[1]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. Bold figures indicate the 95% confidence intervals of odds ratio did not include one or *p* values for trend were <0.05.
[2]KTR, kynurenine to tryptophan ratio.

any biomarker and smoking status for lung cancer risk was detected (all *p*'s for multiplicative interaction >0.05).

When data were analyzed by histological subtype of lung cancer (Supporting Information Table S5), positive associations were observed for KTR and QA and risk of lung squamous cell carcinoma, and for QA and risk of lung adenocarcinoma (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 were not statistically significant.

In the sensitivity analysis, the associations for blood concentrations of kynurenine, KTR and QA were observed for the risk of lung cancer diagnosed within 2 years after blood draw (Table 5 and Supporting Information Table S5). 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

In the largest prospective epidemiological study, we demonstrated the associations for lower levels of tryptophan and higher levels of kynurenine and its metabolites as well as neopterin with risk of developing lung cancer overall. These associations were strongest among current smokers, to a lesser extent, among former smokers and null among never smokers. These positive associations were strongest for lung squamous cell cancer, and for lung cancer cases diagnosed within 2 years of blood draw.

Higher circulating KTR concentrations and risk of lung cancer

Tryptophan is an essential amino acid for immune cell proliferation. Early studies suggested that immune suppressive effect of tryptophan catabolism on T cell is a consequence of decreased concentration of tryptophan.[26] As shown in Figure 1, IDO is the primary enzyme that catalyzes the initial step of the tryptophan metabolism pathway, which converts tryptophan to kynurenine. IDO is upregulated by inflammatory cytokine such as INF-γ and tumor necrosis factor alpha (TNF-α).[27,28] A variety of cells express IDO, including monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts and certain cancer cells. IDO in tumor cells serves as an immunosuppressive enzyme that limits T cell responses against tumors.[29,30] IDO has been found to be overexpressed in several types of cancers including lung cancer.[31] Inhibition of IDO by 1-methyltryptophan significantly delayed the tumor outgrowth in a mouse model of Lewis Lung carcinoma.[31] Clinical studies showed that mRNA expression of IDO was higher in lung cancer tissues than adjacent nonmalignant lung tissues of patients.[32]

Emerging data suggest that kynurenine may play a more direct role than the consumption of tryptophan catabolized by IDO in immune regulation and responses in tumor microenvironment.[33–35] Kynurenine can activate transcription factor aryl hydrocarbon

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)

Histological subtype and biomarker	Adjusted odds ratio (95% confidence interval) by quintiles of biomarker[1]					
	Q1	Q2	Q3	Q4	Q5	p_{trend}
Large cell carcinoma						
Tryptophan	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	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[2]	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	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	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						
Tryptophan	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	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[2]	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	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	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						
Tryptophan	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	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	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	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						
Tryptophan	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	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[2]	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	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	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

[1]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. Bold figures indicate the 95% confidence intervals of odds ratio did not include one or p values for trend were ‹0.05.
[2]KTR, kynurenine to tryptophan ratio.

receptor (AhR). The activation of AhR induces a number of immunosuppressive phenotypes including the generation of immune-tolerant dendritic cells and regulatory T cells, which collectively foster a tumor immunological microenvironment that is defective in recognizing and eradicating cancer cells.[36] In a recent experimental study, kynureninase administration, which depleted kynurenine but did not impact the consumption of tryptophan by IDO, significantly reduced tumor growth. Kynureninase treatment also significantly increased CD8$^+$ tumor infiltrating lymphocytes (TILs) and the production of IFN-γ, TNF-α and interleukin-2 by CD8$^+$ T cells.[37] These data suggest that accumulation of kynurenine renders the immunosuppression in tumor microenvironment. High levels of circulating kynurenine and KTR in humans could be the consequence of enhanced IDO by inflammatory cytokines and/or the reduced metabolism of downstream kynurenine pathway. Previous studies have found that lung cancer patients had higher serum KTR concentration than healthy controls.[38] Our results with higher kynurenine or KTR with higher risk of lung cancer are consistent with findings from the prospective European Prospective Investigation into Cancer and Nutrition (EPIC),[14] through the present study has much larger sample size and diverse populations. In addition, the stronger associations between kynurenine or KTR concentration and risk of lung cancer for individuals within 2 years of blood draw support the notion that cancer cells at subclinical stage may contribute to the elevation of circulating kynurenine in our patient population.

