Hyperhomocysteinemia Is Associated With the Presence of Left Atrial Thrombus in Stroke Patients With Nonvalvular Atrial Fibrillation

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Background and Purpose—Blood stasis is the fundamental mechanism leading to thrombus formation in the venous system. Homocysteine also poses a significant risk for venous thrombosis through its endothelial toxic and prothrombotic properties. In the present study, we hypothesized that high homocysteine might be associated with thrombus formation in another stasis-related condition, atrial fibrillation.

Methods—Forty-two consecutive patients with ischemic stroke caused by nonvalvular atrial fibrillation and admitted within the first day of symptom onset were included. Total fasting plasma homocysteine, serum folic acid, and vitamin B12 levels were measured. All patients were evaluated by transesophageal echocardiography for the presence of a left atrial (LA) thrombus. Homocysteine and vitamin levels were compared between groups with or without LA thrombus.

Results—Transesophageal echocardiography revealed LA thrombus in 20 patients. Mean homocysteine levels were significantly higher in patients with LA thrombus (20.75 versus 13.34 μmol/L, \( P<0.001 \)). Multivariate logistic regression analysis showed that the effect of high homocysteine was independent of other clinical or echocardiographic variables known to increase LA thrombus (\( P=0.017 \)). There was no difference in vitamin B12 levels between groups (\( P=0.118 \)), whereas the mean folic acid level was significantly lower in patients with LA thrombus (\( P=0.004 \)).

Conclusions—High plasma homocysteine conveys an independent risk for LA thrombus formation in patients with stroke caused by nonvalvular atrial fibrillation. This finding further supports the thrombogenic role of high homocysteine in conditions associated with blood stasis. (Stroke. 2003;34:909-912.)

Key Words: atrial fibrillation • homocyst(e)ine • stroke • thrombosis

Nonvalvar atrial fibrillation (AF) is a common problem in the elderly, occurring in 2% to 4% of the population >60 years of age. It is 1 of the most common causes of stroke, estimated to be responsible for 1 of every 10 ischemic strokes in this age group.1 Transesophageal echocardiography (TEE) reveals a thrombus lodged within the left atrial appendage (LAA) in up to 43% of patients with a recent embolic event.2 Even if not seen in TEE, a minute but still clinically important–sized thrombus trapped within the trabeculae of the LAA can frequently be identified at autopsy.3 The fundamental mechanism that leads to thrombus formation in AF is blood stasis in the LAA. Stagnant flow or stasis not only is unique to AF but also is a key feature of thrombus formation in the venous system.4 LA and venous thrombi also resemble each other microscopically. Therefore, factors in addition to stasis contributing to the formation of thrombus in the venous system might also operate in AF.

Homocysteine, an intermediate amino acid formed during the metabolism of dietary methionine, has been linked to a variety of vascular diseases. Large case-control and prospective studies have shown that elevated fasting and post–methionine-loading homocysteine levels are an independent risk factor for arterial and venous thrombotic events.5–7 Meta-analyses have found odds ratios (ORs) of 2.5 (95% CI, 1.8 to 3.5) to 2.95 (95% CI, 2.08 to 4.17) for venous thrombosis in patients with high homocysteine levels.6,7 Hyperhomocysteinemia is also a risk factor for recurrent venous thrombosis, increasing the risk >2-fold.8 Given that elevated homocysteine enhances venous thrombosis, we hypothesized that it may also convey a risk for the formation of LA thrombus in AF.

Methods

Over a 1-year period, 71 consecutive ischemic stroke patients with nonvalvular AF who were admitted within the first 24 hours after symptom onset were included. Baseline clinical characteristics—including age, sex, and history of hypertension, diabetes mellitus, and congestive heart failure—were recorded. A complete diagnostic evaluation was performed for each patient; this included blood chemistry, blood cell counts, erythrocyte sedimentation rate, ECG, coagulation panel (antiphospholipid and anticardiolipin antibodies, activated partial thromboplastin time, prothrombin time, international normalized ratio, protein C, protein S, antithrombin III, fibrinogen, protein C resistance, and factor 5 Leiden), carotid duplex
ultrasonography, transcranial Doppler ultrasonography, CT, MRI, and MR angiography.

