JNCI J Natl Cancer Inst (2015) 107(4): djv010

doi:10.1093/jnci/djv010
First published online February 24, 2015
Article

ARTICLE

A Prospective Study of the Immune System Activation Biomarker Neopterin and Colorectal Cancer Risk

Krasimira Aleksandrova, Shu-Chun Chuang, Heiner Boeing, Hui Zuo, Grethe S. Tell, Tobias Pischon, Mazda Jenab, Bas Bueno-de-Mesquita, Stein Emil Vollset, Øivind Midttun, Per Magne Ueland, Veronika Fedirko, Mattias Johansson, Elisabete Weiderpass, Gianluca Severi, Antoine Racine, Marie-Christine Boutron-Ruault, Rudolf Kaaks, Tilman Kühn, Anne Tjønneland, Kim Overvad, J. Ramón Quirós, Paula Jakszyn, María-José Sánchez, Miren Dorronsoro, Maria-Dolores Chirlaque, Eva Ardanaz, Kay-Tee Khaw, Nicholas J. Wareham, Ruth C. Travis, Antonia Trichopoulou, Pagona Lagiou, Dimitrios Trichopoulos, Domenico Palli, Sabina Sieri, Rosario Tumino, Salvatore Panico, Anne M. May, Richard Palmqvist, Ingrid Ljuslinder, So Yeon J. Kong, Heinz Freisling, Marc J. Gunter, Yunxia Lu, Amanda J. Cross, Elio Riboli, Paolo Vineis

Affiliations of authors: Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbrueke, Nuthetal, Germany (KA, HB); Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK (SCC, BBdM, MJG, YL, AJC, ER, PV); Division of Health Policy Translation, Institutes of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan (SCC); Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway (HZ, GST); Molecular Epidemiology Group, Max Delbrueck Center for Molecular Medicine, Berlin-Buch, Germany (TP); International Agency for Research on Cancer, Lyon, France (MJe, SYJK, HF, MJo); National Institute for Public Health and the Environment, Bilthoven, the Netherlands (BBdM); Department of Gastroenterology and Hepatology, University Medical Center, Utrecht, the Netherlands (BBdM); Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway (SEV); Division of Epidemiology, Norwegian Institute of Public Health, Bergen, Norway (SEV); Bevital AS, Bergen, Norway (ØM); Section for Pharmacology, Department of Clinical Science, University of Bergen, Bergen, Norway (HZ, PMU); Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway (PMU); Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA (VF); Winship Cancer Institute, Emory University, Atlanta, GA (VF); Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway (EW); Department of Research, Cancer Registry of Norway, Oslo, Norway (EW); Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden (EW); Samfundet Folkhälsan, Helsinki, Finland (EW); Human Genetics Foundation (HuGeF), Torino, Italy (GS, PV); Inserm, Centre for research in Epidemiology and Population Health, U1018, Nutrition, Hormones and Women's Health team, F-94805, Villejuif, France (AR, MCBR); Univ Paris Sud, UMRS 1018, F-94805, Villejuif, France (AR, MCBR); IGR, F-94805, Villejuif, France (AR, MCBR); Division of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany (RK, TK); Diet, Genes and Environment Danish Cancer Society Research Center Copenhagen, Denmark (AT); Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark (KO); Public Health Directorate, Asturias, Oviedo, Spain (JRQ); Unit of Nutrition, Environment and Cancer Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Hospitallet de Llobregat, Barcelona, Spain (PJ); Escuela Andaluza de Salud Pública. Instituto de Investigación Biosanitaria de Granada (Granada.ibs), Granada, Spain (MJS); Consortium for Biomedical Research in Epidemiology and Public Health (CIBER Epidemiología y Salud Pública-CIBERESP), Spain (MJS, MDC, EA); Epidemiology and Health Information, Public Health Division of Gipuzkoa, Basque Regional Health Department, San Sebastian, Spain (MD); Department of Epidemiology, Murcia Regional Health Authority, Spain (MDC); Navarre Public Health Institute, Pamplona, Spain (EA); Clinical Gerontology Unit, Addenbrooke's Hospital, University of Cambridge School of Clinical Medicine, Cambridge, UK (KTK); MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge School of Clinical Medicine, Cambridge, UK (NJW); Cancer Epidemiology Unit Nuffield Department of Population Health University of Oxford, Oxford, UK (RCT); Bureau of Epidemiologic Research, Academy of Athens, Athens, Greece (AT, DT); Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece (AT, PL, DT); Department of Epidemiology, Harvard School of Public Health, Boston, MA (PL); Hellenic Health Foundation, Athens, Greece (PL, DT); Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy (DP); Epidemiology and Prevention Unit Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy (SS); Cancer Registry and Histopathology Unit, "Civic - M.P. Arezzo" Hospital, ASP Ragusa, Italy (RT); Dipartamento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy (SP); Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands (AMM); Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden (RP); Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden (IL).

Correspondence to: Krasimira Aleksandrova, MPH, PhD, Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114–116, 14558 Nuthetal, Germany (e-mail: krasimira.aleksandrova@dife.de).

Received: May 29, 2014; **Revised:** November 20, 2014; **Accepted:** January 9, 2015

Abstract

Background: Neopterin may be relevant for colorectal cancer (CRC) development, as a biomarker of cellular immune activity exerting pleiotropic effects on cellular ageing, oxidative stress, and inflammation. So far, the association between prediagnostic neopterin and colon and rectal cancer risk has not been evaluated in human populations.

Methods: A nested case-control study was conducted within the European Prospective Investigation into Cancer and Nutrition cohort using data on plasma concentrations of total neopterin (T-N, sum of neopterin and 7,8-dihydroneopterin) in 830 incident CRC case patients (561 colon and 269 rectal) matched within risk sets to 830 control participants. A subsequent replication study used data from the Hordaland Health Study, where 173 CRC case patients have been diagnosed among 6594 healthy participants over 12 years of follow-up.

Results: After multivariable adjustment for a priori chosen CRC risk factors, a "U-shaped" association of T-N with CRC was revealed. Compared with the second quintile of the T-N distribution, the relative risks for the first, third, fourth, and fifth quintiles were 2.37 (95% CI = 1.66 to 3.39), 1.24 (95% CI = 0.87 to 1.77), 1.55 (95% CI = 1.08 to 2.22), and 2.31 (95% CI = 1.63 to 3.27), respectively. Replication of these associations within the Hordaland Health Study yielded similar results. No differences have been observed when the associations were explored by colon and rectal cancer site (two-sided $P_{difference}$ = .87) and after excluding case patients diagnosed within the first four follow-up years.

Conclusions: These novel findings provide evidence of the role of both suppressed and activated cell-mediated immunity as reflected by prediagnostic T-N concentrations in the development of CRC.

Chronic inflammation and immunity are suggested to play a role in the pathogenesis of colorectal cancer (CRC) (1,2); however, evidence from human studies on the plausible pathways through which inflammation promotes CRC carcinogenesis is scarce. Biomarkers of cellular immune activation may act as potential mediators linking immunity, inflammation, and CRC.

One such candidate biomarker is neopterin. Neopterin and its reduced form 7,8-dihydroneopterin are synthesized and released primarily by monocyte-derived macrophages and dendritic cells upon stimulation with interferon-gamma (INF-γ), a cytokine produced by the Type 1 helper T (Th1) cells (3,4). Higher levels of neopterin in body fluids have been associated with advanced age (5,6), as well as with diseases related to activation of the cellular immune mechanisms, such as certain malignancies, autoimmune diseases, and viral infections, and infections by intracellularly living bacteria or parasites (7–10). High neopterin concentrations are associated with increased production of reactive oxygen species and with low serum concentrations of antioxidants; thereby, neopterin can also be regarded as a biomarker of oxidative stress formed by the activated cellular immune system (11). Taken together, neopterin may play a role for CRC development through exerting pleiotropic effects on cellular aging (6,12), inflammation (13), and oxidative stress (14), but may also serve as an indicator for an infection by external pathogens (ie, bacterial or viral) (15).

