

Hyperhomocysteinaemia and Vitamin B12 Deficiency: The Long-Term Effects in Cardiovascular Disease

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Key Words

Homocysteine · Vitamin B12 · Ischaemic heart disease · Long-term effects

Abstract

Background: An elevated plasma homocysteine level is an established risk factor for cardiovascular disease. Vitamin B12 plays a key role in homocysteine metabolism and could be the main factor in causing cardiovascular disease as well.

Objectives: The aim of this study was to assess whether vitamin B12 deficiency or hyperhomocysteinaemia is associated with recurrent cardiovascular events. **Methods:** Overall, 211 patients discharged alive from our Coronary Care Unit were recruited from February till May 1998. Serum vitamin B12 and plasma homocysteine levels were measured in fasting blood samples. Patient characteristics, medical information and cardiovascular risk factors were assessed from medical files. Patients were followed for 5 years and the prevalence of cardiovascular mortality and morbidity was collected. **Results:** In the follow-up period of 810 person-years, 48 (21%) of the patients experienced a nonfatal recurrent cardiovascular event and another 14 (7%) died of a cardiovascular cause. Among those with ischaemic heart disease at discharge, no difference in survival was found between the patients with a low (<250 pmol/l) or a high vita-

min B12 level ($p = 0.21$). In patients with hyperhomocysteinaemia ($>16 \mu\text{mol/l}$), an increased risk of a recurrent cardiovascular event ($p = 0.05$) in comparison to those with normal plasma homocysteine levels was proven (adjusted hazard ratio of 2.22 (95% CI: 1.40–3.04)). **Conclusions:** In conclusion, high plasma homocysteine concentration, but not a low serum vitamin B12 concentration, increases the risk of cardiovascular morbidity and mortality in patients with ischaemic heart disease.

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Introduction

Many studies have been undertaken to examine causality between plasma homocysteine and coronary heart disease. The general outcome supports the hypothesis that an elevated plasma homocysteine plasma concentration leads to an increased risk of ischaemic heart disease and mortality [1–6].

In the development of hyperhomocysteinaemia, B-vitamins play an important role. Folic acid, vitamin B12 and B6 are all involved in the remethylation of homocysteine. Low levels of even one B-vitamin can increase the plasma homocysteine levels in humans. Patients with hyperhomocysteinaemia are best treated with folic acid.

However, patients with hyperhomocysteinaemia due to vitamin B12 deficiency still remain vitamin B12 deficient.

In this study, we analysed the relationship of vitamin B12 deficiency with plasma homocysteine in cardiovascular patients [7, 8]. Vitamin B12 deficiency and cardiovascular diseases are both common in the elderly [9], but the association between serum vitamin B12 levels and cardiovascular diseases has not been studied before.

The purpose of this study was to test whether a low serum vitamin B12 concentration is responsible for an increased cardiovascular related mortality and morbidity in relation to plasma homocysteine levels.

Methods

Subjects

This study was conducted at the Departments of Cardiology and Gastroenterology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Patients with cardiovascular disease discharged alive from the Coronary Care Unit between February and May 1998 were included. Patient characteristics (gender, age, height, weight and blood pressure), medical history and the pre-existence of cardiovascular risk factors, e.g., hypertension, decreased high-density lipoprotein level, hyperlipidaemia, cigarette smoking, family history of cardiovascular disease and diabetes mellitus were assessed at the moment of alive discharge. Cardiovascular disease was subdivided in different categories depending on the discharge diagnosis: ischaemic heart disease (myocardial infarction and (un)stable angina pectoris), arrhythmia (like atrial fibrillation and ventricular arrhythmia), heart failure and others.

Fasting blood samples were taken and serum vitamin B12 and plasma homocysteine concentrations were measured with Immulite method (DPC, Los Angeles, Calif., USA). Samples were run at the same time to minimize assay variability. For subdivision of the assessments done in the laboratory, cut-off points were solely based on laboratory measurements.

Definitions

Patients with a vitamin B12 deficiency were defined as having serum concentration <250 pmol/l, following the manufacturer's directions. If plasma homocysteine concentrations were >16 µmol/l, patients were defined as hyperhomocysteinaemic.

