Hyperhomocysteinemia and venous thrombosis : studies into risk and therapy

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Chapter 8

Homocysteine lowering by B vitamins and the secondary prevention of deep-vein thrombosis and pulmonary embolism. A randomized, placebo-controlled, double blind trial

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Abstract

The VITRO (VItamins and ThROmbosis) study investigated the effect of homocysteine lowering by daily supplementation of B-vitamins on the risk reduction of deep-vein thrombosis and pulmonary embolism. Patients between 20 to 80 years old with a first objectively confirmed proximal deep-vein thrombosis or pulmonary embolism in the absence of major risk factors and a homocysteine concentration above the 75th percentile of a reference group were asked to participate (hyperhomocysteinemic group). A similar study was conducted in a random sample of patients with a homocysteine below the 75th percentile of the reference group (normohomocysteinemic group). After informed consent patients were randomized to daily multivitamin supplementation (5 mg folic acid, 50 mg pyridoxine and 0.4 mg cyanocobalamin) or placebo and were followed for 2.5 years. End-points were objectively diagnosed recurrent deep-vein thrombosis or pulmonary embolism.

A total of 701 patients were enrolled (360 in the hyper- and 341 in the normohomocysteinemic group). The number of recurrent events of venous thrombosis was 43 out of 353 in the vitamin group (54/1000yr) and 50 out of 348 in the placebo group (64/1000yr). The hazard ratio associated with vitamin treatment was 0.84 (95% CI 0.56 to 1.26): 1.14 (95% CI 0.65 to 1.98) in the hyperhomocysteinemic group and 0.58 (95% CI 0.31 to 1.07) in the normohomocysteinemic group. The results of our study do not show that homocysteine lowering by B-vitamin supplementation prevents recurrent venous thrombosis.

Introduction

Plasma homocysteine levels are associated with an increased risk of deep-vein thrombosis and pulmonary embolism. Up to now 24 case-control studies have been published with an overall relative risk for venous thrombosis of 1.60 (95% CI 1.10 to 2.34) for a 5 µmol/l higher homocysteine level[1]. Moreover, three prospective studies showed an overall relative risk for venous thrombosis of 1.27 (95% CI 1.01 to 1.59) for a difference of 5 umol/l[1]. Recent meta-analyses on the effect of the MTHFR 677TT genotype on cardiovascular disease (2) and venous thrombosis[1,3] showed a modest increase in risk, supporting a hypothesis that homocysteine levels are causally related to thrombotic risk.

Elevated homocysteine levels can be easily treated with B-vitamin supplementation (folic acid, vitamin B6 and vitamin B12). Daily use of folic acid gives a 25% reduction in homocysteine levels even at low doses of 0.5 mg[4,5]. The question is whether lowering of homocysteine by use of B-vitamin supplementation also lowers the risk for venous thrombosis.

In the VITRO (VItamins and ThROmbosis) study, the primary aim was to investigate the effect of a combination preparation of 5 mg folic acid, 50 mg of pyridoxine and 0.4 mg cyanocobalamin in the secondary prevention of deep-vein thrombosis and pulmonary embolism in patients with a first event of venous thrombosis and hyperhomocysteinemia in a randomized, double-blind and placebo controlled setting.

A secondary aim was to study the effect of vitamin supplementation in patients with a first event of venous thrombosis and a ‘normal’ homocysteine concentration in an identical setting[6].

Patients and methods

Study participants

Patients were selected through anticoagulation clinics in The Netherlands. Anticoagulation clinics monitor the anticoagulant treatment of virtually all patients in well-defined geographical areas. Participating anticoagulation clinics asked all patients with a first venous thrombosis to donate a blood sample for homocysteine determination. Patients with a homocysteine plasma concentration in the top quartile of its distribution in the general population (homocysteine ≥12.6 µmol/l)[7] and who met the entry criteria formed the hyperhomocysteinemic group. Enrollment started in March 1996. Because the rate of inclusion was lower than expected, the trial was extended in 1998 with the Thrombosis Centers of Milan and Vienna who included patients with homocysteine above the 75th percentile based on reference population of the respective countries (homocysteine ≥10.6 µmol/l in Milano and homocysteine

