COR ET VASA 55 (2013) E248–E258

Available online at www.sciencedirect.com
SciVerse ScienceDirect
journal homepage: www.elsevier.com/locate/crvasa

Review Article

Homocysteine and venous thromboembolism—Is there any link?

*Jana Hirmerová**

II.interní klinika LFUK a FN Plzeň, přednosta prof. MUDr. Jan Filipovský, CSc, Dr. E. Beneše 13, 30599 Plzeň Czech Republic

ARTICLE INFO

Article history:
Received 2 December 2012
Received in revised form
17 January 2013
Accepted 20 January 2013
Available online 31 January 2013

Keywords:
Homocysteine
Venous thromboembolism
Trombophilia
Risk reduction

ABSTRACT

Homocysteine is an intermediary product of methionine metabolism. The level of homocysteine is controlled by two pathways—remethylation and transsulphuration. Elevated homocysteine level may result from deficiency or impaired function of enzymes and cofactors in these pathways. Homocystinuria is a rare genetic disease with extreme hyperhomocysteinemia and is associated with the occurrence of arterial and venous thrombotic events at young age. Therefore, homocysteine has been considered a risk factor for vascular diseases.

Plasma homocysteine level is influenced by many factors, genetic as well as environmental. Mild hyperhomocysteinemia is quite common. The role of homocysteine in venous thrombosis has been studied less extensively than its role in arterial diseases and nowadays it seems quite controversial. In vitro, it is possible to demonstrate multiple prothrombotic action of homocysteine. However, the results of epidemiologic studies are not so clear. Most of them found an association of hyperhomocysteinemia with venous thromboembolism (VTE) but the association was quite weak and moreover, it was much weaker in prospective than in retrospective studies. It is not quite clear whether elevated homocysteine level is the cause of thromboembolic event or the consequence of it. It is also possible that hyperhomocysteinemia plays a role in the pathogenesis of VTE only as an additional risk factor in the presence of other thrombophilic disorders.

However, some data confirm hyperhomocysteinemia as a risk factor for recurrent VTE. Some smaller studies have also found association of hyperhomocysteinemia with venous thrombosis at unusual sites.

Homocysteine level can be lowered by vitamin supplementation, especially with folic acid and vitamin B12. So far, the benefit of lowering homocysteine level in primary and secondary VTE prevention has not been clearly proven.

Currently, there is not enough evidence to support the necessity of testing homocysteine level in VTE patients, neither is sufficient evidence of the benefit of vitamin supplementation in mild or moderate hyperhomocysteinemia. Therefore, such testing and supplementation should be performed only in selected cases.

*Tel.: +420 377402375; fax: +420 377402374.
E-mail address: hirmerova@fnplzen.cz

0010-8650/$ - see front matter © 2013 The Czech Society of Cardiology. Published by Elsevier Urban & Partner Sp.z.o.o. All rights reserved.
http://dx.doi.org/10.1016/j.crvasa.2013.01.007

Contents

1. Introduction

Venous thromboembolism (VTE) is a multifactorial disease, with many inherited and acquired risk factors and their interaction playing a role in the etiopathogenesis. One of important risk factors is thrombophilia. It is defined as a tendency to form thrombi in veins or arteries but the term is used more frequently in association with venous thrombosis. Hyperhomocysteinemia is often included in the list of thrombophilic disorders [1]. However, there is much disagreement concerning the importance of homocysteine level testing and potential management of hyperhomocysteinemia in VTE patients.

2. Homocysteine metabolism

Homocysteine, a sulfur-containing amino acid, is an intermediary product of methionine metabolism (methionine is an essential amino acid, abundant in animal proteins). Homocysteine is formed intracellularly by demethylation of dietary methionine. Plasma homocysteine level is controlled by two metabolic pathways – remethylation to methionine and transsulphuration to cysteine – Fig. 1. Remethylation is the predominant metabolic pathway which is important for the regulation of fasting level while transsulphuration pathway regulates elevated homocysteine levels, e.g. postprandially or after methionine load.

Remethylation has two alternative pathways in humans.

(a) The predominant one is vitamin B12 (cobalamin) dependent, it occurs in all tissues including vascular endothelium. This pathway is connected with folate cycle.
(b) The additional remethylation pathway is less important, it is bound to hepatocytes.

In transsulpuration pathway, vitamin B6 is required as a cofactor [2–4].

3. Hyperhomocysteinemia and its causes

The determinants of homocysteine level include genetic and physiologic factors, life style, nutrition, various diseases and medication. The level increases with age (from childhood to old age approximately doubles), after adolescence it is slightly higher in men, decreases in pregnancy. Homocysteinemia also depends on renal function and creatinine synthesis. Healthy life style and sufficient vitamin consumption in the diet or in the form of vitamin supplements (especially those containing folic acid) lead to reduction of homocysteine level, as well as folate-fortified diet (e.g., folate fortification of grain products, implemented in the US) [5]. However, mild hyperhomocysteinemia is found in individuals with alternative diet, vegetarians and vegans, respectively, as a consequence of vitamin B12 deficiency [6]. There are also ethnic and regional differences in homocysteine level. Therefore, the reference limits for homocysteinemia should not be strictly defined but should be interpreted with respect to the mentioned determinants. The value of 15 μmol/L is considered as the upper reference limit for the age of 15–65 years; 20 μmol/L for the age above 65 years; and 10 μmol/L for children and pregnant women while these limits should be

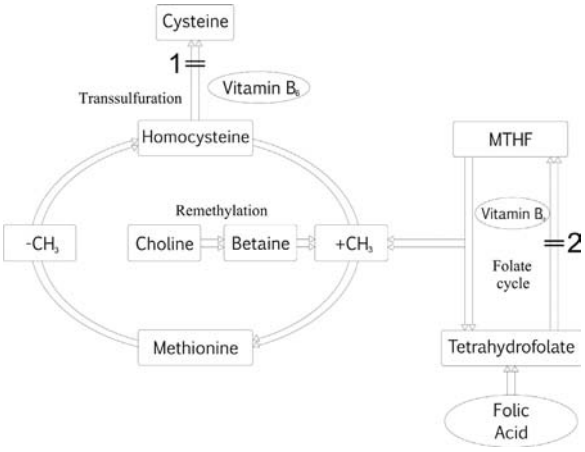

Fig. 1 – Scheme of homocysteine metabolism—according to Key NS, Mc Glennen RC [4]. 1—catalyzed by cystathionine beta-synthase (CBS). 2—catalyzed by methylentetrahydrofolate reductase (MTHFR). In the remethylation pathway, betaine (trimethylglycine) or N-5-methyltetrahydrofolate. (MTHF) serve as methyl (CH3) group donors. Folic acid is a source of tetrahydrofolate; vitamin B12 is a cofactor in the folate cycle. In the transsulphuration pathway, vitamin B6 works as a cofactor.

