The American Journal of Clinical Nutrition 118 (2023) 77–84

The American Journal of CLINICAL NUTRITION

journal homepage: https://ajcn.nutrition.org/

Original Research Article

B-vitamin Treatment Modifies the Mortality Risk Associated with Calcium Channel Blockers in Patients with Suspected Stable Angina Pectoris: A Prospective Cohort Study

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A B S T R A C T

Background: Calcium channel blockers (CCBs) are used for the treatment of cardiovascular disease (CVD), including angina pectoris, and hypertension; however, the effect on survival remains uncertain. CCBs impair fibrinolysis and have been linked to elevated plasma homocysteine (Hcy), a CVD risk marker.

Objective: We explored the association between CCB use and mortality in a large prospective cohort of patients with suspected stable angina pectoris (SAP), and potential effect modifications by Hcy-lowering B-vitamin treatment (folic acid, B_{12}, and/or B_6) as 61.8% of the patients participated in a randomized placebo-controlled B-vitamin intervention trial.

Methods: Patient baseline continuous characteristics according to CCB treatment were tested by linear regression. Hazard ratios (HRs) for mortality associated with CCB treatment, also according to B-vitamin intervention, were examined using Cox regression analysis. The multivariable model included CVD risk factors, medical histories, and the use of CVD medications.

Results: A total of 3991 patients (71.5 % men) were included, of whom 907 were prescribed CCBs at discharge. During 10.3 years of median follow-up, 20.6% died and 8.9% from cardiovascular- and 11.7% from non-cardiovascular causes. Patients treated with CCBs had higher plasma Hcy, fibrinogen levels, and erythrocyte sedimentation rate (all $P<0.001$). Furthermore, CCB use was positively associated with mortality, also after multivariable adjustments (HRs [95% CIs]: 1.34 [1.15,1.57], 1.35 [1.08,1.70], and 1.33 [1.09,1.64] for total, CVD, and non-CVD death, respectively). Numerically stronger associations were observed among patients not treated with B-vitamins (HR [95% CI]: 1.54 [1.25, 1.88], 1.69 [1.25, 2.30], and 1.41 [1.06, 1.86] for total, CVD deaths, and non-CVD deaths, respectively), whereas no association was seen in patients treated with B-vitamins (HR [95% CI]: 1.15 [0.91, 1.46], 1.09 [0.76, 1.57], and 1.20 [0.88, 1.65]).

Conclusions: In patients with suspected SAP, CCB treatment was associated with increased mortality risk primarily among patients not treated with B-vitamins.

Keywords: calcium channel blockers, mortality, coronary artery disease, B-vitamin treatment

Introduction

Calcium channel blockers (CCBs) are widely used in patients with stable angina pectoris (SAP) for symptom relief or blood pressure control [1]. However, there is conflicting evidence that CCBs improve prognosis in the former condition or other clinical manifestations of coronary artery disease (CAD). Notably, while most studies showed no clinical benefit on survival [2–4], some have linked CCB treatment to higher mortality risk [5–7].

The B-vitamins folic acid, vitamin B_{12}, and B_6 are water-soluble nutrients essential for diverse physiological processes, including homocysteine (Hcy) metabolism [8,9]. A deficiency of these vitamins may lead to elevated circulating total Hcy (tHcy) concentrations [8], which is a risk factor for atherothrombosis [8,9]. Moreover, treatment

Abbreviations used: ACEi, ACE inhibitors; ARB, angiotensin receptor blocker; AMI, acute myocardial infarction; CCB, calcium channel blocker; eGFR, estimated GFR; Hcy, homocysteine; PCI, percutaneous coronary intervention; SAP, stable angina pectoris; RCT, randomized controlled trial; WENBIT, Western Norway B Vitamin Intervention Trial.

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https://doi.org/10.1016/j.ajcnut.2023.04.033
Received 12 January 2023; Received in revised form 4 April 2023; Accepted 27 April 2023
Available online 28 April 2023

with these vitamins is reported to have anti-inflammatory [10,11] and anti-coagulant effects [12,13], and vitamin B_6 may inhibit sympathetic tone [14]. However, randomized clinical trials (RCTs) failed to reduce cardiovascular disease (CVD) risk with Hcy-lowering B-vitamins [15,16], although treatment effects may have been heterogeneous according to certain subgroup phenotypes. Notably, CCB treatment has been associated with increased systemic Hcy concentrations [17,18]. Moreover, CCBs promote proinflammatory responses [19,20], decrease fibrinolytic function [21,22], and increase sympathetic activation [23], thus potentially increasing CVD risk.

