Hyperhomocysteinemia and venous thrombosis : studies into risk and therapy

Willems, H.P.J.

Chapter 2

Homocysteine and venous thrombosis: outline of a vitamin intervention trial

HPJ Willems, M den Heijer, GMJ Bos

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Abstract

In the past years several case-control studies established the association of an elevated plasma homocysteine concentration and the risk of venous thromboembolism. It is still unclear if elevated homocysteine concentrations can cause venous thrombosis. The VITRO (VItamins and ThROmbosis) trial is the first multicenter, randomized, doubleblind and placebo-controlled study to evaluate the effect of homocysteine-lowering therapy by means of 5 mg folic acid, 0.4 mg vitamin B12 and 50 mg vitamin B6. The study is a secondary prevention trial in 600 patients who suffered from a first episode of idiopathic deep vein thrombosis (DVT) or pulmonary embolism (PE), or both. There will be 300 hyperhomocysteinemic and 300 normohomocysteinemic patients included, all with an objectivated venous thrombosis. The end point is recurrence of venous thrombosis.

Introduction

Venous thromboembolism is a common illness with an incidence of 1 to 2 per 1000 per year[1,2]. Common causes of venous thromboembolism are acquired factors (cancer, immobility, fractures of the leg, knee or hip operations and use of oral contraceptives) or hereditary factors (deficiencies of protein C, protein S, and antithrombin[3], high levels of Factor VIII[4], mutations in the Factor II gene[5] and in the Factor V gene resulting in activated protein C resistance[6]). In the past two decades much emphasis has been laid on the role of mild hyperhomocysteinemia as a possible risk factor of venous as well as arterial thromboembolism. In this article we will discuss the association of hyperhomocysteinemia and venous thrombosis, and we will give the design of the VITRO study, a secondary prevention study on the effect of homocysteine-lowering therapy on recurrence of venous thrombosis.

Homocysteine and venous thrombosis

Given the high incidence of arterial thrombotic disease in patients with classic homocystinuria, Wilcken and Wilcken[7] investigated the association of homocysteine and arterial thrombotic disease in patients with premature arterial disease. The association they found was later confirmed by a number of retrospective and prospective studies[8]. These studies established hyperhomocysteinemia as a possible risk factor for arterial thrombotic disease. Hwever, patients with homocystinuria are afflicted not only by arterial thrombosis but also by venous thromboembolism (VTE). By analogy to the hypothesis of Wilcken and Wilcken[7] in arterial disease, several studies have been performed since 1991 to investigate the association of homocysteine and venous thrombotic disease. The epidemiological evidence of homocysteine as a risk factor for venous thrombosis, however, is not as abundant as for arterial thrombotic disease (Table 2.1).

Table 2.1 Published studies on the relation of homocysteine and venous thrombosis.

Authors	Publication Year	Study Method	Age	Cut-Off tHcy	Fasting/ MLT[a]	Cases (N)	Controls (N)	Elevated tHcy Cases (N)	Elevated tHcy Controls (N)	Odds Ratio (95% CI)
Brattstrom et al.[9]	1991	case-control	<50	mean +2 SD	fasting	42	42	4	3	1.4 (0.3–6.5)[b]
					MLT			6	2	3.3 (0.6–17.6)[b]
Bienvenu et al.[10]	1993	case-control	<57	mean +2.7 SD	Fasting[c]	23[f]	49	7	0	—
Falcon et al.[11]	1994	case-control	<50	mean +2 SD	fasting	80	51	7	0	—
					MLT	79	40	14	1	8.4 (1.1–66.4)[b]
Amundsen et al.[14]	1995	case-control	<57	mean +2 SD	fasting	35	39	2	1	2.3 (0.2–26.6)[b]
					MLT			2	1	2.3 (0.2–26.6)[b]
Fermo et al.[15]	1995	case-control	mean 36	95th percentile	fasting	107	60	10	3	2.0 (0.5–7.4)[b]
					MLT	58	60	11	3	3.7 (1.0–13.8)[b]
den Heijer et al.[12]	1995	case-control	<88	90th percentile	fasting	185	220	46	21	3.1 (1.8–5.5)
					MLT			44	20	3.1 (1.7–5.5)
Cattaneo et al.[16]	1996	case-control	?	95th percentile	fasting	89	89	7	4	1.8 (0.5–6.4)
					MLT			7	4	1.8 (0.5–6.4)
den Heijer et al.[13]	1996	population based case-control	<70	95th percentile	fasting[c]	269	269	28	13	2.5 (1.2–5.2)
Simioni et al.[17]	1996	case-control	<92	90th percentile[d]	fasting	60	148	15	17	2.6 (1.1–5.9)
Ridker et al.[18]	1997	prospective, nested case-control	mean 60	95th percentile	fasting[c]	145	646	10	29	1.6 (0.8–3.3)
						?	?	?	?	3.4 (1.6–7.3)[e]
Eichinger et al.[19]	1998	prospective	<85	95th percentile[d]	fasting	28	236	12	54	2.7 (1.3–5.8)

