Homocysteine in Vascular Behçet Disease
A Meta-Analysis

Micaela La Regina, Francesco Orlandini, Domenico Prisco, Francesco Dentali

Objective—Behçet disease (BD) is a vasculitis of unknown etiology, characterized by oral or genital aphthous ulcerations and uveitis. Homocysteine (hcy) is an independent risk factor for venous and arterial thrombosis. The association between hyperhomocysteinemia and thrombosis has been investigated in some studies in BD patients. However, information on this association is based only on the results of small studies with conflicting results. To overcome such limitations, we performed a metaanalysis comparing the plasma levels of hcy in BD patients with and without history of thrombosis.

Methods and Results—We searched for all published studies using the Medline and Embase databases. Two reviewers performed study selection independently. Studies were included if hcy was measured in adult BD patients with and without thrombosis. Two reviewers independently extracted data on study and population characteristics. The mean value of hcy in BD patients and the presence of hyperhomocysteinemia in patients with and without thrombosis were collected. Association between thrombosis and hyperhomocysteinemia, and the mean difference of hcy levels in BD patients with and without thrombosis were calculated. Sixteen studies, for a total of 979 patients, were included. Hyperhomocysteinemia was more prevalent in patients with thrombosis than in those without (odds ratio 3.14; 95% CI 1.26 to 7.80) Mean levels of hcy were significantly higher in patients with thrombosis in comparison with patients without (mean difference 3.30 μmol/L; 95% CI 2.09 to 4.51).

Conclusion—Our results suggest that hyperhomocysteinemia may be considered to be associated with thrombosis in BD patients. (Arterioscler Thromb Vasc Biol. 2010;30:2067-2074.)

Key Words: homocysteine ■ thrombosis ■ Behçet disease ■ metaanalysis ■ vascular manifestations

Behçet disease (BD) is a vasculitis of unknown etiology, characterized by oral or genital aphthous ulcerations and uveitis. However, it can affect any organ and system, including the cardiovascular apparatus.1 Vascular manifestations (venous and arterial thrombosis, aneurysms, and pseudoaneurysms) occur in about one third of patients. Venous thromboses, superficial or deep, are predominant.2 This high prevalence of thromboses, known since the 1950s,3 promoted several studies aimed at investigating their pathobiology. Endothelial injury itself, common to other vasculitis, cannot clearly account for thrombosis development, so a hypercoagulable state was early hypothesized and investigated.4

In the last several years, several inherited or acquired factors causing hypercoagulability have been studied in patients with venous and arterial thromboembolic events.5–7 These thrombophilic factors have also been studied in patients with BD with a history of thrombosis, and conflicting results were obtained.8–11 Recently, Ricart et al performed a metaanalysis evaluating the role of FV Leiden, prothrombin G2021A mutation, and the homozygous 677TT mutation of the methyltetrahydrofolate reductase as risk factors for venous thrombosis in BD patients.12 In this study, FV Leiden and G2021A prothrombin mutation were found to increase the risk of thrombosis in these patients, whereas 677TT mutation of methyltetrahydrofolate reductase did not.

These findings are consistent with the results of recent studies performed in general populations in which the presence of 677TT mutation of methyltetrahydrofolate reductase is not a risk factor for venous thromboembolism in the absence of hyperhomocysteinemia.13

On the other hand, in the general population, increased levels of homocysteine (hcy) have been reported to be an independent risk factor for venous thromboembolism in addition to coronary artery disease and peripheral vascular disease.14–16

The association between hyperhomocysteinemia and thrombosis has been investigated in some studies in patients with BD. However, information on this association is scarce and based only on the results of small studies. Furthermore, the results of the available studies are conflicting and did not allow definitive conclusions on the role of hyperhomocysteinemia in these patients.

Objectives
To overcome such limitations, we performed a metaanalysis that collected all the existing studies comparing the mean plasma levels of hcy measured in BD patients with and without thrombosis.

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2067
Methods

A protocol was prospectively developed. Specific objectives, criteria for study selection, approach to assess study quality, outcomes, and statistical methods were a priori defined.17

Study Identification

We tried to identify all published studies that evaluated the role of hcy in BD patients with vascular manifestations using the Medline (1950 to July 2009) and Embase (1980 to July 2009) databases. The search strategy used the keywords and medical subject headings presented in Appendix 1. We supplemented our search by searching the International Society of Thrombosis and Hemostasis and European League Against Rheumatism scientific meeting abstracts from 2002 to 2009 using Behçet, homocysteine, and hyperhomocysteinemia as search terms and by manually reviewing the reference list of all articles retrieved for additional published or unpublished trials.

