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Serum bilirubin and 10-year mortality risk in a Belgian population

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Abstract

Objective: The endogenous antioxidant serum bilirubin may scavenge free radicals and protect against free radical-related diseases.
Methods: Using the 10-year follow-up mortality data from the Belgium Inter-university Research on Nutrition and Health (BIRNH) study the association between serum bilirubin and all-cause, cardiovascular, and cancer mortality in 5460 men and 4843 women was investigated.
Results: In men, with the highest ($\geq$0.6 mg/dl) compared with the lowest serum bilirubin concentration ($\leq$0.2 mg/dl), the adjusted relative risk (RR) was 0.73 (95% confidence interval (CI) 0.57–0.94) for all-cause and 0.42 (95% CI 0.26–0.68) for cancer mortality. The risk for cancer mortality decreased with increasing concentrations of serum bilirubin (p for trend = 0.004) especially for non-lung cancer mortality (p for trend = 0.02). The associations persisted after adjusting for smoking. In women the associations between serum bilirubin and cancer mortality were in the same direction, but did not reach statistical significance (RR = 0.76, 95% CI 0.39–1.5). No significant associations were found between serum bilirubin and cardiovascular mortality in men and women.
Conclusions: In this population high serum bilirubin, however, within normal ranges, was associated with low cancer mortality, especially in men. This may be due to the antioxidant activity of bilirubin. Measurement of serum bilirubin concentrations may contribute to cancer risk estimation.

Introduction

During the past decade, interest in the possible role of exogenous antioxidant compounds in health and disease has virtually exploded [1–3]. Besides the diet-derived exogenous oxidant defense system, humans also possess a variety of endogenous antioxidant compounds that may prevent oxidative damage. *In-vitro* studies have shown that bilirubin is a powerful endogenous antioxidant that scavenges peroxyl [4] and hydroxyl [5] radicals. Bilirubin thereby protects linoleic acid [4], lipid membranes [4], its own carrier protein albumin [5] and other proteins from oxidation. Other *in-vitro* studies pointed to bilirubin as an important cytoprotector for tissues that are poorly equipped with antioxidant defense systems such as myocardium [6] and nervous tissue [7].

Toxic free radicals have been reported to play an important role in the etiology of various diseases such as cardiovascular diseases, autoimmune disorders, Alzheimer disease, rheumatoid arthritis and cancer [1–3]. Patients with coronary artery disease (CAD) had lower serum bilirubin concentrations than control subjects [8]. Moreover, the presence of CAD in asymptomatic male Air Force pilots, referred for coronary angiography, inversely correlated with serum bilirubin concentrations [9, 10]. To date, no prospective studies have been carried out on the relation between serum bilirubin and mortality from free radical-associated diseases.

In this prospective study, we investigated the association between serum total bilirubin concentrations and all-cause, cardiovascular, and cancer mortality in a representative sample of the Belgian population. The

present report is part of a larger project on the relation between endogenous antioxidants and mortality.

Materials and methods

Study population

The baseline survey of the Belgian Inter-university Research on Nutrition and Health (BIRNH study) was performed from 1981 to 1984 in subjects aged 25–74 years. On average, 10 years after the subjects' enrollment, the vital status of each subject was ascertained. The aims, design, and methodology of the BIRNH study have been described elsewhere [11]. Briefly, the study was designed to determine dietary composition, prevalence of major risk factors, for cardiovascular diseases, and their relationship in a representative sample of the Belgian population aged 25–74 years. In addition, the association between diet, risk factors, and mortality on population level was a study purpose. Subjects originated from the general population and were sampled from communal population registries. From each of the 42 Belgian counties, three to five communes were randomly selected, with the most populated commune always included. In each of the selected communes, a random population sample was drawn from the voting lists. Subjects in this sample received information on the study and screening procedure and were invited to participate. They were asked to return a reply form to show their interest. Beforehand, all general practitioners of the area had been given information on the study and were asked to motivate interested subjects. Standardized questionnaires were then sent to the participating subjects to survey age, educational level, smoking, menopausal status, use of oral contraceptives, and food consumption (24-h food record method). The questionnaires completed at home were returned to the clinic by the participant. During the clinic visit, the questionnaires were fully reviewed, together with the participant, by a trained dietician. In addition, anthropometric and blood pressure measurements were carried out and a blood sample was taken. The cohort consists of a total of 5949 men (38.6% of the invited men) and 5353 women (34.4% of the invited women) who completed baseline questionnaires and the clinic visit. Subjects with incomplete data on blood chemistry, blood pressure, anthropometry, dietary variables, and mortality (a total of 489 men and 510 women) were excluded from the statistical analysis. The analyses were thus based on data available from 5460 men and 4843 women.