[a] MLT, methionine loading test: post-methionine loading tHcy or increase of tHcy compared with baseline after loading; [b] odds ratio calculated from the published data; [c] non-fasting; [d] from a previously selected reference group; [e] only idiopathic thrombosis are analyzed; number of patients is not published; [f] venous and arterial thrombosis.

Retrospective Studies

In 1991 Brattstrom et al.[9] published a study in which average homocysteine concentrations did not differ between patients with VTE and controls. Patients, however, had an elevated level more often, although it was not significant because of low sample size. Bienvenu et al.[10] in 1993 found a distinct difference in homocysteine concentrations between patients with either arterial or venous thrombosis and healthy controls. This finding was confirmed by Falcon et al.[11] Our group published a study in 1995: fasting and post-methionine homocysteine concentrations of 185 patients with a history of recurrent VTE and 220 controls from a general practice were compared[12]. Odds ratios of 3.1 were found for both preload and postload homocysteine concentrations above the 90th percentile of the control group (18.6 µmol/l). An increase of the risk was already seen at homocysteine concentrations of 14.0 µmol/l. A second study was performed to estimate the risk of homocysteine for a first episode of VTE. Homocysteine concentrations of a subgroup of patients participating in the Leiden Thrombophilia Study were analyzed[13]. All patients had had a first, objectively confirmed DVT. Baseline but no postload homocysteine levels were available. An odds ratio of 2.5 was calculated for homocysteine concentrations above the 95th percentile of the control group. Also, in this group the odds ratios increased with higher cut-off concentrations. The effect was independent from known hereditary risk factors for VTE such as protein C, protein S, or antithrombin deficiency and, unexplainably, more pronounced in women than in men. No interrelation between Factor V Leiden and hyperhomocysteinemia could be established as a result of the small number of subjects with both abnormilities.
More case-control studies were published subsequently that confirmed the association of hyperhomocysteinemia and venous thrombosis[14-17].

Prospective Studies

Only two prospective studies have been published so far. The U.S. Physicians Health Study is a prospective cohort study in male physicians[18]. Homocysteine concentrations were determined in 14,916 men at baseline. From these men 158 developed a venous thrombosis during the subsequent follow-up years (mean, 12 years). These cases were matched with 646 controls from the same cohort. When analyzing all VTE cases with controls, the investigators found no association between an elevated homocysteine concentration and VTE. However, when only the cases who had suffered an idiopathic VTE were analyzed, they found a relative risk of 3.4. Furthermore, this study demonstrated an even more increased risk of VTE when both hyperhomo-

cysteinemia and Factor V Leiden were present, suggesting a synergy. Also, this finding was more pronounced when only idiopathic cases of VTE were analyzed.

A second prospective study was performed by Eichinger et al.[19] They selected patients with a first episode of idiopathic VTE. After the event total homocysteine (tHcy) was measured. Patients were followed for recurrence of VTE. Hyperhomocysteinemic patients were found to be at greater risk for recurrence than were the normohomocysteinemic patients. This resulted in a relative risk of 2.6 when corrected for age, sex, and the presence of Factor V Leiden.

In a meta-analysis of 10 published case-control studies on the risk of hyperhomocysteinemia and venous thrombosis, pooled estimates of the odds ratios were calculated[20]. The authors found odds ratios of 2.5 (95% confidence interval (CI) 1.8 to 3.5) for fasting levels and 2.6 (95% CI 1.6 to 4.4) for postmethionine increased concentrations, supporting the hypothesis that hyperhomocysteinemia is a risk factor for venous thromboembolism.