Table 1 – Causes of hyperhomocysteinemia [2,5,7].

Genetic causes	1. CBS deficiency 2. Inherited defects of folate metabolism 3. Inherited defects of cobalamin absorption, transport and metabolism 4. Polymorphisms of folate and cobalamin metabolism, including polymorphisms of following enzymes: (a) MTHFR (b) Methionine synthase (c) Methionine synthase-reductase
Acquired factors	1. Folate deficiency 2. Vitamin B12 deficiency 3. Vitamin B6 (pyridoxine) deficiency 4. Some diseases and disorders: (a) Renal insufficiency (b) Proliferative disorders: malignancy, psoriasis (c) Rheumatoid arthritis, systemic lupus erythematosus (d) Hypothyreoidism 5. Some medicaments: sex hormones, insulin, antiepileptics, fibrates, metformin, D-penicillamine, proton pump inhibitors, methotrexate, L-dopa, 6-mercaptopurin, sulfasalazin, cyclosporin, megadoses of vitamin C 6. Factors of life style: smoking, alcohol, sedentary life style, high consumption of animal proteins rich in methionine 7. Other: age, male sex, gastroplasty, Down syndrome, postmenopause

CBS—cystathionine beta-synthase.
MTHFR—methylentetrahydrofolate reductase.

20–25% lower in the case of folate supplementation (fortified diet or vitamin supplement use) [5].

The causes of hyperhomocysteinemia may be inherited or acquired or the combination of both—Table 1.

According to the degree of homocysteine elevation, hyperhomocysteinemia may be classified as:

(1) *Mild*: 15–30 μmol/L (prevalence in population <10%). Possible causes may be found in life style but also in vegeterian diet, polymorphism of methylentetrahydrofolate reductase (MTHFR) 677 T, mild folate or cobalamin deficiency, renal insufficiency, medication influencing homocysteine metabolism or the level of folate or cobalamin
(2) *Moderate*: 30–100 μmol/L (prevalence <1%). It may occur in moderate to severe folate or cobalamin deficiency or in renal insufficiency
(3) *Severe*: above 100 μmol/L (prevalence <0.02%). It may occur in severe cobalamin deficiency or in homocystinuria [5].

The prevalence of congenital homocystinuria is about 1:200,000–300,000 in general population, most cases (90–95%) are the consequence of a homozygous defect in cystathionine beta-synthase (CBS) gene (the rest of cases is associated with some rare mutations in MTHFR, methionine synthase or methionine synthase–reductase genes). Homocysteine levels are high, in the range of 100–500 μmol/L. The disease manifests in most cases in childhood with eye lens dislocation, myopia, marphanoid habitus, mental retardation but also VTE and premature atherosclerosis [2,4,8]. Leading causes of mortality in these patients are VTE, stroke, peripheral arterial thrombosis and myocardial infarction [9]. Early diagnosis and supplementation of pyridoxine and/or folate and betaine, optimally since childhood, may prevent cardiovascular events and other clinical manifestations [5].

Severe deficiency of MTHFR as a possible cause of homocystinuria is quite rare. However, several polymorphisms of MTHFR genes have been reported (polymorphisms means a mutation with the prevalence of the mutated allele in population ≥1%). The most studied was the mutation 677C→T (substitution alanine→valine) [2]. This variant of MTHFR is characteristic with reduced activity in the temperature of 37 °C and increased thermolability in 46 °C, therefore it is called thermolabile. As a consequence of this mutation in a homozygous trait, the specific MTHFR activity is reduces by 50–60% [4,9]. In a homozygous trait, its prevalence is 10–15% in the whites in North America; even 25% in Hispanic Americans while only 0–1% in Afro-Americans [2]. The presence of this mutation in a homozygous trait may lead to mild hyperhomocysteinemia in the case of simultaneous folate deficiency but after folate supplementation the level drops to normal [4]. Mutation in MTHFR gene 1298 A→C (substitution glutamine→alanine) is another common polymorphism with the prevalence of about 40% in a heterozygous trait and 10% in a homozygous trait [4], it leads to the decrease of MTHFR activity by 35% [9].

4. Prothrombotic effects of homocysteine

The association of hyperhomocysteinemia with thrombosis may be explained by multifactorial mechanism, generally by the impaired balance between procoagulation and anticoagulation factors. Nevertheless, it is important to realize that most of our knowledge about homocysteine effects originated

from the studies on cell cultures using supraphysiologic hymocysteine concetrations and therefore, it is questionable whether the results of those in vitro studies might be extrapolated into clinical practice. Another research source is animal models with diet-induced hyperhomocysteinemia. In this case, potential metabolic differences between animals and humans should be taken into consideration. Prothrombotic properties of homocysteine were also documented in blood samples obtained from individuals with various homocysteine levels.

Various effects of hyperhomocysteinemia have been reported in the literature so far—Table 2; however, not all of them are supported by convincing and consistent evidence. Hyperhomocysteinemia may cause endothelial dysfunction to the extent comparable to dysfunction induced by hypercholesterolemia and arterial hypertension. The mechanism is not completely understood, the most probable is the link with increased oxidative stress. Further on, hyperhomocysteinemia may influence multiple components of hemostatic process [3,4,10,11].

On the other hand, there are also some data that do not confirm procoagulant effect of mild hyperhomocysteinemia [11].

5. Hyperhomocysteinemia as a risk factor for VTE

As mentioned above, thromboembolic events are leading cause of morbidity and mortality in patients with congenital homocystinuria, a rare disorder with extremely high homocysteine levels. In the affected individuals, both arterial and venous thrombotic events occur at young age (under 30 years), even in early childhood, as was documented in case reports from the end of the sixties of the last century [12,13].

Even mild to moderate hyperhomocysteinemia may have clinical consequences, there are reports about association with coronary artery disease (CAD), stroke, VTE, placentar vasculopathy, neural tube defects and some neuropsychiatric diseases [5,14].

The association of hyperhomocysteinemia with VTE has been studied less extensively than the association with arterial diseases. The number of studies is lower and the proved association is weaker, respectively [3]. Moreover, homocysteine testing in most studies was performed after a thromboembolic event. Therefore, it remains unclear whether homocysteine is a direct etiologic factor or a laboratory marker. The studies dealing with the association of homocysteine with VTE can be divided into several groups and subgroups:

1. *The association of homocysteine levels with venous thromboembolic events*
   The first case-control study, published in 1991, paradoxically did not confirm the association between homocysteine level and VTE [15].
   Metaanalysis from1998 [16], including 9 case-control studies, demonstrated a significant association between hyperhomocysteinemia and the first or recurrent thromboembolic event (hyperhomocysteinemia was defined as a level above the mean value of control group+2 standard deviations or as a level above the 95th percentile of a control group). The authors calculated an odds ratio (OR) as 2.95 with 95% confidence interval (CI) 2.08–4.17. The risk was even more significant in patients with the history of VTE under the age of 60 years—OR 4.37 (95% CI 1.94–9.84).

