DOI:10.1093/jnci/djt315
Advance Access publication November 18, 2013

ARTICLE

Elevated Plasma Vitamin B12 Levels as a Marker for Cancer: A Population-Based Cohort Study

Johan Frederik Berg Arendt, Lars Pedersen, Ebba Nexo, Henrik Toft Sørensen

Manuscript received February 6, 2013; revised September 22, 2013; accepted September 30, 2013.

Correspondence to: JFB Arendt, BSc, Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Allé 43–45, DK-8200 Aarhus, Denmark (e-mail: johan.frederik.berg.arendt@sun.au.dk).

Background A substantial proportion of patients referred for plasma vitamin B12 (cobalamin [Cbl]) measurement present with high Cbl levels, which have been reported in patients with different cancer types. However, the cancer risk among patients with newly diagnosed high Cbl levels has not been adequately examined.

Methods We conducted this cohort study using population-based Danish medical registries. Patients referred for Cbl measurement with levels greater than the lower reference limit (≥200 pmol/L) were identified from the population of Northern Denmark during the period of 1998 to 2009 using a database of laboratory test results covering the entire population. Data on cancer incidence (follow-up 1998–2010), Cbl treatment, and prior diagnoses were obtained from medical registries. Patients receiving Cbl treatment were excluded. Cancer risks were calculated as standardized incidence ratios (SIRs) with 95% confidence intervals (CIs), stratified by plasma Cbl levels. All statistical tests were two-sided.

Results We identified 333 667 persons without prevalent cancer and not receiving Cbl treatment. Six percent had Cbl levels greater than the upper reference limit (≥601 pmol/L). Cancer risk increased with higher Cbl levels and was highest during the first year of follow-up (Cbl 601–800 pmol/L: SIR = 3.44, 95% CI = 3.14 to 3.76; Cbl >800 pmol/L: SIR = 6.27, 95% CI = 5.70 to 6.88; both $P < .001$). The risks were particularly elevated for hematological and smoking- and alcohol-related cancers for persons with high Cbl levels.

Conclusions High Cbl levels were associated with the risk of subsequently diagnosed cancer, mostly within the first year of follow-up. This may have clinical implications for the interpretation of high Cbl levels.

J Natl Cancer Inst;2013;105:1799–1805

Vitamin B12 (cobalamin [Cbl]) is an essential nutrient involved in one-carbon metabolism and cell division. Daily intake of 2 to 5 μg, together with efficient absorption, transportation, and transformation, are needed to maintain health (1). In clinical practice, measurement of total plasma Cbl is requested widely for the biochemical assessment of Cbl deficiency (2). Three studies have shown that a substantial proportion of patients for whom Cbl measurement is requested have plasma Cbl levels greater than the upper limit of the reference range (3–5), and two of these studies have shown an association between high Cbl levels and cancer (3,5).

The association between elevated plasma Cbl levels and cancer risk is poorly understood. On one hand, a high prevalence of elevated Cbl levels has been reported in patients with liver cancer (6,7), other solid tumors (8,9), and hematological malignancies (10,11). On the other hand, some studies have indicated a high prevalence of cancer, both hematological and solid tumors, among patients with high Cbl levels (3,5). However, the latter studies are limited by their cross-sectional design, and only one study included a comparison group of patients with normal and low plasma Cbl levels (3). Most studies on normal or low Cbl levels in relation to cancer have been negative (12–16), except for some studies showing associations between increasing Cbl levels and lung and prostate cancer (17,18).

Elevated plasma Cbl levels have also been associated with several nonmalignant diseases, including liver diseases, alcoholism, and renal, autoimmune, and infectious diseases (19). Only a few patients with these diseases have high Cbl levels (19). Moreover, these diseases have been reported in a small proportion of patients with high Cbl levels (3–5).

To assess the possible clinical implications of elevated Cbl levels in diagnosing cancer, we conducted a population-based cohort study, examining the incidence of cancer diagnoses among patients with elevated plasma Cbl levels.

Methods

Design, Study Cohort, and Data Sources

We conducted this population-based cohort study using data from medical registries in Northern Denmark over the period from 1998 to 2010. Data were obtained from the Clinical Laboratory

Information System Research Database (LABKA) (20), the Aarhus University Prescription Database (AUPD) (21), the Danish Cancer Registry (DCR) (22), and the Danish National Registry of Patients (DNRP) (23). The Danish Civil Registration System, established in 1968, assigns a civil registration number to all residents, allowing for unequivocal individual-level data linkage among all Danish registries (24).