So far, one epidemiological study recently reported data on the possible link between neopterin and CRC risk (16). However, that study was designed to investigate associations between neopterin with overall cancer and did not differentiate between CRC subtypes. This information may be important, given the known differences in the etiologies of colon and rectal cancers (17). Furthermore, this previous study did not account for potential factors on the causal pathway for CRC risk such as inflammatory, metabolic, and oxidative stress biomarkers, thereby it remains unclear whether neopterin acts independently or merely reflects related etiological pathways. Therefore, we aimed to investigate the association between prediagnostic concentrations of total neopterin (T-N, sum of neopterin and 7,8-dihydroneopterin) with risk of colorectal cancer in a prospective nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) study cohort. In an attempt to validate our findings, we repeated the main association analyses in an independent replication sample within the Hordaland Health Study.

Methods

Study Population

The EPIC study population and recruitment procedures have been described in detail elsewhere (18–20). All participants gave written informed consent. The study was approved by the Ethics Committee at the International Agency for Research on Cancer (IARC), Lyon, France, as well as by the local ethics committees of the study centers (Supplementary Table 1, available online). Concrete details of the EPIC cohort and follow-up are provided in the Supplementary Methods (available online).

Selection of Case Patients and Control Participants

Case subjects were men and women who developed colon or rectal cancers after their recruitment into the EPIC study and before the end of the study period (defined for each study center by the latest end date of follow-up). For the present study, cancers were defined according to the 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death with coding C18.0-C18.7, C18.8, and C18.9 for colon cancer and C19 and C20 for rectal cancer (21). A total of 830 incident case patients with CRC (561 colon, 269 rectal) with available measurements on T-N were included in the present analyses. For each case patient, one control participant was chosen at random among appropriate risk sets consisting of all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. Matching characteristics were: the study center, sex, age at blood collection, time of the day at blood collection, and fasting status, and among women menopausal status. Premenopausal women were further matched on phase of menstrual cycle at blood collection, and postmenopausal women were matched on current hormone replacement therapy (HRT) use (22).

ARTICLE

Lifestyle and Dietary Assessment

Participants provided written informed consent, underwent anthropometric measurements, and completed questionnaires on sociodemographic and lifestyle characteristics, medical history, alcohol consumption, physical activity, and diet, as described elsewhere (23). Further details regarding lifestyle and dietary assessment in the EPIC cohort are provided in the Supplementary Methods (available online).

Laboratory Analyses

Detailed information on blood collection and storage protocols within EPIC is provided in the Supplementary Methods (available online). Plasma concentrations of T-N were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at Bevital A/S (http://www.bevital.no), Bergen, Norway (24). This method yields T-N as a sum of neopterin and 7,8-dihydroneopterin, and the concentrations are therefore higher than those obtained using assays, which measure only plasma neopterin (25,26). Since INF-γ induce a step that precedes formation of 7,8-dihydroneopterin in the neopterin pathway, both neopterin and T-N have been suggested to be of equal value for the assessment of immune activity (24,26). Samples were analyzed in batches of 86, and quality control included six calibration samples, two control samples, and one blank sample in each batch. Samples from case and control participants were kept at -80^{0}C and analyzed within the same batches in random order. The within-day coefficients of variance (CV-s) were 3% to 5%, between-day CVs were 6% to 10%, and the limit of detection was 0.7 nmol/L for T-N (24).

The laboratory staff performing the biochemical analyses was blinded to the case-control status of the blood samples. The laboratory procedures for the measurements of other biomarkers included in the analysis, C-reactive protein (CRP), C-peptide, glycated haemoglobin (HbA_{1c}), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), reactive oxygen metabolites (ROM), and ferric-reducing ability of plasma (FRAP), have been described elsewhere (22,27–30).

Replication Study – The Hordaland Health Study

In order to validate the observed association between plasma T-N and CRC, we conducted a subsequent replication study using data on plasma neopterin measurements from the Hordaland Health Study, a prospective cohort study in Norway where 173 CRC (124 colon, 49 rectal) case patients have been diagnosed among 6594 participants over a follow-up of 12 years. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All participants provided written informed consent. Concrete details on study design, recruitment protocols, case ascertainment, and follow-up have been described elsewhere (15,31) (also see the Supplementary Methods, available online). The Hordaland Health Study was used as an independent replication study, and the data were not pooled together with the EPIC data.

Statistical Analysis

The associations between T-N and risk of CRC were analyzed using multivariable conditional logistic regression. Relative risks (RRs), estimated from the hazard ratios as derived from the risk set sampling design (32) and 95% confidence intervals (CIs), were computed. The associations were examined according to quintiles based on T-N distributions among control participants. In analyses aimed to assess potential nonlinearity of the associations, a cubic nonlinear term added statistically significant information to the models for CRC, colon and rectal cancer, thus suggesting that the shape of the observed associations was curvilinear. We then used regression splines (with five knots at the 5th, 25th, 50th, 75th, and 95th percentiles of the T-N distribution) to model the shape of the association between T-N and CRC.

Multivariable models accounted for matching factors with additional adjustment for a priori–chosen CRC risk factors, including smoking status, physical activity, alcohol, fiber, red and processed meat, fruits and vegetables, fish and shellfish, and waist circumference. We additionally adjusted the multivariable model for factors potentially on the causal pathway of the association between T-N and CRC—inflammatory (CRP), metabolic (C-peptide, Hba1c, HDL-C, adiponectin, leptin, soluble leptin receptor), and oxidative stress (ROM and FRAP)—and evaluated the potential mediating effects of these factors as described in the Supplementary Methods (available online). The associations were also analyzed according to different strata of CRC risk factors, and effect modification was tested using interaction terms of the variables for T-N (in quintiles) multiplied by the stratum variables. Similarly, we examined whether the associations differed by sex or length of follow-up (continuously). To test whether the associations were different by cancer site (colon vs rectum, proximal colon vs distal colon) we performed competing risk analyses by using the model of Lunn-McNeil (33). In addition, to evaluate any difference between the risk estimates across EPIC countries and the Hordaland Health Study, we estimated the proportion of total variation in study estimates that is because of heterogeneity (I^2). To account for potential reverse causality, we repeated main analyses after excluding cases that occurred in the first two years (n = 114), three years (n = 360), and four years (n = 486) of study follow-up. Finally, we repeated the main multivariable-adjusted analyses after excluding individuals with extreme biomarker levels defined as values below or above the first and last decile of T-N distribution and those with self-reported diabetes at study baseline.

P values of less than .05 were considered to indicate statistical significance. All statistical tests were two-sided. All statistical analyses were performed using Statistical Analysis System version 9.2 software (SAS Institute, Inc., Cary, NC).

Results

Descriptive Characteristics of Study Population

The median study follow-up time was 7.2 years and ranged between 3.4 months to 9.7 years. Table 1 presents the baseline characteristics of CRC case patients and their corresponding control participants. Compared with control participants, case patients had higher median T-N concentrations, higher body mass index (BMI) and waist circumference, and higher intakes of alcohol. Among control participants, T-N concentrations were increasing with age, BMI and waist circumference, CRP, and FRAP concentrations and were decreasing with current smoking, HRT use, and HDL-C concentrations (Table 2).