Our study found that the association for KTR or kynurenine metabolite QA with lung squamous cell carcinoma was stronger than that with lung adenocarcinoma. One possible explanation is the interaction between kynurenine and polycyclic aromatic hydrocarbons (PAH), specifically benzo[a]pyrene (B[a]P), in cigarette smoke, on the activation of AhR. Cigarette smoking is more strongly associated with risk of lung squamous cell carcinoma than adenocarcinoma in humans.[39] Experimental studies have shown that exposure to airborne particulate matters (mainly contains PAH) primarily induced lung squamous cell carcinoma in mice with intact AhR gene but no tumors at all in mice without AhR, suggesting that AhR is critical for the development of PAH-induced lung squamous cell carcinoma.[40] As described above, kynurenine and its downstream metabolites may be able to activate AhR, which would enhance the carcinogenic effect of

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)

Time from blood draw to cancer diagnosis and biomarkers	Adjusted odds ratio (95% confidence interval) by quintiles of biomarker[1]					
	Q1	Q2	Q3	Q4	Q5	p_{trend}
<2 years						
Tryptophan	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	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[2]	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	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	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
2–4.9 years						
Tryptophan	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	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[2]	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	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	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						
Tryptophan	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	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[2]	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	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	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

[1]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. Bold figures indicate the 95% confidence intervals of odds ratio did not include one or p values for trend were <0.05.
[2]KTR, kynurenine to tryptophan ratio.

PAH. These data may explain why our observed associations for KTR and QA were stronger with lung squamous cell carcinoma than that with lung adenocarcinoma.

Higher circulating quinolinic acid concentrations and risk of lung cancer

Ours is the first study to evaluate the association between QA and lung cancer risk. We found that a higher concentration of QA in prediagnostic blood samples was associated with higher risk of lung cancer. QA, a downstream metabolite of kynurenine, is a known neurotoxin *via* stimulation of the presynaptic receptor which induces oxidative stress and enhances the production of proinflammatory cytokines in the brain.[41] In the current study, circulating QA concentrations were highly correlated with KTR (r = 0.57), which is consistent with the fact that QA concentrations are correlated with IDO expression.[17] Previous studies showed that during inflammation, QA synthesis occurs mainly in immune cells.[17] 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.[17] 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.[42] 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.

Other notable findings

In the present analysis, high levels of KTR and QA were associated with higher risk of lung cancer in both current and former smokers, but no risk was observed in never smokers. Former smokers had significantly higher levels of kynurenine, KTR and QA than never and current smokers. Individuals often gained weight after they quit smoking.[43] In our study, all five analyzed biomarkers were significantly associated with BMI in a dose-dependent manner. Our results were consistent with previous study results.[44] It is possible that the alternations of kynurenine metabolism in former smokers may contribute to their continued high risk of lung cancer after smoking cessation. It is interesting to note that the levels of kynurenine metabolites in current smokers were comparable with those in never smokers, but the associations for these biomarkers with lung cancer risk were seen in current smokers only. These results suggest that cigarette smoking may be a prerequisite for kynurenine pathway to impact on the risk of developing lung cancer, but smoking is less likely to directly confound the kynurenine metabolites-lung cancer risk association.

Strengths and limitations

The strengths of our study include (*i*) prospective design, (*ii*) usage of prediagnostic plasma/serum samples and (*iii*) large sample size that provided sufficient power for stratified analysis. We also measured concentrations of metabolites of the kynurenine pathway, including QA as a novel inflammatory marker. In addition to matching on smoking status, intensity and duration, we also controlled for circulating cotinine concentration, a biomarker of recent tobacco exposure,[44] and eGFR, a renal function measurement that is strongly related to circulating concentrations of kynurenine and its metabolites.[43] We also measured KTR and neopterin, novel biomarkers for cellular immune activation as shown in prior work to have high intraclass correlation (ICCs, 0.74 and 0.67, respectively) in four different sampling visits over 3.5 years.[45] This indicated that a single time point measurement is a relatively reliable biomarker for long-term levels, and these biomarkers may be better than traditional cytokine biomarkers such as IFN-gamma and interleukins whose ICCs were lower.[46] The present study had some limitations. Although our analysis was based on a hypothesis that markers of immune modulation may be important in lung cancer etiology, the specific mechanisms underlying the observed associations are not clear. Given the complexity of immune response and their interconnectedness, our studied biomarkers had relatively modest associations with lung cancer risk which limits their clinical utility for lung cancer screening and management. As in any observational study, our results could be confounded by other factors, including smoking, which is an established risk factor for lung cancer. Concentrations of all biomarkers except tryptophan varied among three groups of smokers—highest in former smokers and lowest in current smokers (Supporting Information Table S1). Lung cancer risk was only significantly associated with KTR and QA concentrations in former and current smokers. Although smoking status, density and duration were matched for cases and controls in the present study and circulating cotinine concentration 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

Our study demonstrates that lower circulating concentration of tryptophan and higher concentrations of kynurenine (i.e., higher KTR) and kynurenine downstream metabolite QA, biomarkers for immune regulation are associated with increased risk of lung cancer overall, in particular, among current smokers. Stronger associations for kynurenine, KTR and QA with imminent cancer occurring within the initial years after blood draw suggest that immune suppression in tumor microenvironment may play a more important role in the progression from a subclinical to clinical stage of lung cancer.

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