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Short Report

Is high vitamin B12 status a cause of lung cancer?

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

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Vitamin B supplementation can have side effects for human health, including cancer risk. We aimed to elucidate the role of vitamin B12 in lung cancer etiology *via* direct measurements of pre-diagnostic circulating vitamin B12 concentrations in a nested case–control study, complemented with a Mendelian randomization (MR) approach in an independent case–control sample. We used pre-diagnostic biomarker data from 5183 case–control pairs nested within 20 prospective cohorts, and genetic data from 29,266 cases and 56,450 controls. Exposures included directly measured circulating vitamin B12 in pre-diagnostic blood samples from the nested case–control study, and 8 single nucleotide polymorphisms associated with vitamin B12 concentrations in the MR study. Our main outcome of interest was increased risk for lung cancer, overall and by histological subtype, per increase in circulating vitamin B12 concentrations. We found circulating vitamin B12 to be positively associated with overall lung cancer risk in a dose response fashion (odds ratio for a doubling in B12 [$OR_{log2B12}$] = 1.15, 95% confidence interval (95%CI) = 1.06–1.25). The MR analysis based on 8 genetic variants also indicated that genetically determined higher vitamin B12 concentrations were positively associated with overall lung cancer risk (OR per 150 pmol/L standard deviation increase in B12 [OR_{SD}] = 1.08, 95% CI = 1.00–1.16). Considering the consistency of these two independent and complementary analyses, these findings support the hypothesis that high vitamin B12 status increases the risk of lung cancer.

What's new?

Several B-complex vitamins have been linked to cancer risk. In this study, high serum levels of vitamin B12 were associated with an increased risk of lung cancer. The authors first ran a nested case-control study, then confirmed their findings using a Mendelian randomization approach based on genetic data from a much larger database including both lung-cancer patients and controls. The authors conclude that these findings support the hypothesis that high circulating vitamin B12 concentrations increase the risk of lung cancer.

Introduction

The potential role of B vitamins in relation to cancer risk has been reported previously.[1–3] Two large randomized controlled trials of B vitamin supplementation in Norway identified an increased risk for overall cancer among subjects who received both vitamin B12 and B9 (folate), a result that was primarily driven by lung cancer.[4] More recently the Vitamins and Lifestyle (VITAL) cohort study[5] reported increased lung cancer risks among men who used high amounts of vitamin B12 and B6 supplementation. These results[4,5] argue against any chemo preventive effect of vitamin B12 in lung cancer, and instead are consistent with high concentrations of vitamin B12 increasing risk.

To further elucidate the role of vitamin B12 in lung cancer etiology, we conducted two large and complementary analyses based on (*i*) directly measured circulating vitamin B12 concentrations in pre-diagnostic samples from over 5000 case–control pairs, and (*ii*) a Mendelian randomization (MR) analysis based on genetic data on close to 30,000 cases and 60,000 controls.

Materials and Methods

The first analysis was based on 5364 lung cancer cases and 5364 controls that were individually matched by age, sex, cohort and smoking status. This sample was nested within 20 individual prospective cohort studies participating in the Lung Cancer Cohort Consortium (LC3), which was initially

Table 1. Baseline and sample characteristics of study participants

	LC3 participants		TRICL-ILCCO participants	
	No.(%) of participants in group		No.(%) of participants in group	
Discrete variables	Cases (n = 5183)	Matched controls (n = 5183)	Cases (n = 29,266)	Controls (n = 56,450)
Sex				
Men	2827 (54.5%)	2827 (54.5%)	18,208 (62.2%)	27,178 (48.1%)
Women	2356 (45.5%)	2356 (45.5%)	11,058 (37.8%)	24,072 (51.9%)
Smoking status				
Never	1267 (24.4%)	1267 (24.4%)	2355 (8.0%)	7504 (13.3%)
Ever (Former and current)	3916 (75.5%)	3916 (75.5%)	23,223 (79.3%)	16,964 (30.1%)
Former	1458 (28.1%)	1458 (28.1%)		
Current	2458 (47.4%)	2458 (47.4%)		
Education				
Less than high school	1746 (33.7%)	1643 (31.7%)		
Completed high school	735 (14.2%)	754 (14.5%)		
Vocational school	862 (16.6%)	886 (17.1%)		
Some college	651 (12.6%)	698 (13.4%)		
College graduate	499 (9.5%)	480 (9.2%)		
Graduate studies	625 (12.2%)	677 (13.1%)		
Unknown	65 (1.2%)	45 (1.0%)		
Continuous variables, median (5th–95th percentile)				
Age at recruitment (years)	60 (44–72)	60 (44–72)	88% higher than 55	
Vitamin B12 (pmol/L)	432 (239–747)	425 (231–733)		
Clinical characteristics, case participants only				
Age at diagnosis, median (range), (years)	69.7 (53.4 81.7)			
Time from blood draw to diagnosis (years)	6.4 (1.0–16.0)			
Histology, No. (%)				
Large cell carcinoma	166 (3.4%)			
Small cell carcinoma	481 (10.1%)		2664 (9.1%)	
Squamous cell carcinoma	813 (17.0%)		7426 (25.4%)	
Adenocarcinoma	1972 (41.2%)		11,273 (38.5%)	
Missing/Unknown	1751 (29.3%)		7903 (27.0%)	

established to interrogate a potential inverse relation between circulating concentrations of B6 and B9 with lung cancer risk.[6,7]

