Hyperhomocysteinemia Is a Risk Factor of Recurrent Venous Thromboembolism

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Summary

Hyperhomocysteinemia is a risk factor of venous thromboembolism. The risk of recurrence in patients with hyperhomocysteinemia is unknown, and the optimal therapy for these patients after acute venous thromboembolism is uncertain.

In a multicenter study, 264 patients with an objectively documented single episode of idiopathic venous thromboembolism were prospectively followed after discontinuation of oral anticoagulants. Patients were classified as hyperhomocysteinemic if their homocysteine levels exceeded the 95th percentile of the controls. The outcome events studied were objectively confirmed deep-vein thrombosis and/or pulmonary embolism.

Homocysteine levels were elevated in 66 patients (25%) and normal in 198 patients (75%). Recurrent venous thromboembolism occurred in 12 of 66 patients with hyperhomocysteinemia (18.2%) and in 16 of 198 patients without hyperhomocysteinemia (8.1%). The cumulative probability of recurrence 24 months after discontinuation of oral anticoagulants was 19.2 percent (95 percent confidence interval 8.7-27) in patients with hyperhomocysteinemia and was 6.3 percent (95 percent confidence interval 2.4-10.1; p = 0.001) in those without hyperhomocysteinemia. The relative risk of recurrent thrombosis was higher in patients with hyperhomocysteinemia [RR 2.7 (1.3-5.8), p = 0.009].

Patients with hyperhomocysteinemia are at high risk of recurrent venous thromboembolism. The high prevalence of hyperhomocysteinemia in thrombosis patients together with the increased risk of recurrence warrants extended patient screening. The impact on the risk of recurrence of prolonged anticoagulation, supplementation of folate and vitamin B12, or both have to be investigated.

Introduction

A high incidence of recurrent venous thromboembolism has been reported in patients shortly after an acute venous thrombotic event and treatment with oral anticoagulants is therefore generally recommended for three to six months. After discontinuation of secondary thromboprophylaxis, the risk of recurrent venous thromboembolism remains higher than the risk of a primary venous thromboembolic event for several years (1-3). A particularly high incidence of recurrence has been demonstrated in patients with cancer or the antiphospholipid antibody syndrome (1, 4). These patients are candidates for long-term oral anticoagulation, since it is believed that the risk of oral anticoagulants is offset by the benefit of secondary thromboprophylaxis. For patients with a congenital thrombophilia, such as a deficiency of antithrombin, protein C, or protein S, the same therapeutic principles are applied, although – due to the low prevalence of these abnormalities – the benefit/risk ratio of long-term anticoagulant therapy has never been clearly established. For patients with the Factor V Leiden mutation conflicting data on the incidence of recurrence have been reported (5-7) and prospective clinical trials to assess the optimal duration of secondary thromboprophylaxis in carriers of the mutation are needed.

Hyperhomocysteinemia is an established risk factor of venous thrombosis (8-12). In a retrospective analysis, den Heijer and colleagues studied patients with recurrent venous thrombosis and found elevated homocysteine levels in about 25 percent of the cases but in only nine percent of the controls (13). Recommendations for secondary thromboprophylaxis after an acute venous thromboembolic event are mainly based on an appraisal of the risk of recurrent thrombosis and the risk of treatment. Since the actual incidence of recurrence in patients with hyperhomocysteinemia is unknown, guidelines for the management of patients with hyperhomocysteinemia after a venous thromboembolic event are lacking.

We prospectively followed patients with a first venous thromboembolic event after discontinuation of oral anticoagulants and documented all recurrent thromboembolic events. We established the prevalence of hyperhomocysteinemic patients and investigated whether elevated plasma homocysteine levels are associated with an increased risk of recurrent venous thromboembolism.

Methods

Patients and Study Design

The Austrian Study of Recurrent Venous Thromboembolism (AUREC) is an ongoing prospective multicenter trial. Consecutive patients older than 18 years who had been treated with oral anticoagulants for at least three months after an objectively documented first episode of idiopathic venous thromboembolism, and who consented to participate in the study were included. Diagnosis of deep vein thrombosis was established by venography or by color-coded duplex sonography. In case of venography one of the following direct or indirect criteria had to be fulfilled: a constant filling defect; an abrupt discontinuation of the contrast filled vessel at a constant level of the vein; an absence of filling in the entire deep vein system without an external compressing process, with or without venous flow through collateral veins. Diagnostic criteria for color-coded duplex sonography were: visualization of an intraluminal thrombus in a deep vein; lack of or incomplete compressibility; absence of flow spontaneously and following distal manipulation. The diagnosis of pulmonary embolism was made by perfusion/ventilation lung scan according to the criteria of the PIOPED study investigators (14). Patients with both documented deep thrombosis and pulmonary embolism were included.
vein thrombosis and pulmonary embolism were categorized as pulmonary embolism. Patients were excluded because of the following conditions: previous venous thromboembolism; surgery or trauma within the last three months; deficiency of antithrombin, protein C, protein S, or plasminogen; systemic lupus erythematosus and/or an antiphospholipid antibody syndrome; cancer; pregnancy; or requirement of long-term treatment with antithrombotic drugs for reasons other than venous thromboembolism.