Taken together, B-vitamin treatment may potentially mitigate adverse CVD effects of CCBs, influencing the prognosis associated with their use. We, therefore, investigated the associations of CCB use with mortality risk in a large cohort of patients with suspected SAP, also focusing on potential effect modifications by B-vitamin treatment.

Methods

Study design and population

A total of 4166 patients undergoing coronary angiography for suspected SAP during 2000–2004 at 2 university hospitals in Western Norway were included [24]. Among these patients, 2573 (61.8%) were enrolled in the Western Norway B-vitamin Intervention Trial (WENBIT) (ClinicalTrials.gov Identifier: NCT00354081) and received daily treatment with either *1*) folic acid (0.8 mg) plus vitamin B_{12} (0.4 mg) plus vitamin B_6 (40 mg), *2*) folic acid (0.8 mg) plus vitamin B_{12} (0.4 mg), *3*) vitamin B_6 (40 mg), or *4*) placebo until the end of 2006 [15]. Because CCBs are relatively contraindicated in patients with heart failure with reduced ejection fraction [25], we excluded patients with left ventricular ejection fraction (LVEF) $< 40\%$ ($n = 159$). Also, subjects with unspecified data on CCBs ($n = 4$) or missing baseline covariables included in risk models were excluded ($n = 12$), resulting in a total of 3991 subjects eligible for the final analyses (Figure 1). All participants provided written informed consent. The study fulfilled the Declaration of Helsinki and was approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Medicines Agency, and the Norwegian Data Inspectorate.

Baseline data and biochemical analyses

Hypertension (yes vs. no), LVEF (continuous), smoking status (yes vs. no), and the extent of significant CAD at angiography (0–3) were defined as described previously [24]. Baseline diabetes mellitus (yes vs. no) was originally classified by self-reports or by plasma glucose criteria (i.e., fasting plasma glucose $\geq$ 7.0 mmol/L, or random plasma glucose $\geq$ 11.1 mmol/L, or by glycated hemoglobin $\geq$ 6.5% [26]). Blood samples were obtained by study personnel at baseline before or immediately after coronary angiography and stored at $-80°C$ until analysis. Previous reports have described the biochemical analyses for relevant clinical indices [24].

Follow-up and study end points

The primary outcomes for the present study were all-cause, CVD, and non-CVD mortality. Study subjects were followed up from enrollment until death or the end of 2012. Information on mortality was obtained from the Cause of Death Registry at Statistics Norway (www.ssb.no/en).