Table 2 – Multiple effects of hyperhomocysteinemia on endothelium and hemostasis [3,4,10,11].

Vascular endothelium	Endothelial dysfunction – Impaired endothelium-dependent vasodilation – Prothrombotic and proinflammatory phenotype of endothelium
Platelets	Increased thromoboxane synthesis Increased platelet reactivity
Fibrinolysis	Impaired fibrinolysis – Decreased binding of tissue plasminogen activator (tPA) – Decreased plasmin generation – Increased level of thrombin activatable fibrinolysis inhibitor (TAFI)
Coagulation factors and natural inhibitors of coagulation	Increased synthesis of tissue factor (TF) Increased activity of factor VII Decreased inactivation of factor Va Increased activation of factor V Decreased activity of antithrombin Increased thrombin generation Fibrinogen modification Inhibition of thrombomodulin activity Inhibition of protein C activation

Later on, further studies were published, including case-control studies demonstrating a significant association of hyperhomocysteinemia and VTE [17,18] but, on the other hand, also prospective studies that had not proved higher risk of VTE in the individuals with hyperhomocysteinemia [19]. Quite recent Spanish study of younger VTE patients (under the age of 55, respectively) without other thrombophilia has not confirmed hyperhomocysteinemia (the level >15 μmol/L) as an independent VTE risk factor [20].

2. *The association of the polymorphisms in MTHFR genes and VTE*
Most studies focused on potential association of a relatively common MTHFR gene mutation 677C→T which, if inherited in a homozygous trait, means a predisposition to mild hyperhomocysteinemia. The findings are inconsistent.
American authors performed a case-control study [21] and did not confirm this mutation in a homozygous trait as a genetic predisposition to VTE, neither isolated nor in combination with factor V Leiden (FVL).
Italian study [22] confirmed that MTHFR gene 677 TT mutation increased the risk of VTE in the cases without known clinical provoking factor and without other thrombophilia (i.e. FVL, prothrombin gene mutation G20210A, protein C, protein S or antithrombin deficiency) with resultant OR 2.57 ($p=0.017$).
To the contrary, a Japanese study [23] found out that MTHFR gene mutation 677TT is a VTE risk factor only in the presence of other thrombophilia (protein C, protein S or plasminogen deficiency and lupus anticoagulant, respectively) with OR 5.99 (95% CI 1.56–22.96), compared to a control group.
Metaanalysis, published in 2002 [24] included 31 studies and calculated an OR for MTHFR gene mutation 677 TT and VTE as 1.2 (95% CI 1.1–1.4), while a little higher OR was found in the absence of other thrombophilia—OR 1.5 (95% CI 1.2–1.9). The authors evaluated the association of MTHFR gene mutation 677 TT with VTE as weak and did not recommend including MTHFR testing to routine thrombophilia workup.
Similar results were obtained in a large Dutch MEGA study (Multiple Enviromental and Genetic Assessment of Risk Factors for Venous Thrombosis) which included 4375 patients with the first thromboembolic event [25]. The authors did not confirm a significant association between MTHFR 677 TT and VTE—OR 0.94 (95% CI 0.81–1.08).
Neither further common MTHFR gene mutation—MTHFR 1298 A→C has been proven as a significant VTE risk factor [26].

3. *The association of VTE with both homocysteine level and MTHFR gene polymorphism*
The results are again ambiguous.
A Polish case-control study (with 146 patients) failed to confirm an association with MTHFR genotypes as well as with homocysteine level [27].
In another case-control study, including 240 patients with VTE, hyperhomocysteinemia (level >20 μmol/L) was significantly more frequent in the individuals above the age of 50 years and those with idiopathic (i.e. otherwise unexplained) thromboembolic event. However, there was no significant difference in the prevalence of MTHFR gene mutation 677 TT in VTE patients and in the control group [28].
LITE, a large prospective American study (Longitudinal Investigation of Thromboembolism Etiology) did not prove MTHFR gene mutation 677 TT as a VTE risk factor as well [29]. However, it found a weak, statistically nonsignificant association of homocysteine level and VTE risk—adjusted OR 1.55 (95% CI 0.93–2.58) for the comparison of the highest to the lowest quintile of homocysteine levels, while the association was significant in younger individuals (45–64 years)—adjusted OR 2.05 (95% CI 1.10–3.83).
Another large prospective study, Danish "Copenhagen City Heart Study" found 25% higher homocysteine levels in the carriers of MTHFR gene mutation 677 TT, compared to MTHFR 677 CT or CC genotype; however, the study did not confirm higher VTE risk in the carriers of this mutation [30].
Metaanalysis published in 2005 revealed interesting findings. Hyperhomocysteinemia as a VTE risk factor was more significant in retrospective studies (according to the results of 24 studies)—the increase of homocysteinemia by 5 μmol/L was associated with 60% higher VTE risk (OR 1.6, 95% CI 1.10–1.34) while in 3 prospective studies the increase of homocysteinemia by 5 μmol/L resulted in 27% higher risk (OR 1.27, 95% CI 1.01–1.59). The metaanalysis evaluated the impact of MTHFR 677 TT genotype as well—it was slightly associated with VTE risk, with the increase by 20%, respectively (OR 1.2, 95% CI 1.08–1.32). The association of VTE risk and MTHFR 677 TT genotype was influenced by geographical differences—in North America the genotype was not significantly associated with VTE risk which might be explained by higher folate and riboflavin intake [31].
EDITH was a French prospective observational study evaluating an interaction between genetic and environmental VTE risk factors. The authors compared 467 patients with the first unprovoked thromboembolic event to sex- and age-matched controls. They revealed an independent association of mild hyperhomocysteinemia, folate deficiency and vitamin B12 deficiency with VTE—OR in multivariate analysis was 1.48 (95% CI 1.05–2.08) for homocysteine >15 μmol/L; 3.14 (95% CI 1.35–7.32) for folate level ≤4,9 nmol/L; and 1.42 (95% CI 1.03–1.98) for vitamin B12 level≤253 pmol/L. MTHFR 677 TT genotype was not significantly associated with VTE risk—OR 1.13 (95% CI 0.70–1.81). Thus, these results do not prove MTHFR gene mutation 677 TT as a VTE risk factor but may indicate potential influence of the deficiency of B vitamins independently on homocysteine level [32].
HUNT2, a prospective Norwegian–Dutch study followed a cohort of 66,140 individuals for 7 years, evaluated the incidence of VTE and the association of VTE risk with homocysteine level (measured at inclusion) and MTHFR gene polymorphism 677C→T. While MTHFR 677 TT genotype was not a risk factor for VTE, hyperhomocysteinemia (level above the 95th percentile) was a predictor of a thromboembolic event in men (OR 2.17, 95% CI 1.20–3.91) but not in women [33].