≥8.5 µmol/l in women and ≥10.4 µmol/l in men in Vienna). The latest patient was included in May 2001. Parallel to the study in the hyperhomocysteinemic group we performed a study in the normohomocysteinemic group, which was done only in the Netherlands. During the study there was no folate fortification in these three countries.
For all patients who consented in donating blood for homocysteine measurement information was retrieved from the general practitioner or specialist of the patients about the diagnosis and circumstances in which patients developed their thrombosis. Patients were eligible when they had objectively confirmed proximal deep-vein thrombosis or pulmonary embolism in absence of major risk factors (major surgery, known malignant disease, pregnancy and puerperium or immobility for more than three weeks), are aged between 20 to 80 years at time of diagnosis and without obligatory use of vitamin B. When patients met all entry criteria, they were asked to give their informed consent in accordance with the current revision of the declaration of Helsinki (2000).

Randomization and intervention

Eligible patients were randomized to receive high-dose multivitamin daily or identicalappearing placebo. The high-dose multivitamin capsule contained 5 mg folic acid, 0.4 mg cyanocobalamin and 50 mg pyridoxine. The randomization was performed with 4 and 6 random permuted blocks, stratified by homocysteine status (hyperversus normohomocysteinemia), sex and by anticoagulation clinic or study center. The study medication was based on an earlier study on the homocysteine lowering effects of B-vitamins[5]. The medication was tested for stability for the duration of the trial through determination of the vitamin contents of the vitamin capsules. The ranges found during 42 months were 0.4-0.5 mg/capsule for cobalamin, 48.3-59.1 mg/capsule for pyridoxine and 5.1-7.0 mg/capsule for folic acid. Placebo's were made for this trial and capsules were identical for both placebo and vitamins.
Duration of treatment and follow-up was intended for 2.5 years. Participants were seen (after overnight fasting) at the start of the study (before randomization) and 3, 6, and 24 months after randomization. Blood was collected at each visit, for determination of homocysteine. Patients received their study medication at these follow up visits or by mail every 3 or 6 months. The participants started with their study medication as soon as they were randomized i.e. within the period of anticoagulant treatment in order to achieve the homocysteine lowering effect in the vitamin group before the cessation of anticoagulant treatment. Compliance of the drugs was monitored by measuring homocysteine levels.

End-points

The primary endpoint of the study was recurrent symptomatic DVT or recurrent PE. This endpoint was defined as the decision of the treating physician to restart anticoagulant medication. The treating physician was not informed about study medication or homocysteine concentration. Because it might be difficult to make an accurate diagnosis of recurrent deep-vein thrombosis or pulmonary embolism because of residual thrombi, we provided a tool for the treating physicians to make the diagnosis of recurrent deep-vein thrombosis more accurate. In patients with a deep-vein thrombosis of the leg a compression ultrasonography (CUS) was done 3 months after the thrombotic event. If a residue of the old thrombus was seen on the CUS, the CUS was repeated 6 and if necessary 12 months after the thrombosis. In patients with a PE, CUS of both legs was performed to exclude a DVT. The ultrasonographies were performed in one hospital or institution in every participating center. The results of these tests were noted down in a so-called 'patient passport', a booklet which patients were instructed to take with them if they visited their physician with symptoms of a recurrent thrombosis. By using this passport, data on residual thrombosis were available, even if the patient visited another hospital with complaints of recurrent thrombosis. The recommended definition of 'recurrent DVT' was when a previously normal or normalized venous segment could not be compressed with CUS, or when there was an increment in the diameter of residual thrombus with 4 mm[8,9]. The diagnosis of recurrent PE was according to standard clinical practice.

Laboratory measurements

To screen patients with first-time venous thrombosis for hyperhomocysteinemia, homocysteine has to be measured in a large number of patients. To avoid homocysteine increase after blood sampling, we used blood collection tubes with acidic citrate as anticoagulant[10]. After entering the intervention study blood was taken at 0, 3, 6 and 24 months after start of the study. This blood was collected after an overnight fast in EDTA-tubes and directly placed on ice and centrifuged within one hour. The total homocysteine concentration in EDTA-plasma was measured in one central laboratory (Laboratory of Pediatrics and Neurology in Nijmegen) by an automated high-performance liquid chromatography method with reverse phase and fluorescent detection (Gilson 232-401 sample processor, Spectra Physics 8800 solvent delivery system and Spectra Physics LC 304 fluorometer), essentially according to the method by Fiskerstrand *et al.*[11], with modifications[12].

