Open Access Scientific Reports

Open Access Scientific Reports

| Research Article | Open Access |

Elevated Plasma Total Homocysteine and Vascular Disease About Seventy-Two Cases

F Ben Fredj Ismail*, A Mzabi, A Ghrairi, M Karmani, A Rezgui, H Mhiri and C Laouani Kechrid

Department of Internal Medicine, Sahloul Hospital Souse, Tunisia

Abstract

Elevated plasma total homocysteine is considered as a risk factor for occlusive cardiovascular disease. However, some observations have raised questions about elevated plasma total homocysteine as a risk factor. Also, it is considered as a risk factor for venous thromboembolism, but other authors deny this harmful effect of homocysteine. We studied 72 patients' files affected of hyperhomocysteinemia: 48 men and 24 women, middle-age 65 years. The main circumstances of discovery were thrombophily (27 cases) and associated cardiovascular risk factors (44 cases). The causes were essentially the nutritional deficiencies in particular deficit in vitamin B12 (14 cases), deficit in folates (2 cases), type 1 and 2 diabetes (32 cases) and renal insufficiency (11 cases). Elevated plasma total homocysteine was of indefinite cause in 8 cases. The associated manifestations were: a deep venous thrombosis in 13 cases, a superficial venous thrombosis in one case, ischemic vascular recidivism in 3 cases, an acute ischemic stroke in one case, atheromatous stenosis of the renal artery in one case and a coronary syndrome in 16 cases. Elevated plasma total homocysteine was asymptomatic in 37 cases. From our study and a review of the literature we discuss the relationship between elevated plasma total homocysteine and vascular disease.

Keywords: Homocysteine; Cardiovascular risk; Venous thromboembolism

Introduction

Cardiovascular disease is a major cause of mortality worldwide [1-3]. While high blood pressure, high cholesterol, type 2 diabetes, obesity and smoking are known risk factors, epidemiological data collected during the last thirty years suggest that other biological factors may be associated with increased cardiovascular risk [1-3]. Of these, one is increasing interest in moderate hyper homocysteinemia (HH), numerous studies attempt to clarify the relationship moderate HH, and cardiovascular disease and thromboembolism [1-4].

Patients and Methods

We report a retrospective study performed on 72 cases of patients with HH and discuss the potential profile associated vascular diseases. Inclusion criteria were patients collected in Department of Internal Medicine of Sahloul Hospital, Sousse, Tunisia; age superior to 16 years old; homocystein level greater than 15 µmol/l and method used for measurement of plasma homocysteine levels was "immunologic dosage by fluorescence polarization (FPIA)" made in the laboratory of the same hospital. Exclusion criteria were patients taking medications, which could have influenced plasma homocysteine levels.

Results

They were 48 men and 24 women. The average age was 65 years and 3 months with extremes of 22 and 99 years. The main circumstances of the discovery of HH were either part of a thrombophilia (27 cases) or in the assessment of cardiovascular risk factors (44 cases), including three cases of diffuse atherosclerosis early either as part of a megaloblastic anemia vitamin B 12 (1 case). HH was moderate (between homocysteine levels from 15 to 30 µmol/l) in 55 cases, it was of intermediate grade (from 30 to 100) in 15 cases and severe (greater than 100) in 2 cases.

The causes were selected multiple nutritional deficiencies including vitamin B12 deficiency in 14 cases and folate deficiency in 2, diabetes type 1 and 2 was implicated in 32 cases, kidney failure dans11 cases, hypothyroidism in 3 cases and an iatrogenic cause (methotrexate) was found in 2 cases. The HH was undetermined in 8 cases. The events were selected from the HH: deep vein thrombosis (DVT) in 13 cases,

superficial venous thrombosis (SVT) in a case of recurrent ischemic stroke in 3 cases, acute ischemia of the toes in one case, a atherosclerotic stenosis of the renal artery in one case and a coronary syndrome in 16 cases. In addition, several studies describe an association between HH and moderate cardiotoxicity during following clinical practices. In our study, the HH was asymptomatic in 37 cases. The cardiovascular risk factors were associated with the HH type 2 diabetes in 29 cases, diabetes type 1 in 3 cases, hypertension in 38 cases, 7 cases of hypercholesterolemia, hyper triglyceridemia in 22 cases and smoking in 23 cases. It was isolated in 9 cases.