The LABKA database (20) contains all test results from routine tests performed in hospital laboratories in Northern Denmark, which has a total population of 2.2 million inhabitants. Results of more than 1700 different types of analyses are included in the database. For each analysis, the database stores the test result (or indicates that it is missing), civil registration number, date, and the international Nomenclature, Properties and Units code. For some analyses, a local analysis code is recorded. We identified all patients in the LABKA database with a plasma Cbl measurement of greater than 200 pmol/L [lower reference limit in Northern Denmark = 271 pg/mL (27)] recorded from 1998 through 2009. If a patient had more than one Cbl test result, only the first test was included in our analysis. (See Supplementary Table 1, available online, for all codes used in this study.)

The AUPD (21) collects data on all prescriptions reimbursed to patients in the study area. Most prescription medications in Denmark are eligible for full or partial tax-paid reimbursement. Only over-the-counter drugs and a few prescription medications, such as oral contraceptives and sedatives, are normally not eligible for reimbursement, although reimbursement can be granted on a case-by-case basis. All records in the AUPD include the date of dispensing, the patient's civil registration number, codes for the prescribing physician/clinic/hospital department, the Anatomical Therapeutic Chemical code, and the medication name, pack size, dose units, and manufacturer. Patients were classified as having received Cbl therapy if they had one or more relevant prescriptions recorded in the AUPD up to 2 years before measurement of their plasma Cbl level. These patients were excluded from the analysis. In Denmark, Cbl therapy in pharmacological doses is available only by prescription.

The DCR (22) includes all cancer diagnoses at the individual level in Denmark since 1943, coded according to the *International Classification of Diseases*, *10th revision* (ICD-10). The DCR was used to identify subsequent diagnoses of cancer from 1998 to 2010 among patients with a Cbl test result in the LABKA database. Patients were excluded if they had a cancer diagnosis before the date of the plasma Cbl measurement.

The DNRP (23), established in 1977, contains information on all inpatient hospitalizations and, since 1995, all hospital outpatient clinic visits. The DNRP includes the patient's civil registration number, hospital admission and discharge date, date of hospital outpatient visit, and up to 20 diagnoses coded by physicians, including a primary diagnosis representing the main reason for the inpatient or outpatient hospital contact. All diagnoses are coded according to the *International Classification of Diseases*, *8th revision* during the period from 1977 to 1993 and ICD-10 since 1994. To assess possible confounding from underlying diseases, we obtained data on all diagnoses before Cbl measurement from the DNRP and grouped them according to ICD-10 codes. Patients were classified as inpatients or outpatients if they were admitted and/or had a hospital outpatient clinic visit up to 30 days before or 7 days after Cbl measurement. If no hospital contact was recorded during the defined period, patients were classified as outpatients.

Statistical Analyses

We calculated the expected number of cancers, based on national incidence rates by age, sex, and year of diagnosis in 1-year intervals (25). The number of cancers to be expected if the patients had the same risk as the general population was calculated by multiplying person-years of follow-up by the population-based cancer incidence rates. The risk of cancer in the study cohort was calculated as the standardized incidence ratio (SIR; ie, the ratio of observed cancers to expected cancers). The 95% confidence intervals (CIs) for the SIR estimates were calculated assuming a Poisson distribution of the observed number of specific cancers in the follow-up period. Byar's approximation was used unless the observed number was less than 10, in which case, exact 95% confidence intervals were calculated (26). All statistical tests were two-sided.

In the analyses, we first excluded patients receiving Cbl therapy. A priori, we grouped incident cancers through 2010 into four different categories: smoking and alcohol-related cancers, hematological cancers, immune-related cancers, and hormone-related cancers (see Table 1). We also obtained results for specific cancers. We divided patients into three groups according to plasma Cbl level: 200 to 600 pmol/L (regional reference interval (27) = 271–813 pg/mL); 601 to 800 pmol/L (regional reference interval = 814–1084 pg/mL), and greater than 800 pmol/L (regional reference level = >1084 pg/mL).

Table 1. Cancer groups

Smoking and alcohol-related cancers	Hematological cancers	Immune-related cancers	(Sex) hormone-related cancers
Lip	Non-Hodgkin lymphoma	Cervix uteri	Breast
Mouth	Hodgkin lymphoma	Malignant melanoma	Corpus uteri
Oro- and nasopharynx	Multiple myeloma	Nonmelanoma skin cancer including basal cell and squamous cell carcinoma	Ovary
Larynx	Leukemia	Anus	Prostate
Lung	Unspecified cancer of lymph	Penis	Testes
Esophagus	Unspecified cancer of blood		
Pancreas			
Liver			
Colon and rectum			
Kidney			
Urinary bladder			

Patients were also stratified according to age (0–50 years, ≥51 years), sex, length of follow-up (≤1, >1 year, and >5 years), and receipt of inpatient or outpatient care. Standardized incidence ratios were assessed in 100 to 200 pmol/L Cbl level intervals to examine a possible Cbl cutoff level for high cancer risk. Because high plasma Cbl levels also have been associated with other conditions (19), we assessed cancer risk in relation to selected morbidities, categorized according to ICD-10 codes (see Supplementary Table 1, available online).