4. *The association of homocysteine with recurrent VTE*
Some older studies confirmed hyperhomocysteinemia as a risk factor for recurrent thromboembolic events.
In a Dutch study (including 185 patients with a history of recurrent VTE and 220 controls) the revealed adjusted OR was 2.0 (95% CI 1.5–2.7) for homocysteine level above the

90th percentile of a control group, a similar result was obtained for the level measured after methionine load [34]. AUREC (Austrian Study of REcurrent Venous Thromboembolism), a multicenter prospective Austrian study [35], followed 264 patients after the first idiopathic thromboembolic event (i.e. without any association with surgery, injury, pregnancy, protein C or protein S deficiency, antithrombin or plasminogen deficiency, antiphospholipid syndrome, systemic lupus erythematosus or malignancy). Hyperhomocysteinemia was defined as a level above the 95th percentile of a control group, i.e. 8.8 μmol/L in women and 11.6 μmol/L in men. The authors evaluated the incidence of recurrent VTE after anticoagulation withdrawal. There was a significant increase of VTE risk in the patients with hyperhomocysteinemia with relative risk (RR) 2.6 (95% CI 1.1–6.1), adjusted for age, sex and the presence of FVL.

5. *Potential synergic effect of homocysteine or MTHFR 677 TT genotype and other thrombophilias*

Again, the results of various studies are inconsistent.

"Physicians Health Study", a large prospective cohort study brought quite convincing evidence. It followed 14,916 healthy men. Laboratory tests at inclusion comprised also homocysteine level and genetic testing for FVL. Within 12 years, 145 thromboembolic events occurred. Hyperhomocysteinemia (level > the 95th percentile, i.e. 17.25 μmol/L) was associated with the increased risk of idiopathic VTE (not provoked by malignancy, surgery or injury) with RR 3.4 ($p=0.002$), the presence of FVL was associated with RR 2.3 ($p=0.005$) for any VTE event and 3.6 for an idiopathic event ($p=0.0002$). The concomitant presence of hyperhomocysteinemia and FVL lead to a significant risk increase—RR 9.65 ($p=0.009$) for any VTE and 21.8 ($p=0.0004$) for idiopathic VTE [36].

One smaller Italian case-control study in 1997 confirmed a synergic effect of MTHFR gene mutation 677 TT and FVL [37]. While MTHFR gene mutation 677 TT itself was not a significant VTE risk factor, the coexistence with FVL lead to significant increase of the risk associated with FVL: OR for MTHFR gene mutation was 0.8, adjusted OR for FVL 6.3 (95% CI 1.6–25.3) and adjusted OR for concomitant FVL and MTHFR 677 TT genotype was 17.3 (95% CI 2.0–152.9).

Dutch authors evaluated in the group of 171 patients with the history of recurrent VTE potential interaction between hyperhomocysteinemia (level > the 90th percentile of a control group), MTHFR gene mutation 677C→T, FVL and prothrombin gene mutation 20210 G→A [38]. The values of adjusted OR for recurrent VTE were: 1.8 (95% CI 1.1–3) for hyperhomocysteinemia; 5.1 (95% CI 3.0–8.6) for FVL; 1.8 (95% CI 0.7–4.2) for prothrombin gene mutation; and 1.4 (95% CI 0.7–2.8) for MTHFR gene mutation 677 TT. Significant increase of VTE risk was observed if hyperhomocysteinemia was combined with FVL—OR 11.6 (95% CI 3.2–42.5); as well as if FVL combined with MTHFR gene mutation 677 TT—OR 18.7 (95% CI 3.3–108).

However, a more recent retrospective study, also Dutch, found only nonsignificantly increased VTE risk for hyperhomocysteinemia (>17 μmol/L)—adjusted RR 1.6 (95% CI 0.6–4.5). Though the risk was significantly higher in the presence of other thrombophilia, a comparable risk increase was observed even in the individuals with other thrombophilia and normal homocysteine level. The authors summarized that the main determinant of VTE risk was rather concomitant thrombophilia than hyperhomocysteinemia itself [39].

Quite recently published metaanalysis (including 5 studies) then concluded that it was impossible to prove any interaction (neither additive nor multiplicative) between FVL and hyperhomocysteinemia as well as FVL and MTHFR gene mutation 677 TT, concerning VTE risk [40].

6. *Homocysteine and thrombosis at unusual sites*

The mentioned studies dealt with potential association of hyperhomocysteinemia and the most frequent location of venous thrombosis, i.e. in the legs. In the literature, there are also some reports about the prevalence of hyperhomocysteinemia in the patients with thrombosis at unusual sites. Most of published data, however, are derived from small studies, isolated case reports or case series.

- In a small group (31 patients) with *thrombosis of the upper extremity*, 5 patients (16.1%) had elevated homocysteine level [41].
- In a case-control study (121 patients and 242 controls), hyperhomocysteinemia (level > the 95th percentile of the control group, i.e. >19.2 μmol/L in men and >15.2 in women) was associated with fourfold increase of the risk of *cerebral vein thrombosis* [42]. Even more significantly increased risk was observed for the combination of hyperhomocysteinemia and hormonal contraception—OR 19.5 (95% CI 5.7–67.3).

A significant association of hyperhomocysteinemia and cerebral vein thrombosis was found also in two smaller case-control studies [43,44]—the values of OR were 4.18 (95% CI 1.58–11.16) and 6.88 ($p=0.002$), respectively.

- In a case-control study, searching for the risk factors of retinal vein occlusion [45], the authors found significantly higher prevalence of hyperhomocysteinemia (>13.5 μmol/L) in 132 patients with *central retinal vein thrombosis* in comparison to 105 healthy controls, with resultant OR 8.64 (95% CI 1.96–38.0).
- Some authors also investigated potential association between hyperhomocysteinemia and *visceral vein thrombosis*.

In a study of 65 patients with *extrahepatic portal vein obstruction*, the prevalence of hyperhomocysteinemia was higher in the patients than in the control group [46] but the difference did not reach statistical significance—OR 2.0 (95% CI 0.9–4.9).