Study size

Sample size was calculated for the hyperhomocysteinemic group: With alpha=0.05 and beta=0.2 and with an expected recurrence rate of 20% in patients with idiopathic thrombosis (based on the study of Eichinger *et al.*[13]) and hyperhomocysteinemia in 2.5 year, and a 50% risk reduction due to the vitamin therapy (based on a relative risk of more than two for hyperhomocysteinemia[7,13] and the assumption that a 90% of those with homocysteine levels above the 90th percentile could be reduced to less than the 90th percentile with multivitamin treatment[5], 155 patients in each treatment group were required in the hyperhomocysteinemic group[6]. It was decided to randomize the same number of patients in the normohomocysteinemic group. So the intended total sample size was 620.

Statistics

We compared the high-dose vitamin group and the placebo group with respect to age, sex, type of first event (DVT versus pulmonary embolism), initial homocysteine levels for both the hyperhomocysteinemic patients and normohomocysteinemic patients respectively.

Relative risk estimates (hazard ratios) and their 95% confidence intervals (CI) were calculated with a Cox proportional hazard model to assess the effects of high-dose multivitamin supplementation. Variables included in the model were treatment regimen (vitamin versus placebo) and the variables on which the randomization was stratified i.e. sex, anticoagulation clinic and initial homocysteine levels (hyperhomocysteinemic or normohomocysteinemic).

The primary analysis was an intention-to-treat analysis starting at the day of randomization and a follow-up of 2.5 year. We did an on-treatment analysis with restriction of the observation time to the time that patients had reported to take their capsules. A second on-treatment analysis was performed by stratifying the homocysteine reduction in three categories (more than 50% reduction, 50-0% reduction and no reduction in homocysteine level) and calculating the hazard ratio for the first two categories compared to no homocysteine reduction.

Because the treatment regimen started while patients were on anticoagulation treatment (which has a great influence on the risk of recurrence) we also did an analysis without taking in to account the recurrences that occurred before two months after cessation of anticoagulation . In all these three models the data of randomization (and start of the treatment regimen) was the starting time in the Cox model. Finally we used a Cox model with the date of cessation of anticoagulation as starting time.

To assess the role of baseline homocysteine levels as a risk predictor of recurrent events we also performed a Cox model with homocysteine as

continuous variable (in µmol/l) and age, sex and study medication as covariates.

Results

The participating anticoagulation clinics screened 4382 patients (Figure 8.1). Of these patients, 2000 had a homocysteine plasma concentration ≥12.6 µmol/l (75th percentile in a general Dutch population). 1522 of these 2000 patients did not meet the entry criteria and 153 refused participation. The remaining hyperhomocysteinemic patients (n=325) were randomized in the hyperhomocysteinemic group. The Thrombosis Centers of Milan and Vienna included an additional 35 patients with homocysteine above the 75th percentile based on reference population of the centers. Of the 4382 patients screened in the Netherlands 2382 had homocysteine values below 12.6 µmol/l. 1886 patients did not meet the entry criteria or were randomly excluded and 155 refused participation. A total of 341 patients were randomized in the normohomocysteinemic group.

