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Is hyperhomocysteinaemia a risk factor for recurrent venous thrombosis?

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Summary

Several studies have shown a relation between hyperhomocysteinaemia and arterial vascular disease. We looked at the association between hyperhomocysteinaemia and venous thrombosis which could be clinically important as hyperhomocysteinaemia is easily corrected by vitamin supplementation.

We studied 185 patients with a history of recurrent venous thrombosis and 220 controls from the general population. Homocysteine concentrations were measured before and 6 h after oral methionine loading. We defined hyperhomocysteinaemia as the homocysteine concentration above the fasting or the postmethionine value found for the 90th percentile of the controls. Of the 185 patients with recurrent thrombosis, 46 (25%) had fasting homocysteine concentrations above the 90th percentile or the controls (odds ratio is 3·1 [1·8–5·5]). After adjustment for age, sex, and menopausal status the odds ratio was 2·0 (1·5–2·7). Similar results were found for the post-methionine value (unadjusted odds ratio 3·1 [1·7–5·5], adjusted 2·6 [1·9–3·5]).

Hyperhomocysteinaemia is a common risk factor for recurrent venous thrombosis and can lead to a two-fold or three-fold increase in risk.

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Introduction

Hyperhomocysteinaemia is a disorder of methionine metabolism. Several studies have shown a relation between mild hyperhomocysteinaemia and vascular disease.[1,2] However, little attention has been directed towards venous thrombosis, which is surprising because of the high frequency of venous thrombosis among patients with classical homocysteinuria.[3] We present the results of a study on the relation between mild hyperhomocysteinaemia and venous thrombosis in patients with recurrent venous thrombosis.

Patients and methods

Patients were selected from the files of the anticoagulant clinic of The Hague. In the Netherlands, virtually all patients with a history of recurrent venous thrombosis have long-term coumarin therapy and are registered at an anticoagulant clinic. All patients between 20 and 90 years who had had two or more episodes of venous thrombosis were invited to take part. Pregnancy was the only exclusion criterion. Of the 473 patients approached, 185 participated (39%).

We recruited the control group through a general practice in The Hague. We invited 2812 people aged 20–90 from this practice to take part in a health survey of risk factors for cardiovascular disease. 532 people agreed to participate and the first 220 formed the control group.

We obtained a short medical history of all patients by interview and all controls by questionnaire. A methionine-loading test was done on all subjects. This test consisted of a basal homocysteine measurement (after overnight fasting) and a second homocysteine measurement 6 h after oral methionine loading (0·1 g L-methionine per kg bodyweight in 200 mL orange juice).[4] A diet poor in protein was given to the patients during the tests.

We obtained blood samples from the antecubital vein in 5 mL Vacutainer tubes and 4·5 mL EDTA vacuum glass tubes for determination of the homocysteine and vitamin concentrations and routine laboratory measurements (creatinine, aspartate aminotransferase, alanine aminotransferase, gammaglutamyl

