The risk of venous and arterial thrombosis in hyperhomocysteinemic subjects may be a result of elevated factor VIII levels

Willem M. Lijfering, Nic J.G.M. Veeger, Jan-Leendert P. Brouwer, Jan van der Meer

From Division of Haemostasis, Thrombosis and Rheology, Department of Hematology (WML, J-LPB, JvdM); Department of Epidemiology, University Medical Center Groningen, Groningen, The Netherlands (NJGMV).

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Correspondence:
Willem Lijfering, Division of Haemostasis, Thrombosis and Rheology, Department of Hematology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.
E-mail: w.lijfering@int.umcg.nl

ABSTRACT

In a large retrospective study of thrombophilic families, we analyzed 405 relatives of patients, hypothesizing that hyperhomocysteinemia and elevated factor VIII levels are closely related. Median factor VIII levels in hyperhomocysteinemic relatives were 169 IU/dL, compared with 136 IU/dL in normohomocysteinemic relatives (p=0.007), and were more often elevated (>150 IU/dL; p=0.006). Hyperhomocysteinemia was associated with an increased risk of venous and arterial thrombosis; relative risk (RR) 2.6 (CI 1.3-4.8) and 3.7 (CI 1.5-8.4) respectively. Relatives with elevated FVIII were also at risk; RR 2.3 (CI 1.4-4.0) for venous thrombosis and 2.3 (CI 1.0-5.1) for arterial thrombosis. After excluding all relatives with elevated factor VIII, RR for hyperhomocysteinemia and venous thrombosis dropped to 1.3 (CI 0.2-9.8) and no relatives had arterial thrombosis. We conclude that it is likely that the increased risk of venous and arterial thrombosis in hyperhomocysteinemia is mainly related to elevated FVIII levels.

Key words: thrombosis, hyperhomocysteinemia, factor VIII

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Hyperhomocysteinemia is a disorder of methionine metabolism. Since 1969, when McCully made the clinical observation that elevated plasma homocysteine levels are linked with vascular disease,[1] many clinical and epidemiologic studies have demonstrated that hyperhomocysteinemia is a risk factor for both arterial and venous thrombosis.[2-3] Experimental studies suggest that the thrombogenic propensity associated with hyperhomocysteinemia results from endothelial dysfunction and injury.[4] However, it is still not clear whether homocysteine itself or a related metabolite or cofactor is primarily responsible for the thrombogenic effects of hyperhomocysteinemia. In particular, the recent observation that lowering homocysteine levels with vitamin B6, vitamin B12 and folic acid did not reduce the risk of venous and arterial thrombosis in large prospective randomized clinical trials,[5-7] supports the assumption that an associated condition, rather than hyperhomocysteinemia itself, is responsible for the thrombotic event. As high factor VIII levels are associated with venous and arterial thrombosis, and with endothelial injury[8-10] we hypothesize that hyperhomocysteinemia and factor VIII levels are closely related to each other. In a retrospective study, we assessed the contribution of hyperhomocysteinemia and elevated factor VIII levels to the absolute risk of venous and arterial thrombosis.

Design and Methods

Subjects were taken from a previous large single center family cohort study, designed to evaluate the risk of thrombosis associated with hereditary deficiencies of antithrombin, protein C or protein S.[11,12] In that study, probands were consecutive patients with documented venous thrombosis in whom one of these deficiencies had been confirmed. Relatives, 15 years of age or older, were identified by pedigree analysis and enrolled after informed consent was obtained. Clinical data were collected prior to blood sampling.[11,12] Clinical outcome events were classified by objective criteria. Relatives were tested for all currently known thrombophilic defects.[12] Levels of homocysteine were measured by high-performance liquid chromatography.[13] Between May 1995 and July 2001, homocysteine samples were collected after overnight fast-