As yet there is no clear evidence how hyperhomocysteinemia could lead to venous thrombosis. How hyperhomocysteinemia might cause thrombosis is subject of a different article in this issue.

Therapy of hyperhomocysteinemia

Folic acid, hydroxycobalamin (vitamin B12), and pyridoxine (vitamin B6) form the key elements in the therapy of hyperhomocysteinemia. These vitamins function as cosubstrate or as cofactor in the metabolic pathways of homocysteine. High-dose pyridoxine, which as pyridoxal-‘5-phosphate acts as cofactor with cystathionine beta-synthase (CS) in the transsulphuration pathway, is the vitamin that is used in the treatment of classical homocysteinuria. Therapy with pyridoxine is suggested to decrease the incidence of vascular complications in these patients[21,22]. A meta-analysis of 10 studies by the Homocysteine Trialists’ Collaborators showed that in mild hyperhomocysteinemia folic acid is the main homocysteinelowering vitamin[23]: 0.5 to 5 mg of folic acid daily reduced homocysteine concentrations by about 25%. An extra 7% decrease was produced by 0.5 mg/d of vitamin B12. Not only elevated levels of homocysteine but also concentrations usually regarded as normal (<16 µmol/l) were decreased by vitamin supplementation[24]. Similar decreases were found in patients with venous thrombosis as in controls[24].

VITRO study

Study rationale

The clinical key question is whether a decrease of homocysteine concentrations will prevent thromboembolic events[25].This is a question that needs to be answered: It will make us understand more about homocysteine as a possible causal agent of thromboembolic disorders, and it could have great impact on the prevention of vascular diseases[8]. (Although homocysteine is not a strong risk factor, it is, when defined as levels above the 75th or 90th percentile, a very prevalent one.)
In order to answer this question, intervention studies are needed. These studies should be randomized and placebo controlled and therefore not susceptible to bias or confounding factors such as vitamin supplementation or changing dietary habits that can easily influence homocysteine levels. Some investigators argue the need for randomized trials because a substantial amount of evidence is available from retrospective and prospective studies and vitamin therapy appears to be safe. However, every therapeutic intervention deserves a proper evaluation before it is used on a broad scale. Furthermore, although vitamin therapy appears to be safe, the risk of unsuspected adverse effects should be taken into account in every intervention.
In 1996 the VITRO trial was started in the Netherlands. The VITRO trial is a study on the effect of vitamin B in the secondary prevention of venous thrombosis. It is a multicenter, randomized, double-blind and placebocontrolled trial. The study is a collaborative project of the Department of Hematology of the Leyenburg Hospital in The Hague, the Department of Clinical Epidemiology of the Leiden University Hospital, the Laboratory of Pediatrics and Neurology of the Nijmegen University Hospital, and the anticoagulation clinics of The Hague, Rotterdam, Amsterdam, Utrecht, Leiden, Amersfoort, and Delft, The Netherlands. Furthermore, there are four participating centers outside the Netherlands: in Vienna, Austria, and in Milano, Bolzano, and Bologna, Italy.

Study objective

The trial was designed to evaluate whether the effect of homocysteine-lowering therapy by means of multivitamin B in patients with a primary venous thromboembolism and hyperhomocysteinemia leads to a reduction of recurrent thrombosis.

Patient selection

In the Netherlands almost all patients treated with oral anticoagulants are registered at anticoagulation clinics after hospital discharge or directly after

diagnosis when treated in an outpatient setting. The anticoagulation clinics monitor the treatment by international normalized ratio (INR) measurements and adjust the dose of the coumarins. Patients are recruited for the VITRO study by means of these anticoagulation clinics (Figure 2.1).

