Original Article

Interferon-γ–Induced Inflammatory Markers and the Risk of Cancer: The Hordaland Health Study

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Background: It has been reported that interferon-γ (IFN-γ)–induced inflammatory markers, such as circulating neopterin and kynurenine-to-tryptophan ratio (KTR), are increased in patients with cancer and are also a predictor of poor prognosis. However, whether baseline levels of these makers are associated with subsequent cancer risk in the general population remains unknown. **Methods:** We conducted a prospective analysis of the Hordaland Health Study in 6594 adults without known cancer at baseline who were enrolled between April 1998 and June 1999. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using multivariate Cox proportional hazards regression models adjusted for sex, age, body mass index, smoking status, and renal function. **Results:** A total of 971 incident cancer cases (507 men and 464 women) were identified over a median follow-up time of 12 years. Baseline plasma neopterin, KTR and C-reactive protein (CRP) were significantly associated with an increased risk of overall cancer in models adjusted for covariates (*P* for trend across quartiles = .006 for neopterin, .022 for KTR, and .005 for CRP). The multivariate-adjusted HR (95% CI) per SD increment in similar models were 1.09 (1.03-1.16) for neopterin, 1.07 (1.01-1.14) for KTR, and 1.04 (0.98-1.10) for CRP. The associations between the inflammatory markers and risk of major specific cancer types were also provided. **Conclusions:** Our findings indicate that plasma neopterin, KTR, and CRP are associated with a significantly increased risk of overall cancer. Our study revealed novel evidence regarding the role of IFN-γ–induced inflammation in human carcinogenesis. ***Cancer* 2014;120:3370-7.** © 2014 The Authors. *Cancer* published by Wiley Periodicals, Inc. on behalf of *American Cancer Society*. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

KEYWORDS: immune activation, inflammation, neopterin, kynurenine-to-tryptophan ratio, CRP, cancer, risk, cohort study.

Chronic inflammation is perceived to predispose to different forms of cancer[1] and impacts each stage of tumorigenesis, from initiation, promotion, and malignant conversion to invasion and metastasis.[2]

C-reactive protein (CRP) is a commonly used nonspecific biomarker of systemic inflammation. Evidence on the association between CRP and cancer risk is currently inconsistent. A recent meta-analysis found elevated levels of CRP to be associated with an increased risk of overall cancer, lung cancer, and possibly breast, prostate, and colorectal cancer.[3] However, results from Mendelian randomization studies suggest that elevated CRP levels are unlikely to cause cancer.[4]

Neopterin is a metabolite of guanosine triphosphate and is synthesized by activated macrophages upon stimulation with proinflammatory cytokines, particularly interferon-γ (IFN-γ). Therefore, elevated concentrations of neopterin in body fluids reflect cellular immune activation involving T cells and an endogenous release of INF-γ.[5] Studies have observed increased levels of neopterin in patients with malignant diseases such as lung,[6] breast,[7] and pancreatic cancer.[8] The extent of neopterin elevation depends on tumor type and stage,[9] and neopterin has been proposed as a potential biomarker of cancer diagnosis and prognosis.[8,10] Whether prediagnostic neopterin is associated with future cancer risk is, however, unknown.

IFN-γ can also up-regulate enzymatic activity of indoleamine 2,3-dioxygenase (IDO), which catalyzes the conversion of tryptophan to kynurenine followed by further metabolism via the kynurenine pathway.[11,12] As a result, the plasma kynurenine-to-tryptophan ratio (KTR) increases during inflammation. Several studies have demonstrated IDO

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activation, increased tryptophan degradation, and subsequently elevated level of KTR in established cancer, and these indices also predict poor prognosis in patients with cancer such as lung cancer,[13,14] gynecological cancer,[15] and malignant melanoma.[16] It has been postulated that IDO plays a critical role in cancer immunosurveillance.[17] Results from in vitro experiments indicate that IDO overexpression by colorectal tumor cells is significantly correlated with the quantity of tumor-infiltrating T cells.[18] Based on the role of IDO in immunosuppression and immune escape, selective IDO inhibitors (eg, 1-methyltryptophan) have been developed and tested as an adjuvant chemotherapeutic agent in vitro in animal studies and phase 1 trials.[19-22]

Previous publications from human studies on the relation between neopterin, KTR, and cancer have focused on patients with existing disease. However, whether levels of these inflammatory makers, particularly neopterin and KTR, are associated with subsequent cancer risk among apparently healthy individuals remains unknown. The purpose of this cohort study, therefore, was to examine the associations of the systemic inflammatory markers including neopterin, KTR, and CRP with overall cancer risk among community-dwelling men and women.