Study Selection

Study selection was performed independently by 2 reviewers (MLR, FD), with disagreements resolved through discussion and by the opinion of a third reviewer, if necessary. Studies were included if they met the following criteria: (1) diagnosis of BD was objectively confirmed according to International Study Group for Behçet’s Disease criteria18; (2) patients were 18 years or older; and (3) hcy levels were compared in BD patients with and without a history of venous or arterial thrombosis. Case series of patients were excluded. When multiple articles for a single study had been published, we decided to use the latest publication and to supplement it, if necessary, with data from the earlier publications. To assess the agreement between reviewers for study selection, we used the κ statistic, which measures agreement beyond chance.19 According to Maclure and Willett, κ values higher than 0.6 are considered to represent a substantial agreement and values higher than 0.8 an almost perfect agreement.20

Study Validity Assessment

The same 2 unmasked investigators independently completed the assessment of study validity. Because in observational studies the use of quality scoring systems or quality scales is controversial,17 we decided to assess the study quality using the following items: objectivity of thrombosis diagnosis (1 point was given when objective diagnostic test of thrombosis was reported); reporting of exclusion criteria (1 point was given if exclusion criteria were reported, 2 points were given if subjects taking drugs interfering with hcy levels [vitamin supplements or immunosuppressive therapy] were excluded); measurement of hcy levels (1 point was given if hcy levels were measured after an overnight fasting). Only studies that satisfied all the criteria were defined as high-quality studies; studies that satisfied 3 criteria or fewer were defined as low-quality studies.

Data Extraction

Two reviewers (MLR, FD) independently completed data extraction. Disagreement was resolved by consensus and by the opinion of a third reviewer, if necessary. The following baseline characteristics were collected: number of subjects studied, mean age, variation in age, sex, and race. The mean value of hcy±SD or interquartile range from 10th to 90th (or 5th to 95th) percentiles in BD patients with and without thrombosis was extracted. Furthermore, the presence of hyperhomocysteinemia in patients with and without thrombosis was collected. If the required data could not be located in the published report, we contacted the corresponding author by mail, with a reminder e-mail sent in 15 days.

Statistical Analysis

We used Review Manager (RevMan; version 5.0 for Windows; Oxford, England; The Cochrane Collaboration, 2008) to pool data. Association between thrombosis and hyperhomocysteinemia and the mean difference of hcy levels in BD patients with and without thrombosis were calculated using a random-effects model (the DerSimionan and Laird method).21 Pooled results are reported as odds ratio (OR) and mean difference and are presented with 95% CI and with 2-sided probability values. A probability value of 0.05 or less was considered statistically significant. Statistical heterogeneity was evaluated using the I² statistic, which assesses the appropriateness of pooling the individual study results.22 The I² value provides an estimate of the amount of variance across studies due to heterogeneity rather than chance. Finally, funnel plots of effect size against standard error were completed, whenever possible, to assess for the presence of publication bias.23 As a sensitivity analysis, studies of high quality were analyzed separately. Similarly, studies including patients from Middle East and Mediterranean regions and from Central and Far Eastern Asia were analyzed separately as a subgroup analysis to confirm our results in the population in which BD is more common. Finally, because hcy levels have been assessed by different methods in different studies, and results may vary according to method of measurement, we performed subgroup analyses including only studies in which hcy was measured with similar techniques, to explore potential sources of heterogeneity.

Results

Study Identification and Selection

We identified 84 studies using our search strategy: 45 from Medline and 39 from Embase (Figure 1). There were 33 studies identified in duplicate. An additional 69 abstracts from the European League Against Rheumatism and International Society of Thrombosis and Hemostasis scientific meetings were found. We could exclude 94 studies after screening the title and abstract using the predefined inclusion and exclusion criteria; 26 studies were retrieved for more detailed evaluation. The interobserver agreement for the study selection was excellent, with a κ of 0.91. Manual review of references did not reveal any additional study. Ten of the 26 studies were subsequently excluded for the following reasons: 8 did not meet inclusion criteria and 2 contained duplicate data. Sixteen studies were therefore included in our systematic review.4,7,11,24–36 The studies performed by Houman et al35 and Feki et al34 may have
considered in part the same population. However, both the studies were included in our metaanalysis because they considered different end points (Feki et al measured mean hcy levels, and Houman et al considered patients taking vitamin supplements in the last 6 months).