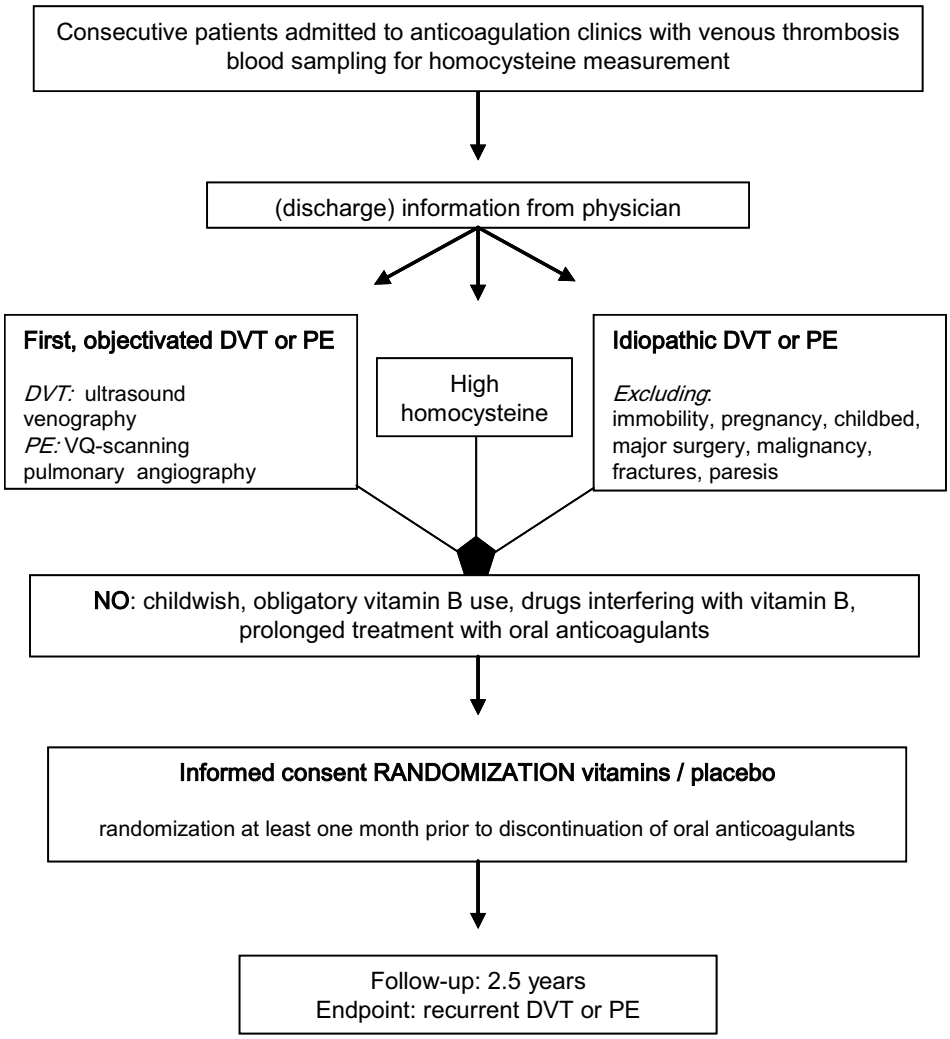

Figure 2.1 VITRO trial: patient selection and randomization.

All new patients registered with the diagnosis of primary DVT or PE are asked to donate an extra blood sample for measurement of their homocysteine concentration. The previously mentioned centers cover a well-defined geographical area of about 3.5 million people in the Netherlands. Because these centers treat nearly all patients with venous thrombosis, the VITRO study is able to include patients without substantial referral bias. The blood is sampled in tubes containing acidic citrate as anticoagulant. In these tubes the blood is stabilized at room temperature for measurement of the homocysteine concentrations[26]. Homocysteine values measured in these tubes correlate very well with those measured in classically used tubes, in other words, tubes containing ethylenediaminetetracetic acid (EDTA) as anticoagulant, that are put on melting ice immediately after blood collection until processing (Willems et al., unpublished data). The blood is processed in the anticoagulation clinic and the plasma is sent to Nijmegen for determination of the homocysteine by regular mail because plasma samples stay stable for several days at room temperature[27]. In the meantime, information is retrieved from the patients' general practitioner or specialist about the diagnosis and circumstances in which the patients developed their thrombosis. Once patients have met all entry criteria, they are asked to participate in the study 1 month before the treatment with the coumarins is terminated. In this month a substantial homocysteine decrease can be achieved[23]. Patients have to give their informed consent in accordance with the current revision of the declaration of Helsinki (1975) in order to participate in the study.

Hyperhomocysteinemic and normohomocysteinemic groups

Two groups of patients are recruited for the VITRO study: a group of patients with hyperhomocysteinemia and a group of similar size with patients with normohomocysteinemia. Because the number of patients with normohomocysteinemia presented by the anticoagulation clinics is greater than the number with hyperhomocysteinemia, a random selection of all normohomocysteinemic patients is made in order to achieve a parallel inclusion: one normohomocysteinemic patient for every hyperhomocysteinemic patient included.