ing. Hyperhomocysteinemia was defined as a fasting level above 18.5 μmol/L, as described in the Dutch population.[14] According to the 2001 Dutch Heart Foundation guidelines, random homocysteine samples (i.e. obtained without overnight fasting) could exclude hyperhomocysteinemia at levels below 10 μmol/L and definitely establish hyperhomocysteinemia when levels were above 20 μmol/L (see *www.hartstichting.nl/uploads/brochures/hartstichting176.pdf*). We screened relatives accordingly until the end of study (July 2004). When they had a random homocysteine level between 10 and 20 μmol/L, they would return to our clinic for a fasting and methionine-loading homocysteine measurement. Recently, we have shown that random homocysteine levels above 20 μmol/L were not associated with an increased risk of venous or arterial thrombosis.[15] Increased fasting levels were appropriate to identify subjects who were at risk of thrombosis associated with hyperhomocysteinemia. Therefore, we excluded relatives from the present analysis if fasting levels were not measured. Factor VIII:C was measured by one-stage clotting assay (Amelung GmBH, Lemgo, Germany) and was increased at levels > 150 IU/dL, as these levels have been identified as an independent risk factor for both venous and arterial thrombosis.[8-9] Annual incidences of venous and arterial thrombosis were calculated by dividing the number of first events and the number of observation years, censored for either arterial or venous thrombosis. Observation time was defined as the period from the age of 15 years until the first thrombotic event or until the end of study. Since recently a potential link between venous and arterial thrombosis has been made,[16] we also calculated annual incidences of successive venous and arterial thrombosis. Observation time in that analysis was defined as the period from the age of 15 years until the second thrombotic event or until the end of study.

Results

The original family cohort comprised 172 probands, with a total number of 1,266 relatives. Of these relatives, 70% (n=891) were eligible (i.e. living and aged 15 years or older). Eighty-eight relatives did not participate due to difficulties in obtaining informed consent. Another 308 relatives were excluded from analysis as they were only screened by random homocysteine tests, and could not be classified by additional measurements of fasting homocysteine levels. Relatives who were not evaluable because of other missing laboratory data were also excluded (n=90). The remaining 405 relatives were analyzed. Their clinical characteristics are summarized in Table 1. Median time interval between venous thrombosis and thrombophilia testing was 12 years (range 0-43) and for arterial thrombosis (range 0-33). Median factor VIII levels in hyperhomocysteinemic relatives were higher than in normohomocysteinemic relatives (169 IU/dL *vs* 136 IU/dL, p=0.007). Elevated factor VIII levels (> 150 IU/dL) were also more often observed in hyperhomocysteinemic relatives compared with normohomocysteinemic relatives (65% *vs* 38%, p=0.006), while other thrombophilic defects were equally divided (Table 2).

Table 1. Clinical characteristics of 405 relatives with or without hyperhomocysteinemia derived from probands with an antithrombin, protein C or protein S deficiency.

	Hyperhomocysteinemia		
	Present (n=26)	*Absent (n=379)*	*p*
Women, n (%)	18 (69)	194 (51)	0.10
Median age at enrollment (range), yrs	41 (20-80)	43 (15-85)	0.59
Venous thrombosis, n (%)	11 (42)	55 (15)	< 0.001
Median age at onset (range), yrs	35 (23-80)	31 (16-68)	0.22
Spontaneous, n (%)	5 (45)	25 (45)	1.0
Secondary to, n (%)	6 (55)	30 (55)	1.0
Oral contraceptives, n	0	12	
Pregnancy, n	3	8	
Surgery, trauma, immobilization, n	3	10	
Arterial thrombosis, n (%)	7 (27)	21 (5)	< 0.001
Median age at onset (range), yrs	61 (32-76)	54 (26-72)	0.31
Classification			
Myocardial infarction, n (%)	1 (4)	6 (2)	0.37
Transient ischemic attack, n (%)	2 (8)	7 (2)	0.11
Ischemic stroke, n (%)	4 (15)	5 (1)	< 0.001
Peripheral arterial thrombosis, n (%)	0 (0)	3 (1)	1.0
Classical risk factors			
Hypertension, n (%)	5 (19)	61 (16)	0.59
Hyperlipidemia, n (%)	5 (19)	49 (13)	0.37
Diabetes mellitus, n (%)	0 (0)	14 (4)	1.0
present smoking, n (%)	5 (19)	118 (31)	0.27
Venous and arterial thrombosis, n (%)	4 (15)	6 (2)	0.002