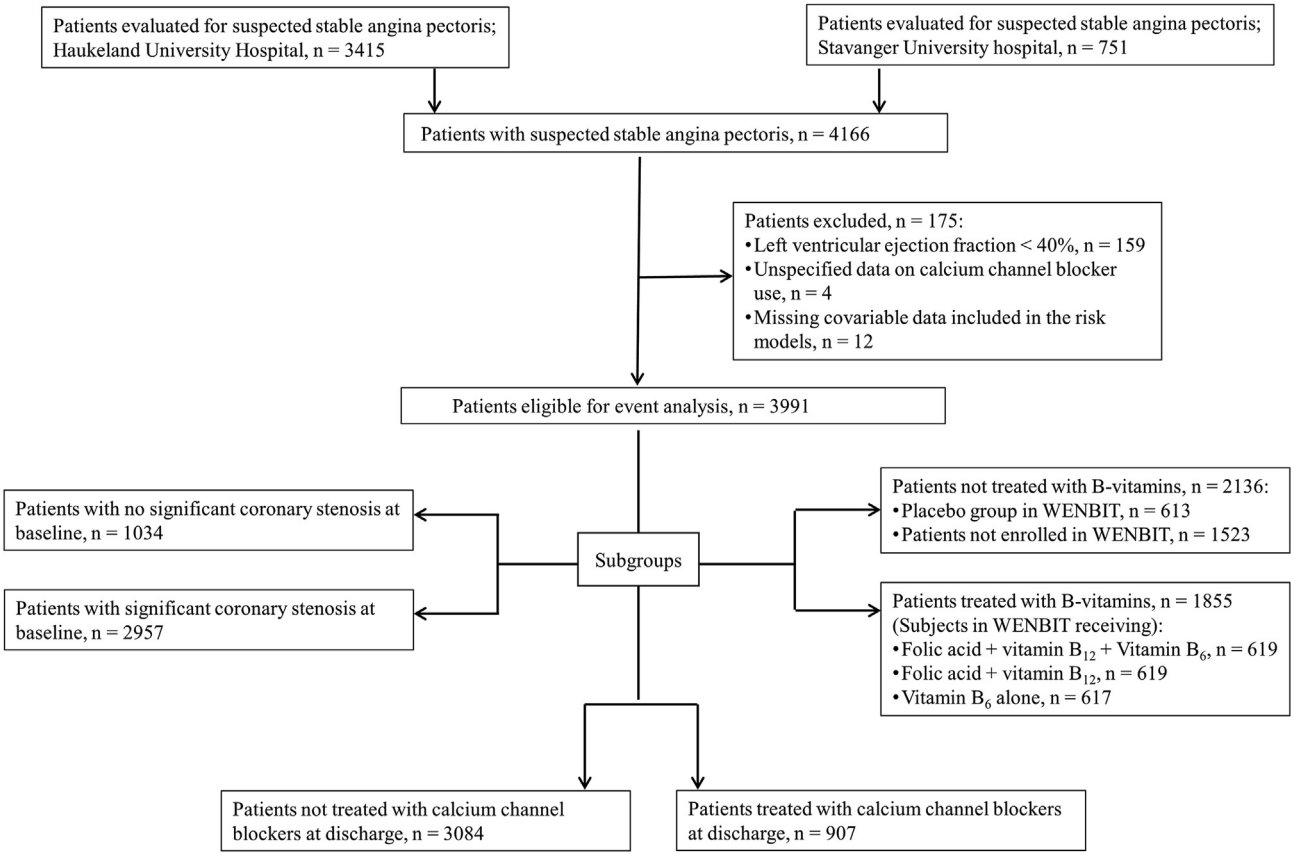

FIGURE 1. Flow-chart showing patient selection in the study cohort. WENBIT, Western Norway B-vitamin Intervention Trial.

Statistical analysis

Baseline categorical variables are reported as counts (percentages), and continuous variables are presented as medians (25th–75th percentiles). Differences in baseline variables according to CCB treatment at discharge were assessed by unadjusted linear regression for continuous and Pearson chi-square test for categorical variables and ordinal data.

Survival was visualized using Kaplan–Meier plots, and the differences across CCB treatment groups were estimated by the log-rank test. Patients who received folic acid plus vitamin B_{12} plus vitamin B_6 or folic acid plus vitamin B_{12} or vitamin B_6 alone in the WENBIT were grouped as B-vitamin treated (yes), whereas those who received placebo in the WENBIT and those not enrolled in the WENBIT were grouped as B-vitamin non-treated (No). The associations between CCB use and mortality, also according to B-vitamin treatment groups were tested using Cox hazards regression models. A simple model (model 1) was adjusted for age (continuous) and gender (male/female). A multivariate model (model 2) was further adjusted for CVD risk factors including the extent of angiographically verified CAD (0–3), diabetes mellitus (yes/no), hypertension (yes/no), smoking (yes/no), estimated GFR (eGFR) (continuous), and medical history including anamnestic heart failure, atrial fibrillation, previous acute myocardial infarction (AMI), and previous percutaneous coronary intervention (PCI) (all yes/no). Model 3 included additional adjustments for CVD medications including ACE inhibitors (ACEi) or angiotensin receptor blockers (ARBs), β-blockers, and statins (all yes/no). Because including non-randomized subjects in the B-vitamin non-treated group may influence results, we additionally included a cohort-control variable (yes [non-randomized] vs. no [WENBIT participants]) in all risk models. Adjustments for BMI, or serum total cholesterol had minor effects on risk estimates and were therefore not included in the models. To account for the competing risk of cause-specific mortality, we also estimated sub-distribution HRs using the Fine and Gray approach [27].

Potential interactions between B-vitamin treatment and CCBs on the outcomes were evaluated by adding interaction product terms to the Cox models. We performed sensitivity analysis by excluding subjects using B-vitamin supplements prior to baseline. Additionally, we evaluated the effect modifications separately according to the presence of angiographically significant CAD (yes [1–3] vs. no [0]) at baseline. We also explored the possibility of reverse causation by excluding the events occurring during the first 365 d of follow-up and conducted analyses using Cox model 2. The statistical analyses were performed in SPSS 27 (SPSS IBM). Competing risks analysis was obtained with the SPSS extension command COMPRISK using the R "cmprsk" package. All reported P values were 2-sided, and $P< 0.05$ was considered statistically significant.

Results

Baseline characteristics

Baseline characteristics according to CCB treatment are shown in Table 1. The median (25th–75th percentile) age was 62 (55–70) y and 22.7% of the patients were prescribed CCBs at baseline. CCB-treated patients had higher plasma tHcy, fibrinogen, and erythrocyte sedimentation rate (all $P<0.001$), but lower pyridoxal 5′-phosphate and eGFR. Patients using CCBs were older, and more often had hypertension and atrial fibrillation. Moreover, CCB treated patients more likely had extensive CAD during angiography, also reflected by higher rates of prior PCI as well as treatment with aspirin, ACEi/ARBs, and statins, although they less often used β-blockers.