Follow-Up

Patients were followed for 5 years. Data on mortality and cardiovascular events were available as of April 23, 2003. The prevalence of (cardiovascular) morbidity and mortality was collected by questionnaires and from medical files. After follow-up, patients with an unknown cause of death were excluded. Recurrent cardiovascular event was defined as new admission to a hospital for cardiovascular disease, including (re)infarction, stroke, coronary angioplasty, coronary surgery, arrhythmia or heart failure.

Statistics

Statistical analysis was undertaken with SAS (version 8.0). Data at baseline were described by means of frequency tables and descriptive statistics. Cox proportional hazards regression was used to analyse the survival in patients with ischaemic heart disease at baseline. Multivariate analysis was used for adjustment by homocysteine, vitamin B12, age, sex, angina pectoris, myocardial infarction, cardiac arrhythmia and heart failure. For better understanding of the survival analysis, an incidence rate (IR) was calculated as incidence of events per person-years of follow-up. Finally, the relation between serum vitamin B12 and plasma homocysteine concentrations was analysed for patients with and without a recurrent event using regression analysis.

Results

There were 211 patients included in the period from February till May 1998. The mean age was 66.9 years (95% CI: 65.3–68.6) and 133 (63%) were male. Overall 141 (67%) had a discharge diagnosis of ischaemic heart disease, 22 (10%) of arrhythmia, 16 (8%) of heart failure and 17 (8%) other. The total follow-up period was 5 years (810 person-years).

Overall, 48 (23%) patients experienced a nonfatal recurrent cardiovascular event during follow-up. Patients with a fatal or nonfatal recurrent cardiovascular event had an average vitamin B12 serum concentration of 250 pmol/l (95% CI: 214–286) and a mean serum homocysteine concentration of 16.2 µmol/l (95% CI: 14.7–17.7). In patients free of cardiovascular events at the end of follow-up, a mean vitamin B12 concentration of 225 pmol/l (95% CI: 203–248) and a mean homocysteine concentration of 15.1 µmol/l (95% CI: 14.2–16.1) were assessed. Other characteristics of the study population categorized for experiencing a recurrent event are shown in table 1. A family history of cardiovascular disease and male gender were found to be statistically significant risk factors for a recurrent cardiovascular event ($p = 0.03$).

A total of 18 (9%) patients died, 14 (7%) from cardiovascular diseases during the follow-up period (table 2). The IR for cardiovascular morbidity is 0.059, where ischaemic heart disease is the main cause. Categorized for vitamin B12 deficiency, IR for overall mortality and cardiovascular morbidity were both lower for patients who were deficient (table 3).

There was no correlation between decreased serum vitamin B12 and increased serum homocysteine concentrations (fig. 1). The (un)adjusted hazard ratios of survival to death from cardiovascular disease or recurrent cardiovascular event in patients with an ischaemic heart disease at baseline are shown in table 4. In the whole co-

Table 1. Characteristics of subjects at baseline

	Recurrent cardiovascular event		OR (95%CI)	p value
	no (n = 149)	yes (n = 62)		
Gender				
Male	83 (59%)	50 (81%)	2.91 (1.43–5.94)	0.0026
Female	58 (41%)	12 (19%)		
Age				
< 65 years	68 (46%)	34 (55%)	0.69 (0.38–1.25)	0.2231
≥ 65 years	81 (54%)	28 (45%)		
Vitamin B12, pmol/l				
< 250	105 (70%)	38 (61%)	0.66 (0.36–1.23)	0.1937
≥ 250	44 (30%)	24 (39%)		
Homocysteine, μmol/l				
> 16	19 (13%)	13 (21%)	1.82 (0.83–3.95)	0.1693
≤ 16	130 (87%)	49 (79%)		
Smoking	29 (19%)	9 (15%)	0.70 (0.31–1.59)	0.3476
Hyperlipidaemia ¹	29 (19%)	13 (21%)	1.10 (0.53–2.29)	0.8031
Hypertension ²	33 (22%)	13 (21%)	0.93 (0.45–1.92)	0.8500
Family history of CVD ³	29 (19%)	5 (8%)	0.32 (0.11–0.90)	0.0260
Diabetes mellitus	26 (17%)	9 (15%)	0.92 (0.37–2.25)	0.8518

¹ Cholesterol >5 mmol/l.