In a small study, including 12 patients with *mesenteric vein thrombosis*, the authors found a significant association of MTHFR 677 TT genotype, in comparison to healthy controls [47].

7. *Correction of hyperhomocysteinemia by vitamin supplementation*

Homocysteinemia correlates inversely with the level of folate, vitamin B12 and, to a lesser extent, vitamin B6 [48]. Supplementation by these vitamins is therefore a possibility how to reduce or even normalize homocysteine level.

Metaanalysis published in 1998 investigated the efficacy of various doses of these vitamins in the reduction of homocysteine level. It evaluated the results of 12 studies including 1114 individuals. The highest effect on homocysteinemia reduction was reached by folic acid administration. The higher was homocysteine level and the lower was folate level prior to supplementation, the higher was the reduction of homocysteine level. There was no difference in the effect in the dose range 0.5–5 mg of folic acid daily—the consequence was a drop in homocysteinemia by 25%.Vitamin B12 in the mean dose 0.5 mg led to an additional level drop by 7% while vitamin B6 (in the mean dose 16.5 mg daily) had no further additional effect [49].

These findings were later confirmed by further, more extensive metaanalysis of 25 studies, including 2596 individuals. Maximal reduction of homocysteine level (by 23–25%) was reached by folic acid, administered in daily dose $\geq$0.8 mg; additional level reduction (by 7%) was achieved by the concomitant medication with vitamin B12 in daily dose 0.4 mg. Again, vitamin B6 did not prove any significant additional effect [50].

In some specific situation, e.g. chronic renal insufficiency, higher doses of folic acid are necessary to reduce homocysteinemia [3]. Uncertainty may also exist in the patients with medication which causes increase of homocysteine level but the data about vitamin dosing in these cases are quite scarce. In a study of the patients with rheumatoid arthritis on metothrexate, supplementation with folic acid 1 mg daily led to a significant drop of homocysteinemia [51]. Fenofibrate use may increase homocysteine level by 40%. German authors demonstrated that this increase may be prevented by comedication with folic acid (0.65 mg daily), vitamin B12 (50 μg daily) and B6 (5 mg daily) [52,53]. A Norwegian study proved the effect of vitamin supplementation (in daily doses of 0.4 mg of folic acid, 120 mg of vitamin B6 and 75 mg of riboflavin) on the reduction of hyperhomocysteinemia induced by antiepileptics [54].

8. *Interventional studies—the effect of reducing homocysteine levels on VTE risk*

Hence, vitamin supplementation is effective in the reduction of homocysteine level but clinical benefit (i.e. the efficacy of vitamin supplementation in VTE prevention) is questionable.

Severe hyperhomocysteinemia—e.g. in homocystinuria requires undoubtedly vitamin therapy. In a multicenter study, 158 patients with homocystinuria (caused by CBS deficiency) were treated by vitamins (folic acid, vitamin B6, vitamin B12, betaine) and a low-methionine diet and the incidence of vascular events was compared to a historic cohort of 629 patients without any supplementation. Vitamin supplementations led to a significant reduction, though not complete normalization of homocysteine levels. It resulted in significant reduction of the risk of vascular events, including thromboembolic events [55].

Nevertheless, the evidence about indication of vitamin therapy in mild to moderate hyperhomocysteinemia is much less convincing. The authors of metaanalysis of 92 studies, evaluating the risk of CAD, stroke and VTE in association with hyperhomocysteinemia calculated that the drop of homocysteine level by 3 μmol/L should result in 25% reduction of VTE risk [56]. However, this assumption has not been proven in prospective studies.

Secondary analysis of HOPE-2 study (including 5,522 individuals at the age above 55 years with cardiovascular disease or diabetes mellitus and at least one vascular risk factor) reported the results of 5 year follow-up of the group with vitamin therapy (2.5 mg of folic acid, 50 mg of vitamin B6 and 1 mg of vitamin B12 daily) in comparison to placebo group [57]. Vitamin supplementaion was effective in homocysteine level reduction but not in VTE risk reduction—hazard ratio (HR) 1.01 (95% CI 0.66–1.53).

The efficacy of vitamin therapy and homocysteine reduction in secondary VTE prevention was evaluated in VITRO study (The Vitamins and Thrombosis), which included 701 patients with the first episode of unprovoked DVT or PE. The effect of vitamin supplementation (in the daily doses of 5 mg of folic acid, 50 mg of pyridoxine and 0.4 mg of vitamin B12) was compared to placebo in two groups—"hyperhomocysteinemic" (with homocysteine level above the 75th percentile of reference group, i.e. $\geq$12.6 μmol/L) as well as "normohomocysteinemic". The follow-up lasted 2.5 years and the observed HR for VTE recurrence associated with vitamin therapy was 1.14 (95% CI 0.65–1.98) for the "hyperhomocysteinemic" group, and 0.58 (95% CI 0.31–1.07) for the "normohomocysteinemic" group [58].

Accordingly, the benefit of vitamin supplementation is uncertain. Though this therapy is not too expensive and is relatively safe it is important to realize some potential risks as well. High doses of vitamin B6 may cause peripheral sensory neuropathy. Further on, long term administration of folic acid in high doses may impair blood–brain barrier. [59].

6. Homocysteine—culprit or innocent bystander?

There are some *hypotheses* trying to explain the unconvincing and inconsistent results of the mentioned studies:

- the studies with vitamin supplementation were mostly performed in the countries with folate-fortified diet, i.e. homocysteine level prior to supplementation was relatively low
- B vitamins may theoretically have a harmful effect which outweighs their potential benefit [7]
- hyperhomocysteinemia
  - is not a cause but a marker of VTE event
  - is only a marker of vitamin B deficiency
  - if mild, is associated only with a small absolute VTE risk
  - is a VTE risk factor only in very high concentrations
  - is a risk factor only if combined with other additional risk factors [2,3,7]
  - is not a real VTE risk factor but the true risk factor is some other pathology associated with hyperhomocysteinemia

For example, Dutch authors discovered an association of hyperhomocysteinemia and high factor VIII level which they considered the proper risk factor of arterial and venous thrombosis [60,61].

In FIELD study, there was a slightly higher incidence of PE after fibrate therapy [62]. It may be theoretically explained by fibrate-induced hyperhomocysteinemia [63]. However, this hypothesis is in contradiction with the results of a French study (including 677 patients with unprovoked VTE event and 677 controls), which revealed significantly higher association of VTE with fibrate use but this association was independent on homocysteine level [64].

7. Guidelines

Insufficient data indicative of a causative association between hyperhomocysteinemia and VTE and unconvincing evidence about the efficacy of homocysteine lowering therapy in primary and secondary VTE prevention result in the absence of clear and actual guidelines for testing homocysteine or therapy of hyperhomocysteinemia in the patients with VTE event, or in the individuals with higher thromboembolic risk.