Measurements

A non-fasting blood sample was taken from the antecubital vein with the subject in the supine position. A few hours later the serum was separated and kept frozen at -30 °C.

Within 1 week the serum samples were brought into the laboratory. Serum total bilirubin concentrations were measured on a Technicon Autoanalyzer II continuous-flow analytical instrument that utilizes the automated method of Gambino and Freda [12], based on the method of Jendrassik and Grof. This method uses a strong alkaline buffer solution and measures azodipyrolles as the end-product. A blank channel runs together with the sample channel to correct for endogenous factors in the sample interfering with total bilirubin concentrations.

Serum total and HDL cholesterol concentrations were measured with an enzymatic colorimetric method (CHOD-PAP method; Boehringer Mannheim, Germany). HDL cholesterol concentrations were measured after heparin–manganese precipitation [13]. Other blood clinical chemistry analyses performed at baseline were uric acid, sodium, potassium, chloride, calcium, total protein, creatinine, and alkaline phosphatase concentrations.

Mortality

Ten years after enrollment of each participant vital status and cause of death was retrieved. The vital status of the subject was checked through the national death register. For the deceased, the doctor who had completed the death certificate, and in case of doubt the family or hospital doctor, was contacted to verify vital status and cause of death. For persons alive the mean time of follow-up time was 10.09 ± 0.01 year. Causes of death were based on clinical grounds and coded according to the *International Classification of Diseases*, 9th revision (total cardiovascular disease ICD-9 390–459 and cancers 140–208). Twenty-five participants of the original cohort ($n = 11{,}302$) were lost to follow-up, and the cause was missing for 44 deaths. The doctors verifying vital status and time and cause of death were not aware of the topic of this present study.

Statistical analyses

All statistical analyses were performed with the SAS version 6.12 software package (SAS Institute, Inc., Cary, North Carolina).

Baseline characteristics of the participants were examined according to serum bilirubin concentrations in

five groups for men ($\leq$0.2, 0.3, 0.4, 0.5, $\geq$0.6 mg/dl) and in four groups for women ($\leq$0.2, 0.3, 0.4, $\geq$0.5 mg/dl). Education level was classified into three groups: low (incomplete or completed primary school), medium (high school), and high (professional higher education or university). Smoking was classified into never, former, and current smokers. Smoking any type of tobacco was defined as current smoker.

Differences in characteristics between groups of serum bilirubin concentrations were tested with analysis of variance. Whenever necessary, Scheffé's method was used for pairwise comparisons. Frequencies of categorical variables were tested with χ^2 tests. Cox proportional hazard survival analysis was applied to investigate the relation between serum bilirubin concentrations and all-cause, cardiovascular and cancer mortality considering individual censoring data. The proportionality assumption was confirmed by the visual inspection of the log–log survival curves. Adjustments were made for age (continuous variable), smoking status, education level, menopausal status, body mass index (BMI) (continuous), total cholesterol concentrations (continuous), and some dietary pro-oxidants (continuous) at baseline. Adjusted variables, except for energy percent of polyunsaturated fatty acids (PUFA), were clearly associated with bilirubin concentrations and with mortality risk. Previous *in-vitro* studies indicated that PUFA might be associated with serum bilirubin concentrations. The p for trend was calculated by including bilirubin concentrations as a continuous variable in the models. All relevant analyses were repeated after excluding cases that occurred within the first 3 years of follow-up, to exclude preclinical diseases that already existed at baseline. Additional analyses were made excluding subjects with above-normal levels of serum bilirubin (>1.0 mg/dl) or γ-glutamyl transferase (γ-GT) (>0.50 μkat/L) from the data set in order to avoid bias by existing liver diseases. A p-value < 0.05 (two-sided) was considered to be statistically significant.