² Systolic BP >160 mm Hg, diastolic BP >95 mm Hg.

³ CVD = cardiovascular disease.

Table 2. Incidence ratios for mortality and cardiovascular morbidity in the patient population

	Number	Incidence ratio ¹
Mortality		
Overall	18	0.023
CVD related	14	0.017
Recurrent cardiovascular event		
Overall	48	0.059
Ischaemic heart disease	27	0.033
Arrhythmia	10	0.012
Heart failure	7	0.009
Other	4	0.005

¹ Analyzed over 810 person-years.

Table 3. Incidence ratios for mortality and cardiovascular morbidity categorized for vitamin B12 deficiency (<250 pmol/l) in patients with a discharge diagnosis of ischaemic heart disease

Serum vitamin B12	Number	Person-years	Incidence ratio
<i>Overall mortality</i>			
<250 pmol/l	9	563	0.016
≥ 250 pmol/l	6	247	0.024
<i>Overall cardiovascular morbidity</i>			
<250 pmol/l	16	563	0.028
≥ 250 pmol/l	11	247	0.045

Table 4. Hazard ratios (HR) for recurrent cardiovascular event analyzed in patients with a discharge diagnosis of ischaemic heart disease

	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Hyperhomocysteinaemia (>16 μmol/l)	2.22 (1.40–3.04)	2.19 (1.35–3.02)
Vitamin B12 deficiency (<250 pmol/l)	0.72 (0.07–1.37)	0.74 (0.08–1.40)
Age (<65 years versus >65 years)	0.84 (0.20–1.47)	0.86 (0.17–1.54)
Gender (M versus F)	2.41 (1.54–3.28)	2.35 (1.43–3.27)

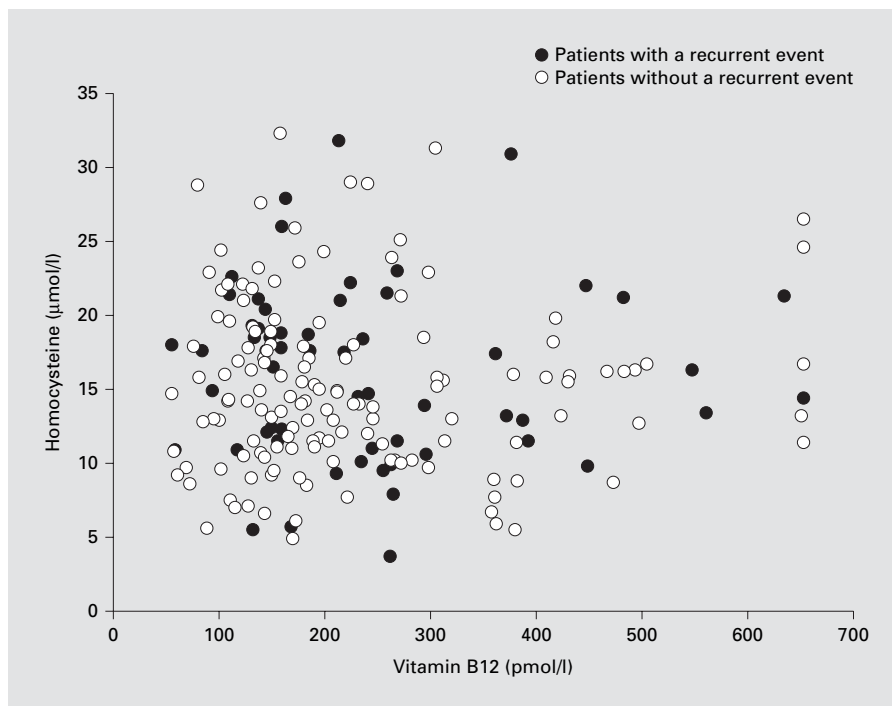


Fig. 1. Correlation between serum vitamin B12 and plasma homocysteine concentrations in all patients ($r = 0.03$; $p = 0.65$).