**Study Characteristics**

Baseline characteristics of patients included in the studies are summarized in Table 1. Nine studies were from Turkey, 3 were from Tunisia, 1 was from Spain, 1 was from Israel, 1 was from Iran, and 1 was from Korea. Studies ranged in size from 24 to 107 patients; a total of 979 patients were included. Homocysteine concentrations have been assessed by different methods: fluorescent polarizing immunoassay, high-performance liquid chromatography, or enzyme-linked immunosorbent assay, which have been demonstrated to correlate well among them.37 The time elapsed

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**Table 1. Characteristics and Results of the Included Studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>No. of Patients</th>
<th>Mean Age and Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aksu et al, 200126</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Presence of hyperlipidemia, hypertension, diabetes, folic acid/vitamin B12 deficiency, vascular atherosclerotic disease, HF, liver/renal disorders, use of vasoactive drugs, vitamin supplements, HRT, impossibility in drug withdrawal</td>
<td>84</td>
<td>36 years (79), M: 54, F: 30</td>
</tr>
<tr>
<td>Nazarinia et al, 200727</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Presence of hyperlipidemia, diabetes, psoriasis, chronic hepatitis, renal failure, chronic alcoholism</td>
<td>123</td>
<td>35.5 years (8.7), M: 46, F: 77</td>
</tr>
<tr>
<td>Feki et al, 200436</td>
<td>Pathology or drugs affecting hcy</td>
<td></td>
<td>59</td>
<td>36.2 years (17 to 67), M: 40, F: 19</td>
</tr>
<tr>
<td>Leiba et al, 20048</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Not reported</td>
<td>107</td>
<td>42.5 years (12 to 78), M: 42, F: 65</td>
</tr>
<tr>
<td>Ricart et al, 200627</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Patients taking drugs interfering with hcy or vitamin supplements</td>
<td>79</td>
<td>45 (12) years, M: 43, F: 36</td>
</tr>
<tr>
<td>Durmazlar et al, 200831</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Autoimmune diseases, drugs affecting hcy, folate/vitamin B12 deficiency, diabetes, hyperlipidemia, chronic hepatitis, renal failure, psoriasis, anemia, patients taking vitamin supplements in the last 6 months</td>
<td>70</td>
<td>33 (7.79) years, M: 40, F: 30</td>
</tr>
<tr>
<td>Dülgu¨ et al, 200837</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Not reported</td>
<td>79</td>
<td>Not reported</td>
</tr>
<tr>
<td>Sentur¨k et al, 20068</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Not reported</td>
<td>55</td>
<td>37.95 (1.25) years, M: 25, F: 30</td>
</tr>
<tr>
<td>Korkmaz et al, 200225</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Cyclosporine treatment, vitamin supplements, hyperlipidemia, renal failure, diabetes, psoriasis, hepatitis, alcoholism</td>
<td>74</td>
<td>35 (8.4), M: 49, F: 25</td>
</tr>
<tr>
<td>Kaykıçıoğlu et al, 200628</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Hyperlipidemia, hypertension, diabetes, folic acid/vitamin B12 deficiency, vascular atherosclerotic disease, heart failure, liver/renal diseases, vasoactive drugs, vitamin supplements, hormone replacement therapy, impossible drug withdrawal</td>
<td>65</td>
<td>38 (9) years, M: 40, F: 25</td>
</tr>
<tr>
<td>Canataroglu et al, 200227</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Not reported</td>
<td>40</td>
<td>40.2 (8.4) years, M: 23, F: 17</td>
</tr>
<tr>
<td>Altınbaş et al, 200027</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Not reported</td>
<td>43</td>
<td>33 (8) years, M: 21, F: 22</td>
</tr>
<tr>
<td>Lee et al, 200227</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Hypertension, diabetes, hyperlipidemia, renal failure, liver disease, other thrombosis risk factors, gastrointestinal resection, vitamin B12 or folate deficiency or supplements, anticoagulant therapy</td>
<td>24</td>
<td>40.2 (2.6) years, M: 15, F: 9</td>
</tr>
<tr>
<td>Ates et al, 200528</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Deficiency of folate or vitamin B12, diabetes, hyperlipidemia, chronic hepatitis, renal failure, alcoholism, patients taking supplements of folate or vitamin B12 in the last 12 months; Patients with SVT were not included</td>
<td>45</td>
<td>38.9 (8.9) years, M: 30, F: 15</td>
</tr>
<tr>
<td>Yesikva et al, 200524</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Hypertension, cancer, anemia, hyperthyroidism, methotrexate, oral contraceptives, vitamin supplements, smoking, alcoholism</td>
<td>32</td>
<td>26.20 (6.8) years, M: 26, F: 6</td>
</tr>
<tr>
<td>Houman et al, 200325</td>
<td>International Study Group for Behçet's Disease criteria</td>
<td>Diseases’ status and drugs that increases hcy</td>
<td>59</td>
<td>39.9 years (17 to 67), M: 40, F: 19</td>
</tr>
</tbody>
</table>