Plasma Concentrations of T-N and Risk for Colorectal Cancer

Table 3 presents the association of T-N concentrations with risk of CRC, colon and rectal cancer in men and in women. In conditional logistic regression analysis, in a multivariable-adjusted

ARTICLE

Table 1. Baseline characteristics of incident colorectal cancer case patients and matched control participants, The European Prospective Investigation into Cancer and Nutrition (1992–2003)

Characteristics	Case patients	Control participants	$P_{difference}$*
No. study participants	830	830	
Female sex†, No. (%)	464 (55.9)	464 (55.9)	
Age†, y	58.4	58.4	.65
Smoking, %			.37
Never	45.3	47.6	
Former	32.5	32.2	
Current	21.2	19.3	
Missing	0.96	0.96	
Education, %			.85
No school degree or primary school	39.5	44.3	
Technical or professional school	20.8	20.3	
Secondary school	17.9	13.7	
University degree	17.3	18.2	
Missing	4.3	3.4	
Physical activity, %			.42
Inactive	12.3	11.1	
Moderately inactive	27.9	26.3	
Moderately active	46.8	47.4	
Active	10.0	11.2	
Missing	3.0	4.1	
BMI, kg/m^2, mean (SD)	26.8 (4.3)	26.2 (3.8)	.003
Waist circumference, cm	89.8 (13.1)	87.8 (12.1)	<.001
Baseline alcohol intake, g/d, median (IQR)	7.7 (0.82–21.8)	6.2 (0.9–18.3)	.02
Red and processed meat intake, g/d, median (IQR)	74.2 (48.8–112.2)	70.7 (45.2–103.4)	.06
Fibre intake, g/d, median (IQR)	22.1 (17.5–27.6)	22.4 (18.2–27.4)	.20
Fruits and vegetables intake, g/d, median (IQR)	393.4 (264.0–557.2)	411.1 (277.5–581.5)	.32
Fish and shellfish, g/d, median (IQR)	22.6 (10.1–39.8)	22.5 (10.2–42.3)	.47
Menopausal status†, %			.70
Premenopausal	12.3	12.7	
Postmenopausal	70.0	69.6	
Perimenopausal/unknown	12.3	12.2	
Surgically postmenopausal	5.4	5.4	
HRT in postmenopausal women†, %	13.8	13.4	.47
Total neopterin, nmol/L, median (IQR)	20.2 (14.2–27.2)	19.6 (15.4–24.2)	<.001
CRP, mg/L, median (IQR)	2.9 (1.1–5.2)	2.3 (1.1–4.3)	.003
C-peptide, ng/mL, median (IQR)	3.5 (3.0–5.0)	3.1 (3.0–3.7)	<.001
HbA_{1c}, %, median (IQR)	5.8 (5.5–6.1)	5.7 (5.5–6.0)	<.001
HDL-C, mmol/L, median (IQR)	1.3 (1.1–1.6)	1.4 (1.-1.6)	.002
Triglycerides, mmol/L, median (IQR)	1.4 (0.9–2.1)	1.4 (0.9–2.0)	0.21
ROM, U/mL, median (IQR)	401.0 (354.0–452.0)	383.0 (335.0–427.0)	<.001
FRAP, µmol/L, median (IQR)	1028.0 (853.0–1226.0)	1007.0 (859.5–1175.5)	0.07

* $P_{difference}$ between case patients and control participants were determined by Student's paired t test for variables expressed as means; by Wilcoxon's signed rank test for variables expressed as medians, by Mc Nemar's test and Bowker's test of symmetry for variables expressed as percentages. All statistical tests were two-sided.
BMI = body mass index; CRP = C-reactive protein; FRAP = ferric-reducing ability of plasma; HbA_1c = glycated haemoglobin; HDL-C = high-density lipoprotein-cholesterol; HRT = hormonal replacement therapy; IQR = interquartile range; ROM = reactive oxygen metabolites.
† Sex, age, menopausal status, and HRT use were among the matching criteria.

model, a "U-shaped" association of T-N with CRC was revealed with a higher risk near both the lowest (median = 12.7 nmol/L) and the highest (median = 30.7 nmol/L) quintile of T-N distribution. Thus, compared with the second quintile of the T-N distribution, the relative risks for the first, third, fourth, and fifth quintiles were 2.37 (95% CI = 1.66 to 3.39), 1.24 (95% CI = 0.87 to 1.77), 1.55 (95% CI = 1.08 to 2.22), and 2.31 (95% CI = 1.63 to 3.27), respectively (Table 3). Restricted multivariable cubic spline plot of CRC for all participants is presented in Figure 1. The associations were not statistically significantly different by sex ($P_{difference}$ = .94). The nonlinear shape of the association was seen both for colon cancer ($P_{nonlinearity}$ < .001) and rectal cancer ($P_{nonlinearity}$ < .001, Figure 2), and no statistically significant differences by cancer site have been detected ($P_{difference}$ = .87). The associations did not seem to differ also according to proximal and distal colon cancer site ($P_{difference}$ = .28). Finally, adjustment for inflammatory, metabolic, and oxidative stress biomarkers did not materially alter the observed associations between T-N and CRC risk (Figure 3). In addition, these factors did not statistically explain the association between T-N and CRC risk, arguing against their role as biological mediators (Supplementary Table 2, available online).

Stratified and Sensitivity Analyses

When we stratified the analyses according to the EPIC participating country, similar elevated risks in the lowest and highest quintiles of T-N distribution for each country were observed

ARTICLE

Table 2. Age- and sex-adjusted characteristics among control participants at baseline by quintiles of total neopterin concentrations, The European Prospective Investigation into Cancer and Nutrition (1992–2003)

Characteristics	Quintiles of total neopterin concentrations (nmol/L)					
	1 (<14.6)	2 (14.6-<17.8)	3 (17.8-<21.1)	4 (21.1-<25.8)	5 (≥25.8)	P_{trend}*
No. of control participants	221	104	122	149	234	
Mean age†, y	55.9	58.1	58.2	58.7	61.0	<.001
Female sex‡, %	55.9	58.3	57.5	55.8	51.9	.21
Smoking status, %						
Never smoker	44.9	43.2	46.7	48.5	45.4	.69
Former smoker	33.1	35.0	27.1	32.2	39.5	.05
Current smoker	21.4	20.5	24.8	18.5	13.7	.02
Education, %						
No school degree or primary school	46.1	38.5	38.7	49.2	50.6	.03
Technical or professional school	20.7	22.7	21.0	18.5	18.3	.19
Secondary school	13.6	14.5	15.9	10.6	9.9	.10
University degree	16.3	20.4	20.5	16.7	19.2	.81
Physical activity, %						
Inactive	9.9	11.9	13.2	9.7	13.2	.68
Moderately inactive	25.4	27.2	27.3	23.1	27.9	.40
Moderately active	40.0	50.3	45.3	49.8	47.3	.09
Active	12.2	8.4	10.3	14.6	10.9	.72
Menopausal status among women, %						
Premenopausal	14.2	11.4	13.7	11.7	12.4	.005
Postmenopausal	75.4	66.9	69.8	71.0	64.3	.10
Perimenopausal/unknown	5.7	14.4	13.3	15.0	13.9	.11
Surgically postmenopausal	5.5	7.2	3.1	2.1	9.2	.51
HRT in postmenopausal women, %	13.6	15.2	14.7	12.6	8.6	.01
Mean BMI§, kg/m^2	25.7	25.5	26.6	26.6	27.2	<.001
Mean waist circumference, cm	87.3	87.0	88.8	89.4	90.6	<.001
Mean alcohol consumption, g/d	10.9	16.7	14.2	18.2	11.6	.65
Mean fiber intake, g/d	23.3	23.0	22.9	24.6	23.8	.21
Mean fish and shellfish intake, g/d	34.3	29.8	30.6	35.5	32.2	.84
Mean fruit and vegetable intake, g/d	464.0	419.0	443.6	483.5	472.1	.23
Mean red and processed meat intake, g/d	78.7	77.7	82.0	81.6	76.0	.71
Mean CRP, mg/L	2.84	2.70	3.38	3.97	5.40	<.001
Mean C-peptide, ng/mL	3.80	3.50	3.63	3.86	3.78	.11
Mean HbA_{1c}, %	5.7	5.7	5.7	5.7	5.7	.85
Mean HDL-C, mmol/L	1.48	1.53	1.41	1.37	1.30	<.001
Mean triglycerides, mmol/L	1.49	1.57	1.65	1.71	1.70	.004
Mean ROM, U/mL	380.5	377.2	378.8	384.9	380.1	.74
Mean FRAP, µmol/L	975.0	1032.5	1053.7	1072.4	1102.4	<.001

* P_{trend} from a linear model, calculated by using the median total neopterin concentrations within quintiles as a continuous variable, adjusted for age (years, as a continuous variable), and sex. All statistical tests were two-sided. BMI = body mass index; CRP = C-reactive protein; FRAP = ferric-reducing ability of plasma; HbA_1c = glycated haemoglobin; HDL-C = high-density lipoprotein-cholesterol; HRT = hormonal replacement therapy; IQR = interquartile range; ROM = reactive oxygen metabolites.