After a 12-hour overnight fast, total plasma homocysteine level was measured in subjects within the first 48 hours after stroke onset. Venous blood was drawn into Vacutainer tubes the morning after admission and placed in crushed ice. The blood was immediately centrifuged, and homocysteine levels were measured within the following 2 hours. Plasma homocysteine levels were determined with the Fluorescence Polarization Immunoassay (IMX System, Abbott Diagnostics). The upper limit of the manufacturer and the laboratory was 15 μmol/L. Serum folic acid and vitamin B12 levels were also determined in a subset of patients admitted within the last 8 months of the present study. The levels of these vitamins were measured simultaneously with the blood sample collected for homocysteine measurements. Serum folic acid and vitamin B12 levels were measured with an IMMULITE analyzer (Diagnostic Products Corp). The reference ranges for folic acid and vitamin B12 were 3 to 17 ng/mL and 160 to 900 pg/mL, respectively.

All patients initially underwent transthoracic echocardiography study (Vivid Five Vigmmed Technology, GE Ultrasound Device) to exclude any valvular disease as the cause of AF. Studies were performed with the patient in the left lateral recumbent position with standard parasternal and apical views. This was followed by a standard pulse, continuous, and color Doppler examinations. LA diameter was obtained from the parasternal long-axis view as suggested by the American Society of Echocardiography.6 Ejection fraction, wall motion abnormalities, and other ventricular abnormalities on transthoracic echocardiography were also noted. After an overnight fast, TEE was performed with a multiplane TEE 5-MHz probe. Lidocaine was used for local anesthesia of the hypopharynx. Sedation was not performed. After standard examination of the cardiac chambers and valves, the LAA was visualized in both the basal short-axis view in the transverse and the left ventricular–LA 2-chamber view in the vertical scans. The images were analyzed online by the echocardiographer, who remained blinded to the homocysteine and vitamin levels, for the presence of LA thrombus, spontaneous echo contrast (SEC), and interatrial septal abnormalities. Thrombus was defined as a discrete echodensity distinct from the underlying endocardium and visible in >1 plane. SEC was considered to be present if a nonhomogeneous echo swirling in the left atrium and distinguishable from background noise by manipulation of gain settings was seen.

Differences in categorical variables between patients with and without LA thrombus (history of hypertension, diabetes mellitus, or congestive heart failure; presence of SEC and left atrial dilatation) were compared by use of a χ² test or Fisher’s exact test. Homocysteine, folic acid, and vitamin B12 levels between groups were compared by the Mann-Whitney U test. A multivariate logistic regression analysis was performed to show whether the effect of high homocysteine was independent of other variables known to increase the LA thrombus (variables with a value of P<0.15 were included in the model). The association between high homocysteine levels and LA thrombus was expressed by the use of ORs with 95% CIs. Spearman’s rank correlation coefficients were calculated to describe associations between folic acid, vitamin B12, and homocysteine levels. A value of P<0.05 was considered significant.

### Results

Of the 71 patients with AF, 8 died before a TEE study could be performed, and 12 declined a TEE study. Nine additional patients were also excluded because the cause of their stroke was judged not to be AF: 6 had either severe internal carotid artery stenosis or occlusion ipsilateral to the acute hemispheric infarction; 1 had distal vertebral artery occlusion and a cerebellar infarction in the territory of the ipsilateral posterior inferior cerebellar artery; and 2 had hypertension and a lacunar infarction on MRI (small-vessel disease). The remaining 42 patients with nonvalvular AF as the cause of their stroke met the inclusion criteria. There were 18 female and 24 male patients; their mean age was 68.7 years. Except for 1 patient who presented with transient ischemic attack, all patients had a long-lasting deficit. Diffusion-weighted MRI revealed an acute infarction in each patient, including the one with transient ischemic attack. The cerebral lesions involved the territories of the middle cerebral artery in 33, the anterior cerebral artery in 3, the posterior cerebral artery in 3, the posterior inferior cerebellar artery in 2, and the anterior inferior cerebellar artery in 1 patient. Other baseline characteristics and echocardiography findings are presented in the Table.