HRT indicates hormone replacement therapy; HF, heart failure; DVT, deep venous thrombosis; SVT, superficial venous thrombosis; HHC, hyperhomocysteinemia; BMI, body mass index; NS, not significant.
levels were excluded. Thus, 4 studies were considered of high quality. 

Hyperhomocysteinemia
Eight studies,4,12,26,27,30,32,35,36 for a total of 166 patients with thrombosis and 318 patients without thrombosis, were included in the metaanalysis. Definition of hyperhomocysteinemia varied among the studies. The presence of hcy levels above the 95th percentile of the hcy concentration in the healthy control group4,35,36 was used as a definition of hyperhomocysteinemia whenever possible. Other definitions from the thrombotic event and the blood sampling was not specified in 15 of 16 studies, even if 6 of them8,12,26,27,29,36 refer clearly to “history of thrombosis.”

Study Quality
Quality assessment items are summarized in Table 2. Thrombosis was objectively confirmed in all the included studies. In 14 studies the blood was drawn after 12 hours or overnight fasting. Exclusion criteria were reported in 13 studies, 4 of them specified that patients taking drugs interfering with hcy levels were excluded. Thus, 4 studies were considered of high

Table 1. Continued

<table>
<thead>
<tr>
<th>Country</th>
<th>Method</th>
<th>Outcomes Measured</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunisia</td>
<td>FPIA</td>
<td>Mean plasma hcy concentrations in BD patients with and without DVT (mean 14.36 ± 0.87 vs 12.41 ± 1.12 μmol/L, P &lt; 0.05)</td>
<td>No statistically significant differences in hcy concentrations between patients with (n=74) and without (n=78) thrombosis (mean: 12.6 ± 3.9 vs 12.2 ± 1.7)</td>
</tr>
<tr>
<td>Spain</td>
<td>FPIA</td>
<td>Mean serum hcy levels and the prevalence of HHC in a cohort of BD patients with and without history of arterial or venous thrombosis.</td>
<td>No statistically significant differences in hcy concentrations (mean: SD: 12.7 ± 8.7 vs 11.1 ± 5.0, P = 0.302) and in the prevalence of HHC (9% vs 11%, P = 0.001) between patients with (n=23) and without thrombosis (n=56).</td>
</tr>
<tr>
<td>Turkey</td>
<td>FPIA</td>
<td>Mean serum hcy levels in a cohort of patients with and without vascular involvement (arterial or venous).</td>
<td>Increased hcy levels in patients with vascular lesions (n=37) compared with those without (n=33) (19.9 ± 10.9 vs 13.75 ± 6.4 μmol/L, P = 0.009).</td>
</tr>
<tr>
<td>Turkey</td>
<td>FPIA</td>
<td>Mean serum hcy levels in BD patients with and without DVT.</td>
<td>Plasma hcy levels were significantly higher in patients with thrombosis (n=20) vs patients without thrombosis (n=59) (mean: SD: 14.87 ± 8.99 vs 10.78 ± 3.81 μmol/L, P &lt; 0.05).</td>
</tr>
<tr>
<td>Spain</td>
<td>FPIA</td>
<td>Mean serum hcy levels in BD patients with and without vascular involvement (arterial or venous).</td>
<td>Plasma hcy levels were significantly higher in the subgroup with (n=27) vascular involvement than in those without (n=38) (mean: SD: 15.7 ± 7.2 vs 12.4 ± 4 μmol/L, P = 0.03).</td>
</tr>
<tr>
<td>Turkey</td>
<td>FPIA</td>
<td>Mean plasma hcy levels in BD patients with and without history of DVT.</td>
<td>Plasma hcy levels were significantly higher in BD patients with DVT history (n=14) vs those without DVT history (n=26) (mean: SD: 15.9 ± 4.6 vs 12.1 ± 3.3 μmol/L, P = 0.013).</td>
</tr>
<tr>
<td>Turkey</td>
<td>ELISA</td>
<td>The presence of hyperhomocysteinemia in BD.</td>
<td>The prevalence of thrombosis was 21/2 (16.6%).</td>
