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Evaluation of the prevalence of severe hyperhomocysteinemia in adult patients with thrombosis who underwent screening for thrombophilia

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ABSTRACT

Introduction: Treatment with B-vitamins and betaine reduces the high risk of thrombosis in patients with homocystinuria, a metabolic syndrome that is characterized by severe hyperhomocysteinemia (HHcy). In contrast, there is no clear demonstration that B-vitamins reduce the risk of thrombosis in patients with mild HHcy: for this reason, many question the clinical utility of measuring total Hcy (tHcy) in patients with thrombosis. However, thrombosis may be the first clinical manifestation of homocystinuria in patients reaching adulthood without signs and symptoms of the syndrome.
Aim: 1) to measure the prevalence of severe, previously undiagnosed, HHcy among patients with thrombosis 2) to profile these patients on the basis of their characteristics.
Methods: Six Italian Thrombosis Centers completed a first questionnaire, reporting tHcy levels in patients with thrombosis who underwent thrombophilia screening, and a second questionnaire, reporting the characteristics of patients with severe HHcy (tHcy > 100 μmol/L).
Results: Of 19,678 cross-sectionally collected patients with thrombosis who underwent thrombophilia screening in the last 12.5 years (median value, range 6-17), 38 had severe HHcy (0.2%). Their median age at diagnosis was 47 years (range 19-83) and the median level of tHcy was 130 μmol/L (range 101-262). Venous thromboembolism (71%) was more frequent than arterial thromboembolism (26%); recurrent thrombosis occurred in 42% of cases.
Conclusions: Measurement of tHcy in adult patients with thrombosis may reveal the presence of severe HHcy. Since treatment of patients with severe HHcy decreases the risk of thrombosis, measurement of tHcy in patients with thrombosis may prove clinically useful.

Introduction

Homocysteine (Hcy) is a sulfhydryl aminoacid derived from the metabolic conversion of methionine, which is dependent on vitamins (folic acid, vitamin B12 and B6) as cofactors or cosubstrates. Hyperhomocysteinemia (HHcy) can be clinically classified in 3 groups, based on plasma levels of total homocysteine (tHcy): mild (tHcy 12-30 μmol/L), moderate (tHcy 30-100 μmol/L) and severe (tHcy > 100 μmol/L) [1]. Severe HHcy (homocystinuria), due to inherited severe metabolic defects of Hcy metabolism [2], first described in 1962 [3], is a rare autosomal recessive disorder (prevalence 1/335,000) [4], which is associated with inherited defects of cystathionine-beta-synthase, or, less frequently, of methylene tethrahydrofolate reductase (MTHFR). It is usually diagnosed in pediatric life and is characterized by mental retardation, ectopia lentis, musculoskeletal abnormalities and high risk of arterial and venous thrombosis. Mild-to-moderate forms of HHcy (fasting levels of tHcy between about 12 and 100 μmol/L) are encountered in phenotypically normal subjects with genetic defects, acquired conditions, or, more frequently, a combination of both and are associated with an increased risk of cardiovascular diseases [5]. The risk of thrombosis in patients with severe hyperhomocysteinemia

Abbreviations: Hcy, homocysteine; tHcy, total homocysteine; HHCy, hyperhomocysteine; MTHFR, methylene tethrahydrofolate reductase; CBS, cystathionine β-synthase; CI, Confidence Intervals.

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is dramatically decreased by B-vitamins, which, in some patients are given in combination with the methyl donor betaine, which contributes to the remethylation of Hcy to methionine [6]. In contrast, no information is available on the clinical effect of correction of moderate HHcy, while the majority of clinical trials that have been performed so far failed to demonstrate that correction of mild HHcy by B-vitamins reduces the risk of recurrences of thrombosis [7,8]. As a consequence, the clinical utility of measuring plasma tHcy in patients with thrombosis has been questioned.

Despite its typical clinical features, homocystinuria may not be easily recognized. Some patients may reach adulthood undiagnosed [9–13] and thromboembolic events may be the first clinical manifestation of the disease in these patients [13]. Therefore, failure to measure tHcy levels in patients with thrombosis would preclude the possibility of diagnosing patients with severe HHcy, who would benefit from therapeutic correction of their metabolic defect.

Based on this background, the primary objective of this cross-sectional study was to gather information on the prevalence of severe, previously undiagnosed, hyperhomocysteinemia among patients with thrombosis who underwent thrombophilia screening, including measurement of fasting plasma tHcy. Secondary objective was to profile these patients on the basis of their clinical characteristics.

Material and methods

Data Collection

A national survey was conducted by means of an online questionnaire sent to six Italian Thrombosis Centers. To create the web-application, a web-site technology with particular attention to data security and privacy through methods of authentication, authorization and accounting was used. The study was approved by the Committee of Medical Ethics of Ospedale San Paolo (Milan, Italy) and review board approval was obtained from all participating Institutions.