MATERIALS AND METHODS

Study Design and Cohort

The Hordaland Health Study is a community-based study that was conducted jointly by the University of Bergen, the National Institute of Public Health, and the Municipal Health Service in Hordaland. The study participants consisted of men and women born during the periods 1925-1927 and 1950-1951 in Hordaland County in western Norway. The individuals in these two specific age groups originated from an earlier study called the Hordaland Homocysteine Study conducted in 1992-1993,[23] which was established to examine the determinants of homocysteine and homocysteine as a risk factor for disease. To be able to examine age effects on homocysteine as well as homocysteine as a risk factor for age-related conditions, such as cardiovascular disease and cancer, the middle-aged cohort was expanded to also include the older cohort, which has a much higher prevalence of disease. Details of the study design have been published elsewhere.[24] The initial study cohort included 7051 participants who were enrolled between April 1998 and June 1999. Data were collected via self-administered questionnaires, anthropometric assessment, and blood analyses. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All participants provided written informed consent.

Of the 7051 participants, we excluded 426 participants who were diagnosed with cancer (other than nonmelanoma skin cancer) before enrollment. Participants with missing data on blood measurements (neopterin, kynurenine, tryptophan, and CRP) (n = 31) were also excluded. A total of 6594 participants (2958 men and 3636 women) were therefore included in the final analysis.

Biochemical Analyses

Nonfasting blood samples were collected at baseline. Aliquots of serum and plasma were frozen at −80°C until analyses. Plasma neopterin, kynurenine, tryptophan, and serum creatinine were measured by liquid chromatography-tandem mass spectrometry.[25,26] Plasma high-sensitive CRP was determined by a novel immuno-MALDI-MS method (unpublished data). All biochemical analyses were performed at Bevital A/S (www.bevital.no). Within-day coefficients of variation for neopterin, kynurenine, and tryptophan were 2.5%-4.7%, and between-day coefficients of variation were 5.7%-10.0%.[25]

Outcome Assessment

Cancer cases were ascertained through linkage with the Cancer Registry of Norway. Cancer incidence diagnoses were coded according to the 3rd edition of the International Classification of Diseases for Oncology (ICD-O-3)[27] and the 10th revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD-10) (http://apps.who.int/classifications/icd10/browse/2010/en). Only the first primary neoplasm was included in the analysis. Mortality data were collected from the Cause of Death Registry at Statistics Norway and coded according to ICD-10.

Additional Data

Self-administered questionnaires provided information on sociodemographic data, health status, and lifestyle factors. Information on smoking (never, former, or current smokers) was coded as categorical variables. Height and weight were measured following standard protocols. Body mass index (BMI) was calculated as kg/m^2 and categorized as normal (BMI <25 kg/m^2), overweight (25 $kg/m^2 \leq$ BMI < 30 kg/m^2), and obese (BMI ≥ 30 kg/m^2) according to the World Health Organization's recommendation. Renal function was assessed using an estimated glomerular filtration rate (eGFR) based on serum creatinine levels.[28]

Statistical Analysis

Continuous variables are presented as medians (interquartile ranges) due to skewed distributions; categorical variables are

given as counts (percentages). Wilcoxon–Mann–Whitney tests were used to compare differences between groups for continuous variables. For each participant, person-years of follow-up were calculated from the date of entry until the date of cancer diagnosis, death, emigration, or the end of follow-up (December 31, 2010), whichever came first.

The association of plasma neopterin, KTR, and CRP with risk of overall cancer was evaluated using Cox proportional hazards regression models with person-years as the underlying time metric. Proportionality was verified using analysis of residuals. Hazard ratios (HR) and 95% confidence intervals (CI) are reported. Models were fitted with neopterin, KTR, and CRP as continuous variables (per SD increment) and as sex-specific quartiles based on the distribution of the study population. Linear trends were tested across increasing quartiles by modeling quartile categories as a continuous variable in the regression models. Multivariable models included the following covariates: age (46-49 years vs 70-74 years), sex, BMI (normal, overweight, and obese), smoking status (never, former, or current smokers) and renal function (normal, eGFR > 60 mL/min/1.73 m^2 or impaired, $1 <$ eGFR ≤ 60 mL/min/1.73 m^2). Risk estimates did not change materially by additional adjustment for physical activity, which therefore was not included in final models. Kaplan-Meier plots were made for cumulative cancer incidence according to neopterin, KTR, and CRP quartiles, and the corresponding *P* values of the log-rank test for possible trends across quartiles are presented.

Multivariable adjusted dose-response relations between inflammatory marker levels and cancer risk were also visualized by generalized additive regression plots.[29] In these plots, biomarker values were fitted with smoothing spline in Cox proportional hazard models including the same covariates as described above.

Interaction analysis was performed between the three markers and sex/age/BMI/smoking status/renal function for cancer risk by including product terms in the regression models. In addition, we also conducted a lag analysis by excluding the first 1 year of follow-up to test for the possibility of reverse causality.