</tr>
<tr>
<td>Korea</td>
<td>FPIA</td>
<td>Mean plasma hcy levels in Korean BD patients with and without thrombosis (DVT, SVT, arterial thrombosis, aneurysms).</td>
<td>Plasma hcy levels were significantly higher in BD patients with thrombosis (n=12) vs those without (n=12) (mean: SD: 11.9 ± 10.3 vs 10.51 ± 5.09 μmol/L).</td>
</tr>
<tr>
<td>Turkey</td>
<td>FPIA</td>
<td>Mean serum hcy levels in BD patients with vascular involvement (arterial and venous thrombosis, arterial aneurysms) and with mucocutaneous involvement.</td>
<td>The serum hcy levels were significantly increased in BD patients with vascular involvement (n=16) compared with those of mucocutaneous BD patients (n=29) (mean: SD: 13.8 ± 5.7 vs 9.9 ± 2.6 μmol/L, P = 0.001).</td>
</tr>
<tr>
<td>Turkey</td>
<td>HPLC</td>
<td>Plasma hcy concentrations in BD patients with and without thrombotic complications.</td>
<td>Plasma hcy concentrations were significantly higher in patients with thrombosis (n=13) vs those without (n=19) (mean: SD: 18.44 ± 3.59 vs 12.42 ± 2.65 μmol/L, P = 0.001).</td>
</tr>
<tr>
<td>Tunisia</td>
<td>FPIA</td>
<td>Plasma hcy levels in BD patients with and without occurrence of DVT in the past.</td>
<td>Comparison between patients with and without DVT showed no significant differences in the median hcy (mean: 14.36 vs 12.41 μmol/L) and the prevalence of HHC even after the adjustment to age, sex, disease duration, disease activity, tobacco consumption, BMI was 20.8% and 14.2%, in the two groups, respectively (P=NS).</td>
</tr>
</tbody>
</table>

HHC was defined as being above the 95th percentile of the control group4,35,36 as a definition of hyperhomocysteinemia.
include levels for healthy control, suggested by assay manufacturer,32 >15 mmol/L,34 >15 mmol/L in accordance with kit reference values,27,30 2 standard deviations (SD) above the mean serum hcy concentration in the healthy group as the cutoff value.12 In 1 study, the cutoff level for hyperhomocysteinemia is not reported. 26

In these studies, the presence of hyperhomocysteinemia was found significantly more often in patients with thrombosis than those without (OR 3.14; 95% CI 1.26 to 7.80; Figure 2), suggesting an association between the high levels of hcy and the development of thrombosis. Heterogeneity among the studies was significant (I²=63%). The funnel plot was asymmetrical, with an absence of studies in the bottom left side of the plot. This suggests that we did not include small studies that failed to demonstrate an association between hyperhomocysteinemia and thrombosis in BD patients (Appendix 2).

Analysis was repeated including only 6 studies4,12,25,32,36 in which hyperhomocysteinemia was defined as hcy levels above the 95th percentile of the hcy concentration in the healthy control group; this analysis yielded similar results (data not shown).

### Mean Levels of Homocysteine

Eleven studies,4,8,12,24,25,27–30,32,36 for a total of 236 patients with thrombosis and 518 patients without thrombosis, were included in the metaanalysis. In these patients, mean levels of hcy were significantly higher in patients with thrombosis compared with patients without thrombosis (mean difference 3.30 μmol/L; 95% CI 2.09 to 4.51; Figure 3), again suggesting an association between the high levels of hcy and the development of thrombosis. Heterogeneity among the studies was significant (I²=48%).

The funnel plot appeared symmetrical, suggesting the absence of publication bias (Appendix 2).