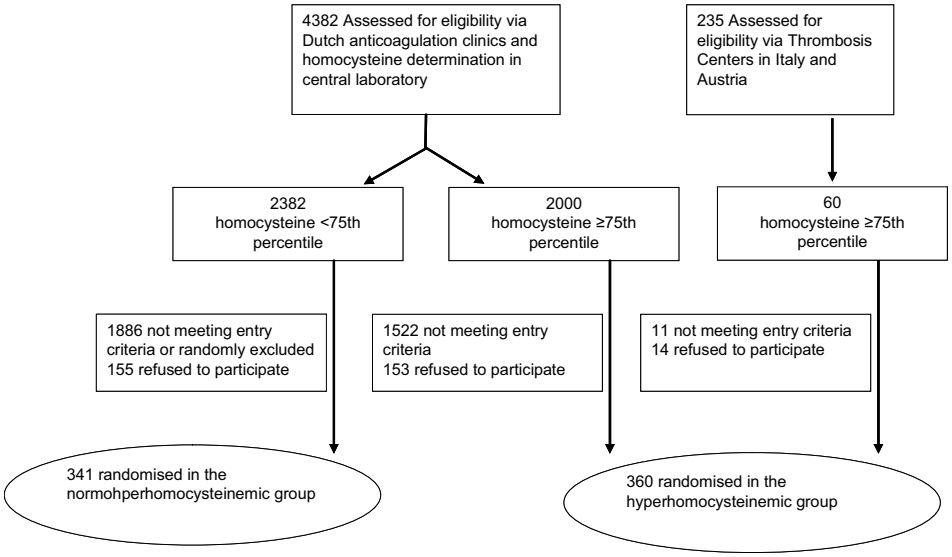

Figure 8.1 Study design

The baseline characteristics for the vitamin and placebo groups according to their homocysteine level (hyperhomocysteinemic and normohomocysteinemic) are shown in Table 8.1. The differences in homocysteine levels between the hyperhomocysteinemic and the normohomocysteinemic group based on the

homocysteine measurement at time of screening remained high at the start of the treatment study. The hyperhomocysteinemic group was slightly older than the normohomocysteinemic group and included more men, due to the use of a uniform cut-off value. However, the vitamin and placebo groups were very similar in both the hyper- and normohomocysteinemic groups.

Table 8.1 Baseline characteristics.

	Hyperhomocysteinemic group (n=360)		Normohomocysteinemic group (n=341)	
Variable	multivitamin (n=177)	placebo (n=183)	multivitamin (n=176)	placebo (n=165)
Sex (M/F)	103/74 (58/41%)	105/78 (57/43%)	80/96 (45/55%)	74/91 (45/55%)
Median age in years (range)	56.4 (18.1-79.9)	57.2 (17.9-79.8)	48.2 (20.2-75.5)	46.3 (19.1-78.5)
Type first event				
deep-vein thrombosis	119 (76%)	126 (69%)	97 (55%)	100 (61%)
pulmonary embolism	43 (24%)	40 (22%)	60 (34%)	51 (31%)
both	15 (8%)	17 (9%)	19 (11%)	14 (8%)
Median duration of anticoagulation in months (range) after randomization	1.6 (0-30)	1.8 (0-30)	1.5 (0-18)	1.6 (0-30)
Geometric mean baseline homocysteine in µmol/l 95% CI)	15.1 (14.3-16.0)	15.9 (14.9-17.0)	9.0 (8.7-9.3)	9.0 (8.7-9.3)
[range]	[6.3-84.8]	[7.4-108.3]	[4.0-23.0]	[4.1-15.5]
Geometric mean homocysteine after three months in µmol/l 95% CI)	8.5 (8.1-8.9)	15.6 (14.5-16.8)	6.5 (6.2-6.7)	9.7 (9.4-10.1)
[range]	[4.1-21.3]	[6.0-91.7]	[2.9-11.6]	[5.5-25.6]

We analyzed the effect of the vitamin/placebo treatment 3 month after start of the intervention. These data demonstrated no effect of placebo on the homocysteine values, whereas a 46% reduction of homocysteine values could be demonstrated in the hyperhomocysteinemic group and a 33% reduction was observed in the normohomocysteinemic group.

During the course of the study, 43 out of 353 (12.2%) patients suffered from a recurrent event of venous thrombosis in the multivitamin group and 50 out of 348 (14.3%) patients had a recurrent venous thrombosis in the placebo group.

Figure 8.2 shows the recurrent thrombosis cumulative incidence curves of patients treated with multivitamins versus those treated with placebo. The overall hazard ratio was 0.84 (95% CI 0.56 to 1.26), e.g. a risk reduction of 16% (95% CI –26 to 44). The hazard ratio associated with vitamin supplementation was 1.14 (95% CI 0.65 to 1.98) in the hyperhomocysteinemic group and 0.58 (95% CI 0.31 to 1.07) in the normohomocysteinemic group. The hazard ratio for men versus women was 1.6 (95% CI 1.05 to 2.45). There was no significant effect for the other covariates.

The results of the on-treatment analysis were similar to the intention to treat analysis (Table 8.2). However, when we stratified the homocysteine reduction

in three categories (more than 50% reduction, 50-0% reduction and no reduction in homocysteine level) we found a hazard ratio of 0.82 (95% CI 0.51 to 1.32) for a 50-0% reduction and 0.43 (95% CI 0.15 to 1.24) for a more than 50% reduction in homocysteine compared to no reduction.