The therapeutic management of HH was based on folic acid alone in 27 cases or in combination with vitamin B12 in 9 cases. Six patients were started on vitamin B12 alone. Thirty patients have not yet received treatment. The homocysteine control, performed in patients who received folic acid, was normal. In patients who received vitamin B 12, homocysteine control was normal in 3 cases, still high in one case and not yet controlled in the other two.

Discussion

Homocysteine is a sulfur amino acid that is involved in cellular metabolism of methionine. The normal plasma homocysteine levels are between 5 and 15 µmol/l. Physiologically, they increases with age and are usually higher in males. Among female subjects, homocysteine rises significantly after menopause. Diverses pathologies may increase, to a varying degree of plasma homocysteine. This is essentially genetic diseases (involving key enzymes of its metabolism) and/or nutritional deficiencies [5,6]. HH of genetic origin is most severe. The main one

follows a cystathionine-syntethase deficiency resulting in a blockage of the trans-sulfuration with, upstream accumulation of homocysteine, leading to HH and homocystinuria. In the homozygous form of this hereditary disease, homocysteine levels are generally higher than 100 µmol/l. Its evolution is characterized by early onset of stroke. Patients heterozygous for the mutation usually have homocysteine levels between 20 and 40 µmol/l, and are also predisposed to develop vascular macro-angiopathic early. The other mutation is that of gene 5, 10-methylene tetrahydrofolate reductase, resulting in the formation of a variant enzyme with reduced activity, thereby limiting the path of partially remethylation with, as a result, a degree of mild HH or intermediate. A mostly moderate hyperhomocysteinemia may result from a deficiency of folate or other cofactors, such as vitamins B6 and/or B12 [5-7]. Kidney failure is another frequent cause of an increase in homocysteine, which can then be up to three or four times the normal values [5-6]. Several authors have considered logical that HH secondary to renal failure may contribute to the development of atherosclerotic lesions commonly encountered in this context [8]. HH has been associated with other diseases such hypothyroidism, pernicious anemia and certain proliferative diseases [5,6]. Several drugs have been implicated in its genesis especially those that interfere with folate metabolism [5,6]. Finally, homocysteine tends to rise moderately in smokers and in cases of chronic alcohol and/or coffee.

In our series, the causes were many: nutritional deficiencies including vitamin B12 and/or folate deficiency, diabetes type 1 and 2, kidney failure, hypothyroidism and iatrogenic (methotrexate). The HH was undetermined in 8 cases. Numerous experimental data, clinical and epidemiological evidence that increased plasma level of homocysteine is considered a risk factor independent of cardiovascular ischemic [9,10]. Indeed, under normal conditions, the vascular endothelium plays an important role in the prevention of atherosclerosis by producing nitric oxide (NO). Homocysteine causes endothelial cell damage and proliferation of smooth muscle cells of blood vessel walls, that it hinders the release of NO and thus inhibits the vascular relaxation [11]. Homocysteine has many other actions on the cardiovascular system: modification of the extracellular matrix and lipoprotein oxidation. It initiates the production of tumor necrosis factor-alpha (TNF-α) and many cellular signals involved in the inflammatory process: it binds to lipoproteins, thereby increasing their ability to bind to fibrin, potentiating the risk of atherogenesis [12]. Homocysteine inhibits the expression and activity of thrombomodulin on the surface of endothelial cells, which allows the activation of protein C, powerful natural anticoagulants, and inhibits the binding of antithrombin III to membrane heparin sulphates [13]. The first clinical observations which an opportunity to discuss a link between hyperhomocysteinemia and cardiovascular disease in young adults with hereditary hyperhomocystinurie [14]. These patients have plasma levels of homocysteine very high and have frequently early thromboembolic stroke or atheromatous, it's actually Mc Cully [14] who was the first to suggest the hypothesis of a relationship between the excess homocysteine and atherosclerosis. Since then, many epidemiological studies have confirmed this postulated original. In our study, recurrent ischemic strokes were observed in 3 cases, atherosclerotic stenosis of the renal artery in one case and a coronary syndrome in 16 cases.