In the ACCP guidelines for antithrombotic therapy (American College of Chest Physicians—ACCP Conference on Antithrombotic and Thrombolytic Therapy) from 2004, hyperhomocysteinemia was mentioned as risk factor for VTE recurrence. For the patients with the first thromboembolic event and some thrombophilic disorders – including hyperhomocysteinemia – the authors recommended prolonged anticoagulation (6–12 months), and this recommendation was labeled with 1A [65]. However, folic acid supplementation was recommended only in pregnant women with hyperhomocysteinemia, but not for VTE prophylaxis but because of the risk of spontaneous abortion [66].

However, ACCP Guidelines have been later updated every 4 years. In the following edition in 2008, neither hyperhomocysteinemia nor other thrombophilic disorders were considered a significant factor in making the decision about the length of anticoagulation [67]. Hyperhomocysteinemia was mentioned as a potential risk factor of abortion but recommendation concerning vitamin supplementation is absent in this edition [68].

Similarly, in the recent edition of ACCP Guidelines from 2012, hereditary thrombophilias are considered only additional risk factor for VTE recurrence and hyperhomocysteinemia itself is not particularly mentioned [69]. In the chapter about VTE and thrombophilic disorders in pregnancy, the authors deal with homozygous MTHFR 677 TT mutation. They note that the association of this mutation with VTE in pregnancy was not proved which might be explained by physiologic decrease of homocysteine level in pregnancy and by common folic acid supplementation in pregnancy (in the indication of neural tube defect prevention). The association with pregnancy morbidity is also mentioned but the authors do not comment on the necessity of vitamin B supplementation in this indication [70].

Due to the absence of recent guidelines concerning homocysteine and VTE, it might be useful to take into consideration some older recommendations.

Back in 2002, the American consensus about thrombophilic disorders considered testing homocysteine level in VTE as controversial. From the laboratory point of view, two methods were suggested—high performance liquid chromatography as well as immunoanalysis [4].

Homocysteine testing is mostly recommended in the fasting state. However, level fluctuation usually does not exceed 10% of the basal value. Therefore, in the practice, fasting might not be so strictly required [1]. In the case of abnormal homocysteine level in non-fasting state, testing the fasting level could be performed later. Neither homocysteine measuring after methionine load is routinely recommended in the practice [2].

It is not quite clear whether a thromboembolic event itself does not induce elevated homocysteine level. Therefore, it seems prudent to postpone the testing after the acute VTE event and to perform it 6 months later [4].

Testing MTHFR gene 677 or 1298 mutations in VTE patients is still sometimes performed but should be abandoned [71].

Testing homocysteine level might be indicated in the cases of idiopathic or recurrent VTE, DVT or PE at young age or thrombosis at unusual sites. Vitamin supplementation might be suitable in the patients with proven folate or vitamin B12 deficiency [31], and in those with some additional VTE risk factor [2]. However, the patients should be informed about the uncertain benefit of this treatment in the prevention of VTE recurrence [72]. Rational diet with sufficient content of fruit and vegetables is a possible alternative of pharmacologic vitamin supplementation [2]. The value up to10–12 μmol/L is recommended as a target homocysteine level [3].

In the last British guidelines for diagnosis and treatment of thrombosis at unusual sites, hyperhomocysteinemia is mentioned as a risk factor of retinal vein occlusion [73] but the authors do not recommend thrombophilia testing (hence, neither homocysteine testing).

8. Conclusion

The association of hyperhomocysteinemia and VTE is controversial. It is possible that thrombosis risk is influenced by the interaction of homocysteine metabolism disorders, vitamin levels and further prothrombotic factors. So far, the data have not brought enough evidence about the benefit of vitamin supplementation in the individuals with mild hyperhomocysteinemia in the primary and secondary VTE prevention [2,3]. Further studies might be required to prove the causative association between hyperhomocysteinemia and VTE, as well as randomized clinical trials to confirm significant VTE risk reduction due to homocysteine lowering by vitamin therapy. Meanwhile, it is necessary to choose quite a conservative approach to the problem of homocysteine and venous thrombosis and to consider carefully homocysteine level management in selected cases.

Acknowledgements

The research was supported by the Charles University Research Fund (Project no. P36).