† The analysis for age is adjusted for sex only.

‡ The analysis for sex is adjusted for age only.

§ Weight (kg)/height (m)^2.

(Supplementary Figure 1, available online). In analyses stratified according to established CRC risk factors, statistically significant interaction was suggested for stratification by HDL-C ($P_{interaction}$ = .03), but not for the rest of the CRC risk factors (Supplementary Table 3, available online); however, because of the low number of case patients in stratified analyses, these results should be interpreted with caution.

Replication Study – The Hordaland Health Study

In order to determine whether the U-shaped association between neopterin and CRC risk was restricted to the EPIC study populations and whether it may also be true for plasma neopterin, as compared with T-N, we repeated the main association analyses in an independent sample of 173 CRC case patients diagnosed over a median follow-up of 12 years among 6594 participants in the Hordaland Health Study in Norway with available baseline measurements of plasma neopterin. In logistic regression analysis, in a multivariable-adjusted model, compared with the second quintile, the risk ratios for the first and last quintiles of T-N distribution were 1.18 (95% CI = 0.67 to 2.09) and 1.66 (95% CI = 1.01 to 2.70), respectively (Table 4). The consistency of the associations was supported by the lack of statistical heterogeneity for the elevated risks in the first and last quintile within the EPIC centers, as well as in the Hordaland Health Cohort (I^2 = 0%; P = .53 and I^2 = 22.1%; P = .25 for the first and last quintile, respectively) (Supplementary Figure 2, available online).

ARTICLE

Table 3. Relative risks and 95% confidence intervals* of colorectal cancer across quintiles of total neopterin concentrations by sex and cancer subsite, The European Prospective Investigation into Cancer and Nutrition Study (1992–2003)

	Quintiles of total neopterin concentrations (median, nmol/L)				
Cancer subsite, sex	1 (12.7)	2 (16.1)	3 (19.6)	4 (22.8)	5 (30.7)
Colorectal cancer, overall					
No. of case patients/control participants	221/166	104/166	122/165	149/167	234/166
Crude model†	2.23 (1.58 to 3.13)	1.00 (referent)	1.21 (0.86 to 1.69)	1.44 (1.02 to 2.03)	2.27 (1.63 to 3.17)
MV-adjusted model (95% CI)‡	2.37 (1.66 to 3.39)	1.00 (referent)	1.24 (0.87 to 1.77)	1.55 (1.08 to 2.22)	2.31 (1.63 to 3.27)
Colorectal cancer, men					
No. of case patients/control participants	100/72	44/69	49/70	65/74	108/81
Crude model†	2.66 (1.55 to 4.58)	1.00 (referent)	1.14 (0.68 to 1.92)	1.46 (0.86 to 2.49)	2.38 (1.40 to 4.03)
MV-adjusted model‡	2.91 (1.60 to 5.30)	1.00 (referent)	1.47 (0.82 to 2.65)	1.71 (0.94 to 3.11)	2.48 (1.37 to 4.47)
Colorectal cancer, women					
No. of case patients/control participants	121/94	60/97	73/95	84/93	126/85
Crude model†	1.99 (1.27 to 3.11)	1.00 (referent)	1.24 (0.79 to 1.94)	1.43 (0.91 to 2.26)	2.23 (1.46 to 3.43)
MV-adjusted model‡	2.29 (1.43 to 3.69)	1.00	1.22 (0.76 to 1.96)	1.58 (0.98 to 2.55)	2.31 (1.48 to 3.61)
Colon cancer, overall					
No. of case patients/control participants	146/101	69/122	77/115	102/117	167/106
Crude model†	2.58 (1.69 to 3.94)	1.00 (referent)	1.20 (0.78 to 1.83)	1.52 (0.99 to 2.34)	2.84 (1.88 to 4.27)
MV-adjusted model‡	2.74 (1.76 to 4.27)	1.00 (referent)	1.17 (0.75 to 1.82)	1.68 (1.07 to 2.63)	2.79 (1.81 to 4.31)
Colon cancer, men					
No. of case patients/control participants	68/45	24/50	30/46	48/46	68/54
Crude model†	4.18 (2.03 to 8.60)	1.00 (referent)	1.41 (0.68 to 2.90)	2.47 (1.23 to 4.98)	3.44 (1.69 to 7.00)
MV-adjusted model‡	4.95 (2.23 to 11.00)	1.00 (referent)	1.71 (0.76 to 3.86)	3.24 (1.44 to 7.29)	3.31 (1.49 to 7.33)
Colon cancer, women					
No. of case patients/control participants	146/101	69/122	77/115	102/117	167/106
Crude model†	1.89 (1.11 to 3.23)	1.00 (referent)	1.02 (0.60 to 1.76)	1.11 (0.64 to 1.94)	2.60 (1.56 to 4.34)
MV-adjusted model‡	2.11 (1.18 to 3.75)	1.00 (referent)	0.95 (0.53 to 1.68)	1.30 (0.72 to 2.35)	2.59 (1.52 to 4.42)
Rectal cancer, overall					
No. of case patients/control participants	75/65	35/44	45/50	47/50	67/60
Crude model‡	1.55 (0.85 to 2.80)	1.00 (referent)	1.14 (0.65 to 2.00)	1.20 (0.66 to 2.16)	1.41 (0.79 to 2.51)
MV-adjusted model‡	1.58 (0.82 to 3.08)	1.00 (referent)	1.21 (0.64 to 2.30)	1.18 (0.61 to 2.24)	1.49 (0.78 to 2.82)
Rectal cancer, men					
No. of case patients/control participants	32/27	20/19	19/24	17/28	40/30
Crude model‡	1.55 (0.85 to 2.80)	1.00 (referent)	1.14 (0.65 to 2.00)	1.20 (0.66 to 2.16)	1.41 (0.79 to 2.51)
MV-adjusted model‡	1.14 (0.35 to 3.64)	1.00 (referent)	1.32 (0.46 to 3.74)	0.58 (0.18 to 1.79)	2.24 (0.70 to 7.16)
Rectal cancer, women					
No. of case patients/control participants	43/38	15/25	26/26	30/22	27/30
Crude model‡	1.54 (0.85 to 2.80)	1.00 (referent)	1.14 (0.65 to 2.00)	1.20 (0.66 to 2.16)	1.41 (0.79 to 2.51)
MV-adjusted model‡	2.83 (1.07 to 7.46)	1.00 (referent)	1.77 (0.67 to 4.69)	2.08 (0.84 to 5.10)	1.56 (0.63 to 3.89)

* Relative risks, estimated from the odds ratios as derived from the risk set sampling design (32) and 95% confidence intervals based on conditional logistic regression. CI = confidence interval; MV = multivariable-adjusted; RR = relative risk.