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC). The study was approved by the Danish Data Protection Agency (record number: 2009-41-3866).

Results

Study Cohort

We identified 352 831 persons without prevalent cancer who had plasma Cbl levels ≥200 pmol/L, of which 19 164 patients were receiving Cbl therapy at the time of plasma Cbl measurement and were therefore excluded. Of the patients included in the study, 19,665 (6%) had levels greater than the upper reference limit (≥601 pmol/L). The total number of person-years of follow-up was 1 421 512 and median follow-up time was 3.5 years (interquartile range [IQR]= 1.9–6.2 years). The median age was 55.1 years (IQR = 40.2–69.2 years). The distribution of outpatients and inpatients was 276 229 (83%) and 57 438 (17%), respectively. A total of 22 652 (7%) patients in the study cohort subsequently developed cancer during the period from 1998 to 2010.

Cancer Risks

The overall standardized incidence ratio was 1.26 (95% CI = 1.24 to 1.28; $P < .001$) (Table 2). The overall cancer risk increased with higher Cbl levels and with a peak during the first year of follow-up (Cbl 601–800 pmol/L: SIR = 3.44, 95% CI = 3.14 to 3.76, $P < 0.001$; Cbl >800 pmol/L: SIR = 6.27, 95% CI = 5.70 to 6.88, $P < 0.001$). Overall Cbl-associated cancer risk decreased with increasing age. The risk was higher for men than for women. For all cancer groups, the first year risk increased with higher Cbl levels, most substantially for Cbl levels greater than 800 pmol/L. After the first year, the risk remained elevated for patients with Cbl levels greater than 800 pmol/L for smoking and alcohol-related cancers and for hematological cancers. The standardized incidence ratios also remained elevated after the first year in patients with Cbl levels in the 601 to 800 pmol/L range for smoking- and alcohol-related cancers (Table 2). For hematological cancers and smoking- and alcohol-related cancers, the standardized incidence ratios remained elevated for more than 5 years of follow-up, although for hematological cancers, this was the case only for patients with Cbl levels greater than 800 pmol/L (Supplementary Table 2, available online). The numbers and percentages of patients diagnosed with cancer according to plasma Cbl levels and length of follow-up are shown in Table 3 and are as follows: overall: 200 to 600 pmol/L: 6.7%, 601 to 800 pmol/L: 7.8%, and greater than 800 pmol/L: 11.0%; first year: 200 to 600 pmol/L: 2.3%, 601 to 800 pmol/L: 3.7%, and greater than 800 pmol/L: 6.6%; after the first year: 200 to 600 pmol/L: 4.4%, 601 to 800 pmol/L: 4.1%, and greater than 800 pmol/L: 4.4%.

Table 2. Cancer risk diagnosed after a plasma cobalamin (Cbl) measurement according to sex, follow-up interval, age, cancer group, and plasma Cbl levels*