REFERENCES

[1] S. Moll, Thrombophilias—practical implications and testing caveats, Journal of Thrombosis and Thrombolysis 21 (2006) 7–15.
[2] M.M. Eldibany, J.A. Caprini, Hyperhomocysteinemia and thrombosis: an overview, Archives of Pathology and Laboratory Medicine 131 (2007) 872–884.
[3] A. Undas, J. Brozek, A. Szczeklik, Homocysteine and thrombosis: from basic science to clinical evidence, Thrombosis and Haemostasis 94 (2005) 907–915.
[4] N.S. Key, R.C. McGlennen, Hyperhomocyst(e)inemia and thrombophilia, Archives of Pathology and Laboratory Medicine 126 (2002) 1367–1375.
[5] H. Refsum, A.D. Smith, P.M. Ueland, et al., Facts and recommendations about total homocysteine determinations: an expert opinion, Clinical Chemistry 50 (2004) 3–32.
[6] M. Krajčovičová-Kudláčková, P. Blažíček, Homocysteinaemia – nutritional determinants, Cor et Vasa 43 (6) (2001) 289–293.
[7] E. Lonn, Homocysteine in the prevention of ischemic heart disease, stroke and venous thromboembolism: therapeutic target or just another distraction?, Current Opinion in Hematology 14 (5) (2007 Sep) 481–487.
[8] M. Orendáč, Clinical picture of homocystinuria with cystathionine beta-synthase deficiency in 19 Czech and Slovak patients, Časopis lekařů českych 139 (2000) 500–507.
[9] R. Carmel, R. Green, D.S. Rosenblatt, D. Watkins, Update on cobalamin, folate, and homocysteine, Hematology (2003) 62–81.
[10] M. Colucci, M. Cattaneo, I. Martinelli, et al., Mild hyperhomocysteinemia is associated with increased TAFI levels and reduced plasma fibrinolytic potential, Journal of Thrombosis and Haemostasis 6 (2008) 1571–1577.
[11] V.E. Gerdes, H.A. Hovinga, H. ten Cate, et al., Homocysteine and markers of coagulation and endothelial cell activation, Journal of Thrombosis and Haemostasis 2 (2004) 445–451.
[12] J.B. Gibson, N.A. Carson, D.W. Neill, Pathological findings in homocystinuria, Journal of Clinical Pathology 17 (1964) 427–437.
[13] K.S. McCully, Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis, American Journal of Pathology 56 (1969) 111–128.
[14] A. Žák, M. Zeman, Consequences of moderate hyperhomocysteinemia in internal medicine, Časopis lekařů českych 143 (2004) 367–374.
[15] L. Brattstrom, L. Tenghorn, C. Lagerstedt, et al., Plasma homocysteine in venous thromboembolism, Haemostasis 21 (1991) 51–57.
[16] J.G. Ray, Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease, Archives of Internal Medicine 158 (1998) 2101–2106.
[17] L.J. Langman, J.G. Ray, J. Evrovski, et al., Hyperhomocyst (e) inemia and the increased risk of venous thromboembolism: more evidence from a case-control study, Archives of Internal Medicine 160 (2000) 961–964.
[18] Y. Unlü, S. Keleş, N. Becit, et al., Hyperhomocysteinaemia as a risk factor for deep-vein thrombosis, European Journal of Vascular and Endovascular Surgery 30 (2005) 315–318.
[19] A.B. Mäkelburg, W.M. Lijfering, S. Middeldorp, et al., Low absolute risk of venous and arterial thrombosis in hyperhomocysteinaemia—a prospective family cohort study in asymptomatic subjects, Thrombosis and Haemostasis 101 (2009) 209–212.
[20] A. Vayá, I. Gómez, Y. Mira, et al., Homocysteine levels in patients with deep vein thrombosis lacking thrombophilic defects, Thrombosis and Haemostasis 99 (2008) 1132–1134.
[21] I.T. Ocal, A. Sadeghi, R.D. Press, Risk of venous thrombosis in carriers of a common mutation in the homocysteine regulatory enzyme methylenetetrahydrofolate reductase, Molecular Diagnostics 2 (1997) 61–68.
[22] D. Gemmati, M.L. Serino, C. Trivellato, et al., C677T substitution in the methylenetetrahydrofolate reductase gene as a risk factor for venous thrombosis and arterial disease in selected patients, Haematologica 84 (1999) 824–828.
[23] H. Fujimura, T. Kawasaki, T. Sakata, et al., Common C677T polymorphism in the methylenetetrahydrofolate reductase gene increases the risk for deep vein thrombosis in patients with predisposition of thrombophilia, Thrombosis Research 98 (2000) 1–8.
[24] J.G. Ray, D. Shmorgun, W.S. Chan, Common C677T polymorphism of the methylenetetrahydrofolate reductase gene and the risk of venous thromboembolism: meta-analysis of 31 studies, Pathophysiology of Haemostasis and Thrombosis 32 (2002) 51–58.
[25] I.D. Bezemer, C.J. Doggen, H.L. Vos, F.R. Rosendaal, No association between the common MTHFR 677C->T polymorphism and venous thrombosis: results from the MEGA study, Archives of Internal Medicine 167 (2007) 497–501.
[26] O. Salomon, N. Rosenberg, A. Zivelin, et al., Methionine synthase A2756G and methylenetetrahydrofolate reductase A1298C polymorphisms are not risk factors for idiopathic venous thromboembolism, The Hematology Journal 2 (2001) 38–41.
[27] T.B. Domagala, L. Adamek, E. Nizankowska, et al., Mutations C677T and A1298C of the 5,10-methylenetetrahydrofolate reductase gene and fasting plasma homocysteine levels are not associated with the increased risk of venous thromboembolic disease, Blood Coagulation and Fibrinolysis 13 (2002) 423–431.
[28] P. Hainaut, C. Jaumotte, D. Verhelst, et al., Hyperhomocysteinemia and venous thromboembolism: a risk factor more prevalent in the elderly and in idiopathic cases, Thrombosis Research 106 (2002) 121–125.
[29] A.W. Tsai, M. Cushman, M.Y. Tsai, et al., Serum homocysteine, thermolabile variant of methylene tetrahydrofolate reductase (MTHFR), and venous thromboembolism: Longitudinal Investigation of Thromboembolism Etiology (LITE), American Journal of Hematology 72 (2003) 192–200.
[30] J. Frederiksen, K. Juul, P. Grande, et al., Methylenetetrahydrofolate reductase polymorphism (C677T), hyperhomocysteinemia, and risk of ischemic cardiovascular disease and venous thromboembolism: prospective and case-control studies from the Copenhagen City Heart Study, Blood 104 (2004) 3046–3051.
[31] M. Den Heijer, S. Lewington, R. Clarke, Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies, Journal of Thrombosis and Haemostasis 3 (2005) 292–299.
[32] E. Oger, K. Lacut, G. Le Gal, et al., Hyperhomocysteinemia and low B vitamin levels are independently associated with venous thromboembolism: results from the EDITH study: a hospital-based case-control study, Journal of Thrombosis and Haemostasis 4 (4) (2006) 793–799.
[33] I.A. Naess, S.C. Christiansen, P.R. Romundstad, et al., Prospective study of homocysteine and MTHFR 677 TT genotype and risk for venous thrombosis in a general population—results from the HUNT 2 study, British Journal of Haematology 141 (2008) 529–535.
[34] M. den Heijer, H.J. Blom, W.B. Gerrits, et al., Is hyperhomocysteinaemia a risk factor for recurrent venous thrombosis?, Lancet 345 (1995) 882–885.
[35] S. Eichinger, A. Stümpflen, M. Hirschl, et al., Hyperhomocysteinemia is a risk factor of recurrent venous