Two studies reported information on mean hcy levels in patients with and without thrombosis, but they did not provide data on the standard deviations of the means.31,33 Unfortunately, we were unable to get these data also after contacting corresponding author of the original studies. Thus, these 2 studies were not included in our metaanalysis. In the first of these studies, Durmazlar et al33 enrolled 70 untreated patients with BD. Mean hcy levels were similar in patients with and without vascular involvement. Likewise, Sentürk et al31 found similar hcy levels in a group of BD patients with an objectively diagnosed deep vein thrombosis and in a group of BD patients without thrombosis.

### Sensitivity and Subgroup Analyses

The mean difference of hcy levels between patients with and without thrombosis in the pooled analysis of the 4 high-quality studies4,24,25,28 was 4.00 μmol/L (95% CI 2.12 to 5.89; I²=42%). Only 1 study of the 4 reported information on hyperhomocysteinemia. Thus, a sensitivity analysis could not be performed for hyperhomocysteinemia.

Given the presence of significant heterogeneity among the studies in the primary analyses, we explored potential sources of heterogeneity, repeating the analyses including only 3 studies4,35,36 that assessed hcy levels using fluorescent polarizing immunoassay.

In this subgroup, we obtained similar results with similar heterogeneity when we considered the association between thrombosis and hyperhomocysteinemia (OR 2.72; 95% CI 1.67 to 6.91; I²=65%). On the other hand, when we considered the mean difference of hcy levels in BD patients with and without thrombosis, we obtained similar results but with
lower heterogeneity (mean difference 3.77 μmol/L; 95% CI 2.31 to 5.23; $I^2=25\%$).

Subgroup analyses including only studies that enrolled patients from Middle East and Mediterranean regions and from Central and Far Eastern Asia confirmed the results of primary analyses. The presence of hyperhomocysteinemia was found significantly more often in patients with thrombosis than in those without (OR 3.82; 95% CI 1.48 to 9.83; $I^2=61\%$), and mean levels of hcy were significantly higher in patients with thrombosis in comparison with patients without thrombosis (3.42 μmol/L; 95% CI 2.14 to 4.70; $I^2=50\%$). The only study excluded in this analysis was the one performed by Ricart et al, in which mean levels of hcy and the presence of hyperhomocysteinemia were not significantly different in BD patients with and without thrombosis.12

**Discussion**

This is the first systematic review and metaanalysis that has assessed the role of hcy as a contributing factor for thrombosis in BD patients.

The results of this study indicate that there is an association between hyperhomocysteinemia and thrombosis in patients with BD and that mean Hcy levels are significantly higher in patients with thrombosis in comparison with patients without. The strength of this association is further increased by the results of a subgroup analysis that considered only patients from Middle East and Mediterranean regions and from Central and Far Eastern Asia, where Behçet disease is more common. Moreover, sensitivity analysis that considered only high-quality studies confirmed our findings.

Thus, taken together, our observations suggest that the presence of hyperhomocysteinemia should be evaluated in patients with BD presenting with thrombosis.

The mechanism of hyperhomocysteinemia underlying thrombosis may be multifactorial and is not completely understood. hcy levels can damage endothelial cells directly or indirectly.38 Excess hcy leads to loss of endothelial function through the generation of toxic hydrogen peroxide, superoxide, and hydroxyl radicals.39 It may also predispose to thrombosis by initiating the proliferation of vascular smooth muscle cells and by interfering with the antithrombotic and fibrinolytic mechanisms of the endothelium.38 Furthermore, increased plasma hcy may promote thrombosis by enhancing the secretion of plasminogen activator inhibitor-1 from vascular endothelial and smooth muscle cells40 and by affecting the function of other endothelial anticoagulant mechanisms, such as interactions involving heparin sulfate and antithrombin.41 Finally, hcy inhibits the expression...
and activation of thrombomodulin, which is a cofactor for protein C activation.42

Because plasma hcy levels are determined by genetic and nutritional factors, some experts have suggested that these patients should be treated with vitamin supplements. However, although randomized controlled trials in patients with venous thrombosis and in healthy volunteers,43 either with or without hyperhomocysteinemia, have demonstrated that combined vitamin supplementation reduces hcy levels effectively, whether BD patients with hyperhomocysteinemia should be treated with vitamins remains to be established. A small randomized controlled trial in BD patients with hyperhomocysteinemia and with uveal involvement found a significantly lower number of uveitis attacks in patients treated with folic acid, suggesting a potential beneficial in reducing hcy levels in these patients.44 However, no study that demonstrated a reduction in venous or arterial thrombosis in patients with BD with hyperhomocysteinemia treated with folic acid or vitamins has been conducted yet.