Characteristic	Persons, No.	Incident cancers, No.	Overall SIR (95% CI)	Plasma Cbl levels, SIR (95% CI)		
				200–600 pmol/L	601–800 pmol/L	>800 pmol/L
All Cancers	333 667	22 652	1.26 (1.24 to 1.28)	1.23 (1.21 to 1.24)	1.61 (1.51 to 1.71)	2.38 (2.22 to 2.56)
≤1 year SIR		8103	2.17 (2.13 to 2.22)	2.04 (1.99 to 2.09)	3.44 (3.14 to 3.76)	6.27 (5.70 to 6.88)
>1 year SIR		14 549	1.02 (1.00 to 1.04)	1.01 (1.00 to 1.03)	1.09 (1.00 to 1.18)	1.24 (1.10 to 1.39)
Men	135 485	10 815	1.36 (1.34 to 1.39)	1.32 (1.29 to 1.35)	1.93 (1.75 to 2.12)	2.94 (2.64 to 3.27)
Women	198 182	11 837	1.18 (1.16 to 1.20)	1.15 (1.13 to 1.17)	1.43 (1.31 to 1.55)	2.05 (1.86 to 2.27)
Age						
0–50 years	142 000	2411	1.29 (1.24 to 1.34)	1.26 (1.20 to 1.31)	1.63 (1.32 to 2.00)	2.99 (2.36 to 3.72)
≤1 year SIR		752	2.26 (2.10 to 2.43)	2.14 (1.98 to 2.30)	3.23 (2.24 to 4.52)	9.04 (6.46 to 12.31)
>1 year SIR		1659	1.08 (1.03 to 1.14)	1.07 (1.01 to 1.12)	1.28 (0.97 to 1.64)	1.77 (1.26 to 2.42)
≥51 years	191 667	20 241	1.26 (1.24 to 1.27)	1.22 (1.21 to 1.24)	1.61 (1.50 to 1.71)	2.33 (2.15 to 2.51)
≤1 year SIR		7351	2.17 (2.12 to 2.22)	2.03 (1.98 to 2.08)	3.46 (3.14 to 3.79)	6.09 (5.51 to 6.71)
>1 year SIR		12 890	1.01 (1.00 to 1.03)	1.01 (0.99 to 1.03)	1.07 (0.97 to 1.17)	1.19 (1.05 to 1.34)
Cancer groups						
Smoking- and alcohol-related		9501	1.46 (1.43 to 1.49)	1.40 (1.37 to 1.43)	2.13 (1.95 to 2.33)	3.05 (2.74 to 3.39)
≤1 year SIR		3799	2.75 (2.67 to 2.84)	2.56 (2.47 to 2.65)	4.89 (4.30 to 5.54)	8.37 (7.31 to 9.55)
>1 year SIR		5702	1.11 (1.08 to 1.14)	1.09 (1.07 to 1.12)	1.33 (1.17 to 1.52)	1.44 (1.20 to 1.71)
Hematological		1748	1.85 (1.76 to 1.94)	1.72 (1.63 to 1.81)	2.27 (1.79 to 2.85)	7.96 (6.66 to 9.44)
≤1 year SIR		912	4.52 (4.23 to 4.82)	4.03 (3.75 to 4.32)	6.82 (5.08 to 8.97)	24.14 (19.51 to 29.54)
>1 year SIR		836	1.12 (1.05 to 1.20)	1.10 (1.02 to 1.18)	0.94 (0.60 to 1.40)	2.99 (2.12 to 4.11)
Immune-related		3565	0.93 (0.90 to 0.96)	0.92 (0.89 to 0.95)	1.17 (1.00 to 1.37)	0.97 (0.74 to 1.23)
≤1 year SIR		834	1.08 (1.01 to 1.16)	1.04 (0.97 to 1.12)	1.89 (1.42 to .2.46)	1.37 (0.84 to 2.12)
>1 year SIR		2731	0.89 (0.85 to 0.92)	0.88 (0.85 to 0.92)	0.97 (0.80 to 1.18)	0.85 (0.62 to 1.14)
Hormone-related		5116	1.10 (1.07 to 1.13)	1.10 (1.06 to 1.13)	1.12 (0.96 to 1.29)	1.19 (0.96 to 1.45)
≤1 year SIR		1555	1.62 (1.54 to 1.70)	1.59 (1.51 to 1.67)	1.96 (1.53 to 2.47)	2.61 (1.92 to 3.47)
>1 year SIR		3561	0.96 (0.93 to 0.99)	0.97 (0.94 to 1.00)	0.88 (0.72 to 1.05)	0.78 (0.57 to 1.03)

* All statistical tests were two-sided. SIR = standardized incidence ratio; CI = confidence interval.

Table 3. Percentages and numbers of patients diagnosed with cancer during the study period disaggregated according to plasma cobalamin levels*

	Plasma Cbl levels		
Group	**200–600 pmol/L (n = 314 002)**	**601–800 pmol/L (n = 12 909)**	**>800 pmol/L (n = 6756)**
Overall, % (No.)	6.7 (20899)	7.8 (1013)	11.0 (740)
≤1 year, % (No.)	2.3 (7180)	3.7 (480)	6.6 (443)
>1 year, % (No.)	4.4 (13719)	4.1 (533)	4.4 (297)

* Percentages are the fraction of patients diagnosed with cancer in each cobalamin (Cbl) level group, presented as overall percentages and according to follow-up interval.