All statistical tests were 2-sided and were considered statistically significant at $P < 0.05$. All statistical analyses were conducted using the SAS (version 9.2, SAS Institute Inc, Cary, NC) and figures generated using R (version 2.15 for Windows).

RESULTS

Population Characteristics

Baseline characteristics of the participants are presented in Table 1. Plasma neopterin concentration was significantly higher in women, in the older age group, in participants with higher BMI, and in those with impaired renal function ($P<.01$). Plasma concentrations of KTR and CRP were significantly higher in participants in the older age group, in participants with high BMI, and in participants with impaired renal function ($P<.01$), whereas no significant sex differences were observed with regard to KTR or CRP levels. Plasma neopterin and KTR were lower, and CRP levels higher in current smokers as compared with former and never smokers ($P<.01$). Additional details regarding the distribution of neopterin and KTR are described elsewhere.[30] The three markers were intercorrelated (Spearman correlation coefficients were 0.56, 0.27, and 0.24 [$P<.001$] for the pairs neopterin/KTR, CRP/KTR, and CRP/neopterin, respectively).

Classical Risk Factors And Future Cancer Risk

A total of 971 incident cancer cases (507 men and 464 women) among the 6594 participants were identified over a median follow-up time of 12 years.

Age as a categorical variable was positively associated with overall cancer risk (adjusted HR, 4.18; 95% CI, 3.60-4.86). Women had a lower risk than men (adjusted HR, 0.71; 95% CI, 0.62-0.82). Compared with never smokers, the adjusted HR (95% CI) were 1.35 (1.16-1.58) for former smokers and 1.62 (1.36-1.93) for current smokers. BMI and renal function, assessed by eGFR, were not significantly associated with cancer risk (data not shown).

Inflammatory Markers at Baseline and Future Cancer Risk

Figure 1 shows the cumulative incidence of overall cancer according to quartiles of neopterin, KTR, and CRP levels, respectively. The unadjusted cancer risk increased significantly in higher quartiles for each of the inflammatory markers ($P<.001$). For instance, the cumulative hazard of overall cancer increased much more rapidly over follow-up time in the fourth quartile of neopterin compared with the first quartile (left panel).

The results from multivariate Cox models are shown in Table 2. Baseline neopterin, KTR, and CRP were associated with an increased risk of overall cancer both in unadjusted analyses and analyses adjusted for age, sex, BMI, smoking status, and renal function (*P* for trend across quartiles = .006 for neopterin, .022 for KTR and .005 for CRP). The multivariate-adjusted HR (95% CI) per SD increment in similar models were 1.09 (1.03-1.16) for neopterin, 1.07 (1.01-1.14) for KTR, and 1.04 (0.98-1.10) for CRP. As shown in Figure 2, positive dose-response relations were observed between neopterin, KTR and CRP and cancer risk.

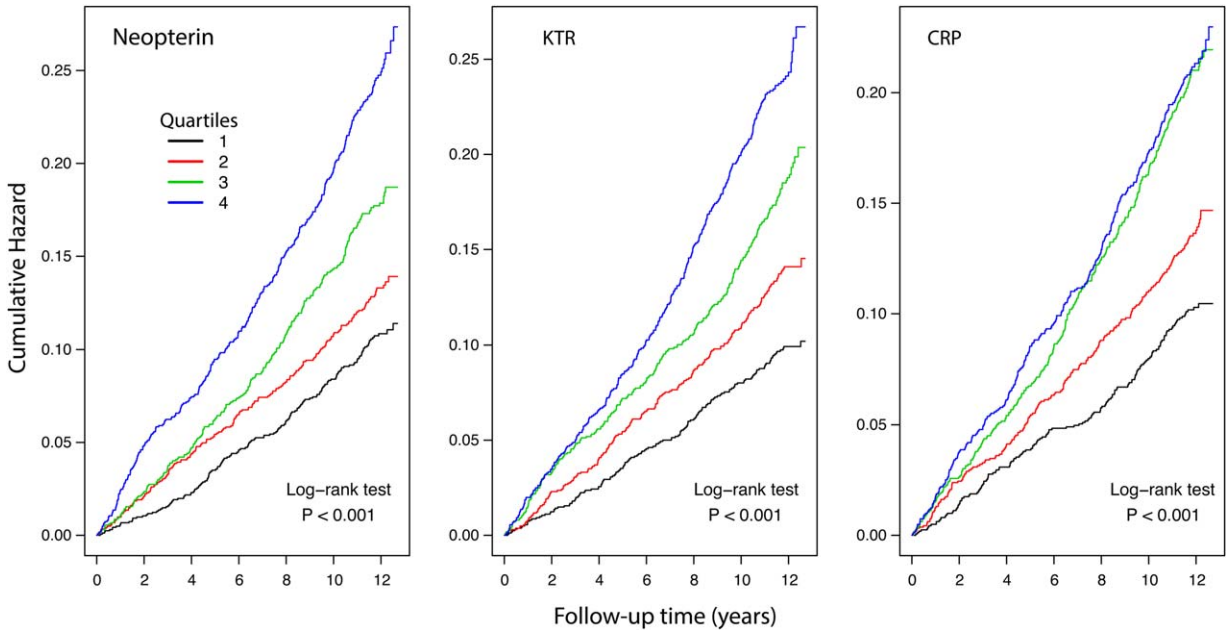

Figure 1. Kaplan-Meier survival curves for cumulative incidence of overall cancer according to sex-specific quartiles of neopterin, kynurenine-to-tryptophan ratio (KTR), and C-reactive protein (CRP) levels.

