

The predictive value of vitamin B12 concentrations and hyperhomocysteinaemia for cardiovascular disease

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Background. Cardiovascular disease has been associated with both homocysteine and vitamin B12 levels. However, little information is available about the mutual relation in cardiovascular patients. The aim of this study was to assess the prevalence of vitamin B12 deficiency in patients with cardiovascular disease, and to study the correlation with homocysteine levels.

Methods. Blood samples were taken from 229 patients who had been admitted to the Coronary Care Unit of the Heart-Lung Centre of the Radboud University Medical Centre in Nijmegen, the Netherlands. Patient demographics and clinical characteristics were assessed from medical files. Adjusted logistic regression was used to study the associations between vitamin B12, homocysteine and ischaemic heart disease.

Results. In 70 patients (33%) serum vitamin B12 levels were below the lower limit of normal (<203 ng/l). Sixty-nine patients (33%) had vitamin B12 concentrations in the lower normal range (between 203 and 339 ng/l). Plasma homocysteine levels above the upper limit of normal were found in 83 out of the 229 patients (36%). Adjusted odds ratios for both vitamin B12 (0.76, 95% CI 0.44-1.30) and homocysteine (1.27, 95% CI 0.74-2.18) levels did not show a statistical association with ischaemic heart disease. No association was found between serum vitamin B12 levels and plasma homocysteine.

Conclusion. Our data suggest that hyperhomocysteinaemia and low serum vitamin B12 concentrations are independent and cannot be used as a diagnostic tool for ischaemic heart disease. (*Neth Heart J* 2007;15:291-4.)

Keywords: vitamin B12, cardiovascular disease, homocysteine

Low serum vitamin B12 concentrations are common in elderly patients in the general population.¹⁻⁵ Humans are dependent on dietary intake of vitamin B12, mainly from animal food products, such as meat, fish and dairy products. Vitamin B12 deficiency may lead to a wide range of complications, of which megaloblastic anaemia is the most common.⁶⁻⁸

Absorption of vitamin B12 is a complex process, in which the stomach plays an important role. First, digesting enzymes liberate vitamin B12 from its binding protein. Free vitamin B12 subsequently binds to intrinsic factor, produced by parietal cells, to form an IF-B12-complex. Only the IF-B12-complex can be absorbed in the terminal ileum. Because of the complexity of this absorption process, there are several causes of vitamin B12 deficiency, such as low dietary intake, low production of protein digesting enzymes, low production of intrinsic factor, as a result of atrophic gastritis (whether or not induced by an infection with *Helicobacter pylori*) or impaired absorption by ileal pathology, respectively.^{1,9}

Several studies have shown that plasma homocysteine concentrations are significantly higher in patients with coronary artery disease, compared with the general population.^{10,11} Vitamin B12 is, besides folate and vitamin B6, an important factor involved in homocysteine metabolism. Therefore, deficiencies of these vitamins may account for hyperhomocysteinaemia.¹² The results of the Framingham study indicate that folate, vitamin B6 and vitamin B12 are important determinants of plasma homocysteine concentration in the healthy population.² However, direct comparison between serum vitamin B12 concentrations and homocysteine levels in patients admitted for cardio-

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vascular disease has not been performed. Moreover, recent studies have found that interventions with the B-vitamins do not result in decreased incidence of cardiovascular outcomes.¹³⁻¹⁵

The aim of this study was therefore to assess the prevalence of vitamin B12 deficiency in patients with cardiovascular disease and its correlation with plasma homocysteine levels. Moreover, the association between reason for admission and both vitamin B12 deficiency and hyperhomocysteinaemia was analysed to test for diagnostic usability.

Patients and methods

Subjects

This study was conducted in the Departments of Cardiology and Gastroenterology & Hepatology of the Radboud University Nijmegen Medical Centre in Nijmegen, the Netherlands. Consecutive patients were recruited on the Coronary Care Unit of the Heart-Lung Centre between February and May 1998 and overnight fasting blood samples were taken the day after admission.