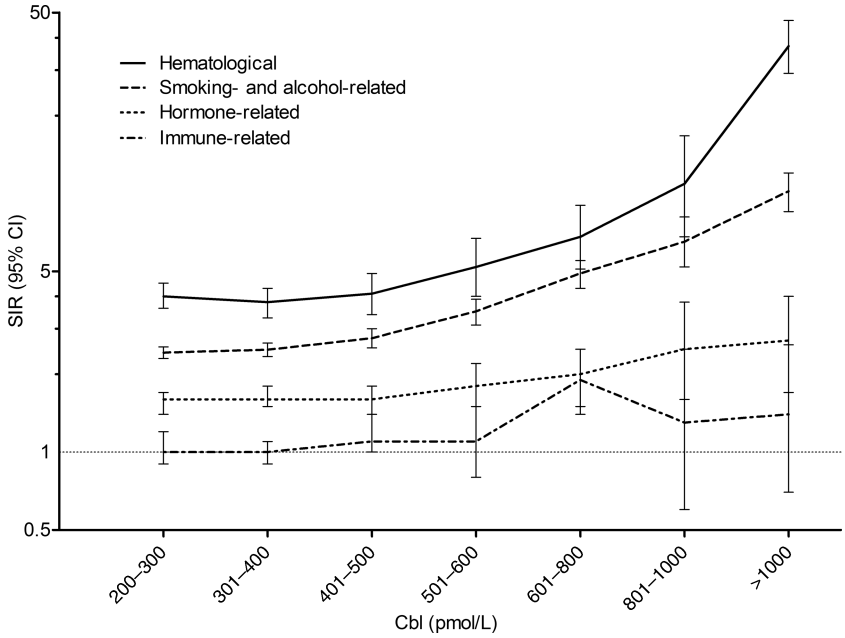

Figure 1. One-year risk of cancer in groups according to plasma cobalamin (Cbl) levels in 100 to 200 pmol/L intervals. The figure shows the 1-year standardized incidence ratios (SIRs) with corresponding 95% confidence intervals (CIs; **vertical bars**) disaggregated according to Cbl levels for hematological cancers (**solid line**), smoking- and alcohol-related cancers (**dashed line**), immune-related cancers (**dotted/dashed line**), and hormone-related cancers (**dotted line**). Note the logarithmic scale for standardized incidence ratio on the y-axis. The **horizontal gray line** indicates a standardized incidence ratio of 1. All statistical tests were two-sided.

Figure 1 presents the first year standardized incidence ratios in detailed Cbl-level intervals. The figure indicates that only risks of smoking- and alcohol-related cancers and hematological cancers substantially increased with higher Cbl levels. Also, it shows that a specific Cbl level cutoff for high cancer risk could not be established. The standardized incidence ratios were elevated fourfold to 37-fold for hematological cancers, twofold to 10-fold for smoking- and alcohol-related cancers, and twofold to threefold for hormone-related cancers; they were not elevated for immune-related cancers.

Patient and Cancer Subtypes

The standardized incidence ratios for inpatients and outpatients showed similar associations, both overall and by cancer group. The highest risks again were found for smoking- and alcohol-related cancers and for hematological cancers. The estimates were highest for inpatients in all cancer groups, although cancer risk was elevated also for outpatients with high Cbl levels (data not shown).

When we examined the association between high Cbl levels and specific cancer types within the first year of follow-up (Figure 2) (SIRs and 95% CIs for Cbl levels >800 pmol/L), we found associations with gastric (SIR = 13.24; 95% CI = 7.23 to 22.21; $P < .001$), colorectal (SIR = 5.48; 95% CI = 4.01 to 7.31; $P < .001$), liver (SIR = 40.70; 95% CI = 25.50 to 61.62; $P < .001$), pancreatic (SIR = 15.57; 95% CI = 10.34 to 22.50; $P < .001$), lung (SIR = 9.27; 95% CI = 7.24 to 11.69; $P < .001$), renal (SIR = 9.56; 95% CI = 4.58 to 17.58; $P < .001$), urinary bladder (SIR = 5.34; 95% CI = 3.11 to 8.55; $P < .001$), lymphatic leukemia (SIR = 8.88; 95% CI = 3.56 to 18.29; $P < .001$), myeloid malignancies (SIR = 105.73; 95% CI = 81.24 to 135.28; $P < .001$), non-Hodgkin lymphoma (SIR = 6.64; 95% CI = 3.31 to 11.89; $P < .001$), and multiple myeloma (SIR = 16.08; 95% CI = 7.70 to 29.58; $P < .001$). The associations increased with higher Cbl levels and were greatest among patients with the highest Cbl levels (>800 pmol/L). The cancer risk remained elevated after the first year of follow-up for liver, pancreatic, lung cancer, and myeloid malignancies, with highest standardized incidence ratios observed for patients with Cbl levels greater than 800 pmol/L (Supplementary Table 3, available online).

The associations between cancer risk and high Cbl levels remained robust in patients with selected morbidities, with no differences among specific morbidities (data not shown).