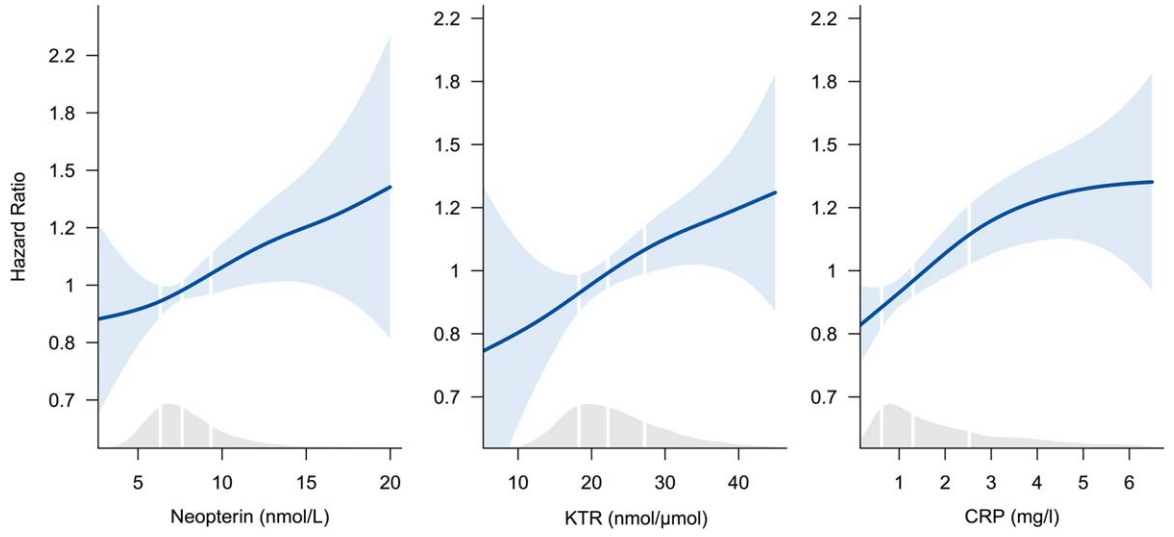

Figure 2. Dose–response relations between inflammatory marker levels and cancer risk by generalized additive regression. Models were adjusted for age, sex, body mass index, smoking status, and renal function. The solid lines represent hazard ratios; the shaded areas represent 95% confidence intervals. Density plots show the distribution of biomarkers, and white vertical lines denote the 25th, 50th, and 75th percentiles.

There were no interactions between the three markers and age, BMI, smoking status, or renal function in association with cancer risk. However, significant interactions were found between the markers and sex (*P* interaction = 0.011, 0.002, and 0.063 for neopterin, KTR, and CRP, respectively). Stratified analysis by sex showed consistently stronger associations in men than in women (data not shown).

Results from lag analyses for the associations of neopterin (HR, 1.09; 95% CI, 1.02-1.16), KTR (HR, 1.07; 95% CI, 1.01-1.14), and CRP (HR, 1.04; 95% CI, 0.98-1.11) with overall cancer risk excluding the first 1 year of

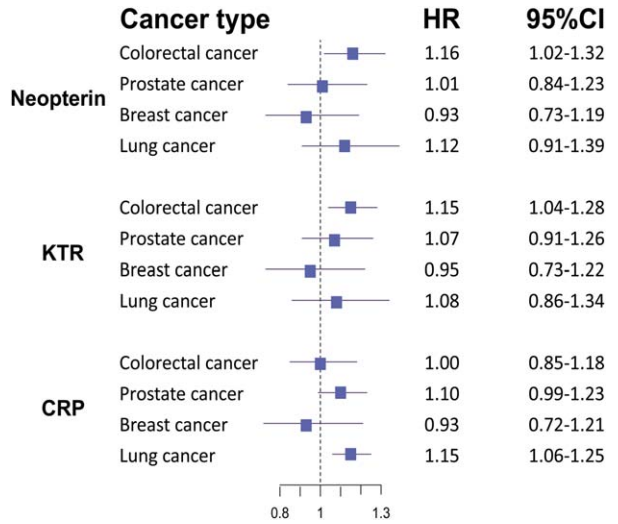

Figure 3. Forest plot showing risk of different cancer types (colorectal cancer [n = 175], prostate cancer [n = 140], breast cancer [n = 108], and lung cancer [n = 88]) according to plasma inflammatory markers. The blue squares represent hazard ratios (HR); the horizontal bars represent 95% confidence intervals (95% CI). The Cox models were adjusted for age, sex, body mass index, and renal function.

follow-up were not materially different from those including the whole follow-up period.