Furthermore, vitamin supplementation was not effective in reducing recurrences even in patients with hyperhomocysteinemia and a previous episode of deep vein thrombosis or pulmonary embolism. Several large randomized controlled trials have failed to show the efficacy of vitamin supplementation in reducing venous and arterial cardiovascular events in patients at high risk of cardiovascular events, and some studies suggest an increased risk of cardiovascular events in patients treated at the same time with folic acid vitamin B12 and vitamin B6.45–47 Thus, elevated total hcy levels may be a marker for but not a cause of vascular disease risk.

Randomized controlled trials to evaluate the safety and efficacy of low doses of vitamins in reducing cardiovascular events in patients with BD with hyperhomocysteinemia are still lacking.

Our systematic review has several potential limitations. First, the application of formal metaanalytic methods to observational studies is controversial, because bias implicit in the study design may misrepresent the strength of associations within the data.47 Moreover, in our metaanalysis, heterogeneity among the studies was significant. However, this may be partially explained by the different methods used to assess hcy levels and subgroup analysis considering only studies that used fluorescent polarizing immunnoassay found a lower heterogeneity among the studies that evaluated mean hcy levels in BD patients with and without thrombosis. Second, studies included in our metaanalysis have different inclusion and exclusion criteria, and to combine results across studies may be inappropriate. Furthermore, baseline characteristics, concomitant therapies, and risk factors for venous and arterial thrombosis are different in patients with and without thromboembolic complications in most of the studies included in our systematic review, suggesting caution in interpretation of the results. However, the association between hcy and thrombosis remains statistically significant in many of the studies that considered in the analysis the impact of other potential risk factors, such as male sex or smoking habits.5,8,12,15,25,27–30,32,34,35 Furthermore, hcy appeared to be a risk factor for thrombosis even in the 2 studies in which immunosuppressive therapy had been withdrawn before meas-urement.28,36 Third, 3 studies1.25,28 have included also some patients with aneurysms as vascular manifestations. We believe that the inclusion of this small number of patients did not significantly affect the results of our metaanalysis. Furthermore, thrombosis of vasa vasorum may be in many cases responsible for the arterial dilatations culminating in aneurysms formation. Lastly, the presence of publication bias for studies that considered hyperhomocysteinemia as a risk factor for thrombosis in BD patients could not be excluded. However, we extensively researched unpublished studies, evaluating the references list of all the included studies and searching in the abstract books of European League Against Rheumatism and International Society of Thrombosis and Hemostasis. Thus, is extremely unlikely that an unpublished study with negative results, if one really exists, was not located by our search strategy.

In conclusion, our results suggest that hyperhomocysteinemia may be considered to be associated with thrombosis in patients with BD coming from Middle East and Mediterranean regions and from Central and Far Eastern Asia. Additional studies including patients from other countries should clarify its role in other populations. Whether treatment with low doses of folic acid and B vitamins could be efficacious in reducing thromboembolic complications in patients with BD and hyperhomocysteinemia remains to be established.

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Disclosures
None.

References


Supplemental material

I. Medline and Embase search strategies.

MEDLINE (1950 to July Week 5 2009)
1 Behcet Syndrome/ (6132)
2 behcet syndrome.mp. (6147)
3 behcet disease.mp. (648)
4 1 or 2 or 3 (6263)
5 Hyperhomocysteinemia/ (2671)
6 Homocysteine/ (9873)
7 Hyperhomocysteinemia.mp. (4128)
8 homocysteine.mp. (14246)
9 8 or 6 or 7 or 5 (15109)
10 4 and 9 (39)

EMBASE (1980 to 2009 Week 32)
1 Behcet disease/ (6037)
2 behcet disease.mp. (6096)
3 1 or 2 (6096)
4 hyperhomocysteinemia/ (4635)
5 homocysteine/ (11508)
6 hyperhomocysteinemia.mp. (5197)
7 homocysteine.mp. (13939)
8 6 or 4 or 7 or 5 (15214)
9 8 and 3 (45)

II. Funnel Plots

IIa) Hyperhomocysteinemia
IIb) Mean levels of homocysteine