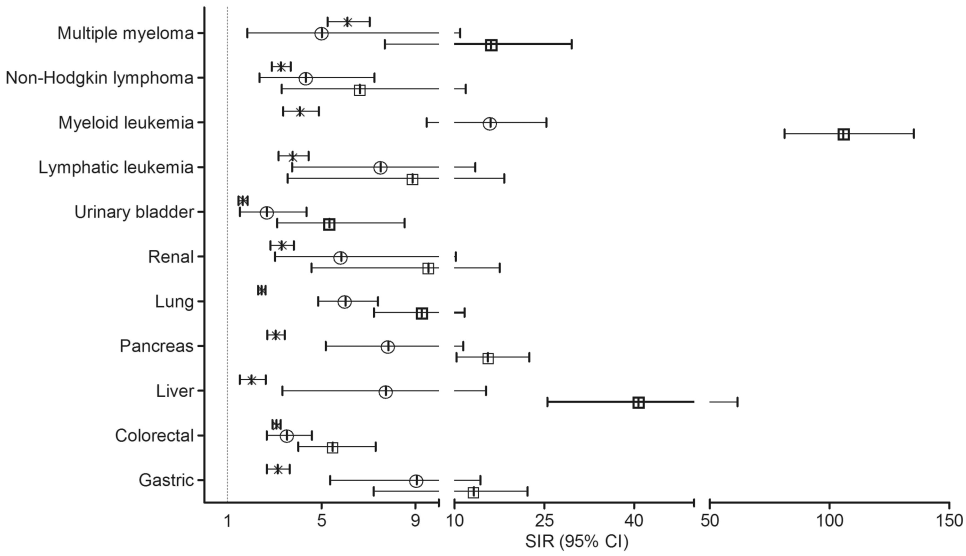

Figure 2. Risk of specific cancer types within the first year after plasma cobalamin (Cbl) measurement. The figure shows the 1-year standardized incidence ratios (SIRs) with corresponding 95% confidence intervals (CIs) disaggregated according to Cbl levels: x: 200 to 600 pmol/L; o: 601 to 800 pmol/L; and □: greater than 800 pmol/L. The **vertical gray line** indicates standardized incidence ratio of 1. All statistical tests were two-sided.

Discussion

Our study shows that elevated plasma Cbl levels are markers for various types of cancers, most notably hematological cancers within the first year after Cbl measurement. These findings remained robust in the stratified analyses.

Our results extend those of earlier research (3,5–11) by showing a strong association between elevated Cbl levels and cancer in a large study with a longitudinal design. Earlier studies have examined Cbl levels in patients with selected cancers. The majority of these previous studies have been negative (12–16), except for lung and prostate cancer (17,18). We focused on high Cbl levels and thereby avoided the limitations of these earlier studies. Our study was based on a large cohort of patients referred for plasma Cbl measurement rather than patients with a particular type of cancer or persons from the general population. Second, unlike the earlier studies, we assessed risk among patients with Cbl levels above a given reference range. Because we specifically focused on high Cbl levels, we were able to demonstrate associations with cancer, including types not previously associated with high Cbl levels, such as cancers of the pancreas and urinary bladder and multiple myeloma.

Our study also was able to examine clinical routine practice. We showed that approximately 15% of the population in Northern Denmark has had a plasma Cbl measurement during the study period and that these measurements show Cbl levels within or above the reference range. This suggests that plasma Cbl measurements are used routinely and frequently to screen for Cbl deficiency in the presence of symptoms or other risks. Although our study cannot directly assess use of high Cbl levels as a nonspecific marker of cancer, our findings may be relevant to the clinical interpretation of such high levels.

A number of different diseases have been suggested as causing high Cbl levels (19), but common to most of them is that the pathogenesis involving elevated Cbl levels is not fully understood. In principle, some of these diseases might be responsible for the long-term associations observed in our study, thereby possibly confounding the associations between high Cbl levels and cancer. For example, the association between liver cancer and high Cbl levels could, in theory, be caused by nonmalignant liver disease underlying the liver cancer. However, when we stratified the analyses according to prior diseases, the results remained robust. This indicates that high Cbl levels could be a marker for cancer both in the short- and long-term, exceeding 5 years for hematological cancers (Supplementary Table 2, available online). Such persistent long-term associations are more likely to be affected by unrecorded confounders, such as lifestyle factors. Although smoking is not associated with disruptions in Cbl status (28), alcoholism and alcohol-related liver disease have previously been linked to high Cbl levels (3,29). This could influence the long-term risk that we observed for some cancers, such as liver cancer, whereas the long-term association with hematological cancers is more peculiar.

Despite its large size and the use of registries to ensure complete follow-up, several possible limitations of our study require consideration. Misclassification of diagnosis is one concern. However, Danish medical registries have proven to be of high validity and completeness (20–23,30), and any potential misclassification of information is likely to be minor and nondifferential (ie, not associated with Cbl levels). Another concern is that we could not directly assess the clinical criteria for measuring plasma Cbl levels because we did not have access to medical files and there are currently no specific guidelines for requesting plasma Cbl measurement. Mainly speculative, unspecific symptoms (eg, anemia, fatigue, weight loss) could be the reason for requesting plasma Cbl measurement, and the same symptoms may also be related to the suspicion of cancer. This may explain why we found elevated standardized incidence ratios also for patients within normal Cbl levels. However, we find it unlikely that the finding of elevated Cbl levels in itself led to increased surveillance for cancer, although this statement cannot