We conducted secondary analyses on major specific cancer types (ie, colorectal cancer [n = 175], prostate cancer [n = 140], breast cancer [n = 108] and lung cancer [n = 88], as shown in Figure 3. Neopterin and KTR were positively associated with risk of colorectal cancer (HR per SD increment [95% CI]: 1.16 [1.02-1.32] for neopterin, 1.15 [1.04-1.28] for KTR), whereas CRP was found to be associated with an increased risk of lung cancer (HR per SD increment [95% CI]: 1.15 [1.06-1.25]).

DISCUSSION

Principal Findings

In this prospective cohort study, we observed a positive association of IFN-γ–induced inflammatory markers (neopterin and KTR) and CRP with risk of overall cancer among 6594 adults followed for a median of 12 years. These associations were largely unaffected by adjustment for sociodemographic and lifestyle factors, including smoking.

Inflammatory Markers and Incident Cancer Risk

Prospective studies on the association between IFN-γ–induced inflammatory markers and incident cancer risk have not been reported previously, except for a recent study focusing on KTR and lung cancer risk.[31] Reported associations between CRP and cancer risk in prospective cohort studies are inconsistent.[32-34] Although having substantial heterogeneity, a recent meta-analysis[3] including 11 prospective cohort studies reported that the pooled HR (95% CI) per natural log unit change in CRP was 1.11 (1.03-1.18) for overall cancer, 1.31 (1.10-1.52) for lung cancer, 1.04 (0.91-1.17) for breast cancer, 1.06 (0.97-1.16) for prostate cancer, and 1.06 (0.93-1.18) for colorectal cancer. Our results support positive associations of circulating CRP levels with risk of overall cancer and lung cancer. More importantly, our study goes beyond this commonly used nonspecific inflammatory parameter by addressing systemic inflammatory markers reflecting IFN-γ–mediated immune activation with regard to cancer risk. We report that elevated baseline levels of neopterin and KTR were both significantly associated with an increased risk of overall cancer and colorectal cancer.

Possible Mechanisms

Chronic inflammation due to infections, autoimmune disease, environmental irritants, or obesity plays a crucial role in each step of tumorigenesis[4] through induction of oncogenic mutations, genomic instability, early tumor promotion, and enhanced angiogenesis.[2]

As a classic acute-phase protein, CRP levels are moderately elevated in response to chronic inflammation.[4] Precise mechanisms binding the association of CRP with cancer risk remain uncertain. The association between CRP and cancer incidence may reflect the production of various cytokines and chemokines by occult tumor cells that attract leukocytes. Some cancerous cells express CRP and secrete interleukin-6 and interleukin-8, which stimulate CRP production in the liver.[32] From the available data, it is not possible to elucidate whether elevated CRP is a marker of occult cancer or is causative in carcinogenesis.[3,32]

Neopterin is produced by oxidation of 7,8-dihydroneopterin, and the amounts of neopterin produced by activated macrophages not only reflect IFN-γ activity but also correlate with their capacity to form and release reactive oxygen species. Neopterin can enhance the toxic effects induced by reactive oxygen species during cell-mediated immune response.[9,35] In addition, epidemiological studies show that elevation of neopterin production correlates with increasing age in healthy individuals.[9] Furthermore, plasma neopterin and KTR have been positively associated with risk of coronary events in an apparently healthy population[36] and mortality in patients with stable coronary artery disease.[37] Taken together, these findings suggest that the pathological process associated

TABLE 1. Baseline Characteristics of the Study Participants in the Hordaland Health Study