Hyperhomocysteinemia was associated with an increased risk of venous or/and arterial thrombosis; relative risks were 2.6 (95% CI, 1.3-4.8) for venous thrombosis, 3.7 (95% CI, 1.5-8.4) for arterial thrombosis, and 7.3 (95% CI 2.1-25.9) for both events (Table 3). Relatives with elevated factor VIII levels were also at risk; relative risks were 2.3 (95% CI, 1.4-4.0) for venous thrombosis, 2.3 (95% CI, 1.0-5.1) for arterial thrombosis, and 9.6 (95% CI, 1.2-76.1) for both. Excluding relatives with elevated factor VIII levels, relative risk of venous thrombosis in hyperhomocysteinemic relatives dropped to 1.3 (95% CI, 0.2-9.8), and no hyperhomocysteinemic relatives had arterial thrombosis, or both venous and arterial thrombosis. Excluding relatives with normal factor VIII levels, relative risks in hyperhomocysteinemic relatives were 2.6 (95% CI, 1.3-5.2), 4.0 (95% CI, 1.6-9.9), and 5.4 (95% CI, 1.4-20.0) respectively.

Discussion

Given the small numbers involved, these results should be handled with caution. However, this study suggests that the absolute risk of venous and arterial thrombosis in hyperhomocysteinemic subjects was not a result of

hyperhomocysteinemia itself but depended on elevated factor VIII levels. Hyperhomocysteinemia did not influence the risk when factor VIII levels were normal. However, the already higher absolute risk in relatives with elevated factor VIII levels was reinforced by hyperhomocysteinemia, suggesting an interaction. These findings agree with large prospective randomized clinical trials that showed no effect of homocysteine lowering therapy with B-vitamins on the risk of venous and arterial thrombosis.[5-7] Although it has been known for some time that hyperhomocysteinemia and elevated factor VIII levels are closely related to endothelial injury,[4,10,17] it is surprising that none of the previous studies which identified hyperhomocysteinemia as an independent risk factor for venous or arterial thrombosis adjusted the risk of thrombosis for elevated factor VIII levels.[2-3,14] It is also interesting that in our study, a history of both venous and arterial thrombosis was associated with elevated levels of factor VIII and hyperhomocysteinemia. This supports the assumed relationship between both diseases.[16]

As our study cohort was made up from a high-risk thrombophilic population, annual incidences of venous thrombosis were higher than those reported in the normal population (i.e. 0.1-0.3%).[18,19] However, antithrombin, protein C and protein S deficiencies were equally divided among hyperhomocysteinemic and normohomocysteinemic subjects, and survival analysis showed that non-deficient hyperhomocysteinemic relatives remained at an increased risk of venous thrombosis compared with non-deficient, normohomocysteinemic relatives (*data not shown*). Therefore, it is less likely that these inherited deficiencies alone explain the higher risk of venous thrombosis in hyperhomocysteinemic subjects, as shown in another study.[14]

Some aspects of our study do require further comment. First, since our study was retrospective, we did not measure fasting homocysteine at time of thrombosis. We are, however, confident that this has not influenced our results because we found no correlation between age and homocysteine levels at time of enrollment (r^2=0.012, *data not shown*). Second, despite maximum efforts, approximately 10% of subjects were not enrolled due to unavailable fasting homocysteine values. This demonstrates the

Table 2. Prevalence of concomitant thrombophilic defects in 405 relatives of probands with an antithrombin, protein S or protein C deficiency.

Variable, n (%)	*Hyperhomocysteinemia*		
	Present (n=26)	*Absent (n=379)*	*P*
Antithrombin deficiency	4 (15)	32 (8)	0.27
Protein C deficiency	2 (8)	34 (9)	1.0
Protein S deficiency	3 (12)	37 (10)	0.73
Elevated factor VIII	17 (65)	144 (38)	0.006
Factor V Leiden	3 (12)	50 (13)	1.0
Prothrombin G20210A	2 (8)	35 (9)	1.0

Table 3. Annual incidences of first episodes of venous and arterial thrombosis in relatives of probands with an antithrombin, protein C or protein S deficiency.