Vitamin B12

The assessment of serum vitamin B12 concentrations in the study population was performed with the DPC Immulite (Diagnostic Products Corporation, Los Angeles, CA). The vitamin B12 assay serum samples were chemically pretreated in an automated cycle to release vitamin B12 from binding proteins. An aliquot of pretreated sample, hog intrinsic factor (HIF) and an alkaline phosphatase-labelled monoclonal antibody against HIF (anti-HIF MAb) were introduced into the reaction tube containing a vitamin B12-coated bead. B12 from the patient sample and B12 on the bead competed for HIF. A fraction of the enzyme-labelled anti-HIF MAb became complexed with the HIF that bound to the vitamin B12 on the bead. The chemiluminescent substrate was added and underwent hydrolysis in the presence of the enzyme label. This reaction yielded a result that related inversely to the vitamin B12 concentration in the patient sample. The anti-HIF MAb used in this assay was specifically produced and selected to bind HIF without interfering with the latter's ability to bind vitamin B12. In the assay, unbound materials were removed by a patented centrifugal wash technique. The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, yielded an unstable intermediate in the presence of the alkaline phosphatase label. The continuous production of this intermediate resulted in a sustained emission of photons, allowing multiple readings for enhanced precision. The results were automatically adjusted for dilution. Samples were run at the same time to minimise assay variability. Normal values for vitamin B12 in the healthy population had previously been assessed in the laboratory. In healthy volunteers, the lower limit of normal, defined as mean minus twice the standard

deviation was 203 ng/l. Based on these values, vitamin B12 concentrations <203 ng/l were classified as lower than normal, between 203 and 339 ng/l as low-normal, and above 339 ng/l as normal.

Homocysteine

The analysis of homocysteine was performed by high performance liquid chromatography with fluorescent detection and is essentially as described by Fortin et al. with some modifications.¹⁶ In short, 100 µl plasma was reduced by adding 10 µl tris(2-carboxyethyl)-phosphine hydrochloride for 30 minutes. Proteins were precipitated with 100 µl perchloric acid for five minutes followed by centrifugation for five minutes at 10,000 xg. Subsequently the supernatant (100 µl) was incubated with 20 µl sodium hydroxide, 200 µl borate buffer and 20 µl 7-fluorbenzofurazane-4-sulphonic acid at 60 °C for one hour. Of the derivative sample 20 µl was injected and thiols were eluted with an isocratic eluent at flow rates of 350 µl/min for five minutes and 600 µl/min for another five minutes. Concentration of homocysteine was determined using calibration curves containing mixtures of all thiols. Hyperhomocysteinaemia was defined as homocysteine concentrations higher than 17 µmol/l, since this was the upper limit of normal, defined as mean plus twice the standard deviation, in the healthy population derived from our geographical area.

Statistical analysis

Demographics and reasons for hospitalisation were assessed on admission by a standard form filled in by the cardiologist. Reasons for hospitalisation were classified into three main categories: ischaemic heart disease (myocardial infarction or (un)stable angina pectoris), arrhythmia (cardiac arrhythmia, such as atrial fibrillation or ventricular arrhythmia) and other.

The differences between demographic characteristics, reasons for hospitalisation and serum vitamin B12 and plasma homocysteine concentrations were evaluated with a χ^2 test. Adjusted regression analysis was used to assess the association of age, gender and the presence of vitamin B12 deficiency or hyperhomocysteinaemia and an ischaemic event. Finally, the correlation between serum vitamin B12 and plasma homocysteine concentrations was studied using Spearman's test for correlation.

Results

A total of 229 consecutive cardiovascular patients (151M, 78F), with a mean age of 62 (SD=13) years, were studied. Low vitamin B12 serum concentrations were found in 70 (33%) patients, low-normal vitamin B12 concentrations in 69 (33%) patients, while a total of 70 (33%) patients were considered to have normal serum vitamin B12 concentrations. Almost half of the patients aged ≥ 65 years ($n=65$; 48%) had lower vitamin B12 levels, compared with only 40% ($n=38$) of the patients aged <65 years ($p=0.22$). Infection with