		Median (IQR)		
	No. (%)	Neopterin (nmol/L)	KTR (nmol/μmol)	CRP (mg/L)
Overall	6594 (100.0)	7.6 (6.3-9.2)	22.4 (18.4-27.7)	1.6 (0.7-3.6)
Sex				
Men	2958 (44.9)	7.4 (6.2-9.0)	22.4 (18.6-27.5)	1.6 (0.7-3.5)
Women	3636 (55.1)	7.7 (6.4-9.4)[a]	22.4 (18.2-27.8)	1.5 (0.6-3.6)
Age, y				
46-49	3632 (55.1)	6.9 (5.9-8.2)	20.0 (17.1-23.7)	1.1 (0.5-2.6)
70-74	2962 (44.9)	8.6 (7.3-10.5)[a]	26.1 (21.8-31.7)[a]	2.2 (1.1-4.4)[a]
BMI				
Normal	2976 (45.2)	7.5 (6.3-9.1)	21.4 (17.9-26.5)	1.1 (0.5-2.4)
Overweight	2809 (42.7)	7.6 (6.4-9.3)	22.9 (18.6-28.1)	1.8 (0.9-3.8)
Obese	797 (12.1)	7.8 (6.4-9.7)[a]	25.0 (19.9-30.4)[a]	3.3 (1.7-6.6)[a]
Smoking				
Never smoker	2597 (40.6)	7.7 (6.5-9.4)	22.6 (18.3-28.0)	1.4 (0.6-3.2)
Former smoker	2131 (33.3)	7.9 (6.6-9.6)	23.4 (19.3-29.0)	1.6 (0.7-3.6)
Current smoker	1668 (26.1)	7.1 (5.9-8.6)[a]	21.0 (17.5-25.4)[a]	1.9 (0.8-4.0)[a]
Renal function				
Normal	6000 (91.0)	7.4 (6.3-8.9)	21.8 (18.1-26.6)	1.5 (0.6-3.4)
Impaired	594 (9.0)	10.1 (8.5-12.6)[a]	31.4 (25.3-38.4)[a]	2.6 (1.2-5.4)[a]

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; KTR, kynurenine-to-tryptophan ratio.
Not all sums are equal to the total number of the participants due to missing values.
[a] $P<.01$ for difference between groups.

TABLE 2. HRs and 95% CIs for Incident Cancer in the Hordaland Health Study (n=6594)

	Unadjusted		Sex, age-adjusted		Multivariate-adjusteda	
	HR (95% CI)	*P* trend	HR (95% CI)	*P* trend	HR (95% CI)	*P* trend
Neopterin (mmol/L)						
Quartile 1	1.00 (ref.)	<.001	1.00 (ref.)	.012	1.00 (ref.)	.006
Quartile 2	1.24 (1.01-1.52)		0.97 (0.79-1.19)		1.01 (0.82-1.25)	
Quartile 3	1.67 (1.37-2.03)		1.08 (0.88-1.32)		1.11 (0.90-1.36)	
Quartile 4	2.33 (1.94-2.81)		1.23 (1.01-1.50)		1.29 (1.05-1.59)	
Continuous[a]	1.21 (1.16-1.27)	<.001	1.07 (1.01-1.14)	.024	1.09 (1.03-1.16)	.007
KTR (nmol/μmol)						
Quartile 1	1.00 (ref.)	<.001	1.00 (ref.)	.044	1.00 (ref.)	.022
Quartile 2	1.42 (1.15-1.75)		1.17 (0.95-1.45)		1.17 (0.94-1.45)	
Quartile 3	1.92 (1.57-2.34)		1.24 (1.01-1.53)		1.25 (1.01-1.54)	
Quartile 4	2.51 (2.07-3.04)		1.25 (1.02-1.54)		1.29 (1.04-1.60)	
Continuous	1.17 (1.13-1.20)	<.001	1.05 (0.99-1.11)	.142	1.07 (1.01-1.14)	.037
CRP (mg/L)						
Quartile 1	1.00 (ref.)	<.001	1.00 (ref.)	<.001	1.00 (ref.)	.005
Quartile 2	1.36 (1.10-1.68)		1.01 (0.82-1.24)		0.98 (0.80-1.22)	
Quartile 3	2.04 (1.68-2.48)		1.34 (1.09-1.63)		1.31 (1.07-1.61)	
Quartile 4	2.10 (1.73-2.55)		1.31 (1.07-1.60)		1.25 (1.01-1.53)	
Continuous	1.10 (1.05-1.14)	<.001	1.06 (1.00-1.12)	.041	1.04 (0.98-1.10)	.164

Abbreviations: CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; KTR, kynurenine to tryptophan ratio.
Adjusted for sex, age (46-49 years vs 70-74 years), body mass index (normal, overweight, or obese), smoking (never, former, or current smoker), and renal function (normal or impaired).
[a] Biomarkers as continuous variables (per SD increment).

with immune activation may precede the appearance of clinical disease.

Malignant tumors emerge partly because early cancer cells escape from immunosurveillance.[19] IDO and tryptophan-2,3-dioxygenase (TDO) catabolize the essential amino acid tryptophan, which promotes selective apoptosis of T lymphocytes[38] and suppress antitumor immune responses.[39] This has been attributed to tryptophan depletion

and/or formation of immunomodulating kynurenines.[39] TDO is activated in tumor cells and has a similar effect of nonspecific immunosuppression.[40] Plasma KTR reflects the activities of IDO and TDO, both of which may indirectly reflect the antitumor capability of the body.[39]

Methodological Considerations

The risk estimates in the current study were not affected by excluding the first year of follow-up, which suggests that reverse causality (changes in levels of inflammatory markers due to an undetected cancer) was unlikely. Dose–response relationships between all 3 markers and cancer risk further indicated the robustness of the results. However, when specifying cancer types as outcomes, we found similar results in some but not all cancer types, which may be due to reduced sample size and number of cancer cases.