	Venous thrombosis				*Arterial thrombosis*				*Venous and arterial thrombosis*			
	Observation years	*Relatives with event*	*Incidence/ year,% (95% CI)*	*Relative risk (95% CI)*	*Observation years*	*Relatives with events*	*Incidence/ year,% (95% CI)*	*Relative risk (95% CI)*	*Observation years*	*Relatives with events*	*Incidence/ year,% (95% CI)*	*Relative risk (95% CI)*
Hyperhomocysteinemia												
Absent	10408	55	0.53 (0.40-0.69)	Reference	11096	21	0.19 (0.12-0.29)	Reference	11251	6	0.05 (0.02-0.11)	Reference
Present	804	11	1.37 (0.68-2.45)	2.6 (1.3-4.8)	1004	7	0.70 (0.28-1.44)	3.7 (1.5-8.4)	1025	4	0.39 (0.11-1,00)	7.3 (2.1-25.9)
Elevated factor VIII*												
Absent	5889	21	0.36 (0.22-0.55)	Reference	6196	9	0.15 (0.07-0.28)	Reference	6249	1	0.02 (0.004-0.09)	Reference
Present	5133	43	0.84 (0.60-1.13)	2.3 (1.4-4.0)	5712	19	0.33 (0.20-0.52)	2.3 (1.0-5.1)	5835	9	0.15 (0.07-0.29)	9.6 (1.2-76.1)
Hyperhomocysteinemia in relatives with normal factor VIII levels												
Absent	5674	20	0.35 (0.21-0.54)	Reference	5976	9	0.15 (0.06-0.29)	Reference	6029	1	0.02 (0.004-0.09)	Reference
Present	215	1	0.47 (0.01-2.59)	1.3 (0.2-9.8)	220	0	0 (0-1.68)	NA	220	0	0 (0-1.68)	NA
Hyperhomocysteinemia in relatives with elevated factor VIII levels												
Absent	4592	33	0.72 (0.49-1.01)	Reference	4978	12	0.24 (0.12-0.46)	Reference	5080	5	0.09 (0.03-0.23)	Reference
Present	541	10	1.85 (0.89-3.40)	2.6 (1.3-5.2)	734	7	0.95 (0.38-1.96)	4.0 (1.6-9.9)	755	4	0.53 (0.14-1.36)	5.4 (1.4-20.0)

**FVIII levels missing in 4 relatives. NA: not applicable.*

difficulties of homocysteine tests in which individuals have to return to a clinic in a fasting state. They also have to undergo a methionine-loading test which takes six hours and has possible side effects such as nausea and malaise.[20] Given that asymptomatic subjects are less willing to undergo these tests, this would have resulted in an overestimate of annual incidences of thrombosis in our study cohort. However, hyperhomocysteinemic relatives were not at risk of venous and arterial thrombosis when factor VIII levels were normal. Third, relatives who were only tested for hyperhomocysteinemia with random samples and had homocysteine levels <10 μmol/L or >20 μmol/L were excluded from analysis. Their exclusion probably did not influence our results as we previously showed that these subjects were at a similar risk of venous and arterial thrombosis.[15] Fourth, classical cardiovascular risk factors were not taken into account because appropriate analysis was not possible due to the small number of relatives with hyperhomocysteinemia. Finally, our overall event number was small and consequently our findings may be due to a type II error. Because we did a post-hoc analysis of data, we were not able to retrieve missing data which would have enlarged our sample size and possibly could have excluded this statistical error. We emphasize that our findings should be seen as hypothetical. Although pathophysiologically plausible, they need to be established in further studies.

In conclusion, it is likely that the increased risk of venous and arterial thrombosis in hyperhomocysteinemia is mainly related to elevated factor VIII levels.

Authors' Contributions

VdM: design and conduct of the study; LB: acquisition of the data; WL, NV, JvdM: analysis and interpretation of the data: WL, JvdM: drafting of the manuscript; WL, NV, J-LB, JvdM: critical revison of the manuscript for important intellectual content; WL, NV: statistical analysis; JLPB: administrative, technical and material support: JvdM: supervision.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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