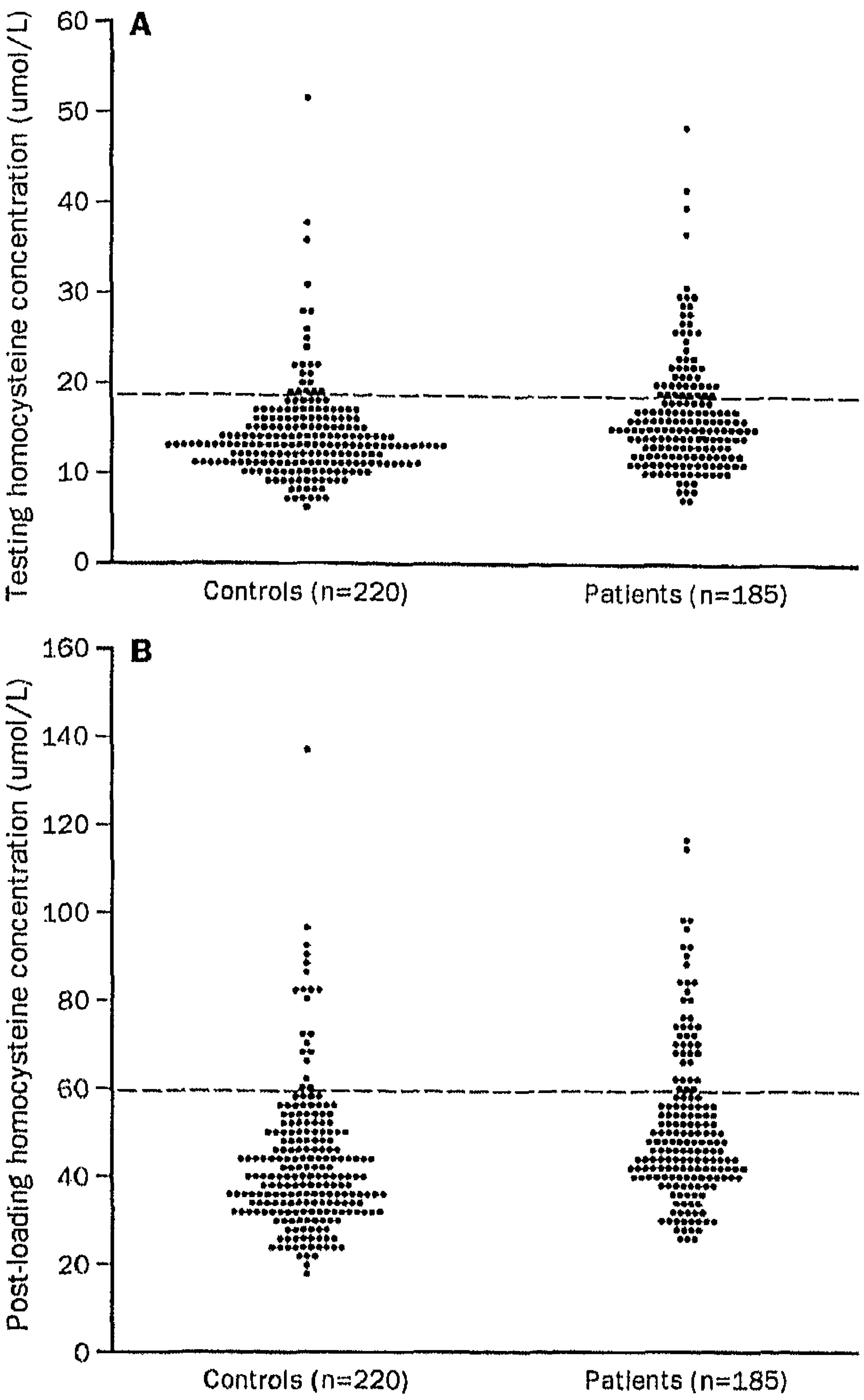

Figure: **Homocysteine concentrations in patients and controls**
Fasting (A) and post-methionine (B) homocysteine concentration in thrombosis patients and controls. Dotted lines indicate 90th percentiles of distribution of control subjects.

transpeptidase, alkaline phosphatase, and a whole blood count). The EDTA-samples for homocysteine measurement were immediately placed on ice and centrifuged at 3500 *g* for 5 min within 2 h. The plasma was separated and stored at −20°C until analysis.

Routine laboratory tests were done with a Kodak Ektachem Processor. Vitamin B12 and folic acid concentrations were measured in serum samples stored at −70°C with the Dualcount SPNB (solid phase no boil) Radioassay (Diagnostic Product Corporation, Los Angeles, USA). The total homocysteine concentration in plasma was measured in the laboratory of Paediatrics and Neurology of the Nijmegen University Hospital (HJB) by an automated high-performance liquid chromatography method with reverse phase separation and fluorescent detection (Gilson 232-401 sample processor, Spectra Physics 8800 solvent delivery system and Spectra Physics LC 304 fluorometer), essentially according to the method by Fiskerstrand et al.[5]

The methionine-loading test was used to detect hyperhomocysteinaemia. We analysed both fasting and post-loading homocysteine concentrations because there is no consensus whether hyperhomocysteinaemia should be defined as an abnormal homocysteine concentration, either in the fasting state or after methionine loading. As a cut-off point we used the 90th percentile of the distribution of the control subjects. We also analysed the data with the 80th, 95th, and 97·5th percentile as cut-off points.