Results

Baseline characteristics

The distribution of serum bilirubin concentrations was skewed to the right. The median serum bilirubin concentration was 0.44 mg/dl (range: 0.1–3.8 mg/dl) in men, and 0.35 mg/dl (range: 0.1–2.8 mg/dl) in women. Subjects with high bilirubin concentrations were younger, heavier (men only), and taller as compared with persons with low bilirubin concentrations (Tables 1 and 2). Subjects with low education predominate in the low, and those with high education in the high, concentration groups of serum bilirubin. Current smoking was more prevalent in the lower than in the higher concentration groups of bilirubin. Diets of men and women with the highest serum bilirubin concentrations contained less iron and less PUFA than diets of those with the lowest serum bilirubin concentrations. Dietary intake of vitamin C and β-carotene did not differ between groups with different serum bilirubin concentrations.

Mortality

In this population sample of 5460 men and 4843 women, 712 men and 288 women died during the 10-year follow-up period of 99,450 person-years. Two hundred forty-six men and 121 women died of cardiovascular diseases, which was 4.5% and 2.5% of the male and female population, respectively. Two hundred thirty-four male and 82 of the female deaths were attributed to cancer, respectively 4.3% of the male and 1.7% of the female population. Lung cancer was the most frequent type of cancer death in men (n = 96), and breast cancer in women (n = 11).

Adjusted for age, smoking status, level of education, BMI, total cholesterol, and dietary intakes of iron and PUFA, serum bilirubin was inversely associated with all-cause mortality in men ($p = 0.0960$) (Table 3). Men in the third (relative risk (RR) = 0.75, 95% confidence interval (CI) 0.60–0.95) and the fifth (RR = 0.73, 95% CI 0.57–0.94) group of serum bilirubin had a significantly lower all-cause mortality compared with men in the first group. The inverse association was evident for cancer ($p = 0.004$), but not for cardiovascular mortality ($p = 0.37$). Men with the highest serum bilirubin concentrations had a lower cancer mortality risk compared with men with the lowest serum bilirubin concentrations (RR = 0.42, 95% CI 0.26–0.68). Inverse associations between serum bilirubin and mortality across groups were observed for lung cancer ($p = 0.09$) and significantly for non-lung cancer ($p = 0.02$) mortality.

Higher serum bilirubin concentrations were not significantly associated with a reduced risk from cancer mortality in women (the highest versus the lowest serum bilirubin groups RR = 0.76, 95% CI 0.39–1.5) (Table 4). Associations of all-cause and cardiovascular disease mortality with serum bilirubin were not evident in women.

Excluding the cases that occurred during the first 3 years of follow-up from the analysis (169 male and 50 female deaths), similar associations were observed. Excluding persons with high γ-GT (>0.50 μkat/L) from the analysis (536 men and 170 women, 23 male and 5 female deaths), the associations were stronger both for

Table 1. Baseline characteristics and serum bilirubin concentrations (mg/dl) in men (n = 5460)

	Serum bilirubin (mg/dl)				
	≤0.2	0.3	0.4	0.5	≥0.6
No.	740	1316	1334	880	1190
Age (years)	52 ± 13	51 ± 14	50 ± 14[1,2]	49 ± 13[1,2]	48 ± 14[1,2,3]
Weight (kg)	76 ± 12	76 ± 11	77 ± 11[2]	78 ± 11[1,2]	78 ± 12[1,2,3]
Height (cm)	171 ± 7	171 ± 7	172 ± 7[2]	172 ± 7[1,2]	173 ± 7[1,2,3,4]
BMI (kg/m^2)	26 ± 4	26 ± 4	26 ± 3	26 ± 4[1,2]	26 ± 3[1,2]
SBP (mmHg)	136 ± 18	137 ± 18	136 ± 18	136 ± 19	136 ± 17
DBP (mmHg)	81 ± 12	82 ± 12	82 ± 12	82 ± 12	82 ± 12
Alcohol intake (%)					
0 En%	39	41	36	37	38
<4 En%	23	21	22	21	20
>4 En%	39	38	42	43	42
Education level (%)[a]					
Low	45	45	37	36	33
Intermediate	38	36	37	39	39
High	16	18	25	24	28
Smoking status (%)[a]					
Never smokers	15	18	21	25	27
Former smokers	23	28	30	31	32
Current smokers	60	53	48	44	40
Diet					
Vitamin C (mg/day)	100 ± 61	101 ± 62	99 ± 59	99 ± 56	102 ± 61
β-Carotene (mg/day)	3.2 ± 3.2	3.3 ± 3.7	3.3 ± 3.5	3.0 ± 3.5	3.4 ± 3.7
Iron (mg/day)	16.8 ± 5.8	16.6 ± 5.9	16.3 ± 5.5[1]	16.1 ± 5.4[1,2]	16.1 ± 5.6[1,2]
PUFA (En%)	8.1 ± 4.6	7.6 ± 4.5[1]	7.4 ± 4.3[1]	7.5 ± 4.3[1]	7.6 ± 4.6[1]
Serum					
Total cholesterol (mmol/L)	6.06 ± 1.08	6.12 ± 1.14	6.03 ± 1.14[2]	6.00 ± 1.18[2]	5.92 ± 1.10[1,2,3]
HDL cholesterol (mmol/L)	1.28 ± 0.34	1.26 ± 0.32	1.26 ± 0.33	1.26 ± 0.33	1.25 ± 0.32