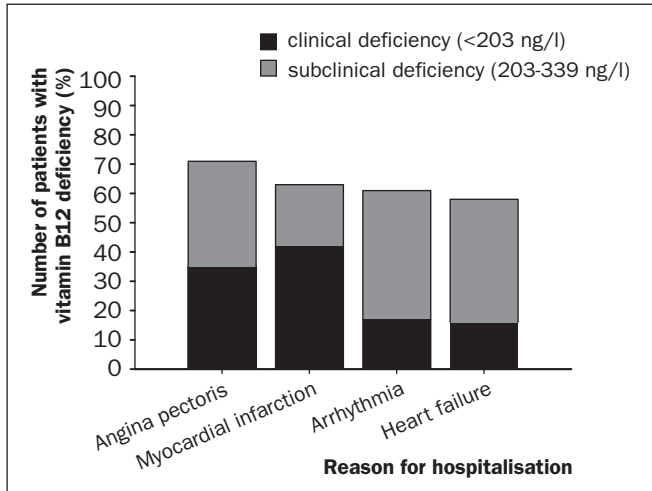


Figure 1. The association between reason for hospitalisation of the patients and vitamin B12 deficiency.

Helicobacter pylori was negatively associated with lower vitamin B12 levels: a total of 43 (38%) *Helicobacter pylori* positive patients had lower vitamin B12 levels, compared with 60 (51%) in negative patients ($p=0.04$).

The patient population was hospitalised because of (un)stable angina pectoris ($n=117$; 51%), acute myocardial infarction ($n=48$; 21%), arrhythmia ($n=25$; 11%), heart failure ($n=21$; 9%) and other reasons ($n=18$; 8%). The reasons for hospitalisation and their association with vitamin B12 deficiency are shown in figure 1. In patients hospitalised for angina pectoris (AP) or myocardial infarction, lower vitamin B12 serum concentrations were measured ($p=0.02$). Plasma homocysteine levels were above the upper limit of normal in 83 of the 229 patients (38%).

We did not find any statistically significant correlation (Spearman's correlation $r=0.00$, $p>0.05$) between serum vitamin B12 concentrations and plasma homocysteine concentrations in our patient population (figure 2).

The risk of ischaemic heart disease is shown in table 1 as (adjusted) odds ratios (OR) for both vitamin B12 deficiency and hyperhomocysteinaemia. Adjusted odds ratios for both vitamin B12 and homocysteine levels

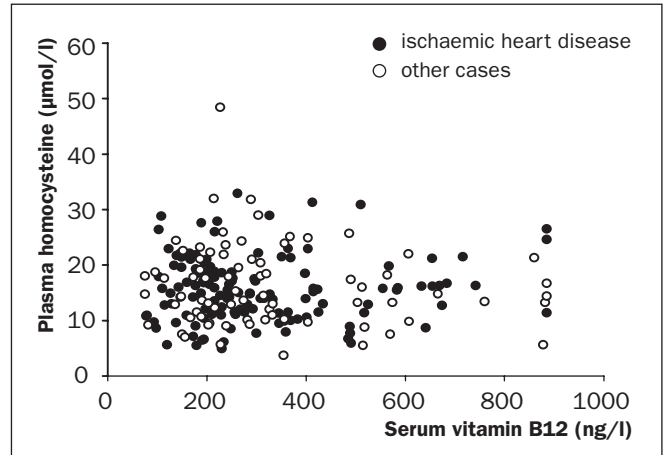


Figure 2. Correlation between serum vitamin B12 and plasma homocysteine concentrations in patients with cardiovascular diseases (Spearman's correlation $r=0.00$, $p>0.05$).

did not show a statistical association with ischaemic heart disease. Only high age was statistically significantly associated with the risk of ischaemic heart disease.

Discussion

In this study we have assessed the prevalence of vitamin B12 deficiency and hyperhomocysteinaemia in patients admitted to our coronary care unit. Our results showed that low serum vitamin B12 concentrations and hyperhomocysteinaemia are extremely common in patients admitted to a coronary care unit, especially in those with ischaemic heart disease, but were not inter-correlated and were not diagnostically accurate.