	Cases n=105	Controls n=220	Odds ratio
Fasting homocysteine concentrations			
>90th percentile (18·6 µmol/L)	46	21	3·1 (1·8–5·5)
≤90th percentile (18·6 µmol/L)	139	199	
Post-methionine homocysteine concentrations			
>90th percentile (58·8 µmol/L)	44	20	3·1 (1·7–5·5)
≤90th percentile (58·8 µmol/L)	141	200	

Table 1: **Homocysteine concentrations and risk of recurrent venous thrombosis**

We calculated crude odds ratios as estimates of the relative risk and used Miettinen's test-based 95% CI.[6] Adjusted odds ratios were calculated with a logistic regression model. These odds ratios reflected the risk of recurrent thrombosis for people with hyperhomocysteinaemia compared with normal individuals when the data were adjusted for age, sex, and menopausal status.[7] To find a dose-response relation we stratified the homocysteine concentrations of both cases and controls into quartiles and calculated the odds ratios for the three higher levels and compared them with the lowest reference level.[8] We also calculated an aetiologic fraction, which represented the proportion of all cases of recurrent thrombosis attributable to hyperhomocysteinaemia, provided that the relation between hyperhomocysteinaemia and thrombosis is a causal one.[9]

The study protocol was approved by the ethics committee of the Leyenburg Hospital.

Results

The mean age of the patient group invited was 67 (range 20–97) years old. For patients who took part mean age was 61 (range 23–88) years compared with 51 (21–84) years in controls. The ratio between pulmonary embolism and deep venous thrombosis as main diagnosis was 1/1·6 in the group of patients invited and 1/1·5 in patients taking part in the study. The median time between the first episode of thrombosis and the study was 17 (range 1–58) years; the median time between the last episode of thrombosis and the study was 7 (range 1–30) years.

The figure shows the individual homocysteine fasting and post-methionine values. The cut-off point was defined as the 90th percentile of the distribution among the control subjects (18·6 µmol/L for the fasting value and 58·8 µmol/L for the post-methionine value). These cut-off points are comparable to the reference values (mean +2 SD) obtained from healthy, younger volunteers in the laboratory of Nijmegen with values of 18, 15, and 19 µmol/L for the fasting homocysteine concentration and 54, 51, and 69 µmol/L for the post-methionine homocysteine concentration for men, premenopausal women, and postmenopausal women, respectively.

Among the patients with thrombosis, 46 (25%) had fasting homocysteine concentrations above the cut-off

	Odds ratio
Fasting homocysteine concentrations	
80th percentile (16·2 µmol/L)	2·7 (1·8–4·3)
90th percentile (18·6 µmol/L)	3·1 (1·8–5·5)
95th percentile (22·2 µmol/L)	3·1 (1·4–6·8)
97·5th percentile (27·7 µmol/L)	2·7 (0·8–9·0)
Post-methione homocysteine concentrations	
80th percentile (51·7 µmol/L)	2·5 (1·6–3·9)
90th percentile (58·8 µmol/L)	3·1 (1·7–5·5)
95th percentile (79·0 µmol/L)	1·7 (0·6–4·3)
97·5th percentile (86·9 µmol/L)	3·2 (0·6–8·4)

Table 2: **Thrombosis risk for different cut-off points of homocysteine concentrations**

point of 18·6 μmol/L, versus 21 in the control group (table 1). This yields a crude odds ratio of 3·1 (1·8–5·5). When the post-methionine homocysteine concentrations were used, 44 out of 185 patients (24%) had levels exceeding the cut-off point of 58·8 μmol/L, versus 20 in the control group, which gave a very similar odds ratio (unadjusted odds ratio 3·1 [1·7–5·5]). Of the 46 patients who exceeded the fasting cut-off point 27 also exceeded the post-methionine 90th percentile value. The odds ratios for several other cut-off points are shown in table 2.