TABLE 1
Baseline characteristics of the patient population according to calcium channel blocker use at discharge ($n = 3991$)[1]

	Patients not treated with CCBs ($n = 3084$)	Patients treated with CCBs ($n = 907$)	P value[2]
Age, y	61 (53-68)	66 (58-73)	<0.001
Male gender, n (%)	2205 (71.5)	647 (71.3)	0.92
BMI, kg/m^2	26 (24-28)	26 (24-29)	<0.001
Hypertension, n (%)	1205 (39.1)	665 (73.3)	<0.001
Diabetes mellitus, n (%)	1164 (37.7)	361 (39.8)	0.26
Current smoking, n (%)	993 (32.2)	267 (29.4)	0.12
Heart failure, n (%)	106 (3.4)	37 (4.1)	0.36
Atrial fibrillation, n (%)	219 (7.1)	105 (11.6)	0.001
Prior AMI, n (%)	1198 (38.8)	345 (38.0)	0.66
Prior PCI, n (%)	537 (17.4)	216 (23.8)	<0.001
eGFR, mL/min per 1.73 m^2	92 (81–100)	87 (75–96)	<0.001
Plasma tHcy, μmol/L	10.2 (8.6–12.3)	10.9 (8.9–13.3)	<0.001
Serum CRP, mg/L	1.67 (0.81–3.4)	2.10 (1.1–4.1)	0.098
Fibrinogen, g/L	3.60 (3.1–4.0)	3.70 (3.3–4.2)	<0.001
Platelet count, $\times 10^9$/L	239 (203–281)	243 (210–281)	0.20
Erythrocyte sedimentation rate, mm/hr	10.0 (5–16)	12.0 (7–20)	<0.001
LVEF, %	67 (60–70)	67 (60–70)	0.73
Serum total cholesterol, mmol/L	5.0 (4.3–5.8)	4.8 (4.2–5.6)	0.01
Extent of CAD, n (%)			<0.001
No stenotic vessels	857 (27.8)	177 (19.5)	
1-vessel disease	741 (24.0)	198 (21.8)	
2-vessel disease	688 (22.3)	201 (22.2)	
3-vessel disease	798 (25.9)	331 (36.5)	
B vitamin status			
Plasma folate, nmol/L	10.0 (7.4–14.6)	10.3 (7.4–15.7)	0.03
Serum cobalamin, pmol/L	362 (275–468)	367 (272–460)	0.19
Plasma PLP, nmol/L	42.1 (30–60)	39 (28–56)	0.004
B-vitamin treatment[3], n (%)	1418 (46.0)	437 (48.2)	0.24
Medications after angiography, n (%)			
Aspirin	2502 (81.1)	774 (85.3)	0.004
β-blocker	2276 (73.8)	616 (67.9)	<0.001
ACEi and ARB	867 (28.1)	332 (36.6)	<0.001
Statins	2434 (78.9)	763 (84.1)	0.001

ACEi, angiotensin-converting enzyme inhibitor; AMI, acute myocardial infarction; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CCBs, calcium channel blockers; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; PLP, phyrodoxal 5'-phosphate; tHcy, total homocysteine.

[1] Continuous variables are presented as medians (25th–75th percentiles), and categorical variables are reported as counts (%).

[2] P values were determined with the use of linear regression for continuous variables and Pearson chi-square test for categorical and ordinal variables.

[3] Subjects receiving folic acid+vitamin B_{12}+ vitamin B_6 or folic acid+vitamin B_{12} or vitamin B_6 alone in the Western Norway B-vitamin Intervention Trial.

Calcium channel blocker use and mortality risk

During a median (25^{th}–75^{th} percentiles) follow-up time of 10.3 (9.3–11.6) y, 822 participants (20.6%) died and 356 (8.9%) from cardiovascular causes and 466 (11.7%) from noncardiovascular causes. Figure 2 shows a significantly lower survival rate for all endpoints among patients treated with CCBs ($P_{\text{log-rank}} \leq 0.001$, for all). In model 1, HRs (95% CI) associated with CCB treatment were 1.51 (1.31, 1.75) for total death, 1.62 (1.30, 2.01) for CVD death, and 1.44 (1.18, 1.75) for non-