Entry criteria

1. Objectively confirmed, primary, proximal DVT (diagnosed with compression ultrasonography or venography) or PE (diagnosed with "high probability" ventilation-perfusion [V/Q] scanning or pulmonary angiography), or both
2. Homocysteine concentrations above the 75th percentile of a reference group of the general population from which the patients are selected (hyperhomocysteinemic group) or homocysteine concentrations below the 75th percentile of this reference group (normohomocysteinemic group)

3. Age 20 to 80 years
4. Absence of other major risk factors for DVT such as major abdominal surgery, major hip and knee operations, fractures of the legs or hips, multitrauma, malignant disease, pregnancy and childbirth, paralysis of the legs, immobility for more than 3 weeks
5. Ability to start the study at least 4 weeks before discontinuation of the coumarins in order to ensure substantial homocysteine decrease at that time Patients with the following conditions are excluded: (obligatory) use of vitamin B, continued use of coumarins, pregnancy or trying to get pregnant, and the use of medications that are influenced by folic acid (phenytoin, L-dopa, methotrexate).

Randomization

Randomization is done by someone not involved in the treatment of the patients. The randomization is stratified by gender and by anticoagulation clinic. Randomization tables with six and four random permuted blocks are used for the randomization.

Treatment schedule and trial medication

The treatment group will be treated with 1 capsule daily containing 5 mg folic acid, 400 μg hydroxycobalamin, and 50 mg pyridoxine. The stability of this combination has been tested and proven valid for the duration of the study. The placebo group will take 1 placebo capsule daily. The study medication is provided by Dagra Farma, Amsterdam, The Netherlands.

Follow-up

Randomized patients are seen at outpatient clinics at the start of the study and 3, 6, and 24 months after randomization. Because the time span between the follow up visits to the outpatient clinics is quite large, patients will be interviewed by telephone every 6 months. The duration of the study for each patient is 2.5 years. Because the last visit to the outpatient clinic is already after 2 years, the trial will end for each patient after an interview
by telephone. At each follow-up visit blood is sampled for determination of tHcy. By means of determination of tHcy, therapy compliance is measured. Patients are seen after overnight fasting. Patients receive their study medication at these follow-up visits, and the leftover capsules will be taken back.

Ultrasonographies

In patients with DVT of the leg, a compression ultrasonography (cUS) is performed 3 months after the thrombosis. The cUS is done to enhance the diagnosis of a possible recurrence. If residue of the old thrombus is seen on the cUS, the cUS is repeated 6 and, if necessary, 12 months after the thrombosis. In patients with a PE, a cUS of both legs is performed to exclude a DVT. The ultrasonographies are performed in one hospital or institution in every participating city. The results of the cUS are noted in a so-called patient passport, a booklet that patients are instructed to give to the physician if signs and symptoms of a thrombosis recur. By using this passport, the data on the residual thrombosis are available, even if patients visit other hospitals. The diagnosis “recurrent DVT” can be made when a previously normal or normalized venous segment cannot be compressed or when there is an increase in the diameter of residual thrombus by 4 mm[28,29].

Endpoint

Endpoint in the study is recurrent DVT or recurrent PE, or both, as diagnosed by the treating physician and scored definitive if anticoagulants are prescibed. The diagnosis might be confirmed by using objective tests as described before. Analysis will be done on the confirmed and nonconfirmed (but indicating clinical relevance) recurrent events and on the confirmed recurrences only.

Study size

The study size is calculated for the hyperhomocysteinemic group. With α=0.05 and β=0.2 and with a recurrence percentage of 20% in the placebo group and a 50% risk reduction because of the vitamin therapy, 155 patients in each treatment group are required if tested one sided. This means that more than 310 patients will be randomized. A similar number of patients will be included in the normohomocysteinemic group, resulting in a total study size of 620 patients.