Our patients were selected from an anticoagulant clinic where they were registered because of recurrent venous thrombosis. In principle, for the diagnosis we relied on information of the referring physicians. We could also verify at least one diagnosis of venous thrombosis in 100 of the 185 patients by means of a positive objective test (ventilation/perfusion scan, ultrasonography, or plethysmography) from the hospitals where patients were treated. In this subgroup we found 24 patients with a fasting homocysteine concentration above 18·6 μmol/L (odds ratio 3·0 [1·6–5·8]) and 21 patients with a post-methionine homocysteine concentration above 58·8 μmol/L (odds ratio 2·7 [1·3–5·3]).

Among the 220 controls, 7 (3·1%) had a history of venous thrombosis. 3 had fasting homocysteine above 18·6 μmol/L, and 2 had post-methionine homocysteine above 58·8 μmol/L, giving an odds ratio of 8·1 (1·5–45·1) and 4·3 (0·4–52·3).

When we adjusted the results for age, sex, and menopausal status the odds ratios were slightly lower (2·0 [1·5–2·7] for the fasting and 2·6 [1·9–3·5] for the post-methionine value).

We stratified the homocysteine measurements of both patients and controls into quartiles and calculated the odds ratios for the three highest levels and compared them with the lowest (table 3). For fasting and post-methionine values the odds ratio increased with homocysteine concentration.

The importance of hyperhomocysteinaemia as a risk factor for venous thrombosis is a function of the odds ratio and the prevalence in the general population. This is reflected by the aetiologic fraction. For the fasting value we calculated an aetiologic fraction of 0·17 (CI 95% 0·09–0·24). This implies that 17% of recurrent thrombosis may be due to hyperhomocysteinaemia (defined as a homocysteine concentration above 18·6 μmol/L). For the post-methionine value we calculated an aetiologic fraction of 0·16 (0·08–0·23, cut-off point: 58·8 μmol/L).

	Cases (n=185)	Controls (n=220)	Total (n=405)	Odds ratio (95% CI)
Fasting homocysteine concentrations				
1st quartile <11·0 μmol/L	36	68	104	1*
2nd quartile 11·0–14·0 μmol/L	35	66	101	1·0 (0·9–1·1)
3rd quartile 14·0–17·0 μmol/L	51	49	100	2·0 (1·1–3·6)
4th quartile >17·0 μmol/L	63	37	100	3·2 (1·8–5·8)
Post-loading homocysteine concentrations				
1st quartile <35·5 μmol/L	24	77	101	1*
2nd quartile 35·5–43·0 μmol/L	47	55	102	2·7 (1·5–5·0)
3rd quartile 43·0–54·0 μmol/L	50	52	102	3·1 (1·7–5·7)
4th quartile >54·0 μmol/L	64	36	100	5·7 (3·1–10·5)

*Reference category, odds ratio=1. Test for trend, p<0·001.

Table 3: **Thrombosis risk for strata of homocysteine concentrations**

Vitamin B12 and folic acid were lower in the control subjects than in the patients, despite more vitamin supplements (median serum folic acid 6·0 [range 2·3–>24] ng/mL in patients and 5·8 [1·9–21·5] ng/mL in controls; median vitamin B12 408 [139–2387] pg/mL in patients and 288 [119–>2400] pg/mL in controls).

Discussion

Our study shows that hyperhomocysteinaemia is a risk factor for recurrent venous thrombosis. Because the enrolment of incident cases with recurrent venous thrombosis would have taken a long time, we invited patients from an anti-coagulant clinic who were registered with a history of recurrent venous thrombosis.