Each participating center was requested to collect data on the number of patients for whom a diagnosis of severe hyperhomocysteinemia (fasting tHcy, >100 μmol/L) was made, among those who underwent thrombophilia screening for previous episodes or venous or arterial thromboembolism. For each patient with severe HHcy, a second, more detailed questionnaire was completed, detailing personal data, fasting plasma tHcy levels, type of molecular defect (when available), clinical characteristics, co-morbidities, type of thrombotic event(s), presence of additional thrombophilic states, use of estrogens, renal function, serum levels of folates and vitamin B12, type and duration of treatment.

Statistical Analysis

The data were analysed using descriptive statistical methods. Data are presented as median and ranges, or percentages and 95% confidence intervals were calculated where appropriate. The statistical analyses were carried out using SPSS for Windows version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Prevalence of severe hyperhomocysteinemia

A total of 19,678 records of patients with thrombosis who underwent thrombophilia screening over a median period of 12.5 years (range 6-17 in different Centers) was cross-sectionally collected and entered into the database. The thrombophilia screening was performed in 54% of cases after a venous thrombotic event and in 46% after an arterial thrombotic event.

Of the 19,678 patients, 38 had severe HHcy (0.2%, 95% CI 0.19-0.25). The frequency of antithrombin deficiency, a rare, severe thrombophilic defect that is commonly included in thrombophilia screening, was 0.6% (95% CI 0.49-0.74) (Table 1). In patients with a venous thrombotic event the prevalence of severe HHCy was 0.25%, whereas in patients with arterial thrombotic event it was 0.11%.

Characteristics of patients with severe hyperhomocysteinemia

Data on the 38 patients with severe HHcy are summarized in Table 1. The median age at diagnosis was 47 years (range 19-83), the median age of the first thrombotic event was 46 years (range 15-83) and the male/female ratio was 1.5:1. The median plasma level of tHcy was 123 μmol/L (range 101-262). The number of patients with associated renal insufficiency was 8/38 (21%): 1 with mild, 1 with moderate and 6 with severe insufficiency. Data on serum folate and B12 levels were available for 17 and 22 patients: folate and B12 deficiency was observed in 8 and 8 patients, respectively with a deficiency of both vitamins in 5 patients. A complete set of data for serum folate, B12 and creatinine was available only for 15 patients: among them, 11 patients had abnormal values of both creatinine and either folate or B12 levels.

None of the patients was screened for molecular abnormalities of cystathionine β-synthase (CBS) or MTHFR known to be associated with homocystinuria. The common partial loss of function mutation C677T of MTHFR was searched in 14 patients (37%): 9 of them (64%) were found to be homozygous for the mutation.

Clinical thrombotic events

Venous thromboembolism was more frequent than arterial thromboembolism (Table 2): deep vein thrombosis (n = 16), pulmonary embolism (n = 2), superficial thrombophlebitis (n = 4), venous thrombosis at unusual sites (n = 5), myocardial infarction (n = 2), stroke (n = 4), carotid arterial thrombosis (n = 1), renal arterial thrombosis (n = 1) and peripheral artery disease (n = 2). One patient had concomitant arterial and venous thrombosis. In total seventeen patients (45%) had recurrent thrombotic events; in 12 patients (31%) recurrence occurred after diagnosis. In 6 patients (17%) severe HHcy was associated with a known thrombophilic defect: heterozygous factor V Leiden (n = 3), heterozygous prothrombin G20210A mutation (n = 2) and lupus anticoagulant (n = 1); two patients (16%) were on treatment with oral contraceptives at the time of their first thrombotic event.

Effects of treatment

All patients were administered folic acid: 75% of them also received cobalamin and vitamin B6. The median daily dosage was 5 mg for folate and 500mcg for cobalamin, while it was unknown for vitamin B6. Median plasma tHcy levels decreased from 120 (range 101-262) to 40 (range 9.9-135) μmol/L after treatment with B vitamins in the 20 patients for whom measurements before and after treatment are available (Fig. 1). After treatment, the plasma levels of tHcy were

Table 1
Prevalence of severe hyperhomocysteinemia and of antithrombin deficiency in the cohort and characteristics of patients with severe hyperhomocysteinemia.

Total number of patients	19,678
Prevalence of severe HHcy(tHcy > 100 μmol/L) (95% CI)	0.2% (0.19-0.25)
Prevalence of antithrombin deficiency (95%CI)	0.6% (0.49-0.74)
Characteristics of patients with severe HHcy	
Men/Women	23/15
Age, median (range), y	47 (19-83)
Age at 1st thrombotic event, median (range), y	46 (15-83)
Plasma tHcy, median (range), μmol/L	123 (101-262)
No. with high serum creatinine	8/38 (21%)
No. with low serum folate	8/17 (47%)
No. with low serum B12	8/22 (36%)

tHcy: total homocysteine.