A meta-analysis of 72 studies of MTHFR (gene for methylenetetrahydrofolate reductase) and 20 prospective studies showed that homocysteine increased 5 µmol/l of homocysteine is associated with increased ischemic heart disease of 42% + and stroke (stroke) of + 59% and a decrease 3 µmol/l of homocysteine is associated with a reduction of ischemic heart disease - 16% and stroke-24% [15].

Numerous studies have shown an abnormal increase in homocysteine after methionine overload in the coronary [16], and/or in patients with peripheral arterial disease (PAD) [17]. The work of Clarke et al. [17] demonstrated that the relative risk of coronary heart disease is 20 times higher than normal when there is excess homocysteine. Boushey et al. [18] conducted a meta-analysis including all studies relating to the association between HH and cardiovascular pathology and published until June 1994. From seventeen studies, it was estimated that an increase in total homocysteine by 5 mmol/l is an increasing risk of coronary disease in men by 1.6 and 1.8 in women. A meta-analysis of same authors [18] of eleven studies on the relationship between HH and cerebrovascular disease had found an increased cardiovascular risk of 1.9 for both sexes when homocysteine increases 5µmol/l. Finally, from three studies, Boushey et al. [18] have calculated that the risk of developing peripheral vascular disease was 6.8 times higher for an increase in homocysteine of 5 µmol/l. Data from the study United British Provident Association Prospective Study [2] showed that the mean homocysteine was higher in patients who died following a stroke than in matched healthy subjects (13.1 vs. 11.8 µmol/l). Souissi et al. also showed that plasma homocysteine levels and the prevalence of HH were significantly (p<0.001) higher in coronary patients (16.3 ± 7.9 and 29%) than controls (12.6 ± 4.0 and 10%) and that this association persisted after adjustment for major cardiovascular risk factors [19]. Moreover, several studies have reported a positive relationship between higher moderate HH and cardiovascular disease among subjects with pre-existing conditions such as kidney failure [20], coronary artery disease [21], peripheral vascular disorders [22], a diabetes [23], lupus erythematosus [24] or venous thromboembolism [25]. Several other studies supported this positive relationship between HH and chronic arterial disease. In practice, Taylor et al. showed that the risk of cardiovascular death increased by nearly 6% when homocysteine level increased by 1 µmol/l [22]. Elevated homocysteine levels of 5 µmol/l appears to increase coronary risk in the same way that an increase in serum cholesterol of 20 mg/dl [18]. Similarly, the prognostic value of homocysteine in coronary artery disease has been highlighted by several studies. The homocysteine has emerged as a powerful predictor of mortality in patients with angiographically proven coronary artery disease [21]. Moderate HH is frequently objectified in diabetics type 1 or type 2. She is best described when there is a preclinical or clinical nephropathy, and/or when plasma levels of folate are reduced [7,26]. In our study, we identified three HH with type 1 diabetes and 29 HH with type 2 diabetics. The association of HH (measured fasting or after methionine load) to a macro-angiopathic pathology was found by several authors. This is particularly the case in the study of Buysschaert et al. [27] who found a macro-angiopathy (in term cerebral, coronary and/or localized to the lower limb arteries) in 70% of a cohort of patients with type 2 diabetes whose homocysteine was greater than 15 mmol/l and in 42% of those with normal homocysteine levels. Smulders et al. [28] found, among type 2 diabetics, a frequency of 57% of macro-angiopathy in case of HH and 19% when homocysteine is normal. It is worth mentioning that Hoogeveen et al. [29] evoked a potentialisation of the pathogenic effects of HH in the genesis of atherosclerosis in the presence of type 2 diabetes. In another study [30], the HH was seen as a cardiovascular risk factor in type 2 diabetes regardless of age and renal function. Lui et al. [31] have shown that HH in type 2 diabetes was associated with a high prevalence of peripheral arterial disease and kidney disease. In populations at high cardiovascular risk, several prospective studies have shown a strong association between moderate HH and cardiovascular risk, and between HH and cardiovascular morbidity and mortality [9,10]. However, in other publications [32,33] in populations free of disease and cardiovascular risk factors,