Our study involved centralized biochemical analyses on pre-diagnostic serum/plasma samples and their individually matched controls using a microbiological assay to measure circulating concentrations of vitamin B12,[8] as well as a Liquid chromatography–tandem mass spectrometry (LC–MS/MS) based assay[9] to measure cotinine. After excluding participants with missing values (n = 7) or extreme values of vitamin B12 (> 850 pmol/L, n = 174), a total of 5183 case–control pairs remained for our study (Table 1). To evaluate the relation between directly measured vitamin B12 and lung cancer risk we used conditional logistic regression, additionally adjusted for educational attainment and tobacco exposure (smoking matched by design, as well as cotinine concentrations). Adjusting for body mass index and alcohol intake status did not alter our estimates, and covariates indicating those risk factors were not included in the final model. P-value for trend was calculated with a continuous variable as base 2 logarithm of the circulating concentrations of vitamin B12.

The second investigation involved an MR analysis based on extensive genome-wide data for lung cancer risk from 29,266 lung cancer cases and 56,450 controls of European descent. This extensive genetic data is available from the Transdisciplinary Research in Cancer of the Lung (TRICL) and The International Lung Cancer Consortium (ILCCO) collaborations (Table 1).[10] In the MR framework, genetic variants that are robustly associated with circulating vitamin B12 can be used as proxies and compared between cases and controls, rather than using direct measures of circulating B12 concentrations (as in LC3). The advantage of the MR methodology is that genetic variants are not affected by reverse causation of the disease and are less sensitive to confounding.[11] Single nucleotide polymorphisms (SNP) for circulating vitamin B12 that were previously identified in European populations,[12]

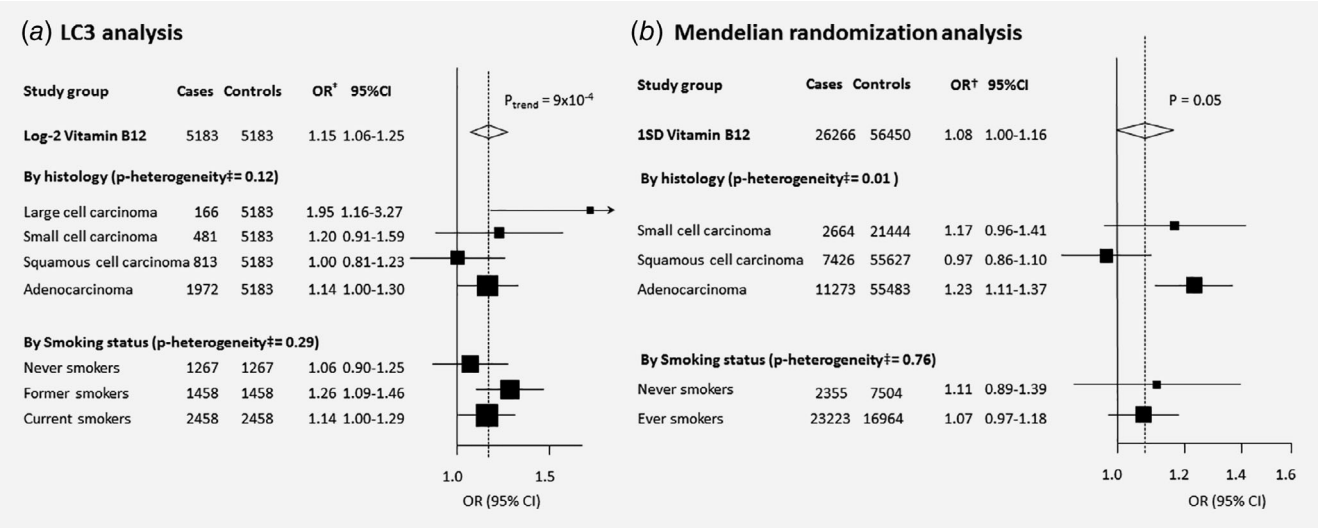

Figure 1. Forest plot showing the relationship between circulating vitamin B12 and lung cancer risk from the LC3 and a Mendelian randomization analysis. Footnote: *LC3 odds ratios (OR) indicate relative risks of a doubling in circulating concentrations (base 2 logarithm transformed) adjusted for cotinine and education when relevant (95%CI: 95% confidence intervals). † Mendelian randomization ORs indicate the odds for a one standard deviation (SD) increase in circulating concentrations (approximately 150 pmol/L). ‡ P heterogeneity indicates results of chi-square test assessing the null hypothesis of ORs being the identical.