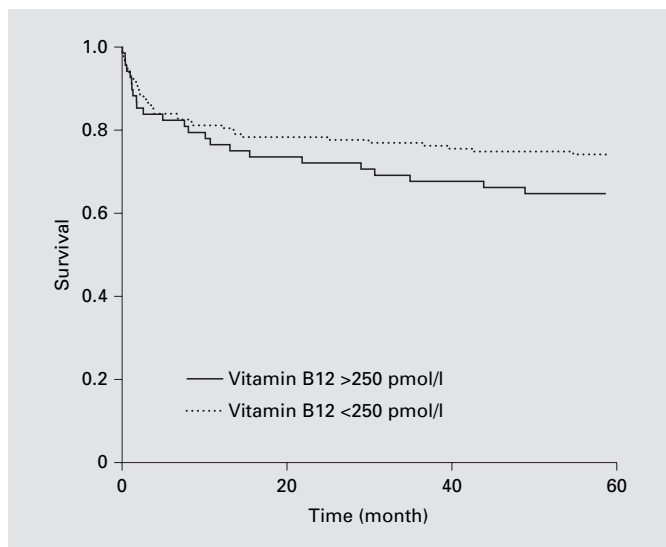


Fig. 2. Survival curve of patients with a discharge diagnosis of ischaemic heart disease with or without vitamin B12 deficiency ($p = 0.32$).

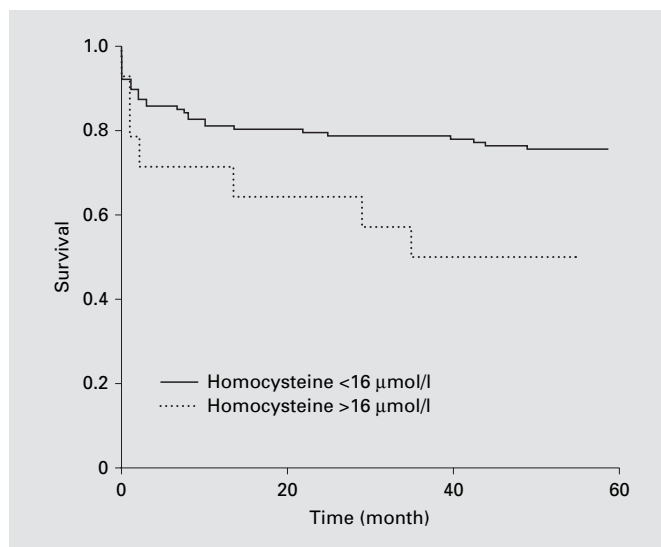


Fig. 3. Survival curve of patients with a discharge diagnosis of ischaemic heart disease with or without hyperhomocysteinaemia ($p = 0.05$).

hort, there was no statistically significant difference in survival between patients with and without vitamin B12 deficiency ($p = 0.21$). This did not change when only the patients with ischaemic heart disease at baseline were included as shown in figure 2. However, figure 3 shows that

patients with hyperhomocysteinaemia and an ischaemic heart disease at baseline had an increased risk for a second cardiovascular event in comparison to those with normal plasma homocysteine levels and ischaemic heart disease at baseline.

Discussion

The present study shows that cardiovascular patients with a low vitamin B12 concentration do not have a higher risk for future cardiovascular morbidity and mortality in comparison to those with a normal vitamin B12 concentration. However, increased plasma homocysteine levels are associated with recurrent cardiovascular events and mortality in patients with ischaemic heart disease at baseline. Apparently, plasma homocysteine concentration is more important than serum vitamin B12 concentrations for prediction of recurrent ischaemic events. This study does not corroborate with the expected correlation between vitamin B12 and homocysteine concentration.