This implicates a consession to diagnostic accuracy. So it is possible that some patients might not have had recurrent venous thrombosis according to present criteria of objective testing. However, if this is the case our results will only be diluted, and the real association between hyperhomocysteinaemia will be stronger. Furthermore, in 100 patients we were able to trace medical records and found a positive objective test for venous thrombosis. In this subgroup we came to the same conclusion.

Up to now there is no consensus about reference values for plasma homocysteine concentrations. Most clinical studies on hyperhomocysteinaemia refer to laboratory reference values, usually obtained from healthy young volunteers. However, we found it more suitable to use a control group from the general population ie, the population where the patients came from.[10] First we analysed our data at different cut-off points. A cut-off point at the 90th percentile of the control group is quite similar to the reference value based on a laboratory staff control group, which is younger and might have less co-morbidity. This means that, with respect to these laboratory reference values hyperhomocysteinaemia is quite common in our control group, which is recruited from a general population.

We also found an increasing odds ratio with an increasing homocysteine concentration. This finding implies that there is no certain sharp cut-off point at all. We feel the issue of reference values concerning hyperhomocysteinaemia resembles the discussion on cholesterol, in so far that it is not very informative if a cholesterol value exceeds the reference value, but if it exceeds a certain concentration above which an increased risk of any clinical event exists. For homocysteine this would be already at the level of 14 μmol/L for the fasting value and at the level of 35·5 μmol/L for the post-methionine homocysteine values (table 3).

Although only a modest odds ratio of 3 was determined, this observation is important because of the high prevalence of hyperhomocysteinaemia in the general population. This is reflected by the aetiologic fraction. We found an aetiologic fraction of 0·17 for the fasting value and 0·16 for the post-methionine value at the 90th percentile, meaning that hyperhomocysteinaemia can account for a substantial proportion of recurrent thrombosis.

Three previous case-control studies focused on the relation between hyperhomocysteinaemia and primary venous thrombosis. Bienvenu et al[11] found increased homocysteine concentration in patients with venous thrombosis. However, this patient group was not homogeneous, since it included patients with Budd-Chiari syndrome and central retinal vein occlusion.

Battström et al[12] reported no significant difference between patients with venous thrombosis and controls which might have been because of the small sample size (42 cases and 42 controls). From their data we were able to calculate an odds ratio for primary thrombosis of 3·1 (0·40–27·7). Falcon et al[13] found a high percentage of hyperhomocysteinaemia (18·8%) in patients with one or more events of venous thrombosis before 40 years. Our study shows that for recurrent thrombosis this observation can be extrapolated to all ages. Since we focused on recurrent thrombosis, the relative risk for a first thrombotic event cannot be inferred from our data.

The underlying mechanism by which hyperhomocysteinaemia can provoke thrombosis is unknown. There is some evidence that homocysteine contributes to endothelial damage in the arterial vessel wall[2,3] but it is not clear whether this mechanism has a role in venous thrombosis. Another mechanism has been postulated by Rodgers et al[14,15] who found an in vitro effect of homocysteine on factor V activation and inhibition of thrombomodulin-dependent protein C activation. Moreover, an effect of homocysteine on thrombocyte aggregation has been reported.[16]

Hyperhomocysteinaemia may be the result of a hereditary defect in the enzymes involved in methionine metabolism or it might be acquired as a result of vitamin deficiency. It is not known whether the two types confer a similar risk of thrombosis. However, we did not find lower folic acid and vitamin B12 concentrations in patients than in controls. In fact we found higher homocysteine concentration at a given folic acid concentration in patients than in controls (data not shown.) This finding may suggest that some form of decreased enzyme activity and not vitamin deficiency leads to thrombosis.[17]

Several studies have shown that hyperhomocysteinaemia can be corrected by vitamin supplementation.[18,19] Whether vitamin supplementation for those with high levels of homocysteine will be beneficial with respect to prevention of (recurrent) venous thrombosis has to be further studied.[17,20]

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