individuals with plasma homocysteine concentrations highest did not show an increased risk of developing a cardiovascular morbid event. Therefore, the hypothesis that moderate HH is an independent risk factor for cardiovascular disease remains the subject of controversy [9, 10]; thereby creating a reserve to use in the interpretation of previous results [6,34]. The authors of these works consider contradictory, on the basis of several prospective analyzes, the HH could be more an indicator of macro-angiopathy and/or consequence of atherosclerosis, rather than an etiological factor. Several reports of the Physicians Health Study [1] showed no significant relationship between homocysteine and myocardial infarction: subjects with myocardial infarction (n=271) had an initial homocysteine little different matched healthy subjects but the difference was significant because of the large number of subjects (11.1 versus 10.5 μmol/l). In the Tromso Health Study [35], the difference between cancer patients and healthy subjects was also low: the average homocysteine was 12.7 ± 4.7 mmol/l in patients and 11.3 ± 3.7 mmol/l in control subjects.

It is important to mention that besides its deleterious role, even controversial; on arterial vascular trunks, HH promotes the development of lesions and venous thromboembolism associated pathologies [36]. The literature data indicate that moderate HH was found with a frequency of 19 to 47% in arterial thrombosis [37,38] and 10 to 25% in venous thrombosis [36,38]. Thus in our series, the HH was associated with deep vein thrombosis (DVT) in 13 cases, a superficial thrombosis vein (STV) in one case and arterial thrombosis in one case. A meta-analysis of available epidemiological studies seems to confirm the role of HH in the development of veunous thromboembolism (VTE) [39]. In case-control studies collected, an increase of 5 μmol/l homocysteine was associated with an increased risk of VTE. The strength of this association was moderate, however: the odds ratio (OR) estimated in this study was 1.60 (confidence interval 95%: 1.10 to 2.34). HH was also a risk factor for recurrence in the cohort studies, with an even lower risk, estimate of 1.24 (confidence interval 95%: 1.01 to 1.59). Thus, HH is a minor risk factor for VTE (OR <2) [40].

The treatment of HH based on oral administration of folic acid alone or in combination with vitamin B6 and/or B12 [41,42]. The folic acid dose to be administered is between 1 to 5 mg/day. In our series, the therapeutic management of HH was based on folic acid alone in 27 cases or in combination with vitamin B12 in 9 cases. Six patients received vitamin B12 alone. Thirty patients have not yet received treatment. The homocysteine control, performed in patients who received folic acid, had returned to normal. In patients who received vitamin B 12, homocysteine control was normal in 3 cases, still high in one case and not yet controlled in the other two.

Many randomized controlled trials showed a decrease in homocysteine levels in patients receiving folate orally at a dose of 1 mg/day, this decrease being more important than starting homocysteine was elevated and low folate levels [43]. Schnyder et al. [44] showed in a cohort of subjects, who underwent coronary angioplasty, that oral supplementation with folate reduces homocysteine levels compared to placebo and significantly reduced the occurrence of vascular restenosis (19.6% versus 37.6%). However, Den Heijer et al. [45] showed that vitamin supplementation (5 mg of folate, 0.4 g of vitamin B12 and 50 mg of vitamin B6 per day) does not decrease the risk of recurrent thromboembolism, or hyperhomocysteinemic patients, nor in patients with normal homocystein level [45]. This lack of effect in secondary prevention of clinical risk has also been reported in arterial disease [46]. As for our daily practice in Tunisia, the homocysteine is essential as part of a thrombophilia, an assessment of cardiovascular risk factors.

An etiological investigation was immediately initiated if biology shows HH. Treatment, based on folic acid, is most often prescribed. Thus, Znazen et al. [47] emphasize the need for homocysteine to determine the etiology of a veinus thrombosis, assess the risk of recurrence and define the duration of oral anticoagulation prophylaxis. However, many teams consider that HH is not associated with an increased risk of coronary artery disease, cerebrovascular disease and peripheral vascular and therefore homocysteine is not required by first intention. This leads us to conclude that HH is still a controversial issue and that the route is still long to finally reach an agreement.