Patients were referred to the principal thrombosis center (Department of Internal Medicine I, University of Vienna, Austria) shortly before the discontinuation of oral anticoagulants for all further clinical and laboratory investigations. Shortly before discontinuation of oral anticoagulant therapy venography of the affected extremity was performed in all patients with deep vein thrombosis. The day of discontinuation of oral anticoagulants was defined as the day of study entry. After normalization of the prothrombin time, patients were screened for the presence of an antithrombin-, protein C-, protein S- or plasminogen-deficiency, or of antiphospholipid antibodies. Patients in whom one of these disorders was detected at this time, were excluded.

Patients were seen at three months intervals for the first 12 months and every six months thereafter. At each clinical visit, a data form recording the patient’s medical history was completed and a physical examination was performed. The patients were advised to report the intake of all medications including over-the-counter-drugs, such as aspirin, herbal medicine and all kind of vitamin supplementation. At study entry, patients were provided with detailed written information on the clinical symptoms of venous thromboembolism. Patients were instructed to report immediately to one of the thrombosis centers if symptoms of deep vein thrombosis and/or pulmonary embolism occurred.

Endpoints

The principal endpoint of the study was recurrence of venous thromboembolism. The diagnosis of recurrent venous thromboembolism was established by an independent investigator who was unaware of the presence or absence of thrombotic risk factors. Recurrent venous thromboembolism had to be confirmed by venography and/or a perfusion/ventilation lung scan according to the criteria mentioned above. Recurrent deep vein thrombosis was diagnosed if the patient had a thrombus in another leg, a thrombus in another deep vein in the same leg as the previous event or a thrombus in the same venous system as the previous event with a proximal extension of the thrombus if the upper limit of the original thrombus had been visible or - if not - the presence of a constant filling defect surrounded by contrast medium.

Laboratory Analysis

For measurement of homocysteine, venous blood collected after overnight fasting was immediately centrifuged at 1600 g for 20 min at 4°C, and the plasma was snap frozen and stored at −80°C. The total homocysteine concentration was measured in the citrated plasma by high performance liquid chromatography (HPLC; column: Superspher RP 18, mesh size 4 µm, Waters, USA) under isocratic conditions at room temperature using an acetate-buffer (flow-rate: 2 ml/min). All procedures before HPLC analysis were performed according to the manufacturer’s recommendations of an assay kit for homocysteine testing (Immundiagnostik, FRG). Hyperhomocysteinemia was diagnosed when the fasting plasma levels were above the 95th percentile (8.8 µmol/l in females, 11.6 µmol/l in males) of those measured in 73 healthy control subjects comparable to the patients with regard to age- and sex-distribution.

Patients were screened for the presence of Factor V Leiden (APC-resistance) according to the method described by Bertina and colleagues (15). Determination of a deficiency of antithrombin, protein C, protein S or plasminogen was performed as previously described (16). The presence of a lupus anticoagulant (antiphospholipid antibody) was established based on the criteria of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society of Thrombosis and Haemostasis (17). Anticardiolipin antibodies were determined by immunological methods (Synelisa Cardiolipin-AK, Elias, Freiburg, Germany).

Statistical Analysis

Times to recurrence of venous thromboembolism (uncensored observations) or follow-up times in patients without recurrence (censored observations) were analyzed using survival time methods (18). The probability of recurrent venous thromboembolism was estimated following the method of Kaplan-Meier (19). To test for homogeneity between strata we applied the log-rank and the generalized Wilcoxon test. Categorical data were checked for homogeneity using contingency table analyses (χ²-test). Simple descriptive statistics were computed to provide a clear presentation of the data. For numerical operations a SAS software package was used.

Results

Patients Characteristics

From July, 1992, until September, 1997, 777 patients were enrolled. 496 Patients were excluded because of the following conditions: previous venous thromboembolism (109); surgery or trauma within the previous three months (118); deficiency of antithrombin, protein C, protein S, or plasminogen (6 patients); systemic lupus erythematosus and/or an antiphospholipid antibody syndrome (9 patients); cancer (85 patients); or because they required long-term treatment with antithrombotic drugs, e.g. for atrial fibrillation, prosthetic heart valves, coronary heart or peripheral arterial disease (169 patients). Seventeen patients in whom a deficiency of antithrombin, protein C, protein S, or plasminogen was detected after discontinuation of oral anticoagulants were also excluded.