Smoking was a risk factor of cancer as consistently demonstrated by others[41], but stratified analysis by smoking status indicated that smoking status was not a significant effect modifier, demonstrating that the associations between inflammatory markers and cancer risk did not depend on the smoking status of the participants. The effects of cigarette smoking on host immunity are complex, and its net effect on immunity depends on many variables, including dose and type of tobacco, mode of exposure, and the presence of other inflammatory mediators.[42-44] Smoking has both proinflammatory and suppressive effects and may impair host immune responses and thereby promote cancer.[45]

Strengths and Limitations

This is the first prospective community-based study evaluating IFN-γ–induced inflammatory markers, neopterin, and KTR, and the risk of overall cancer. The main strengths of the current study are the large sample size, complete and long-term follow-up, and different markers reflecting inflammatory status.

When examining the associations, we adjusted for important confounding factors, including age, sex, BMI, smoking status, and renal function. Such adjustment was undertaken because these factors affect cancer risk, and also because a previous study in the same population showed associations between systemic inflammatory markers and these potential confounders.[30]

This study also has several limitations. The study population was drawn from a small geographic region, representing two narrow age ranges, which may potentially limit generalizability. Furthermore, within-subject reproducibility of the systemic inflammatory markers together with other lifestyle factors over the follow-up period was not considered. This is important because single time point assessment of biomarker status may lead to regression dilution bias, which may attenuate "true" associations.[46] However, we have assessed within-subject reproducibility over 3.5 years for neopterin and KTR in another population. The observed intraclass correlation coefficients were 0.67 and 0.74, respectively, indicating fair to good reproducibility.[47]

In conclusion, elevated plasma neopterin, KTR, and CRP are associated with a significantly higher risk of developing cancer. The current study reveals novel evidence regarding the role of IFN-γ–induced inflammation in human carcinogenesis. These inflammatory markers may assist as early predictors of cancer risk in the general population.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

REFERENCES

1. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*. 2009;30:1073-1081.
2. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;140:883-899.
3. Guo YZ, Pan L, Du CJ, Ren DQ, Xie XM. Association between C-reactive protein and risk of cancer: a meta-analysis of prospective cohort studies. *Asian Pac J Cancer Prev*. 2013;14:243-248.
4. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Crit Rev Clin Lab Sci*. 2011; 48:155-170.
5. Pingle SK, Tumane RG, Jawade AA. Neopterin: biomarker of cell-mediated immunity and potent usage as biomarker in silicosis and other occupational diseases. *Indian J Occup Environ Med*. 2008;12: 107-111.
6. El-Akawi ZJ, Abu-Awad AM, Sharara AM, Khader Y. The importance of alpha-1 antitrypsin (alpha1-AT) and neopterin serum levels in the evaluation of non-small cell lung and prostate cancer patients. *Neuro Endocrinol Lett*. 2010;31:113-116.
7. Murr C, Bergant A, Widschwendter M, Heim K, Schrocksnadel H, Fuchs D. Neopterin is an independent prognostic variable in females with breast cancer. *Clin Chem*. 1999;45:1998-2004.
8. Talar-Wojnarowska R, Gasiorowska A, Olakowski M, Lekstan A, Lampe P, Malecka-Panas E. Clinical value of serum neopterin, tissue polypeptide-specific antigen and CA19-9 levels in differential diagnosis between pancreatic cancer and chronic pancreatitis. *Pancreatology*. 2010;10:689-694.
9. Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. *Curr Drug Metab*. 2002;3:175-187.
10. Melichar B, Solichova D, Freedman RS. Neopterin as an indicator of immune activation and prognosis in patients with gynecological malignancies. *Int J Gynecol Cancer*. 2006;16:240-252.
11. Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy States. *Int J Tryptophan Res*. 2009;2:1-19.
12. Gonzalez A, Varo N, Alegre E, Diaz A, Melero I. Immunosuppression routed via the kynurenine pathway: a biochemical and pathophysiologic approach. *Adv Clin Chem*. 2008;45:155-197.