Over the world, there is much debate whether low serum vitamin B12 and high plasma homocysteine concentrations lead to an increased risk at cardiovascular events [1, 5, 6]. The disparity in result may be due to the different study populations, where studies done in Australia, Korea and Spain show conflicting results [2, 5, 7]. The differences between countries can be explained by diet. Moreover, the incidence of cardiovascular disease differs over the world. We tried to anticipate the latter by presenting IR comparing groups of patients with and without ischaemic heart disease at the start of follow-up.

In previous studies, there was also a wide variety of definition of recurrent events: defined as coronary artery disease related mortality or coronary artery disease related morbidity [2, 5, 6]. Some studies only concentrated on fatal or nonfatal myocardial infarction or ischaemic heart disease (myocardial infarction and angiographically confirmed coronary artery occlusion) [3, 4]. Our results are consistent in that they show that high homocysteine concentrations are a risk factor for recurrent cardiovascular events in patients with ischaemic heart disease.

Vitamin B12 deficiency can easily be treated with oral supplements or by intramuscular injection. Vitamin B12 supplementation has a relatively small effect on plasma homocysteine concentration, especially in comparison with folic acid. Comprehensive literature reviewing observational studies showed that adding 0.5 mg/day of folic acid alone can reduce basal homocysteine levels by about 25%, and the combination of 0.5 mg folic acid with 0.5 mg vitamin B12 further reduces the levels by 7% [1]. Furthermore, vitamin B12 and folic acid are not the only factors influencing the homocysteine metabolism. Kidney dysfunction has also been demonstrated as determinant of elevated homocysteine concentrations [8, 9]. The pathophysiological mechanism of hyperhomocysteinaemia

in chronic renal failure in humans, however, has not been clarified yet. A second explanation may be that vitamin B12 deficiency is uncommon in the general population, being usually related to a problem of absorption rather than nutrition. The cause of similar vitamin B12 concentrations between the groups with and without a recurrent event may be found in the previous history of the patients. Both groups contain patients who were at least once hospitalized for cardiovascular diseases at baseline. There might have been a difference if one group had had a lower rate of history of cardiovascular diseases.

Hyperhomocysteinaemia is associated with endothelial injury and dysfunction promoting platelet activation and thrombus formation, which could result in acute coronary syndromes. Our results show that in the subgroup of patients with ischaemic heart disease at the beginning of follow-up, high plasma homocysteine concentration and male gender more often lead to recurrent cardiovascular events or mortality. These findings are consistent with recent literature. However, since this study shows no statistically significant independent relationship between vitamin B12, homocysteine and cardiovascular morbidity and mortality, other factors seem to be more important. Due to lack of information about folic acid and vitamin B6 concentrations, no adequate evaluation can be made of the correlation between these vitamins and the recurrence of cardiovascular disease.

This study has some limitations. Due to the relatively small population, we did not find high event rates. However, considering the sample size, we did find statistically significant higher recurrent event rates in the hyperhomocysteinaemia group that is consistent with literature. In other words, hyperhomocysteinaemia has been recognized to be a risk factor for recurrent cardiovascular events due to its prothrombotic effect. Consequently, it can be suggested that lowering homocysteine levels with B12, B6 and folic acid would prevent cardiovascular events. However, in the three large prospective trials (VISP, HOPE-2 and NORVIT) in patients with established cardiovascular disease [10–12], there was no clinical benefit of treatment with folic acid and B vitamins for prevention of recurrent cardiovascular events and mortality. Although our population is a small group of 211 patients, the results are in accordance with the results of the three large studies.

In conclusion, our results indicate that a decreased serum vitamin B12 level does not significantly increase the risk of cardiovascular disease related mortality and morbidity in patients discharged from a Coronary Care Unit.

However, an elevated plasma homocysteine level is an important risk factor for recurrent ischaemic cardiovascular events.

Conclusion

This study shows that cardiovascular patients with a low vitamin B12 concentration do not have a higher risk for future cardiovascular morbidity and mortality

in comparison to those with a normal vitamin B12 concentration. However, increased plasma homocysteine levels are associated with recurrent cardiovascular events and mortality in patients with ischaemic heart disease at baseline. Apparently, plasma homocysteine concentration is more important than serum vitamin B12 concentration for prediction of recurrent ischaemic events.

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