Scheffé test for difference from the first[1], second[2], third[3] or fourth[4] group ($p < 0.05$).
PUFA: polyunsaturated fatty acids; En%: percent of energy.
[a] $p < 0.001$ (χ^2 test) for differences among groups.

male (RR = 0.32, 95% CI 0.19–0.55) and female cancer mortality (RR = 0.61, 95% CI 0.29–1.3) comparing the highest with the lowest serum bilirubin concentrations. Excluding persons (121 men and 39 women, 1 male and 2 female deaths) with high serum bilirubin concentrations (>1 mg/dl) the results were less pronounced for male and slightly more pronounced for female cancers. When the number of smoking-years was added in the model the results remained essentially unchanged.

Discussion

In this prospective study low cancer mortality is observed in men and women with high serum bilirubin concentrations. Men in the highest range of serum bilirubin concentrations had a 58% lower risk of developing cancer compared with men in the lowest range. For women a 24% lower risk was not statistically significant. Additional exclusion of the subjects whose serum bilirubin concentrations were outside the normal range yielded similar results.

The BIRNH study was originally set up to study the relation between diet and mortality on a national scale. Therefore, a great effort has been made to draw a random, age- and sex-stratified sample from the Belgian adult population for each district in Belgium. With a participation rate of 36.5%, a somewhat healthier study population sample than the general Belgian population may be expected. A non-response investigation (n = 466) with respect to smoking and nutritional habits, however, indicated no important differences between participants and non-participants for dietary intake and a slightly higher percentage of male smokers and female ex-smokers among the refusals [14]. From the original cohort, nearly all subjects were followed up. The age- and sex-specific mortality rates obtained were comparable to those from official Belgian mortality statistics. The only exception was that women in this study had a lower cancer mortality rate, suggesting a more healthy female study population than the general.

Table 2. Baseline characteristics and serum bilirubin concentrations (mg/dl) in women (n = 4843)

	Serum bilirubin (mg/dl)			
	≤0.2	0.3	0.4	≥0.5
No.	1343	1557	993	950
Age (years)	49 ± 13	50 ± 13	49 ± 13	47 ± 13[1,2,3]
Weight (kg)	67 ± 12	67 ± 12	66 ± 11	65 ± 11[1,2]
Height (cm)	159 ± 6	160 ± 6[1]	160 ± 6[1]	161 ± 6[1,2,3]
BMI (kg/m^2)	26 ± 5	26 ± 5	26 ± 4[1]	25 ± 4[1,2,3]
SBP (mmHg)	132 ± 20	133 ± 22	133 ± 22	129 ± 19[1,2,3]
DBP (mmHg)	80 ± 12	80 ± 12	80 ± 12	79 ± 12
Oral contraceptive users (%)	15	13	13	13
Postmenopausal (%)[a]	48	50	48	41
Alcohol intake (%)				
0 En%	61	62	60	60
<4 En%	22	23	21	20
>4 En%	17	16	19	20
Education level (%)[a]				
Low	48	44	42	38
Intermediate	39	42	41	45
High	13	13	16	17
Smoking status (%)[a]				
Never smokers	73	75	74	70
Former smokers	8	9	8	12
Current smokers	17	15	17	16
Diet				
Vitamin C (mg/day)	97 ± 62	95 ± 56	95 ± 57	93 ± 55
β-Carotene (mg/day)	3.2 ± 4.0	3.4 ± 4.1	3.3 ± 3.5	3.3 ± 3.8
Iron (mg/day)	13.6 ± 4.2	13.6 ± 4.1	13.3 ± 4.2	13.1 ± 4.3[1,2]
PUFA (En%)	8.2 ± 4.7	7.8 ± 4.6[1]	7.6 ± 4.4[1]	7.7 ± 4.8[1]
Serum				
Total cholesterol (mmol/L)	6.20 ± 1.29	6.18 ± 1.29	6.08 ± 1.21[1]	5.93 ± 1.23[1,2,3]
HDL cholesterol (mmol/L)	1.55 ± 0.38	1.53 ± 0.36	1.55 ± 0.38	1.55 ± 0.37