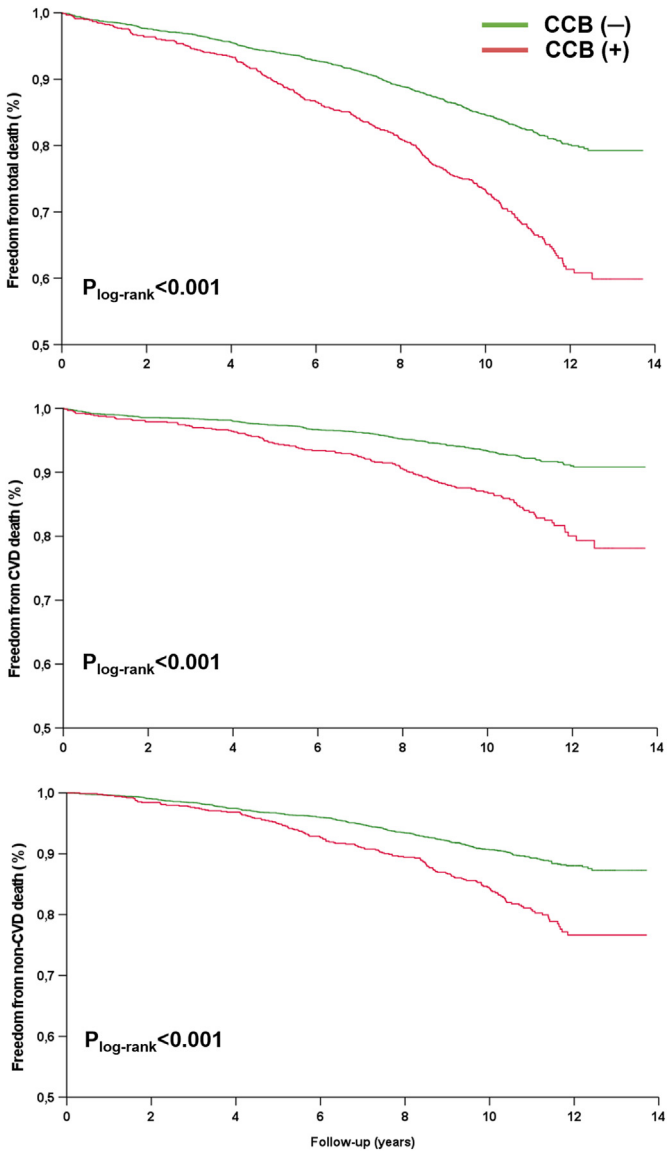

FIGURE 2. Survival curves for total, CVD, and non-CVD mortality according to calcium channel blocker use at discharge. Survival was examined using the Kaplan–Meier plots, and the differences across CCB treatment groups were determined by the log-rank test. CCB (−), calcium channel blockers non-users; CCB (+), calcium channel blockers users; CVD, cardiovascular disease.

CVD death (Table 2). Corresponding HRs (95% CI) were 1.36 (1.17, 1.58), 1.40 (1.12, 1.75), and 1.33 (1.08, 1.63) in model 2, and the associations remained essentially similar in model 3 (Table 2). When performing competing risk analyses according to CVD and non-CVD death, the risk estimates were only slightly attenuated (Supplemental Table 1).

Effect modifications by B-vitamin treatment on CCB use and mortality risk

Table 3 describes the relationship between CCB use and mortality according to B-vitamin treatment. Among patients not treated with B-vitamins, we found pronounced positive associations between CCB use and risk of total and CVD mortality (HR [95% CI]: 1.86 [1.53, 2.25] and 2.17 [1.63, 2.89], respectively, in model 1), whereas there were no associations among those treated with B-vitamins (HR [95% CI]: 1.16 [0.93, 1.46] and 1.11 [0.78, 1.56], respectively) (P-interaction≤0.004 both). These relationships persisted after multivariable adjustments (Table 3). Accordingly, we also found a trend toward a similar effect-modification according to non-CVD mortality (model 1 adjusted HR [95% CI]: 1.65 [1.27, 2.14] in B-vitamin non-treated vs. 1.21 [0.90, 1.63] in B-vitamin treated subjects) (P-interaction = 0.12) (Table 3). We additionally tested the possible influence of non-WENBIT allocation by excluding patients who were not enrolled in the WENBIT trial (n = 1523) and obtained numerically stronger associations between CCB use and risk of total and CVD mortality in the placebo group (Supplemental Table 2)

Interaction of CCBs with B-vitamin treatment in patients with and without significant coronary stenosis

In patients with at least one significantly stenosed epicardial coronary artery (n = 2957), 695 died (314 from CVD and 381 from non-CVD causes), whereas 127 deaths (42 CVD and 85 non-CVD related) occurred in patients without any significant stenosis (n = 1034). Notably, we observed an increased risk for total and CVD mortality with CCB use and B-vitamin non-treatment among patients in the former group (P-interaction=0.02 both) (Table 4).