Of the remaining 264 patients, 66 (25%) patients had homocysteine levels above the 95th percentile of the controls and 195 (75%) patients had normal homocysteine levels. The demographic and clinical characteristics of the 264 patients are given in Table 1. During the course of the study, none of the patients received preparations containing folic acid or vitamin B12. Patients with hyperhomocysteinemia were significantly older at the time of diagnosis of their venous thromboembolic event (56 ± 17 years, range 19 to 84) and at study entry (57 ± 17, range 19 to 84) than patients without hyperhomocysteinaemia (46 ± 15 years, range 14 to 85, and 47 ± 16, range 18 to 86, respectively; p = 0.0001).

Factor V Leiden was present in 25 percent of the patients with hyper-

Table 1 Demographic and clinical characteristics of 264 patients with a history of venous thromboembolism (VTE)

<table>
<thead>
<tr>
<th>Site of thrombotic event</th>
<th>Patients with Hyperhomocysteinaemia (n = 66)</th>
<th>Patients without Hyperhomocysteinaemia (n = 198)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous thrombosis of the leg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>20 (30)</td>
<td>50 (26)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Distal</td>
<td>22 (30)</td>
<td>66 (33)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>2 (3)</td>
<td>12 (6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Anterior venous thrombosis</td>
<td>21 (30)</td>
<td>65 (33)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Duration of oral anticoagulant therapy (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± standard deviation</td>
<td>7 ± 4</td>
<td>8 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>median (range)</td>
<td>6 (3-24)</td>
<td>6 (3-168)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Observation Time (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± standard deviation</td>
<td>20 ± 15</td>
<td>20 ± 18</td>
<td>n.s.</td>
</tr>
<tr>
<td>median (range)</td>
<td>19 (5-50)</td>
<td>25 (5-57)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* percent of total study population
homocysteinemia and in 30 percent of the patients without hyperhomocysteinemia.

In 20 patients the study was terminated because of antithrombotic treatment for causes other than venous thrombosis (8 patients with hyperhomocysteinemia and 12 without hyperhomocysteinemia, respectively), because the patient was diagnosed with cancer (4 without hyperhomocysteinemia), or because the patient was lost for follow-up (8 and 15, respectively). These patients were withdrawn from the study and were censored at the time of withdrawal. None of the study patients died during the observation period.

**Recurrent Venous Thromboembolism**

Twelve of 66 patients with hyperhomocysteinemia (18.2 percent) and 16 of 198 patients without hyperhomocysteinemia (8.1 percent) had recurrent deep vein thrombosis and/or pulmonary embolism. The cumulative probability of recurrence after discontinuation of oral anticoagulant therapy (Fig. 1) was significantly higher in patients with hyperhomocysteinemia than in patients without hyperhomocysteinemia (Wilcoxon p = 0.001, log-rank p = 0.007). Within 12 months, the probability of recurrence was 12.6 percent (95 percent confidence interval 4.4-21) in patients with hyperhomocysteinemia and was 3.5 percent (95 percent confidence interval 0.7-6.2) in patients without hyperhomocysteinemia. At 24 months the incidence of recurrent venous thromboembolism increased to 19.2 percent (95 percent confidence interval 8.7-27) in patients with hyperhomocysteinemia and to 6.3 percent (95 percent confidence interval 2.4-10.1) in patients without hyperhomocysteinemia.

The relative risk (RR) of recurrent venous thromboembolism was 2.7 (95 percent confidence interval 1.3-5.8, p = 0.009) in patients with hyperhomocysteinemia. Hyperhomocysteinemia remained an independent risk factor of recurrent venous thromboembolism after adjustment for age, sex and Factor V Leiden (RR 2.6, 95 percent confidence interval 1.1-6.1; p = 0.02).

Our study population included 16 patients with a combined defect (hyperhomocysteinemia and Factor V Leiden), three of them (19 percent) developed recurrent venous thromboembolism. We further analyzed the influence of Factor V Leiden on the risk of recurrence to exclude the possibility that the increased risk of recurrence in patients with hyperhomocysteinemia is mainly caused by a co-existing Factor V Leiden mutation. We estimated separately the probability of recurrence in patients with and without hyperhomocysteinemia who did not carry the Factor V Leiden mutation. At 12 and 24 months, the probability of recurrence was 11.3 percent (95 percent confidence interval 1.9-20.6) and 17.8 percent (95 percent confidence interval 5.4-30) in patients with hyperhomocysteinemia and no Factor V Leiden (47 patients, 8 recurrences), and was 4.2 percent (95 percent confidence interval 1.1-6.4) and 6.2 percent (95 percent confidence interval 1.7-10.7) in patients with neither hyperhomocysteinemia nor Factor V Leiden (133 patients, 10 recurrences), respectively (Wilcoxon p = 0.02, log-rank p = 0.02).