Scheffé test for difference from the first[1], second[2] or third[3] group ($p < 0.05$).
PUFA: polyunsaturated fatty acids; En%: percent of energy.
[a] $p < 0.001$ (χ^2 test) for differences among groups.

Smoking may confound the relation between serum bilirubin and cancer mortality. In agreement with earlier studies [8–10, 15], serum bilirubin concentrations are lower in male current smokers and intermediate in former smokers compared with never smokers. This may be the result of an excess consumption of bilirubin by free radical species from cigarette smoke [16]. Smoking is also related to cancer mortality. After controlling for smoking status the association was still strongly present across the bilirubin groups for lung and even more so for non-lung cancer in males. The present data, therefore, do not suggest that the relation found between bilirubin and mortality is confined to a specific cancer type. Dietary pro-oxidants and antioxidants can influence bilirubin oxidation. *In-vitro* studies indicate that serum vitamin C [17, 18] lowers bilirubin, and that lipid peroxides from PUFA [19] or iron [20] enhance bilirubin oxidation. Our study indicated that intakes of the pro-oxidants rather than the antioxidants varied with serum bilirubin concentrations. Although we have adjusted for known determinants of bilirubin concentration for which information was available in the present study, residual confounding could still be present from known origins (for example fasting status or light exposure) or unknown origins.

The results of this study should also be interpreted with caution, as other factors could have biased the results. This is a *post-hoc* study, since at the time of the baseline survey the possible importance of bilirubin as an antioxidant and of antioxidants in the prevention of disease was still largely unknown. Serum bilirubin was measured only once as part of the blood clinical chemistry evaluation. Since daily variation is present and intra-subject coefficients of variation are reported of 25% [21], repeated measurements could have improved precision. Secondly, in a clinical setting the focus is on detecting high rather than low bilirubin concentrations to diagnose an impaired liver function. The performance

Table 3. Mortality and serum bilirubin concentrations (mg/dl) in men (relative risk and confidence interval)

	Serum bilirubin (mg/dl)					*p* for trend
	≤0.2	0.3	0.4	0.5	≥0.6	
No. of men	740	1316	1334	880	1190	
Total mortality						
Deaths/1000 person-years	19.2	15.1	12.8	13.0	10.6	
Crude	1.00	0.78 (0.63–0.98)	0.66 (0.53–0.83)	0.68 (0.52–0.87)	0.55 (0.43–0.70)	0.0001
Adjusted[a]	1.00	0.82 (0.65–1.0)	0.75 (0.60–0.95)	0.89 (0.69–1.2)	0.73 (0.57–0.94)	0.0960
Cardiovascular diseases						
Deaths/1000 person-years	6.0	4.7	4.6	4.1	4.6	
Crude	1.00	0.79 (0.53–1.2)	0.78 (0.52–1.2)	0.68 (0.43–1.1)	0.78 (0.52–1.2)	0.4306
Adjusted[a]	1.00	0.81 (0.54–1.2)	0.85 (0.57–1.3)	0.87 (0.55–1.4)	1.02 (0.68–1.5)	0.3667
Total cancer						
Deaths /1000 person-years	7.6	5.1	4.3	4.4	2.4	
Crude	1.00	0.66 (0.46–0.96)	0.57 (0.39–0.83)	0.58 (0.38–0.89)	0.31 (0.20–0.49)	0.0001
Adjusted[a]	1.00	0.70 (0.48–1.0)	0.65 (0.45–0.96)	0.78 (0.51–1.2)	0.42 (0.26–0.68)	0.0040
Lung cancer						
Deaths /1000 person-years	2.9	2.0	2.0	2.0	0.8	
Crude	1.00	0.69 (0.38–1.2)	0.67 (0.37–1.2)	0.70 (0.36–1.3)	0.27 (0.12–0.59)	0.0034
Adjusted[a]	1.00	0.73 (0.40–1.3)	0.80 (0.45–1.5)	0.96 (0.50–1.8)	0.40 (0.18–0.89)	0.0898
Non-lung cancer						
Deaths/1000 person-years	4.7	3.0	2.4	2.4	1.6	
Crude	1.00	0.65 (0.41–1.0)	0.50 (0.31–0.83)	0.51 (0.29–0.89)	0.34 (0.19–0.60)	0.0008
Adjusted[a]	1.00	0.67 (0.42–1.1)	0.56 (0.34–0.92)	0.67 (0.38–1.2)	0.43 (0.24–0.77)	0.0195