be further assessed. Hence, we find it reasonable to believe that the risk of confounding by indication is minimal. Finally, we did not include concurrent blood test results on other biochemical parameters. Other abnormal test results could influence the probability of a subsequent cancer diagnosis. This may explain the decrease in cancer risk after the first year that we found for immune-related and hormone-related cancers, a compensatory deficit. Finally, we chose to classify cancers a priori to analysis according to type (hematological), life-style factors (smoking- and alcohol-related), and two groups based on potential pathogenesis (hormone related and immune related). To circumvent that some specific cancer types could fall in to more than one of the predefined groups, we also analyzed risk estimates for all specific cancers to obtain more detailed information within the predefined groups.

The underlying pathogenesis leading to high Cbl levels is poorly elucidated, with a few exceptions (6,10,11). It is not thought to involve increased Cbl intake because intestinal absorption capacity is saturable (31) and high physiological consumption does not increase plasma Cbl levels substantially. Only Cbl therapy in the form of injections or extremely high oral doses can produce high circulating levels, and in this study, patients treated with Cbl were excluded. We therefore conclude that the mechanisms resulting in high Cbl levels may be related to malignant pathogenesis. Our recent study showed that levels of the circulating Cbl binding protein haptocorrin were high in patients with high plasma Cbl levels (3). Moreover, cancer was associated with high Cbl and high haptocorrin levels. This protein originates from a variety of tissues, but its physiological function remains unknown (32). It is elevated in patients with some cancer types (6,10,11) and has been suggested as a marker for disease progression (6,10). Thus, haptocorrin may be a candidate factor to include in future studies of the possible pathogenic mechanisms leading to high Cbl levels in cancer patients, in particular for the novel associations demonstrated in this study.

In conclusion, our study showed that high plasma Cbl levels increased the risk of subsequently diagnosed cancer, mostly within the first year of follow-up. However, this association was not present for all cancer types. Although our results may have clinical implications for interpreting high Cbl levels, further studies are warranted to examine the possible diagnostic value of high plasma Cbl levels.