A TEE was obtained within a mean of 9 days after stroke (range, 3 to 12 days). LA thrombus was identified in 20 of the 42 patients (47.6%). LA thrombus was limited to within the LAA in 13 of the 20 patients. In the remaining 7 patients, the thrombus extended beyond the limits of the LAA and protruded into the LA cavity. There was no difference between patients with or without LA thrombus with respect to age, sex, and history of hypertension, diabetes mellitus, and congestive heart failure (the Table). Likewise, LA diameter did not differ between groups, whereas SEC was more common in patients with LA thrombus. The mean±SE fasting plasma homocysteine level was higher in patients with than in those without LA thrombus (20.75 versus 13.34 μmol/L; P<0.001; the Figure). Homo-
cysteine values ranged from 11.64 to 40.55 μmol/L in patients with LA thrombus and from 10.47 to 22.00 μmol/L in those without LA thrombus. Sixteen of 20 patients with and 4 of 22 patients without LA thrombus had homocysteine levels exceeding the cutoff value of 15 μmol/L \((P<0.001)\). Multivariate logistic regression analysis identified elevated fasting plasma homocysteine as an independent risk factor for LA thrombus \((P=0.017)\). The OR adjusted for the presence of SEC and LA dilatation was 14.25 \((95\% \text{ CI, } 2.70 \text{ to } 75.14)\) for homocysteine levels >15 μmol/L.

Serum folic acid and vitamin B12 measurements were obtained in a subset of 26 patients admitted consecutively within the last 8 months of the study period. An LA thrombus was found on TEE in 12 of the 26 patients. The Table summarizes the mean, lowest, and highest values of vitamin B12 and folic acid in each group. Vitamin B12 and folic acid levels were below the lower reference value in 1 patient each. Both of these patients exhibited LA thrombus on TEE. There was no difference in vitamin B12 levels with respect to the presence of LA thrombus \((P=0.118)\). In contrast, the mean serum folic acid level was significantly lower in patients with LA thrombus on TEE \((P=0.004)\). The levels of folic acid were inversely correlated with homocysteine levels \((r= -0.477, P=0.014)\).

**Discussion**

Homocysteine is a multipotent molecule exerting various modes of injury to the endothelium. It facilitates oxidative arterial injury, damages the vascular matrix, and induces vascular smooth muscle proliferation.\(^{10,11}\) Moreover, homocysteine alters the thrombotic properties of the endothelium by inhibiting the expression of thrombomodulin, activating protein C, enhancing the activity of factors 12 and 5, and augmenting platelet adhesion to the endothelial cells.\(^{12,13}\) The interference of homocysteine with the coagulation system creates a prothrombotic milieu. Virchow’s postulates for thrombogenesis require abnormalities of blood flow, vessel wall, and blood constituents. High homocysteine fulfills the 2 criteria of Virchow’s triad by its endothelial toxic and prothrombotic properties. Therefore, one can postulate that in conditions associated with abnormal blood flow such as stasis, high levels of homocysteine enhance thrombus formation. Indeed, elevated homocysteine has been linked closely to venous thrombosis, which is almost always associated with blood stasis.\(^{4}\) The present study is the first to question the thrombogenic role of homocysteine in another stasis-related condition, AF. In accordance with the venous thrombosis data, elevated homocysteine exhibited an association with the presence of LA thrombus; the mean fasting plasma homocysteine level was significantly higher in patients with LA thrombus compared with that in patients with no thrombus. Moreover, this association was independent of other clinical and echocardiographic parameters known to be associated with LA thrombus formation. The only other study of homocysteine in patients with AF, by Friedman,\(^{14}\) revealed no difference in homocysteine levels among those with or without AF. However, homocysteine levels were significantly higher in a subset of AF patients with a history of stroke than in those who had AF but no stroke. Although Friedman did not stratify AF patients with respect to the presence of thrombus, his results are still in line with those of the present study because patients with AF and stroke are more likely to harbor an LA thrombus.\(^{1}\)