[a] Adjusted for age, smoking status, education level, BMI, total cholesterol, and dietary intakes of iron and PUFA.

Table 4. Mortality and serum bilirubin concentrations (mg/dl) in women (relative risk and confidence interval)

	Serum bilirubin (mg/dl)				*p* for trend
	≤0.2	0.3	0.4	≥0.5	
No. of women	1343	1557	993	950	
Total mortality					
Deaths/1000 person-years	7.0	5.9	5.6	5.6	
Crude	1.00	0.84 (0.63–1.1)	0.80 (0.58–1.1)	0.78 (0.55–1.1)	0.8414
Adjusted[a]	1.00	0.79 (0.59–1.1)	0.76 (0.54–1.1)	0.87 (0.62–1.2)	0.5771
Cardiovascular diseases					
Deaths/1000 person-years	2.7	2.8	1.9	2.6	
Crude	1.00	1.05 (0.67–1.6)	0.73 (0.42–1.3)	0.96 (0.57–1.6)	0.9274
Adjusted[a]	1.00	0.97 (0.62–1.5)	0.67 (0.38–1.2)	1.04 (0.62–1.8)	0.7392
Total cancer					
Deaths/1000 person-years	2.0	1.7	1.6	1.3	
Crude	1.00	0.89 (0.52–1.5)	0.83 (0.44–1.5)	0.70 (0.36–1.4)	0.3014
Adjusted[a]	1.00	0.82 (0.48–1.4)	0.75 (0.40–1.4)	0.76 (0.39–1.5)	0.1562

[a] Adjusted for age, smoking status, education level, menopausal status, BMI, total cholesterol, and dietary intakes of iron and PUFA.

of bilirubin measurements in lower ranges has not been tested thoroughly. Both factors diminish the measurement precision of bilirubin; however, this will underrather than overestimate the strength of the observed associations with cancer mortality.

The results from the present study do not exclude associations of serum bilirubin and CAD, as were reported earlier [8–10]. Associations of bilirubin concentrations with cardiovascular mortality were probably weaker than with cancer mortality. The number of

deaths may have been too small, and therefore the power too low, to detect associations with cardiovascular mortality among the men and all-cause, cancer, and cardiovascular mortality among the women. The distribution of serum bilirubin concentrations in women, in addition, was narrower than in men.

The putative mechanisms for possible causal associations between serum bilirubin and cancer mortality remain speculative. The serum levels of unconjugated bilirubin, the major fraction of serum bilirubin, depend directly on the production rate of bilirubin and inversely on the removal rate by the liver. For production, heme oxygenase is the rate-limiting enzyme. Heme oxygenase has recently drawn a lot of attention because of its involvement in defense mechanisms against agents that induce oxidative stress [22]. This antioxidant action could be mediated through bilirubin [4, 5, 19, 23]. Bilirubin concentrations are inversely associated with the activity of the enzyme UDP-glucuronosyltransferase (UGT). UGTs eliminate, by glucuronidation, a wide variety of endobiotic and xenobiotic substrates, which include therapeutic drugs and carcinogens. Bilirubin concentrations may, in part, reflect exposure to these compounds.

If the results of the present study are confirmed in other prospective cohorts, serum bilirubin could be considered as a marker for cancer risk. Analyses of bilirubin concentrations are inexpensive and routinely performed in clinical laboratories for other reasons. Even preventive measures to optimalize bilirubin concentrations, *e.g.* smoking cessation and dietary modifications, are theoretically possible and should be further explored.

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