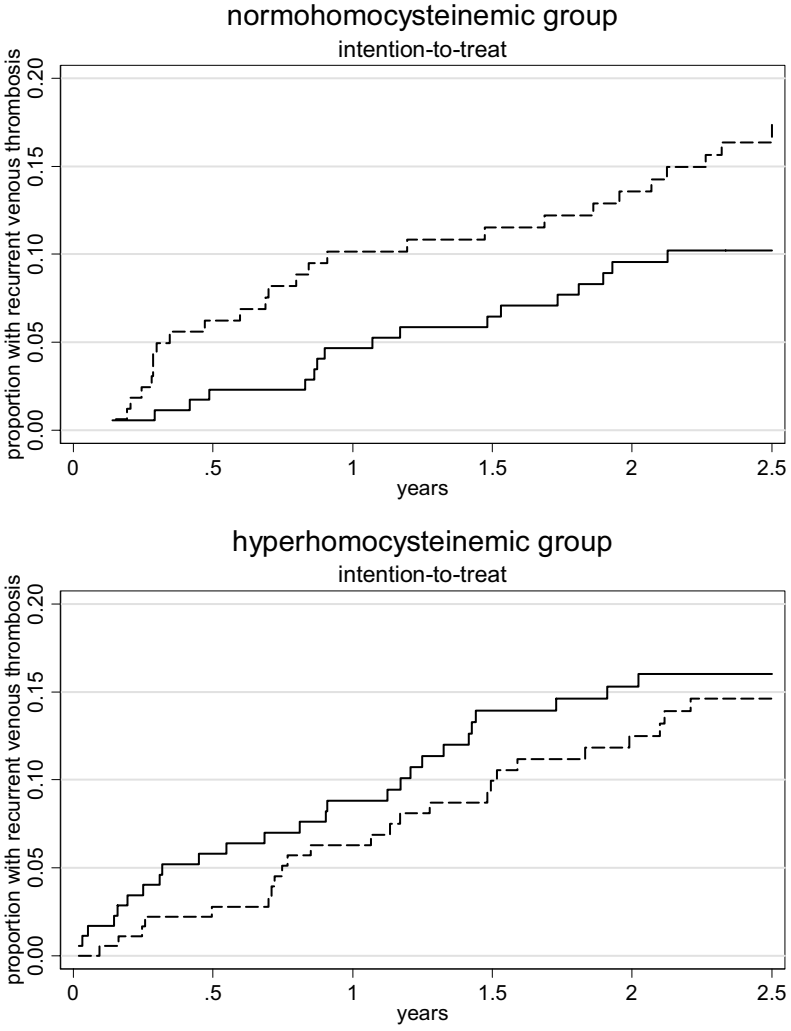

Figure 8.2 Recurrent thrombosis cumulative incidence in patients treated with multivitamin (——) or placebo (- - - -) in a hyperhomocysteinemic and a normohomocysteinemic group.

Because the treatment regimen started while patients were on anticoagulation treatment (which has a great influence on the risk of recurrence) we also did an analysis after exclusion of early recurrences (during anticoagulant treatment or within the first two months after cessation of anticoagulant treatment) (Table 8.2). This subgroup analysis gave similar risk estimates for the hyper- and normohomocysteinemic group. This was also seen in the fourth analysis in which we took the date of cessation of anticoagulation as starting time. in the Cox model.