‡ The crude model takes into account matching factors: age, sex, study center, follow-up time since blood collection, time of the day at blood collection and fasting status. Women were further matched by menopausal status, phase of menstrual cycle at blood collection, and postmenopausal women were matched by hormone replacement therapy use.

‡ The MV-adjusted model is based on the crude model and is further adjusted for smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish and shellfish, and waist circumference.

Sensitivity Analyses

In sensitivity analyses, the strength of the association remained unchanged after excluding individuals diagnosed with cancer within the first two, three, or four years of study follow-up (Table 5). Similarly, the associations were not substantially altered after excluding participants with self-reported prevalent diabetes or with extreme T-N concentrations (data not shown).

Discussion

In this prospective nested case-control study, we found a U-shaped association between T-N concentrations and risk of CRC independent of established CRC risk factors, as well as of inflammatory, metabolic, and oxidative stress biomarkers. Compared with the baseline median T-N concentration of 16.1 nmol/L, the concentrations below 12.7 nmol/L (lowest

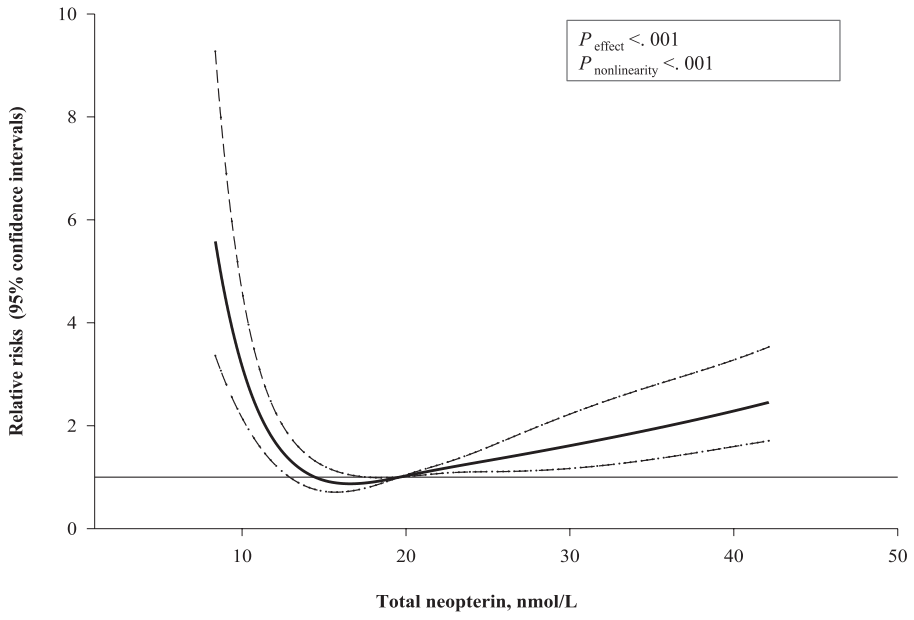

Figure 1. Association of total neopterin with colorectal cancer in a spline regression model including all participants. Based on cubic spline regression with five knots at the 5th, 25th, 50th, 75th, and 95th percentiles of total neopterin distribution in a multivariable-adjusted model taking into account matching factors, age, sex, study center, follow-up time since blood collection, time of the day at blood collection and fasting status. Women were further matched by menopausal status and phase of menstrual cycle at blood collection, and postmenopausal women were matched by hormone replacement therapy use and with additional adjustment for smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish and shellfish, and waist circumference. Median total neopterin concentration among control participants is the reference standard. **Dashed lines** indicate 95% confidence intervals. The two-sided Wald Chi-Square test was used to test for nonlinearity. The null hypothesis is that the effect of total neopterin on colorectal cancer risk is linear. A *P* value of less than .001 indicates a nonlinear association.

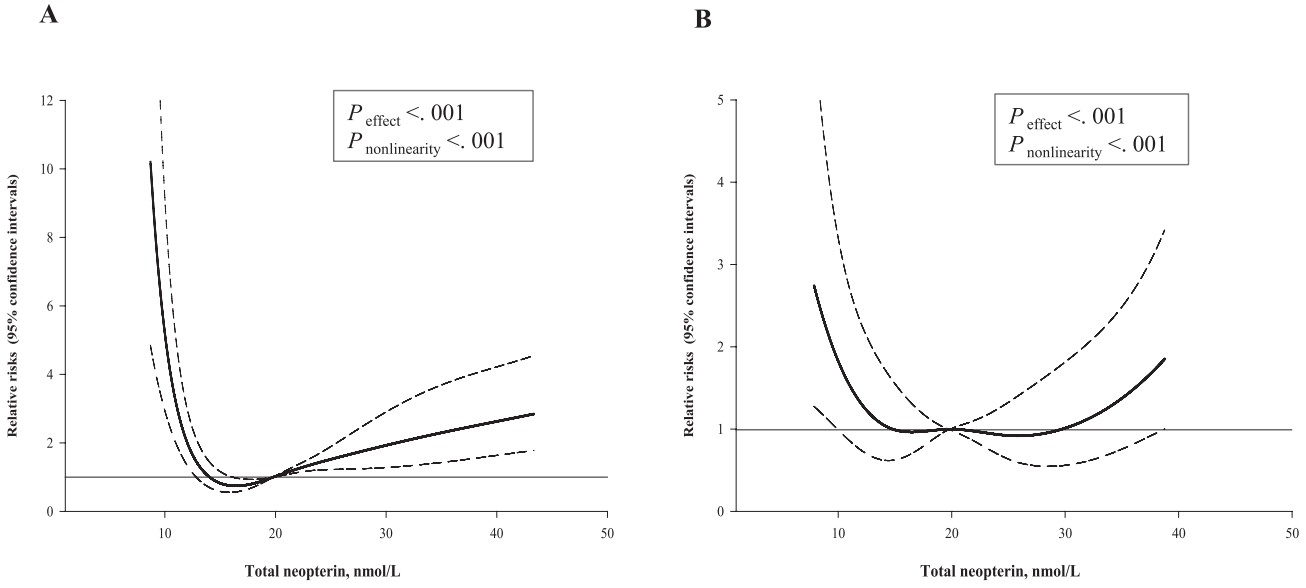

Figure 2. Association of total neopterin with colorectal cancer in a spline regression model by cancer site: **(A)** colon cancer and **(B)** rectal cancer. Based on cubic spline regression with 5 knots at the 5th, 25th, 50th, 75th, and 95th percentiles of total neopterin distribution in a multivariable-adjusted model taking into account matching factors, age, sex, study center, follow-up time since blood collection, time of the day at blood collection, and fasting status. Women were further matched by menopausal status and phase of menstrual cycle at blood collection, and postmenopausal women were matched by hormone replacement therapy use and with additional adjustment for smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish and shellfish, and waist circumference. Median total neopterin concentration among control participants is the reference standard. **Dashed lines** indicate 95% confidence intervals. The Wald Chi-Square test was used to test for nonlinearity. The null hypothesis is that the effect of total neopterin on colorectal cancer risk is linear. A *P* value of less than .001 indicates a nonlinear association. All statistical tests were two-sided.

quintile of T-N distribution) and above 30.7 nmol/L (highest quintile) were associated with a higher CRC risk. The nonlinear shape of the association was present also after excluding case patients diagnosed with cancer within the first four years of study follow-up, arguing against the possibility that results were driven by an existing preclinical disease. These results