References

1. Nielsen MJ, Rasmussen MR, Andersen CB, Nexo E, Moestrup SK. Vitamin B12 transport from food to the body's cells--a sophisticated, multistep pathway. *Nat Rev Gastroenterol Hepatol*. 2012;9(6):345–354.
2. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. *Am J Clin Nutr*. 2011;94(1):348S–358S.
3. Arendt JF, Nexo E. Cobalamin related parameters and disease patterns in patients with increased serum cobalamin levels. *PLoS One*. 2012;7(9):e45979.
4. Carmel R, Vasireddy H, Aurangzeb I, George K. High serum cobalamin levels in the clinical setting—clinical associations and holo-transcobalamin changes. *Clin Lab Haematol*. 2001;23(6):365–371.
5. Chiche L, Jean R, Romain F, et al. Clinical implications of high cobalamin blood levels for internal medicine. *Rev Med Intern*. 2008;29(3):187–194.
6. Lildballe DL, Nguyen KQ, Poulsen SS, Nielsen HO, Nexo E. Haptocorrin as marker of disease progression in fibrolamellar hepatocellular carcinoma. *Eur J Surg Oncol*. 2011;37(1):72–79.
7. Norredam K, Chainuvati T, Gimsing P, Hippe E, Viranuvatti V. Plasma cobalamin and transcobalamin in patients with primary carcinoma of the liver. A study from Thailand. *Scand J Gastroenterol*. 1983;18(2):229–232.
8. Carmel R, Eisenberg L. Serum vitamin B12 and transcobalamin abnormalities in patients with cancer. *Cancer*. 1977;40(3):1348–1353.
9. Wakatsuki Y, Inada M, Kudo H, et al. Immunological characterization and clinical implication of cobalamin binding protein in human gastric cancer. *Cancer Res*. 1989;49(11):3122–3128.
10. Gimsing P, Overballe-Petersen C, Hippe E. Cobalamin and cobalamin-binding proteins in plasma related to the clinical condition in chronic myelogenous leukemia. *Leukemia*. 1995;9(9):1604–1609.
11. Zittoun J, Zittoun R, Marquet J, Sultan C. The three transcobalamins in myeloproliferative disorders and acute leukaemia. *Br J Haematol*. 1975;31(3):287–298.
12. de Vogel S, Schneede J, Ueland PM, et al. Biomarkers related to one-carbon metabolism as potential risk factors for distal colorectal adenomas. *Cancer Epidemiol Biomarkers Prev*. 2011;20(8):1726–1735.
13. Gibson TM, Weinstein SJ, Mayne ST, et al. A prospective study of one-carbon metabolism biomarkers and risk of renal cell carcinoma. *Cancer Causes Control*. 2010;21(7):1061–1069.
14. Schernhammer E, Wolpin B, Rifai N, et al. Plasma folate, vitamin B6, vitamin B12, and homocysteine and pancreatic cancer risk in four large cohorts. *Cancer Res*. 2007;67(11):5553–5560.
15. Vollset SE, Igland J, Jenab M, et al. The association of gastric cancer risk with plasma folate, cobalamin, and methylenetetrahydrofolate reductase polymorphisms in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol Biomarkers Prev*. 2007;16(11):2416–2424.
16. Zhang SM, Willett WC, Selhub J, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst*. 2003;95(5):373–380.
17. Collin SM, Metcalfe C, Refsum H, et al. Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: a case–control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2010;19(6):1632–1642.
18. Johansson M, Relton C, Ueland PM, et al. Serum B vitamin levels and risk of lung cancer. *JAMA*. 2010;303(23):2377–2385.
19. Arendt JF, Nexo E. Unexpected high plasma cobalamin: proposal for a diagnostic strategy. *Clin Chem Lab Med*. 2013;51(3):489–496.
20. Grann AF, Erichsen R, Nielsen AG, Froslev T, Thomsen RW. Existing data sources for clinical epidemiology: The clinical laboratory information system (LABKA) research database at Aarhus University, Denmark. *Clin Epidemiol*. 2011;3:133–138.
21. Ehrenstein V, Antonsen S, Pedersen L. Existing data sources for clinical epidemiology: Aarhus University Prescription Database. *Clin Epidemiol*. 2010;2:273–279.
22. Gjerstorff ML. The Danish Cancer Registry. *Scand J Public Health*. 2011;39(Suppl 7):S42–S45.
23. Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. *Scand J Public Health*. 2011;39(Suppl 7):S30–S33.
24. Pedersen CB. The Danish Civil Registration System. *Scand J Public Health*. 2011;39(Suppl 7):S22–S25.
25. Sorensen HT, Mellemkjaer L, Skriver MV, et al. Fever of unknown origin and cancer: a population-based study. *Lancet Oncol*. 2005;6(11):851–855.
26. Breslow NE, Day NE. Statistical methods in cancer research. Volume II—the design and analysis of cohort studies. *IARC Sci Publ*. 1987;(82):1–406.
27. Laboratory reference intervals for Northern Denmark (updated 24th of January 2011). https://www.itsundhed.dk/laboratorie_liste/Prog/d_udskriftsvenlig_udgave.aspx?id=179. Accessed January 14, 2013.
28. Ulvik A, Ebbing M, Hustad S, et al. Long- and short-term effects of tobacco smoking on circulating concentrations of B vitamins. *Clin Chem*. 2010;56(5):755–763.
29. Himmerich H, Anghelescu I, Klawe C, Szegedi A. Vitamin B12 and hepatic enzyme serum levels correlate in male alcohol-dependent patients. *Alcohol Alcohol*. 2001;36(1):26–28.
30. Thygesen LC, Daasnes C, Thaulow I, Bronnum-Hansen H. Introduction to Danish (nationwide) registers on health and social issues: structure, access, legislation, and archiving. *Scand J Public Health*. 2011;39(Suppl 7):S12–S16.

31. Bor MV, Lydeking-Olsen E, Moller J, Nexo E. A daily intake of approximately 6 microg vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women. *Am J Clin Nutr*. 2006;83(1):52–58.
32. Morkbak AL, Poulsen SS, Nexo E. Haptocorrin in humans. *Clin Chem Lab Med*. 2007;45(12):1751–1759.

Funding

This work was funded by the Faculty of Health Sciences, Aarhus University, Aarhus, Denmark (to JFBA, LP, EN, HTS); Aarhus University Hospital, Aarhus, Denmark (to LP, EN, HTS); Frits, Georg, and Marie Cecilie Gluds Foundation and Else og Mogens Wedell-Wedellborgs Foundation (to JFBA); and The Danish Cancer Society (grant No. R73-A4284-13-S17) and the Karen Elise Jensen Foundation (to HTS). The Department of Clinical Epidemiology, Aarhus University Hospital, receives funding for other studies from companies in the form of research grants to (and administered by) Aarhus University. None of these studies have any relation to this study.

Note

The sponsors of this study had no role in the initiation, planning, design, or conduct of the study, in data acquisition, management, and analyses, in the interpretation of results, in the writing and approval of the manuscript, or in the decision to submit the manuscript for publication. The researchers of this study declare independence from the sponsors of the study.

Affiliations of authors: Department of Clinical Epidemiology (JFBA, LP, HTS) and Department of Clinical Biochemistry (JFBA, EN), Aarhus University Hospital, Aarhus, Denmark.