13. Suzuki Y, Suda T, Furuhashi K, et al. Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. *Lung Cancer*. 2010;67:361-365.
14. Engin AB, Ozkan Y, Fuchs D, Yardim-Akaydin S. Increased tryptophan degradation in patients with bronchus carcinoma. *Eur J Cancer Care (Engl)*. 2010;19:803-808.
15. Schroecksnadel K, Winkler C, Fuith LC, Fuchs D. Tryptophan degradation in patients with gynecological cancer correlates with immune activation. *Cancer Lett*. 2005;223:323-329.
16. Weinlich G, Murr C, Richardsen L, Winkler C, Fuchs D. Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. *Dermatology*. 2007;214:8-14.
17. Curti A, Trabanelli S, Salvestrini V, Baccarani M, Lemoli RM. The role of indoleamine 2,3-dioxygenase in the induction of immune tolerance: focus on hematology. *Blood*. 2009;113:2394-2401.
18. Brandacher G, Perathoner A, Ladurner R, et al. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. *Clin Cancer Res*. 2006;12:1144-1151.
19. Koblish HK, Hansbury MJ, Bowman KJ, et al. Hydroxyamidine inhibitors of indoleamine-2,3-dioxygenase potently suppress systemic tryptophan catabolism and the growth of IDO-expressing tumors. *Mol Cancer Ther*. 2010;9:489-498.
20. Costantino G. New promises for manipulation of kynurenine pathway in cancer and neurological diseases. *Expert Opin Ther Targets*. 2009;13:247-258.
21. Prendergast GC. Immune escape as a fundamental trait of cancer: focus on IDO. *Oncogene*. 2008;27:3889-3900.
22. Hou DY, Muller AJ, Sharma MD, et al. Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. *Cancer Res*. 2007;67:792-801.
23. Refsum H, Nurk E, Smith AD, et al. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr*. 2006;136(6 suppl):1731S-1740S.
24. Vikse BE, Vollset SE, Tell GS, Refsum H, Iversen BM. Distribution and determinants of serum creatinine in the general population: the Hordaland Health Study. *Scand J Clin Lab Invest*. 2004;64:709-722.
25. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2009;23:1371-1379.
26. Midttun O, Kvalheim G, Ueland PM. High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. *Anal Bioanal Chem*. 2013;405:2009-2017.
27. Fritz A. International Classification of Diseases for Oncology: ICD-O. 3rd ed. Geneva, *Switzerland: World Health Organization;* 2000.
28. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604-612.
29. Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model. New York: Springer; 2000.
30. Theofylaktopoulou D, Midttun O, Ulvik A, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study. *Clin Exp Immunol*. 2013;173:121-130.
31. Chuang SC, Fanidi A, Ueland PM, et al. Circulating biomarkers of tryptophan and the kynurenine pathway and lung cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2014;23:461-468.
32. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. *J Clin Oncol*. 2009;27:2217-2224.
33. Heikkila K, Silander K, Salomaa V, et al. C-reactive protein-associated genetic variants and cancer risk: findings from FINRISK 1992, FINRISK 1997 and Health 2000 studies. *Eur J Cancer*. 2011;47:404-412.
34. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities study. *Cancer Epidemiol Biomarkers Prev*. 2011;20:297-307.
35. Mohamed KH, Mobasher AA, Yousef AR, et al. BAL neopterin: a novel marker for cell-mediated immunity in patients with pulmonary tuberculosis and lung cancer. *Chest*. 2001;119:776-780.
36. Sulo G, Vollset SE, Nygard O, et al. Neopterin and kynurenine-tryptophan ratio as predictors of coronary events in older adults, the Hordaland Health Study. *Int J Cardiol*. 2013;168:1435-1440.
37. Pedersen ER, Midttun O, Ueland PM, et al. Systemic markers of interferon-gamma-mediated immune activation and long-term prognosis in patients with stable coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2011;31:698-704.
38. Fallarino F, Grohmann U, Vacca C, et al. T cell apoptosis by tryptophan catabolism. *Cell Death Differ*. 2002;9:1069-1077.
39. Platten M, Wick W, Van den Eynde BJ. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. *Cancer Res*. 2012;72:5435-5440.
40. Opitz CA, Litzenburger UM, Sahm F, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature*. 2011;478:197-203.
41. Gandini S, Botteri E, Iodice S, et al. Tobacco smoking and cancer: a meta-analysis. *Int J Cancer*. 2008;122:155-164.
42. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. *J Dent. Res*. 2011;91:142-149.
43. Rom O, Avezov K, Aizenbud D, Reznick AZ. Cigarette smoking and inflammation revisited. *Respir Physiol Neurobiol*. 2013;187:5-10.
44. Bagaitkar J, Zeller I, Renaud DE, Scott DA. Cotinine inhibits the pro-inflammatory response initiated by multiple cell surface Toll-like receptors in monocytic THP cells. *Tob Induc Dis*. 2012;10:18.
45. Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. *Nat Rev Immunol*. 2009;9:377-384.
46. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. *BMJ*. 2010;340.
47. Midttun O, Townsend MK, Nygard O, et al. Most blood biomarkers related to vitamin status, one-carbon metabolism, and the kynurenine pathway show adequate preanalytical stability and within-person reproducibility to allow assessment of exposure or nutritional status in healthy women and cardiovascular patients. *J Nutr*. 2014;144:784-790.