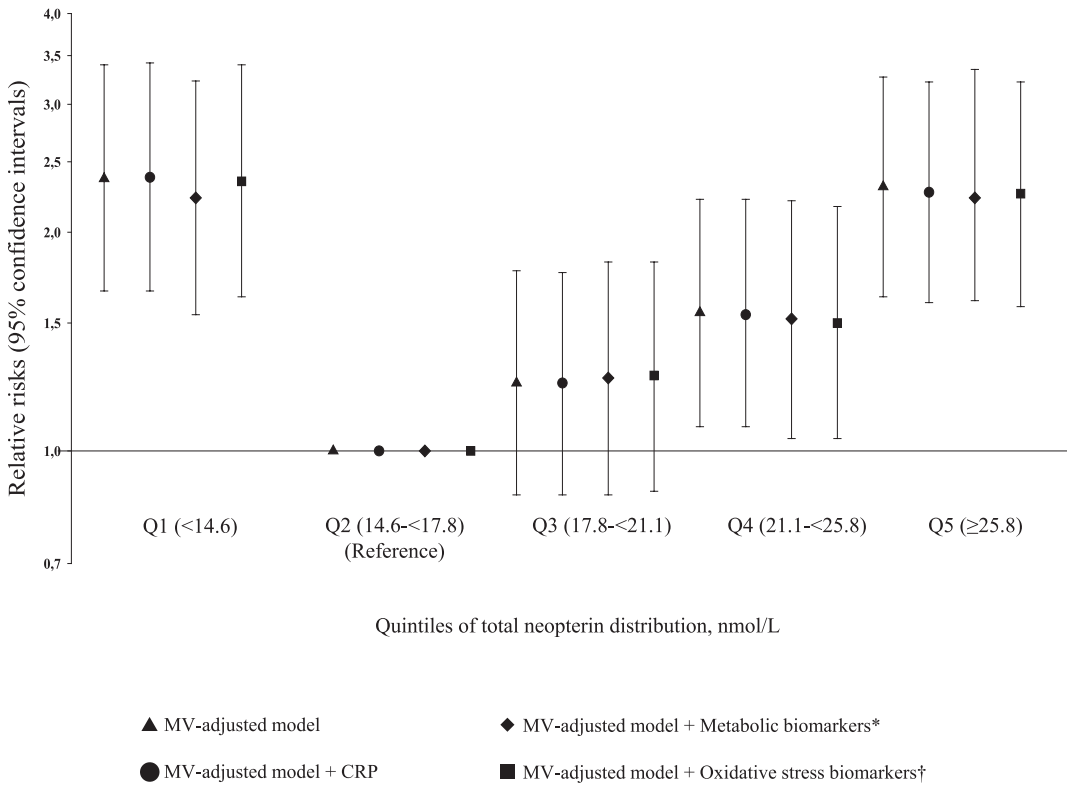

Figure 3. Multivariable-adjusted relative risks and 95% confidence intervals according to quintiles of neopterin concentrations after additional adjustment for C-reactive protein, metabolic and oxidative stress biomarkers. The multivariable-adjusted model takes into account matching factors, age, sex, study center, follow-up time since blood collection, time of the day at blood collection, and fasting status. Women were further matched by menopausal status and phase of menstrual cycle at blood collection, and postmenopausal women were matched by hormone replacement therapy use and with additional adjustment for smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish and shellfish, and waist circumference. The **error bars** represent 95% confidence intervals. Please note median T-N concentrations for the 1st, 2nd, 3rd, 4th, and 5th quintiles are 12.7, 16.1, 19.6, 22.8, and 30.7 nmol/l, respectively. *The list of metabolic biomarkers included: HDL-cholesterol, C-peptide, Hba1c, leptin, solube leptin receptor, adiponectin. †The list of oxidative stress biomarkers included: reactive oxygen metabolites and ferric-reducing ability of plasma. All statistical tests were two-sided. CRP = C-reactive protein; MV = multivariable-adjusted.

ARTICLE

Table 4. Hazard ratios and 95% confidence intervals for incident colorectal cancer across sex-specific quintiles of plasma neopterin concentrations, the Hordaland Health Study, 1998–2010

	Sex-specific quintiles of neopterin concentration*				
Model	1	2	3	4	5
No. of case patients/control participants	27/1281	24/1285	27/1281	36/1273	59/1249
Sex and age-adjusted model	1.36 (0.78 to 2.36)	1.00 (referent)	0.99 (0.57 to 1.73)	1.14 (0.67 to 1.92)	1.74 (1.07 to 2.83)
MV-adjusted model†	1.18 (0.67 to 2.09)	1.00 (referent)	0.93 (0.53 to 1.62)	1.08 (0.64 to 1.84)	1.66 (1.01 to 2.70)

* Median neopterin concentrations (nmol/L) in sex-specific quintiles: 5.34, 6.46, 7.41, 8.61 and 11.31 for men; 5.47, 6.69, 7.71, 8.97 and 11.69 for women, respectively. HR = hazard ratio; MV = multivariable-adjusted.

† The MV-adjusted model in Cox regression analysis is adjusted for sex, age (46–49 years vs 70–74 years), body mass index (normal, overweight, or obese), smoking status (never, former, or current smokers), and physical activity (none/light or moderate/vigorous).

were consistent within each of the EPIC countries and were confirmed also for the associations of plasma neopterin in a replication study within the prospective Hordaland Health Study. To our knowledge, this is the first epidemiological study to report on the existence of a U-shaped association between prediagnostic T-N concentrations and CRC risk.

Previous studies suggested that urinary excretion of neopterin as characteristic of systemic immune activation was elevated in patients with CRC (34) and provided prognostic information for CRC survival (35,36). To account for potential influence of preclinical disease, we excluded cancer case patients diagnosed within the first four years of study follow-up. In these analyses, the associations remained unchanged, suggesting that neopterin metabolism is involved not only in tumor progression, but potentially also in tumor initiation.

In humans, neopterin has been shown to exert pleiotropic effects, therefore we statistically evaluated influences by a number of factors on the potential causal pathway between T-N and CRC. Finding stable associations after accounting for potential influences of inflammatory, metabolic, and oxidative stress biomarkers suggested that T-N represents an independent pathway linking cell-mediated immunity with CRC risk.

Our results suggest both very low and very high levels of T-N distribution to be associated with higher risk of CRC. We

Table 5. Relative risks and 95% confidence intervals of colorectal cancer across quintiles of total neopterin concentrations in sensitivity analyses excluding colorectal cancer case patients in the first years of study follow-up, The European Prospective Investigation into Cancer and Nutrition Study (1992–2003)

Study follow-up years excluded from the analysis	Quintiles of total neopterin concentrations (median, nmol/L)				
	1 (12.7)	2 (16.1)	3 (19.6)	4 (22.8)	5 (30.7)
Two years*					
No. of case patients/control participants	153/166	76/166	88/165	110/166	169/166
MV-adjusted RR (95% CI)†	1.98 (1.32 to 2.96)	1.00 (referent)	1.20 (0.81 to 1.78)	1.42 (0.94 to 2.14)	2.08 (1.42 to 3.06)
Three years*					
No. of case patients/control participants	121/166	57/166	76/165	89/165	127/164
MV-adjusted RR (95% CI)†	2.15 (1.34 to 3.42)	1.00 (referent)	1.29 (0.84 to 2.00)	1.58 (0.99 to 2.52)	1.95 (1.26 to 3.01)
Four years*					
No. of case patients/control participants	91/164	44/164	59/164	69/165	81/164
MV-adjusted RR (95% CI)†	1.95 (1.11 to 3.40)	1.00 (referent)	1.12 (0.68 to 1.83)	1.34 (0.78 to 2.30)	1.40 (0.83 to 2.37)