Table 8.2 Incidences and relative risks for recurrent venous thrombosis

	Vitamin[a] n/py (ir%)	Placebo[a] n/py (ir%)	HR vitamin versus placebo[b]
Intention to treat analysis			
Hyperhomocysteinemic group	26/387 (6.7%)	24/403 (6.0%)	1.14 (0.65 to 1.98)
Normohomocysteinemicgroup	17/412 (4.1%)	26/373 (7.0%)	0.58 (0.32 to 1.08)
Overall	43/799 (5.4)%	50/776 (6.4%)	0.84 (0.56 to 1.26)
On treatment analysis			
Hyperhomocysteinemic group	24/338 (7.1%)	22/344 (6.4%)	1.13 (0.63 to 2.02)
Normohomocysteinemicgroup	16/363 (4.4%)	22/337 (6.5%)	0.65 (0.34 to 1.24)
Overall	40/702 (5.7%)	44/682 (6.4%)	0.88 (0.57 to 1.36)
Intention to treat analysis with exclusion of early recurrences[c]			
Hyperhomocysteinemic group	17/387 (4.4%)	21/403 (5.2%)	0.84 (0.44 to 1.60)
Normohomocysteinemicgroup	15/412 (3.6%)	20/373 (5.4%)	0.66 (0.34 to 1.30)
Overall	32/799 (4.0%)	41/775 (5.3%)	0.76 (0.48 to 1.21)
Intention to treat analysis beginning after cessation of anticoagulation			
Hyperhomocysteinemic group	23/338 (6.8%)	24/347 (6.9%)	0.98 (0.55 to 1.74)
Normohomocysteinemicgroup	17/379 (4.5%)	24/338 (7.1%)	0.62 (0.33 to 1.15)
Overall	40/717 (5.6%)	48/685 (7.0%)	0.80 (0.52 to 1.21)

[a] number of recurrences,py=person years, ir=annual incidence in %; [b] HR=hazard ratio (95% CI), adjusted for study center, sex and hyper-/normohomocysteinemia; c early recurrences: recurrences before two months after cessation of anticoagulation.

Although the duration of anticoagulant treatment was similar for the various groups, there was a relatively high number of early recurrences in the hyperhomocysteinemic vitamin group (9 events) compared with the hyperhomocysteinemic placebo group (3 events). In contrast, in the normohomocysteinemic group early recurrences occurred more often in the placebogroup (6 events) than in the vitamin group (2 events).

To assess the role of baseline homocysteine levels as a risk predictor of recurrent events we performed a Cox model with homocysteine as continuous variable (in µmol/l) and age, sex and study medication as covariates.The hazard ratio for recurrence associated with a 5 µmol/l higher homocysteine level at baseline was 1.13 (95% CI 1.05 to 1.20). This effect was similar in the

placebo group as in the vitamin group. The hazard ratio for a homocysteine concentration above the 90th percentile (20.1 µmol/l) was 1.8 (95% CI 1.1 to 3.2). Homocysteine levels were not associated with early recurrences.

Discussion

Our study is the first clinical trial on the effect of B-vitamins in the prevention of recurrent venous thrombosis. Our study shows that B-vitamin supplementation lowers homocysteine values but it doesn't show a risk reduction in recurrent venous thrombosis. Homocysteine at baseline is a modest risk factor for recurrent events.

The results of our trial showed a difference in effect in the hyperhomocysteinemic group compared to the normohomocysteinemic group. This difference in effect was contrary to what was expected and could not be biologically explained. Therefore we looked for possible explanations for this finding. One explanation is that there is an uneven distribution of early recurrences during or shortly after discontinuation of anticoagulation.

These recurrences might be explained by other risk factors (such as cancer) or may be the result of a rebound phenomenon[14]. In fact, these early recurrences were not associated with basal homocysteine levels (as were the recurrences during follow-up), so the uneven distribution over the various treatment groups could be attributed to chance. When we excluded early recurrences, the overall risk estimate became 0.76, and the effects in the hyper- and normohomocysteinemic group were quite similar. The same occurs after taking the date of anticoagulant cessation as starting point for the survival analysis. Although, these analyses are post-hoc analyses, they support that the overall estimate of 0.84 (95% CI 0.56 to 1.26) is the best summary of the study, despite an initial heterogeneity of effect.

The domain of our trial was idiopathic venous thrombosis. We had very strict inclusion criteria (objectively confirmed first event of proximal deep-vein thrombosis or pulmonary embolism in absence of major risk factors - major surgery, known malignant disease, pregnancy and puerperium or immobility for more than three weeks -, are aged between 20 to 80 years at time of diagnosis and without obligatory use of B-vitamins). Most of the patients were not eligible because thrombosis occurred after surgery, patients were older than 80, had cancer or had a recurrent event. For these reasons many patients had to be screened in order to include the required number of patients for this study.