In AF, the fibrillating LA creates a milieu for blood stasis, most prominently in the LAA. This can be qualitatively detected on echocardiography as SEC, a phenomenon characterized by smokelike echoes swirling in the LA, resulting from a stasis-induced, increased erythrocyte aggregation.\(^{15}\) Likewise, LAA outflow velocity measurements serve as a quantitative marker for blood stasis in LAA. Both the presence of SEC and reduced LAA peak outflow velocity \(<20 \text{ cm/s}\) have been shown to convey an independent risk for thrombus formation in LAA.\(^{16}\) In addition to stasis, various markers of hypercoagulable state such as factor 8, fibrinogen, dimer, prothrombin fragment 1.2, and von Willebrand factor have been shown to increase in AF.\(^{17–20}\) The presence of a prothrombotic state is important because it ties Virchow’s criteria to thrombus formation in AF. However, this is not sufficient to establish a causal relationship because LA thrombus in AF might be a trigger rather than a result of elevated hemostatic markers. Homocysteine is different than conventional hemostatic factors because it is neither a direct contributor to the coagulation pathway such as coagulation factors nor a byproduct of the thrombotic (such as fibrinopeptide A and prothrombin fragment 1.2) or fibrinolytic (such as dimer) systems. Unlike most other hemostatic factors, homocysteine levels do not increase in the acute phase of thrombotic episodes such as acute coronary events and stroke.\(^{21,22}\) Therefore, the independent association between high homocysteine and LA thrombus demonstrated in the present study is highly suggestive of a causal relationship. However, it is not possible to be certain about a causal relationship without confirming such an association in a prospective design.

The present study is the first to demonstrate a relationship between LA thrombus in AF and lower folic acid levels. Folic
acid plays a key role as a cofactor in the process of the remethylation of homocysteine to form methionine. It has previously been shown that as folic acid levels decrease, homocysteine levels increase. Subnormal levels of folic acid are associated with moderately elevated homocysteine levels. Accordingly, homocysteine levels showed an inverse but modest correlation with serum levels of folic acid in the present study. This association suggests that the homocysteine elevations observed in our cohort might have been caused in part by low folic acid levels.

Some limitations may apply to the present study. First, LAA outflow velocities were not measured and therefore were not included in the logistic regression analysis. However, the presence of SEC and the increased LA diameter are also strong markers of LA stasis and were included in the analyses. The persistence of a significant association between high homocysteine and LA thrombus after adjustment for these factors strongly suggests that the effect of homocysteine on the LA thrombus is independent of the degree of LA stasis. Second, homocysteine levels increase after stroke; thus, measurements obtained after stroke may not reflect the levels before stroke. It has previously been shown that homocysteine levels in stroke patients and in control subjects are similar when measured in the acute phase of stroke (mean, 2.2 mmol/L) and in the convalescent phase. Therefore, we performed homocysteine measurements within 48 hours of stroke onset. It is also conceivable that any change with respect to baseline would have an impact on our whole study population because all patients had stroke, thereby reducing the bias that might influence comparisons.

Accurate identification and proper management of risk factors predisposing to thrombus formation in the LA are critical to minimize the embolic complications of AF. Such attempts might be more important in patients who cannot continuously and effectively use warfarin because of contraindications. The link between high homocysteine levels and LA thrombus established in the present study raises the question of whether homocysteine-lowering therapies could be an appropriate candidate for this task. It is possible to reduce plasma homocysteine levels with folic acid therapy at a dosage between 0.5 and 5 mg/day by ~25%, even in people who are not vitamin deficient. The efficacy of such a measure in reducing the stroke rate in AF remains to be tested in future studies.

References

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