Sensitivity analysis

When excluding the first year of follow-up (n = 56), HRs (95%Cl) comparing CCB use with non-use were 1.41 (1.20, 1.65), 1.51 (1.19, 1.91), and 1.34 (1.09, 1.65) for total, CVD, and non-CVD mortality, respectively, in model 2. Corresponding risk estimates were 1.59 (1.29, 1.96), 1.90 (1.38, 2.61), and 1.39 (1.06, 1.84) in patients not receiving B-vitamins and 1.20 (0.95 1.53), 1.18 (0.81 1.72), and 1.23 (0.90, 1.69) in subjects treated with B-vitamins (P-interaction = 0.031, 0.029, and 0.34, respectively)

Similarly, excluding patients reported to be using supplements containing B-vitamins prior to baseline (n = 96) did not materially influence the risk estimates (data not shown).

Discussion

Principal findings

In this large study among patients with suspected stable angina pectoris, the use of calcium channel blockers was associated with increased risk of all-cause, cardiovascular, and non-cardiovascular mortality primarily among patients not receiving B-vitamin treatment.

CCBs, CAD, and mortality

CCBs are widely used for symptomatic treatment of CAD [1]; however, available evidence also suggests that CCB treatment may worsen the risk of some CAD outcomes such as heart failure [25], myocardial infarction [6], or unstable angina [28]. Regarding the effect of CCB on mortality in patients, the randomized ACTION trial failed to demonstrate any improvement in survival [2], and these findings have been supported by a meta-analysis and large observational studies [3, 4]. However, the SPRINT 2 trial performed in AMI patients suggested that CCBs may even increase early mortality risk [5]. Similarly, an overview of small RCTs among patients with CAD indicated a trend toward more deaths with CCB treatment [6], also supported by a meta-analysis of 16 clinical trials including 8350 patients with CAD [7]. In the present study, we found that patients with presumed stable CAD using CCBs had increased mortality risk during very long-term follow-up; however, our findings extend these data and suggest that

TABLE 2
HRs (95% CIs) for mortality according to calcium channel blocker use at discharge among patients with suspected stable angina pectoris ($n = 3991$)[1]

	Total death		CVD death		Non-CVD death	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Events	822		356		466	
Model 1	1.51 (1.31–1.75)	<0.001	1.62 (1.30–2.01)	<0.001	1.44 (1.18–1.75)	<0.001
Model 2	1.36 (1.17–1.58)	<0.001	1.40 (1.12–1.75)	0.003	1.33 (1.08–1.63)	0.01
Model 3	1.34 (1.15–1.57)	<0.001	1.35 (1.08–1.70)	0.01	1.33 (1.09–1.64)	0.01

CCB, calcium channel blockers.
Model 1 is adjusted for age (continuous), gender (male/female), and cohort-control variable (yes/no)
Model 2 is adjusted for age (continuous), gender (male/female), cohort-control variable (yes/no), extent of coronary artery disease (0–3), diabetes mellitus (yes/no), hypertension (yes/no), smoking (yes/no), estimated glomerular filtration rate (continuous), heart failure, atrial fibrillation, previous acute myocardial infarction, and previous percutaneous coronary intervention (all yes/no).
Model 3 is adjusted for variable in Model 2 plus use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β-blockers, and statins (all yes/no)
[1] HR (95% CIs) were obtained by Cox regression models.

TABLE 3
HRs (95% CIs) for mortality according to CCB use at discharge among patients with and without B-vitamin treatment[1]

	Patients not treated with B-vitamins ($n = 2136$)	Patients treated with B-vitamins ($n = 1855$)	*P*-interaction
Total death			
Events	459	363	
Model 1	1.86 (1.53–2.25)	1.16 (0.93–1.46)	0.002
Model 2	1.55 (1.27–1.89)	1.16 (0.92–1.47)	0.035
Model 3	1.54 (1.25–1.88)	1.15 (0.91–1.46)	0.041
CVD death			
Events	200	156	
Model 1	2.17 (1.63–2.89)	1.11 (0.78–1.56)	0.004
Model 2	1.74 (1.29–2.35)	1.12 (0.79–1.61)	0.042
Model 3	1.69 (1.25–2.30)	1.09 (0.76–1.57)	0.041
Non-CVD death			
Events	259	207	
Model 1	1.65 (1.27–2.14)	1.21 (0.90–1.63)	0.12
Model 2	1.40 (1.07–1.85)	1.20 (0.88–1.64)	0.31
Model 3	1.41 (1.06–1.86)	1.20 (0.88–1.65)	0.36

CCB, calcium channel blockers.
Treated with B-vitamins, subjects receiving folic acid+vitamin B_{12}+ vitamin B_6 or folic acid+vitamin B_{12} or vitamin B_6 alone in the Western Norway B-Vitamin Intervention Trial (WENBIT); patients not treated with B-vitamins, patients receiving placebo in WENBIT, and those not enrolled in WENBIT.
Model 1 was adjusted for age (continuous), gender (male/female), and cohort-control variable (yes/no).
Model 2 was adjusted for age (continuous), gender (male/female), cohort-control variable (yes/no), extent of coronary artery disease (0–3), diabetes mellitus (yes/no), hypertension (yes/no), smoking (yes/no), estimated glomerular filtration rate (continuous), heart failure, atrial fibrillation, previous acute myocardial infarction, and previous percutaneous coronary intervention (all yes/no).
Model 3 was adjusted for variable in Model 2 plus use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β-blockers, and statins (all yes/no).
[1] HR (95 % CIs) was obtained by Cox regression models.