An important point in clinical trials with B-vitamins is the difference achieved in homocysteine concentration in the vitamin group and the placebo group. This difference was small in a trial in stroke patients in North-America[15]. In the design of our study we opted for a strong homocysteine lowering effect, which

was found in a schedule with 5 mg folate, 0.4 mg vitamin B12 and 50 mg vitamin B6 [5]

Furthermore our study was done in an area without food-fortification with folate. Therefore, a strong difference in median homocysteine between high-dose multivitamin and placebo of 6.3 µmol/l (42%) in the hyperhomocysteinemic group and 2.9 µmol/l (30%) in the normohomocysteinemic group was found.

An on-treatment analysis based on the percentage of reduction showed a trend to a risk reduction in subjects with the highest reduction in homocysteine. This finding stresses the importance of adequate homocysteine reduction in clinical trials with B-vitamins. The dose-response relationship gives also some indication that our trial does not completely exclude an effect of vitamin supplementation to prevent recurrent venous thrombosis.

Our study was designed in 1995. For the sample size calculation we assumed a risk reduction of 50% that was based on earlier case-control studies and especially on a cohort study in patients with first time venous thrombosis with a relative risk of 2.7 for recurrent thrombosis in patients in the top-quartile of the homocysteine distribution[13]. Findings from others, after the start of this trial, indicated less strong effects of hyperhomocysteinemia on the risk of first thrombosis. In a recent metaanalysis we found a relative risk for venous thrombosis between 1.27 in prospective and 1.60 in retrospective studies for a 5 µmol/l increase in homocysteine[1]. On the basis of a meta-analysis of MTHFR 677TT genotype the risk associated with a 3 µmol/l increase in homocysteine levels was 16% (1,3). So, the main conclusion of our study is that vitamin supplementation for treatment of hyperhomocysteinemia does not results in an apparent decrease in incidence of recurrent events. A second conclusion is that our study has not enough power to detect or rule out a modest risk reduction of 10-20% that is expected now on the base of prospective and genetic studies. However, the question is whether such a modest risk reduction is clinically relevant, because the associated numbers needed to treat are large (75-150 /year) In the field of arterial vascular disease 12 studies on the effect of vitamin treatment on vascular disease are initiated[16] of which three are published now[15,17,18]. None of these trials did show a beneficial effect of vitamin supplementation on the incidence of recurrent vascular events. It should be noted that in these trials vitamin supplementation was added to standard treatment that included generally platelet aggregation inhibitors, cholesterol-lowering drugs and antihypertensive medication, which is not a standard treatment after an event of venous thrombosis. Therefore the effect of vitamin supplementation might be different in a trial in patients with venous thrombosis compared to trials in cardiovascular patients.

Our study was a secondary prevention study. This implies that the risk of recurrent venous thrombosis was the subject of study, which might be different from the risks of first-time venous thrombosis. This is clearly demonstrated by

the observation that factor V Leiden - which is a strong risk factor for first-time venous thrombosis – is not or only weakly associated with recurrent venous thrombosis[19]. Two prospective studies has been published on the risk for a recurrent event of venous thrombosis associated with hyperhomocysteinemia. In the first study elevated homocysteine levels (above the 75the percentile) were associated with a 2.7-fold increase in risk[13]. In the second study no increased risk was found (hazard ratio 0.9 (95% CI 0.5 to 1.6))[19]. In our study baseline homocysteine concentration is a predictor of recurrent venous thrombosis. However, the relative risk is lower than the risk for first time venous thrombosis[1].

One of the problems with secondary prevention studies in venous thrombosis is the diagnosis of a recurrent event. It could be difficult to distinguish between a recurrent event and the persistence of a residual thrombus (especially in DVT). To facilitate uniform diagnosis within the study we did repeated ultrasound examinations after the first event and provided a 'patient passport' with information for the treating physician. There was however no central validation of the diagnosis, which is a potential limitation of the study. We have chosen for the decision of the treating physician to restart anticoagulant treatment as defined endpoint, which is in fact the most clinical relevant parameter.

Several explanations can be given to the observation that baseline homocysteine levels are predictive for a recurrent event but homocysteine lowering does not result in a decrease in incidence. First, it might be a matter of insufficient power of our study to detect small effects. This explanation is supported by the post-hoc analyses and the dose-response relationship. Second, it can be explained by another factor that is related to homocysteine but is not affected by vitamin supplementation. However, based on current knowledge there is no evidence to treat patients with venous thrombosis with B-vitamins in order to prevent recurrent events.

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