* Excluded from the analysis were 114 case patients diagnosed in the first two years of study follow-up; 360 case patients diagnosed in the first three years of study follow-up, and 486 case patients diagnosed in the first four years of study follow-up. CI = confidence interval; MV = multivariable-adjusted; RR = relative risk.
† The MV-adjusted model in conditional logistic regression analysis takes into account matching factors: age, sex, study center, follow-up time since blood collection, time of blood collection, and fasting status), with additional adjustment for smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, consumption of red and processed meat, consumption of fish and shellfish, and waist circumference. Women were further matched by menopausal status and phase of the menstrual cycle at blood collection; postmenopausal women were matched by use of hormone replacement therapy.

could see that this tendency consistently exist within all EPIC participating countries, as well as in an independent replication study in Norway. Given that low neopterin may be indicative of reduced immune response at cellular level (37) and given high neopterin levels, an indicator of activated immune response (ie, because of infection), our findings likely reflect these dual pathways for CRC carcinogenesis. Both animal models and human studies have previously suggested that low immune state may be associated with higher cancer risk (38,40). Thus, in mouse models, the adaptive immune system was not efficiently suppressing gastrointestinal tract tumor formation in immune-deficient mice compared with wild-type mice (38). Furthermore, immunosuppressed patients have been shown to have an increased incidence of CRC and adenomatous polyps (39). In line with these observations, our findings possibly reflect the role of lowered immunity in CRC risk (40).

We also observed that highly elevated, but not moderately elevated, T-N concentrations were associated with higher risk of CRC. High levels, compared with moderately high neopterin levels, have been associated with viral rather than bacterial infections (41), which may provide a basis to speculate on the potential involvement of a viral infection as an underlying explanation of this relation; however, our data does not allow us to test such a hypothesis. By contrast, with other cancers of the gastrointestinal tract (gastric carcinoma, mucosa-associated lymphoid-tissue lymphoma), a direct causal link between microbial infection (bacteria and viruses) and CRC has not been established (42). Thus, despite emerging evidence supporting the involvement of viral organisms in oncogenesis, for CRC clinical data are lacking (42). Further studies—both experimental and in human populations—are needed to test potential plausibility of such an interpretation.

We should also note that the risk seemed at minimum and even reached protective values at median T-N concentration of 16.1 nmol/L. This may be explained by the fact that in contrast to the proinflammatory effects of IFN-γ at relatively high concentrations, low-dose IFN-γ appears to exert global suppressive effects on T cell trafficking and anti-inflammatory effects (43).

Neopterin was evaluated as a single biomarker of cell-mediated immune response reflecting INF-γ production by Th1 cells. Since Th1 cells produce also other cytokines such as interleukin-2 and tumor necrosis factor–beta (44), it will be of interest for the future research to examine the potential interactions of these biomarkers with neopterin in CRC development.

Strengths of our study include its prospective design and large sample size, which allowed performing detailed analyses by CRC subsite. Using the unique availability of multiple exposure information in the EPIC study—anthropometric, dietary, lifestyle, and biomarker data—we were able to control our analyses for important determinants of CRC, as well as to evaluate effect of biomarkers on the potential causal pathway between T-N and CRC. Finally, the main results were reproduced within the EPIC countries and in an independent replication study in Norway, therefore results are likely generalizable to these European populations.

Some limitations should be taken into account when interpreting the results. Our assay measures T-N, which is the sum of 7,8-dihydroneopterin and neopterin, in contrast to the ELISA method, which measures only neopterin. Nevertheless, both neopterin and T-N reflect inflammation and are shown to have similar clinical utility in assessment of immune activity (27) and we were able to observe similar results in an independent replication study using plasma neopterin. Furthermore, the associations between T-N and CRP, as well as the other metabolic biomarkers in our data, were comparable with previous reports (45). A single assessment of T-N concentrations at baseline may be susceptible to short-term variation, which could bias results toward the null. However, T-N showed good reproducibility in a recent validation study, where the within-person and between-person CVs ranged from 24.6% to 25.7% and 19.1% to 27.4%, and the overall intraclass correlation coefficients (ICCs) ranged from 0.52 (95% CI = 0.26 to 0.71) for a period of one to two years and 0.67 (95% CI = 0.64 to 0.70) for a period of over 3.5 years, respectively (46). In our study, we were not able to control the analyses for CRC screening. However, it is unlikely that screening history would have largely influenced our results, because at the time of study recruitment population-wide CRC screening programs were not available in most of the EPIC countries (47).

In conclusion, we found a U-shaped association between T-N concentrations and risk of CRC, independent of established CRC risk factors, as well as of inflammatory, metabolic, and oxidative

stress biomarkers. These novel findings suggest that both suppressed and activated cell-mediated immunity as reflected by the T-N concentrations may play an important role for CRC development. Further research to confirm and extend current findings is warranted for understanding the underlying pathology behind these associations.

Funding

Plasma neopterin measurements within the Hordaland Health Study have been supported by the Research Council of Norway (Project No: 204650).

The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort is supported by the Europe Against Cancer Program of the European Commission (SANCO). The individual centers also received funding from: Denmark: Danish Cancer Society; France: Ligue centre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, and Institut National de la Santé et de la Recherche Médicale (INSERM); Greece: the Hellenic Health Foundation, the Stavros Niarchos Foundation, and the Hellenic Ministry of Health and Social Solidarity; Germany: German Cancer Aid and Federal Ministry of Education and Research; Italy: Italian Association for Research on Cancer and the National Research Council; the Netherlands: Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands; Norway: Helga – Nordforsk centre of excellence in food, nutrition and health and the Norwegian Extra Foundation for Health and Rehabilitation, the Norwegian Cancer Society; Spain: Health Research Fund (FIS) of the Spanish Ministry of Health (Exp 96/0032, RETICC DR06/0020), the Spanish Regional Governments of Andalusia, Asturias, Basque Country, Murcia (N^0 6236), and the Navarra and the Catalan Institute of Oncology; Sweden: Swedish Cancer Society, Swedish Scientific Council, and Regional Governments of Skane and Västerbotten; United Kingdom: Cancer Research UK and Medical Research Council. Grant support for the biochemical measurements: High-density lipoprotein cholesterol and triglycerides were analyzed with additional support from the Ministry of Public Health, Welfare and Sports, the Netherlands, German Cancer Aid, Federal Ministry for Education and Research, European Union, European Union and AIRC-ITALY, German Cancer Aid, Federal Ministry for Education and Research, European Union, Stavros Niarchos Foundation, Hellenic Ministry of Health, Hellenic Health Foundation, MRC and Cancer Research UK; Glycated hemoglobin was analyzed with additional support from the National Cancer Institute grant 1RO1CA102460, and data analyses on C-reactive protein were performed with support from World Cancer Research Fund International and Wereld Kanker Onderzoek Fonds (WCRF NL).

Notes

The funders played no role in designing or conducting the study, in the collection, management, analysis, or interpretation of the data, nor the preparation, review, or approval of this manuscript. The authors have no conflicts of interest to disclose.

We thank the participants, study and administrative staff, and researchers in the European Prospective Investigation into Cancer and Nutrition Cohort for their outstanding cooperation. We would like to acknowledge the contribution of Ellen Kohlsdorf, Wolfgang Bernigau, and Sven Knüppel (German Institute of Human Nutrition Potsdam-Rehbruecke) for data management and assistance during data analysis. The authors thank Prof Dr Dietmar Fuchs (Division of Biological Chemistry, Biocentre, Austria) for sharing his opinion on neopterin data interpretation.