including 8 independent SNPs (linkage disequilibrium $R^2 < 0.1$), explained 5.1% of circulating B12 variance.[12] The strength of this instrument was assessed by estimating an F-statistic (306.2), which, given the size of the instrument discovery sample (N = 45,576) gave sufficient power (80%) to detect OR estimates for lung cancer overall (1.10), adenocarcinoma (1.15), squamous cell (1.17) and small cell carcinoma (1.20). The effects on lung cancer risk for predicted B12 vitamin concentrations were estimated using a likelihood-based approach,[13] and the resulting OR estimates reflect a one standard deviation increase (SD) in vitamin B12 concentrations (150.1 pmol/l) based on the discovery study[12]. The instrumental SNPs could show heterogeneity of the estimated effect of vitamin B12 levels on lung cancer risk due to pleiotropic effects of these SNPs from other potential lung cancer risk factors. Thus, sensitivity analyses were performed to assess potential bias (non-balanced pleiotropic effects) on our initial risk estimates.[14] Additionally, we evaluated the association between the genetic proxies of vitamin B12 concentrations and smoking behavior using summary statistics for genetic association with smoking parameters from the Tobacco and Genetics (TAG) Consortium dataset comprising 74,035 participants[15] using a similar MR approach. Finally, by way of reference with the GWAS catalog (https://www.ebi.ac.uk/gwas/) we sought to identify previously reported associations between the 8 SNPs included in this analysis and other known lung cancer risk factors beyond smoking.

Results

Directly measured circulating vitamin B12 was positively associated with overall lung cancer risk in the LC3 consortium (OR for a doubling in vitamin B12 [$OR_{log2B12}$] = 1.15, 95% confidence interval [95%CI] = 1.06–1.25, Fig. 1). Positive associations were seen for adenocarcinoma ($OR_{log2B12}$ [95%CI] = 1.14 [1.00–1.30]) and small-cell carcinoma ($OR_{log2B12}$ [95%CI] = 1.20 [0.91–1.59]), but no association was seen for squamous cell carcinoma ($OR_{log2B12}$ [95%CI] = 1.00 [0.81–1.23]). Subsequent analyses indicated a positive dose–response relation between directly measured circulating vitamin B12 and lung cancer risk (eTable1 in Supporting Information) that was consistently seen among all women, former and current smokers, participants with time from blood draw <72 months and > 120 months (eFig. 1 in Supporting Information), and European/Australian and Asian cohorts (eTable 1 in Supporting Information).

The MR analysis for circulating vitamin B12 based on 8 genetic variants was consistent with the LC3 results, showing that a one SD genetically predicted higher vitamin B12 concentration was associated with an increase in overall lung cancer risk (OR_{SD} [95%CI] = 1.08 [1.00–1.16]). Similar to the LC3 analysis, the MR analysis stratified by histology suggested stronger associations for adenocarcinoma (OR_{SD} [95% CI] = 1.23 [1.11–1.37]) and small-cell carcinoma (OR_{SD} [95% CI] = 1.17 [0.96–1.41]), but not for squamous cell carcinoma (OR_{SD} [95%CI] = 0.97 [0.86–1.10]; p value for heterogeneity = 0.01, Fig. 1). The MR-Egger test did not indicate bias in the risk estimates due to pleiotropy for lung overall (P value for MR-Egger intercept [p_{Int}] = 0.17), nor for any histological subtype (p_{Int} > 0.11). Furthermore, genetically predicted higher vitamin B12 concentrations were not associated with smoking parameters (OR_{SD} being a smoker [95%CI] = 1.00 [0.91–1.11]; number of extra cigarettes smoked per day [95% CI] = −0.13 [−0.82:0.57]), indicating that our MR results on lung cancer risk were not explained by smoking as a

confounder. Finally, the GWAS catalog did not list any other lung cancer risk factor in association with the 8 SNPs used for the current MR analysis. More specifically, the rs1801222 and rs602662 SNPs were associated with homocysteine levels in the one-carbon metabolism pathway, and pediatric autoimmune diseases, respectively.

Discussion

In summary, we performed two complementary and independent analyses to evaluate if elevated concentrations of vitamin B12 increased lung cancer risk.[5] Circulating concentrations of vitamin B12, based on pre-diagnostic blood samples from the LC3 consortium on over 5000 case–control pairs, were positively associated with lung cancer risk, and in contrast to the VITAL study, this association was consistently seen across sexes, former and current smokers, time from blood draw, and geographic region (eFig. 1, Supporting Information). Confirming these results, the MR analysis based on genetic data indicated that higher concentrations of vitamin B12 increased the risk of lung cancer, especially for adenocarcinoma and small-cell carcinoma, with no association seen for squamous cell carcinoma. Generalizability of our results to populations not represented in the data used for the current analyses should be made with caution.

Conclusions

Considering the consistency of these two independent and complementary analyses, as well as previously published studies,[4,5] these findings support the hypothesis that higher circulating vitamin B12 concentrations increase the risk of lung cancer.

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