Five patients with hyperhomocysteinemia (7.6 percent) and 9 patients without hyperhomocysteinemia (4.5 percent) developed deep vein thrombosis. Pulmonary embolism occurred in seven patients with hyperhomocysteinemia (10.6 percent) and in seven patients without hyperhomocysteinemia (3.5 percent).

The mean time until recurrence of venous thromboembolism was significantly shorter in patients with hyperhomocysteinemia (8.1 ± 8.6 months, range 0.5 to 26.4) than in patients without hyperhomocysteinemia (16.1 ± 9.9 months, range 2.7 to 30.8; p = 0.01).

**Discussion**

Our data provide strong evidence that, in patients with elevated homocysteine levels and a history of venous thrombosis, the risk of recurrence is higher than in thrombosis patients without hyperhomocysteinemia. In a large cohort of consecutive patients with a single episode of objectively documented idiopathic venous thromboembolism, the risk of recurrent venous thrombosis was almost three-fold higher in patients with hyperhomocysteinemia than in patients with normal homocysteine levels. The cumulative probability of recurrence at two years was 19.2 percent in patients with and 6.3 percent in patients without hyperhomocysteinemia.

The individual risk of thrombosis in a patient with a congenital or acquired defect predisposing to thrombosis may vary considerably. There is evidence that the presence of co-existing risk factors in the same subject potentiates the risk of thrombosis (20-22). Conversely, the risk of recurrence is lower in patients with temporary compared to permanent risk factors (23). To eliminate a potential inhomogeneity in our patient population, patients with a history of recurrent venous thrombosis, with antithrombin-, protein C-, protein S-, or plasminogen-deficiency, patients with cancer, the antiphospholipid antibody syndrome, and patients with temporary risk factors, such as surgery, trauma or pregnancy, were excluded. Evidence has been presented that hyperhomocysteinemia and Factor V Leiden have a synergistic effect resulting in an increased risk of future venous thrombosis (24). Factor V Leiden was unknown when our trial was initiated and, therefore, was not considered an exclusion criterion. To exclude a possible potentiating effect of Factor V Leiden on the risk of recurrence in patients with hyperhomocysteinemia, we estimated the probability of recurrence in patients with and without hyperhomocysteinemia who were not Factor V Leiden carriers. After exclusion of carriers of the mutation as well as after adjustment of the relative risk for Factor V Leiden, hyperhomocysteinemia remained a strong and independent risk factor of recurrent venous thromboembolism. In addition, as we have previously shown, the presence of Factor V Leiden does not confer a higher risk of recurrence in our patient population (6).

Basal homocysteine levels were measured in the individual patient after overnight fasting. Patients were not tested after a methionine load and an increased number of hyperhomocysteinemic patients might have been detected when methionine-loading test would have been performed.

Our patients with hyperhomocysteinemia were significantly older at the time of venous thrombosis than the patients with normal homocysteine levels. This observation is in accordance with den Heijer who...
found a sharp increase in the risk of thrombosis associated with hyperhomocysteinemia at increasing age (11). These data imply that hyperhomocysteinemia is an important risk factor also in the elderly population.

How should these data affect current clinical practice with regard to the management of patients with venous thromboembolism? In our study, 25 percent of the patients who had elevated homocysteine levels which is similar to the prevalence of hyperhomocysteinemia in other cohorts of thrombosis patients (9, 13). Therefore, besides Factor V Leiden hyperhomocysteinemia is the most frequent permanent risk factor of venous thrombosis. The high prevalence of hyperhomocysteinemia in thrombosis patients together with the high risk of recurrence warrants extended patient screening in order to facilitate decisions regarding patient counseling and therapeutic management.

Decisions on the duration of secondary thromboprophylaxis with oral anticoagulants are based on the incidence of recurrence and the risk of bleeding. Patients with venous thrombosis and hyperhomocysteinemia are clearly at a high risk of recurrence and, in principle, would be candidates for anticoagulant therapy longer than the currently recommended period of three to six months. It has, however, convincingly been shown that levels of homocysteine can be lowered by folate- and vitamin B12-supplementation in almost all subjects regardless of the cause of hyperhomocysteinemia (25, 26). There is an inverse correlation, especially in elderly subjects, between homocysteine and folate levels in plasma (27). In view of our results hyperhomocysteinemia has to be regarded as an important thrombotic risk factor also in the elderly. Since older patients have an increased risk of bleeding during oral anticoagulation, they may even benefit more from vitamin supplementation following venous thromboembolism than younger patients. Based on the results of this study, randomized prospective clinical trials are needed to investigate the use of prolonged oral anticoagulation, vitamin supplementation or both in reducing the risk of recurrent venous thrombosis in patients with hyperhomocysteinemia.

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References


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