TABLE 4
HRs (95% CIs) between CCB use and mortality across B-vitamin groups according to significant CAD at baseline[1]

	Patients not treated with B-vitamins	Patients treated with B-vitamins	*P*-interaction
Significant stenosis			
n	1311	1646	
Total death	1.71 (1.37–2.14)	1.21 (0.95–1.54)	0.022
CVD death	1.92 (1.38–2.67)	1.14 (0.79–1.63)	0.02
Non-CVD death	1.55 (1.14–2.09)	1.27 (0.91–1.77)	0.32
No stenosis			
n	825	209	
Total death	1.20 (0.74–1.97)	1.03 (0.42–2.54)	0.46
CVD death	1.26 (0.59–2.70)	1.65 (0.08–34.6)	0.99
Non-CVD death	1.18 (0.61–2.26)	0.96 (0.36–2.58)	0.46

CAD, coronary artery disease; CCB, calcium channel blockers.
Patients treated with B-vitamins, subjects receiving folic acid + vitamin B_{12} + vitamin B_6 or folic acid + vitamin B_{12} or vitamin B_6 alone in Western Norway B Vitamin Intervention Trial (WENBIT); patients not treated with B-vitamins, patients receiving placebo in WENBIT, and those not enrolled in WENBIT.
[1] HR (95 % CIs) was obtained by Cox regression models. Model adjusted for age (continuous), gender (female/male), cohort-control variable (yes/no), diabetes mellitus (yes/no), hypertension (yes/no), smoking (yes/no), estimated glomerular filtration rate (continuous), heart failure, atrial fibrillation, previous acute myocardial infarction, and previous percutaneous coronary intervention (all yes or no).

the relationship might be attenuated by concomitant treatment with B-vitamins. Because food fortification with folic acid and B_{12}, as well as B- vitamin supplementation is common in many countries around the world [29], this may explain the null- associations in prior observational studies.

Possible mechanisms

CCBs, B-vitamin treatment, and CVD mortality

The crosstalk between CCBs and B-vitamin treatment on mortality risk is not clear but could be related to the regulation of Hcy

metabolism, sympathetic activity, inflammatory responses, and fibrinolysis. In line with the current study, CCB treatment has been previously related to higher systemic tHcy concentrations [17], which is associated with pro-atherothrombotic changes, such as endothelial dysfunction, smooth muscle cell proliferation, and cardiovascular remodeling [8,9]. Furthermore, an analysis of 63 clinical studies found that dihydropyridine CCBs may increase plasma norepinephrine concentrations and heart rate, typical indicators of sympathetic activation [23], which is implicated in CVDs [30]. Notably, β-blockers might mitigate such effects [31], and β-blocker use was less frequent among patients who used CCBs in the current study. Others have also shown that CCBs induce gene expression of the inflammatory cytokine interleukin-6 (IL-6) [19,20]. Higher IL-6 is linked with CVD risk [32] and is reported to foster proatherogenic effects, including activation of endothelial cells and platelets, stimulation of macrophage lipid accumulation, and induction of CRP expression [33]. Interestingly, we observed a tendency toward higher serum CRP concentrations in CCB users compared with non-users. Moreover, CCBs have been shown to decrease fibrinolytic activity via increasing plasminogen activator inhibitor-1 (PAI-1) concentrations [21,22]. The PAI-1 activity is reported to be upregulated by fibrinogen [34], and it is therefore interesting that we observed a positive association between CCB use and circulatory fibrinogen. Fibrinogen is also known to accelerate red blood cells (RBCs) aggregation, which is reflected by increased erythrocyte sedimentation rate, which has been positively correlated with coronary disease [35]. Interestingly, we also observed that CCB users had an increased erythrocyte sedimentation rate. These concurrent with the observation that RBCs have a particularly high number of Ca^{2+} channels [36], may indicate the involvement of RBCs in atherogenesis, and CCB use may be associated with risk via altering functionality of RBCs. Of note, neovascularization, vascular spasm, and intramural hemorrhage of the vasa vasorum, the microvascular network supporting the outer wall of larger blood vessels, has been linked with plaque instability and the triggering of myocardial infarction as well as aortic dissection [37–39]. Further studies should thus evaluate if these clinical event occurrences reflect an increased requirement of RBC function as a protective mechanism.