Hyperhomocysteinaemia has been associated with cardiovascular disease in several meta-analyses.^{10,11} It is widely believed that most slightly to moderately high homocysteine concentrations in the general population are due to vitamin deficiency and/or enzyme defects, such as the MTHFR C677T mutation. There is limited information available about the association between homocysteine and vitamin B12 in patients with cardiovascular disease.

Few studies have evaluated the relation between homocysteine and vitamin B12. The results of the Framingham study indicate that folate, vitamin B6 and

Table 1. Risk of ischaemic heart disease for vitamin B12 deficiency (serum vitamin B12 concentration <339 ng/l) and hyperhomocysteinaemia (plasma homocysteine concentration <17 mmol/l).

	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)
Vitamin B12 deficiency	0.74 (0.43-1.27)	0.76 (0.44-1.30)
Hyperhomocysteinaemia	1.40 (0.86-2.29)	1.27 (0.74-2.18)
Male gender	1.15 (0.80-1.64)	0.81 (0.47-1.41)
Age ≥ 65 years	1.83 (1.33-2.50)	2.02 (1.13-3.71)

* adjusted for all tested variables listed in the left column. OR=odds ratio, CI=confidence interval.

also vitamin B12 are important determinants of plasma homocysteine concentration in the healthy population.² The reason for this association is not apparent, but several possible explanations can be put forward. In the first place, the correlation between low serum vitamin B12 and hyperhomocysteinaemia is much weaker than the correlation between low serum folic acid and hyperhomocysteinaemia; therefore our patient population might have been too small to demonstrate this association.

Secondly, the use of cardiovascular medication by our patient group or comorbidity might have influenced the correlation, but we have not found any indications for this in the recent literature. Thirdly, it has been suggested that methylmalonic acid concentrations are more meaningful for assessing vitamin B12 deficiency. The importance of methylmalonic acid was specially demonstrated in studies underestimating vitamin B12 deficiency based on serum concentrations. But, our study had already found that 33% of the patients had low vitamin B12 serum concentrations. The true reason for the lack of inverse correlation between serum vitamin B12 and homocysteine concentrations in our patients remains to be elucidated. Therefore, our data suggest that hyperhomocysteinaemia and low serum vitamin B12 concentrations are independent and may not be risk factors for cardiovascular disease.

What other issues are raised by the study? First, homocysteine concentrations and cardiovascular disease both increase, and serum vitamin B12 concentrations decrease with age. It is possible that the current observations are, in reality, an effect of age, rather than disease. However, adjusted for age, only ischaemic heart disease and not age was a significant independent predictor of hyperhomocysteinaemia and low serum vitamin B12 concentrations. Second, there is no universally accepted definition of hyperhomocysteinaemia. In this study we defined hyperhomocysteinaemia as homocysteine concentrations two standard deviations above the mean, although this is controversial. Furthermore, this 'normal range' includes individuals with cardiovascular disease. This implicates that the differences between those with and those without ischaemic heart disease might be even greater than indicated by the data presented. Third, there is continuing debate on whether homocysteine concentrations should be measured in fasting plasma. Despite the ongoing debate it is still the golden standard. However, unless the patient has ingested a large protein-rich meal, non-fasting concentrations will be elevated by only 10% or less.¹⁷ We believe that it is unlikely that use of fasting samples would have significantly affected the present data and their interpretation. Fourth, is the relationship observed between hyperhomocysteinaemia and low vitamin B12 on the one hand and cardiovascular disease on the other associative or causal? And if causal, does cardiovascular disease cause hyperhomocysteinaemia and low serum vitamin

B12 concentrations, or do hyperhomocysteinaemia and low serum vitamin B12 concentrations contribute to development or progression of the disease? These questions need to be answered in future studies.

In conclusion, hyperhomocysteinaemia and low serum vitamin B12 concentrations are not associated and not diagnostically accurate as risk factors for ischaemic heart disease. Hyperhomocysteinaemia is found in 36% of patients with cardiovascular disease, whereas low vitamin B12 concentrations are found in 33% of these patients. Although vitamin B12 plays a role in homocysteine metabolism, hyperhomocysteinaemia and low serum vitamin B12 concentrations seem to be independent in patients hospitalised for cardiovascular disease. ■

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