Conclusion

In addition to traditional cardiovascular risk factors, excess homocysteine could be a risk factor in itself. It is therefore logical to consider prescribing, particularly in the presence of other predisposing factors, drug treatment simple, inexpensive, and especially since folic acid is particularly effective in normalizing the rates of pathological homocysteine. This could help to limit the degenerative macrovascular complications. HH could also be a risk factor for venous thromboembolism. However, it is reasonable to encourage the initiation of other prospective studies to support additional interventional definitively the merits of this particular attitude that many experimental data, clinical and epidemiological studies continue to clear homocysteine in vascular disease. It appears that only intervention trials, designed to study the effects of reducing homocysteine levels by vitamin supplementation on cardiovascular disease, could determine whether moderate HH is a simple indicator or risk factor cardiovascular authentic.

References

1. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, et al. (1992) A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 268: 877-881.

2. Wald NJ, Watt HC, Law MR, Weir DG, McPartlin J, et al. (1998) Homocysteine and ischemic heart disease: results of a prospective study with implications regarding prevention. Arch Intern Med 158: 862-867.

3. Kark JD, Selhub J, Adler B, Gofin J, Abramson JH, et al. (1999) Nonfasting plasma total homocysteine level and mortality in middle-aged and elderly men and women in Jerusalem. Ann Intern Med 131: 321-330.

4. Ridker PM, Manson JE, Buring JE, Shih J, Matias M, et al. (1999) Homocysteine and risk of cardiovascular disease among postmenopausal women. JAMA 281: 1817-1821.

5. Welch GN, Loscalzo J (1998) Homocysteine and atherothrombosis. N Engl J Med 338: 1042-1050.

6. Nygård O, Vollset SE, Refsum H, Brattström L, Ueland PM (1999) Total homocysteine and cardiovascular disease. J Intern Med 246: 425-454.

7. Stabler SP, Estacio R, Jeffers BW, Cohen JA, Allen RH, et al. (1999) Total homocysteine is associated with nephropathy in non-insulin-dependent diabetes mellitus. Metabolism 48: 1096-1101.

8. Chauveau P, Chadefaux B, Coudé M, Aupetit J, Hannedouche T, et al. (1993) Hyperhomocysteinemia, a risk factor for atherosclerosis in chronic uremic patients. Kidney Int Suppl 41: S72-S77.

9. Ueland PM, Refsum H, Beresford SA, Vollset SE (2000) The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 72: 324-332.

10. Brattström L, Wilcken DE (2000) Homocysteine and cardiovascular disease: cause or effect? Am J Clin Nutr 72: 315-323.

11. Guilland JC, Favier A, Potier de Courcy G, Galan PS (2003) Hercberg Hyperhomocysteinemia: a cardiovascular risk factor or a simple marker? Fundamentals. Pharmacoeconomics 51: 101-110.

12. McCully KS, Olszewski AJ, Vezeridis MP (1990) Homocysteine and lipid metabolism in atherogenesis: effect of the homocysteine thiolactonyl derivatives, thioretinaco and thioretinamide. Atherosclerosis 83: 197-206.

13. Harpel PC, Zhang X, Borth W (1996) Homocysteine and hemostasis: pathogenic mechanisms predisposing to thrombosis. J Nutr 126: 1285S-1289S.

14. McCully KS (1969) Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 56: 111-128.

15. Wald DS, Law M, Morris JK (2002) Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 325: 1202.

16. Murphy-Chutorian DR, Wexman MP, Grieco AJ, Heininger JA, Glassman E, et al. (1985) Methionine intolerance: a possible risk factor for coronary artery disease. J Am Coll Cardiol 6: 725-730.

17. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, et al. (1991) Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 324: 1149-1155.

18. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 274: 1049-1057.

19. Souissi M, Feki M, Mourali S, Enneifer M, Omar S, et al. (2006) [Homocysteinemia and coronary artery disease: a case-control study in a Tunisian population]. Arch Mal Coeur Vaiss 99: 781-785.

20. Bostom AG, Culleton BF (1999) Hyperhomocysteinemia in chronic renal disease. J Am Soc Nephrol 10: 891-900.

21. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, et al. (1997) Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 337: 230-236.

22. Taylor LM, Moneta GL, Sexton GJ, Schuff RA, Porter JM (1999) Prospective blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. J Vasc Surg 29: 8–19.

23. Stehouwer CD, Gall MA, Hougaard P, Jakobs C, Parving HH (1999) Plasma homocysteine concentration predicts mortality in non-insulin-dependent diabetic patients with and without albuminuria. Kidney Int 55: 308-314.

24. Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J, et al. (1996) Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. Lancet 348: 1120-1124.

25. Eichinger S, Stümpflen A, Hirschl M, Bialonczyk C, Herkner K, et al. (1998) Hyperhomocysteinemia is a risk factor of recurrent venous thromboembolism. Thromb Haemost 80: 566-569.

26. Wollesen F, Brattström L, Refsum H, Ueland PM, Berglund L, et al. (1999) Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney Int 55: 1028-1035.

27. Buysschaert M, Dramais AS, Wallemacq PE, Hermans MP (2000) Hyperhomocysteinemia in type 2 diabetes: relationship to macroangiopathy, nephropathy, and insulin resistance. Diabetes Care 23: 1816-1822.

28. Smulders YM, Rakic M, Slaats EH, Treskes M, Sijbrands EJ, et al. (1999) Fasting and post-methionine homocysteine levels in NIDDM. Determinants and correlations with retinopathy, albuminuria and cardiovascular disease. Diabetes Care 22: 125-132.

29. Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJ, Jakobs C, et al. (1998) Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulinindependent diabets mellitus: a population-based study. Arterioscler Thromb Vasc Biol 18: 133-138.

30. Vangelder E, Delecourt F, Cardozo MB, Dhondt JL, Forzy G (2006) [Hyperhomocysteinaemia and type 2 diabetes]. Ann Biol Clin (Paris) 64: 485-489.

31. de Luis DA, Fernandez N, Arranz ML, Aller R, Izaola O, et al. (2005) Total homocysteine levels relation with chronic complications of diabetes, body composition, and other cardiovascular risk factors in a population of patients with diabetes mellitus type 2. J Diabetes Complications 19: 42-46.

32. Alfthan G, Pekkanen J, Jauhiainen M, Pitkäniemi J, Karvonen M, et al. (1994) Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study. Atherosclerosis 106: 9-19.

33. Voutilainen S, Lakka TA, Hämelahti P, Lehtimäki T, Poulsen HE, et al. (2000) Plasma total homocysteine concentration and the risk of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Studypdel. J Intern Med 248: 217–22.

34. Meleady R, Graham I (1999) Plasma homocysteine as a cardiovascular risk factor: causal, consequential, or of no consequence? Nutr Rev 57: 299-305.

35. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, et al. (1995) Serum total homocysteine and coronary heart disease. Int J Epidemiol 24: 704-709.

36. den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, et al. (1996) Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med 334: 759-762.

37. Bergmark C, Mansoor MA, Swedenborg J, de Faire U, Svardal AM, et al. (1993) Hyperhomocysteinemia in patients operated for lower extremity ischaemia below the age of 50-effect of smoking and extent of disease. Eur J Vasc Surg 7: 391-396.

38. Bienvenu T, Ankri A, Chadefaux B, Montalescot G, Kamoun P (1993) Elevated total plasma homocysteine, a risk factor for thrombosis. Relation to coagulation and fibrinolytic parameters. Thromb Res 70: 123-129.

39. Den Heijer M, Lewington S, Clarke R (2005) Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost 3: 292-299.

40. Le Gal G, Delluc A (2007) Should we still look for hyperhomocysteinemia in patients with venous thromboembolism? Rev Med Interne 28: 517-519.

41. Aarsand AK, Carlsen SM (1998) Folate administration reduces circulating homocysteine levels in NIDDM patients on long-term metformin treatment. J Intern Med 244: 169-174.

42. Malinow MR, Duell PB, Hess DL, Anderson PH, Kruger WD, et al. (1998) Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. N Engl J Med 338: 1009-1015.

43. (1998) Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. BMJ 316: 894–898.

44. Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, et al. (2001) Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. N Engl J Med 345: 1593-1600.

45. den Heijer M, Willems HP, Blom HJ, Gerrits WB, Cattaneo M, et al. (2007) 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: 139-144.

46. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, et al. (2006) Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med 354: 1567-1577.

47. Znazen R, Guermazi S, Karoui M (2007) Association of tow thrombotic risk factors: factor V Leiden and hyperhomocysteinemia. A case report. Pathol Biol (Paris) 55: 163-166.