Treatment with folic acid, vitamin B_{12}, and B_6, had no beneficial effect on CVD outcomes and mortality in secondary prevention trials [8,16], including the WENBIT [15], although the tHcy-lowering effect by folic acid, vitamin B_{12}, and B_6 has been well documented [8,9]. Coupled with the evidence that folic acid either alone [12] or in combination with other B-vitamins [13] may improve coagulation status, whereas B6 may reduce sympathetic activity [14], these findings indicate a potential mechanism by which B-vitamin treatment mitigates CCB-associated adverse effects. Treatment with B-vitamins has also been associated with an anti-inflammatory status in some studies [10,11]; however, such an effect was not observed in a prior small study from a subsample of the WENBIT cohort [40].

CCBs, B-vitamin treatment, and non-CVD mortality

Another important finding of our study is the positive association between CCB use and non-CVD mortality. In a prospective cohort study of more than 5000 older subjects, the long-term use of CCBs was associated with an increased risk of multiple cancer forms [41]. Furthermore, CCBs have been associated with increased occurrence of gastrointestinal [42] and surgical bleeding [43]. Importantly in the WENBIT, treatment with folic acid was also associated with a non-significant increased risk of cancer [15]. Similar results were obtained in another large secondary prevention trial among patients surviving AMI [16], thus highlighting the need for further studies to pinpoint the underlying mechanisms. Such studies should also investigate the possibility if the effect-modification by B-vitamin treatment on CCB-related prognosis may be due to an excess risk related to B-vitamin treatment in the patient subgroup not receiving CCBs.

Strengths and limitations

The large sample size, detailed characterization of the patient population, and long-term follow-up are strengths of our study. Furthermore, we obtained endpoint data from a health registry with almost 100% national coverage.

We acknowledge some limitations. First, due to the observational nature of our study, residual and uncontrolled confounding cannot be ruled out. Although we controlled for several CVD risk factors, the bias due to unmeasured confounders such as social risk factors and family history still exists. Another limitation is confounding by indication, i.e., patients prescribed with CCBs more likely may have had a higher cardiovascular morbidity burden, influencing long-term mortality outcomes. However, controlling for medical history or CVD medications had no impact on the associations studied. Moreover, patients with heart failure with reduced ejection fraction in whom the adverse effects of CCBs are established [25] were excluded. Additionally, the estimates were not attenuated even after excluding the first year of follow-up. However, a significant effect-modification by B-vitamin treatment on CCB-related prognosis was present in patients with verified CAD only, suggesting that the observed findings were confined to this patient population. Third, we could not account for possible individual changing patterns of drug prescription during follow-up, nor were we able to account for the dosage or compliance with CCB use post baseline. Fourth and importantly, the intervention period of WENBIT was relatively short (3–5 y); however, we observed an effect-modification on survival beyond drug interruption, suggesting a potential legacy effect. Fifth, a few patients in our study (2.4%) were reported to be receiving B-vitamins before baseline, and excluding them had no major impact on associations. Finally, our study has limited ability to draw causal connections.

Conclusions

Among patients with suspected SAP, the use of CCBs was associated with increased long-term risk of all-cause, cardiovascular, and non-cardiovascular mortality. However, these associations were attenuated in patients receiving B-vitamin treatment, which may explain some of the heterogenic results in prior observational studies.

Acknowledgments

We are grateful to all the WENBIT coworkers at Haukeland and Stavanger university hospitals, as well as the laboratory personnel performing biochemical analyses at Bevital A/S, Bergen, Norway. We thank Tomislav Dimoski at the Norwegian Institute of Public Health, Norway, for his contribution by developing the software necessary for obtaining admission data from Norwegian public hospitals and conducting data collection and quality assurance of data in this project.

The authors' responsibilities were as follows – ID and OKN: contributed to the conception or design of the work; ID: conducted the study, analyzed the data, performed the analysis, interpreted the data, and wrote the manuscript; ID, GFTS, and OKN: contributed to the data acquisition; GFTS, EØB, AU, SS, and OKN: contributed to the data

interpretation and the critical revision of the manuscript for intellectual content. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy. The authors report no conflicts of interest.

Funding

This work was supported by the University of Bergen, the Department of Heart Disease, Haukeland University Hospital, the Western Norway Regional Health Authority, the Trond Mohn Foundation (BFS2017NUTRITIONLAB), and the Foundation to Promote Research into Functional Vitamin B12 Deficiency, Bergen, Norway.

Author disclosures

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.04.033.

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