thromboembolism, Thrombosis and Haemostasis 80 (1998) 566–569.
[36] P.M. Ridker, C.H. Hennekens, J. Selhub, et al., Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism, Circulation 95 (1997) 1777–1782.
[37] M. Cattaneo, M.Y. Tsai, P. Bucciarelli, et al., A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (factor V:Q506), Arteriosclerosis, Thrombosis, and Vascular Biology 17 (1997) 1662–1666.
[38] M.B. Keijzer, M. den Heijer, H.J. Blom, et al., Interaction between hyperhomocysteinemia, mutated methylenetetrahydrofolatereductase (MTHFR) and inherited thrombophilic factors in recurrent venous thrombosis, Thrombosis and Haemostasis 88 (2002) 723–728.
[39] W.M. Lijfering, M. Coppens, M.H. van de Poel, et al., The risk of venous and arterial thrombosis in hyperhomocysteinaemia is low and mainly depends on concomitant thrombophilic defects, Thrombosis and Haemostasis 98 (2007) 457–463.
[40] M.B. Keijzer, G.F. Borm, H.J. Blom, et al., No interaction between factor V Leiden and hyperhomocysteinemia or MTHFR 677TT genotype in venous thrombosis. Results of a meta-analysis of published studies and a large case-only study, Thrombosis and Haemostasis 97 (2007) 32–37.
[41] M.F. Hendler, S.S. Meschengieser, A.N. Blanco, et al., Primary upper-extremity deep vein thrombosis: high prevalence of thrombophilic defects, American Journal of Hematology 76 (2004) 330–337.
[42] I. Martinelli, T. Battaglioli, P. Pedotti, et al., Hyperhomocysteinemia in cerebral vein thrombosis, Blood 102 (2003) 1363–1366.
[43] G. Boncoraglio, M.R. Carriero, L. Chiapparini, et al., Hyperhomocysteinemia and other thrombophilic risk factors in 26 patients with cerebral venous thrombosis, European Journal of Neurology 11 (2004) 405–409.
[44] P. Ventura, M. Cobelli, M. Marietta, et al., Hyperhomocysteinemia and other newly recognized inherited coagulation disorders (factor V Leiden and prothrombin gene mutation) in patients with idiopathic cerebral vein thrombosis, Cerebrovascular Diseases 17 (2004) 153–159.
[45] C.J. Glueck, R.K. Hutchins, J. Jurantee, et al., Thrombophilia and retinal vascular occlusion, Clinical Ophthalmology 6 (2012) 1377–1384.
[46] M. Primignani, I. Martinelli, P. Bucciarelli, et al., Risk factors for thrombophilia in extrahepatic portal vein obstruction, Hepatology 41 (2005) 603–608.
[47] L. Amitrano, V. Brancaccio, M.A. Guardascione, et al., High prevalence of thrombophilic genotypes in patients with acute mesenteric vein thrombosis, The American Journal of Gastroenterology 96 (2001) 146–149.
[48] J. Selhub, P.F. Jacques, P.W. Wilson, et al., Vitamin status and intake as primary determinants of homocysteinemia in an elderly population, The Journal of the American Medical Association 270 (1993) 2693–2698.
[49] Homocysteine Lowering Trialists' Collaboration, Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials, British Medical Journal 316 (1998) 894–898.
[50] Homocysteine Lowering Trialists' Collaboration, Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials, The American Journal of Clinical Nutrition 82 (2005) 806–812.
[51] A.E. van Ede, R.F. Laan, H.J. Blom, et al., Homocysteine and folate status in methotrexate-treated patients with rheumatoid arthritis, Rheumatology 41 (2002) 658–665.
[52] J. Dierkes, S. Westphal, C. Luley, Serum homocysteine increases after therapy with fenofibrate or bezafibrate, Lancet 354 (1999) 219–220.
[53] J. Dierkes, S. Westphal, S. Kunstmann, et al., Vitamin supplementation can markedly reduce the homocysteine elevation induced by fenofibrate, Atherosclerosis 158 (2001) 161–164.
[54] T. Apeland, M.A. Mansoor, K. Pentieva, et al., The effect of B-vitamins on hyperhomocysteinemia in patients on antiepileptic drugs, Epilepsy Research 51 (3) (2002 Oct) 237–247.
[55] S. Yap, G.H. Boers, B. Wilcken, et al., Vascular outcome in patients with homocystinuria due to cystathionine β-synthase deficiency treated chronically: a multicenter observational study, Arteriosclerosis, Thrombosis, and Vascular Biology 21 (2001) 2080–2085.
[56] D.S. Wald, M. Law, J.K. Morris, Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis, British Medical Journal 325 (7374) (2002) 1202.
[57] J.G. Ray, C. Kearon, Q. Yi, et al., Homocysteine-lowering therapy and risk for venous thromboembolism: a randomized trial, Annals of Internal Medicine 146 (2007) 761–767.
[58] M. den Heijer, H.P. Willems, H.J. Blom, et al., Homocysteine lowering by B vitamins and the secondary prevention of deep vein thrombosis and pulmonary embolism: a randomized, placebo-controlled, double-blind trial, Blood 109 (2007) 139–144.
[59] A. Siniscalchi, F. Mancuso, L. Gallelli, et al., Increase in plasma homocysteine levels induced by drug treatments in neurologic patients, Pharmacological Research 52 (2005) 367–375.
[60] Lijfering W.M., Veeger N.J., Brouwer J.L., van der Meer J. The risk of venous and arterial thrombosis in hyperhomocysteinemic subjects may be a result of elevated factor VIII levels. Haematologica. 2007;92:1703-1706.
[61] W.M. Lijfering, M. Coppens, N.J. Veeger, et al., Hyperhomocysteinemia is not a risk factor for venous and arterial thrombosis, and is associated with elevated factor VIII levels, Thrombosis Research 123 (2) (2008) 244–250.
[62] FIELD study investigators, Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial, Lancet 366 (2005) 1849–1861.
[63] B. Vergès, Fenofibrate therapy and cardiovascular protection in diabetes: recommendations after FIELD, Current Opinion in Lipidology 17 (2006) 653–658.
[64] K. Lacut, G. Le Gal, J.H. Abalain, et al., Differential associations between lipid-lowering drugs, statins and fibrates, and venous thromboembolism: role of drug induced homocysteinemia?, Thrombosis Research 122 (2008) 314–319.
[65] H.R. Büller, G. Agnelli, R.D. Hull, et al. Antithrombotic therapy for venous thromboembolic disease: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy, Chest, 2004, 126, pp. 401S–428S.
[66] S.M. Bates, I.A. Greer, J. Hirsh, J.S. Ginsberg. Use of antithrombotic agents during pregnancy: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy, Chest, 2004, 126, pp. 627S–644S.
[67] C. Kearon, S.R. Kahn, G. Agnelli, et al., Antithrombotic therapy for venous thromboembolic disease: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition), Chest 133 (2008) 454 S–545 S.
[68] S.M. Bates, I.A. Greer, I. Pabinger, et al., Venous thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th ed.), Chest 133 (2008) 844 S–886 S.

[69] C. Kearon, E.A. Akl, A.J. Comerota, et al., Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed.: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines, Chest 141 (2012) e419S–e494S.
[70] S.M. Bates, I.A. Greer, S. Middeldorp, et al., VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed.: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines, Chest 141 (2012) e691S–736 S.
[71] G. Le Gal, A. Delluc, Faut-il encore se préoccuper de l'homocystéinémie des patients atteints de maladie veineuse thromboembolique?, La Revue de Médecine Interne 28 (2007) 517–519.
[72] M. den Heijer, M.B. Keijzer, Hyperhomocysteinemia as a risk factor for venous thrombosis, Clinical Chemistry and Laboratory Medicine 39 (2001) 710–713.
[73] C. Tait, T. Baglin, H. Watson, et al., Guidelines on the investigation and management of venous thrombosis at unusual sites, British Journal of Haematology 159 (2012) 28–38.