References

1. Grizzi F, Bianchi P, Malesci A, et al. Prognostic value of innate and adaptive immunity in colorectal cancer. *World J Gastroenterol*. 2013;19(2):174–184.
2. Boland CR, Luciani MG, Gasche C, et al. Infection, inflammation, and gastrointestinal cancer. *Gut*. 2005;54(9):1321–1331.
3. Huber C, Batchelor JR, Fuchs D, et al. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. *J Exp Med*. 1984;160(1):310–316.
4. Murr C, Widner B, Wirleitner B, et al. Neopterin as a marker for immune system activation. *Curr Drug Metab*. 2002;3(2):175–187.
5. Spencer ME, Jain A, Matteini A, et al. Serum levels of the immune activation marker neopterin change with age and gender and are modified by race, BMI, and percentage of body fat. *J Gerontol A Biol Sci Med Sci*. 2010;65(8):858–865.
6. Frick B, Schroecksnadel K, Neurauter G, et al. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. *Clin Biochem*. 2004;37(8):684–687.
7. Wirleitner B, Schroecksnadel K, Winkler C, et al. Neopterin in HIV-1 infection. *Mol Immunol*. 2005;42(2):183–194.
8. Berdowska A, Zwirska-Korczala K. Neopterin measurement in clinical diagnosis. *J Clin Pharm Ther*. 2001;26(5):319–329.
9. Sucher R, Schroecksnadel K, Weiss G, et al. Neopterin, a prognostic marker in human malignancies. *Cancer Lett*. 2010;287(1):13–22.
10. Hoffmann G, Wirleitner B, Fuchs D. Potential role of immune system activation-associated production of neopterin derivatives in humans. *Inflamm Res*. 2003;52(8):313–321.
11. Murr C, Fuith LC, Widner B, et al. Increased neopterin concentrations in patients with cancer: indicator of oxidative stress? *Anticancer Res*. 1999;19(3A):1721–1728.
12. Blasko I, Knaus G, Weiss E, et al. Cognitive deterioration in Alzheimer's disease is accompanied by increase of plasma neopterin. *J Psychiatr Res*. 2007;41(8):694–701.
13. Oxenkrug G, Tucker KL, Requintina P, et al. Neopterin, a Marker of Interferon-Gamma-Inducible Inflammation, Correlates with Pyridoxal-5'-Phosphate, Waist Circumference, HDL-Cholesterol, Insulin Resistance and Mortality Risk in Adult Boston Community Dwellers of Puerto Rican Origin. *Am J Neuroprot Neuroregen*. 2011;3(1):48–52.
14. Murr C, Winklhofer-Roob BM, Schroecksnadel K, et al. Inverse association between serum concentrations of neopterin and antioxidants in patients with and without angiographic coronary artery disease. *Atherosclerosis*. 2009;202(2):543–549.
15. Ip M, Rainer TH, Lee N, et al. Value of serum procalcitonin, neopterin, and C-reactive protein in differentiating bacterial from viral etiologies in patients presenting with lower respiratory tract infections. *Diagn Microbiol Infect Dis*. 2007;59(2):131–136.
16. Zuo H, Tell GS, Vollset SE, et al. Interferon-gamma-induced inflammatory markers and the risk of cancer: The Hordaland Health Study. *Cancer*. 2014; In press.
17. Wei EK, Giovannucci E, Wu K, et al. Comparison of risk factors for colon and rectal cancer. *Int J Cancer*. 2004;108(3):433–442.
18. Slimani N, Kaaks R, Ferrari P, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. *Public Health Nutr*. 2002;5(6B):1125–1145.
19. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol*. 1997;26(Suppl 1):S6–S14.
20. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 2002;5(6B):1113–1124.
21. *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision – ICD-10*. In. 2 ed. Geneva, Switzerland: World Health Organisation, 2004.
22. Rinaldi S, Rohrmann S, Jenab M, et al. Glycosylated hemoglobin and risk of colorectal cancer in men and women, the European prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev*. 2008;17(11):3108–3115.
23. Haftenberger M, Lahmann PH, Panico S, et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr*. 2002;5(6B):1147–1162.
24. 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(9):1371–1379.

25. Werner ER, Fuchs D, Hausen A, et al. Simultaneous determination of neopterin and creatinine in serum with solid-phase extraction and on-line elution liquid chromatography. *Clin Chem*. 1987;33(11):2028–2033.
26. Fuchs D, Milstien S, Kramer A, et al. Urinary neopterin concentrations vs total neopterins for clinical utility. *Clin Chem*. 1989;35(12):2305–2307.
27. Aleksandrova K, Jenab M, Boeing H, et al. Circulating C-reactive protein concentrations and risks of colon and rectal cancer: a nested case-control study within the European Prospective Investigation into Cancer and Nutrition. *Am J Epidemiol*. 2010;172(4):407–418.
28. Jenab M, Riboli E, Cleveland RJ, et al. Serum C-peptide, IGFBP-1 and IGFBP-2 and risk of colon and rectal cancers in the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer*. 2007;121(2):368–376.
29. van Duijnhoven FJ, Bueno-De-Mesquita HB, Calligaro M, et al. Blood lipid and lipoprotein concentrations and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Gut*. 2011;60(8):1094–1102.
30. Leufkens AM, van Duijnhoven FJ, Woudt SH, et al. Biomarkers of oxidative stress and risk of developing colorectal cancer: a cohort-nested case-control study in the European Prospective Investigation Into Cancer and Nutrition. *Am J Epidemiol*. 2012;175(7):653–663.
31. 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.
32. Prentice RL BN. Retrospective studies and failure time models. *Biometrika*. 1978;65:153–158.
33. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics*. 1995;51(2):524–532.
34. Hronek M, Zadak Z, Solichova D, et al. The association between specific nutritional antioxidants and manifestation of colorectal cancer. *Nutrition*. 2000;16(3):189–191.
35. Weiss G, Kronberger P, Conrad F, et al. Neopterin and prognosis in patients with adenocarcinoma of the colon. *Cancer Res*. 1993;53(2):260–265.
36. Melichar B, Solichova D, Melicharova K, et al. Urinary neopterin in patients with advanced colorectal carcinoma. *Int J Biol Markers*. 2006;21(3):190–198.
37. Ledochowski M, Murr C, Widner B, et al. Inverse relationship between neopterin and immunoglobulin E. *Clin Immunol*. 2001;98(1):104–108.
38. Rogers LM, Olivier AK, Meyerholz DK, et al. Adaptive immunity does not strongly suppress spontaneous tumors in a Sleeping Beauty model of cancer. *J Immunol*. 2013;190(8):4393–4399.
39. Koudougou C, Bonneville M, Matysiak-Budnik T, et al. Review article: antitumoural immunity in colorectal cancer - current and potential future implications in clinical practice. *Aliment Pharmacol Ther*. 2013;38(1):3–15.
40. Hadden JW. Immunodeficiency and cancer: prospects for correction. *Int Immunopharmacol*. 2003;3(8):1061–1071.
41. Denz H, Fuchs D, Hausen A, et al. Value of urinary neopterin in the differential diagnosis of bacterial and viral infections. *Klin Wochenschr*. 1990;68(4):218–222.
42. Collins D, Hogan AM, Winter DC. Microbial and viral pathogens in colorectal cancer. *Lancet Oncol*. 2011;12(5):504–512.
43. Shachar I, Karin N. The dual roles of inflammatory cytokines and chemokines in the regulation of autoimmune diseases and their clinical implications. *J Leukoc Biol*. 2013;93(1):51–61.
44. Romagnani S. Type 1 T helper and type 2 T helper cells: functions, regulation and role in protection and disease. *Int J Clin Lab Res*. 1991;21(2):152–158.
45. Thewissen MM, Damoiseaux JG, Duijvestijn AM, et al. Abdominal fat mass is associated with adaptive immune activation: the CODAM Study. *Obesity (Silver Spring)*. 2011;19(8):1690–1698.
46. 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(5):784–790.
47. Zavoral M, Suchanek S, Zavada F, et al. Colorectal cancer screening in Europe. *World J Gastroenterol*. 2009;15(47):5907–5915.