Possible outcome and outlook

For the VITRO study only patients with an idiopathic venous thrombosis are selected. It is hypothesized that these patients have no apparent cause of their thrombosis; therefore, the contribution of homocysteine in the etiology of the thrombosis might be greater than in patients with very apparent causes such as bedrest, hip fractures, and so on. This hypothesis was later confirmed by the results of the study by Ridker et al [18], which showed a higher odds ratio in idiopathic thrombosis than it did in nonidiopathic thrombosis. A second

assumption is that patients with idiopathic DVT or PE are at higher risk for recurrent thrombosis in comparison with patients with an environmental and reversible cause [30]. Based on the results of the case-control studies, homocysteine is a risk factor for venous thrombosis. However, it is not a certainty that lowering homocysteine concentrations will have an effect on recurrence of events. Most case-control studies are retrospective and do not address the issue of causality; they only show associations. It could be hypothesized that homocysteine is a result of the thrombosis, in which case homocysteinelowering therapy will not result in a decrease in the number of recurrent events. However, from the results of the two prospective studies, this is not likely. Another, more likely assumption is that the homocysteine increase is an epiphenomenon: an unknown disorder leading to thrombosis as well as to homocysteine increment. If homocysteine is such an epiphenomenon, prospective case-control studies will show an association, but homocysteine-lowering therapy will not take away the causal agent of the thrombosis and intervention will not result in a decease of recurrent events. Even if homocysteine is a causal agent in the development of venous thrombosis, the effect of homocysteine- lowering therapy on *recurrent* thrombosis can only be assumed. Only two of the previously discussed studies address the association of homocysteine and recurrent thrombosis. The study by den Heijer *et al*. [12] on recurrent thrombosis shows an odds ratio that is very much in the range of the odds ratios found in studies on primary thrombosis. One could conclude that homocysteine is a risk factor only for primary thrombosis and not a risk factor for recurrence any more when patients have already had their first thrombotic event. Other risk factors, for instance an insufficient venous system caused by the first thrombosis, then play a much more important role that diminishes a possible effect of homocysteine.

Lowering homocysteine concentrations would not make a great difference or would even be useless. The study by Eichinger *et al*.[19] however, estimated the risk of recurrent thrombosis compared with patients who already had a first thrombotic event and showed an odds ratio of 2.7, supporting the hypothesis that homocysteine-lowering therapy could have an effect on recurrent thrombosis.

In this study two groups of patients were selected, a hyperhomocysteinemic and a normohomocysteinemic group. Based on the results of case-control studies, the risk of disease in this latter group is lower than it is in the hyperhomocysteinemic group [12,13,19].The addition of a normohomocysteinemic group can provide us with important extra information. Four types of outcome can be hypothesized.

1. There is no effect of vitamin therapy on recurrence in both groups. This outcome hypothesis is discussed earlier.

2. Vitamin therapy will only have an effect in the hyperhomocysteinemic group, suggesting that hyperhomocysteinemia above a certain concentration is a causal agent in the development of venous thrombosis.
3. Vitamin therapy will have a similar effect in both the hyperhomocysteinemic and the normohomocysteinemic group, suggesting a pathophysiological mechanism that acts independent of homocysteine (e.g., folic acid mediated).
4. The effect of vitamin therapy is greater in the hyperhomocysteinemic group than it is in the normohomocysteinemic group. This suggests a graded association of homocysteine and venous thrombosis: The effect of vitamins will be more pronounced in patients with higher levels of homocysteine. Because vitamin therapy causes homocysteine decrease, even of previous "normal" concentrations[24] effect of vitamins on recurrence of thrombosis in the normohomocysteinemic group cannot be ruled out. Although there are not sufficient data from the case-control studies done in venous thrombosis, studies in arterial vascular disease suggest such a graded response[8]. Based on the results from the hyperhomocysteinemic group, trend analyses will be performed in the VITRO study to measure the effect in the normohomocysteinemic group.

In 1996, the trial was started in The Netherlands. In 1998, the four centers outside The Netherlands were approached for participation and started randomizing patients. These centers are provided with the same study medication and randomize patients according to the same entry criteria. Homocysteine measurements are done in each respective center and the cut-off level of the homocysteine is based on the 75th percentile of the local reference group. By July 1999, more than 500 patients were randomized. The study is expected to be complete in the beginning of 2000. Results of the study can be expected by the end of 2002.

If homocysteine lowering effectively decreases the number of recurrent venous thromboembolic events, this will be of great benefit for patients. It will not only reduce the risk of recurrence but also diminish morbidity and mortality resulting from the use of long-term anticoagulant therapy in patients after a second event. In addition, if vitamin treatment will reduce the number of events it will prove the relevance of homocysteine metabolism as a risk factor in vascular disease, a conclusion impossible to make from case-control studies and still a subject of debate in the prospective studies.

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