Plasma Thrombomodulin, Fibrinogen, and Activity of Tissue Factor as Risk Factors for Acute Cerebral Infarction

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Key Words: Cerebral infarction; Thrombomodulin; Fibrinogen; Tissue factor; Hypertension; Risk factor

Abstract

Several studies have indicated association between hematologic markers and increased risks of cerebrovascular disease, but few reports referred to their roles together. We studied plasma levels of 16 hematologic markers in 50 cases diagnosed as acute cerebral infarction (ACI) and 54 hospital control subjects. Plasma levels of thrombomodulin, fibrinogen, and activity of tissue factor (aTF) were significantly higher in cases than in control subjects (P < .001, P < .01, and P < .05, respectively). Multivariate logistic regression analysis showed that hypertension and high plasma levels of thrombomodulin, fibrinogen, and aTF were significantly associated with presence of ACI (odds ratio [OR], 143.74, P < .001; OR, 2.05, P < .05; OR, 2.09, P < .05; OR, 1.02, P < .05, respectively). Our findings indicate that hypertension and elevation of plasma thrombomodulin, fibrinogen, and aTF are independent risk factors for ACI.

Many risk factors for cardiovascular diseases have been identified, and cerebrovascular diseases share some of them.1-5 Recent studies also indicated that several factors were associated with increased risk of cerebral infarction,4,6-8 but few reports were concerned about their coeffects on patients with acute cerebral infarction (ACI). In this study, we explored the relationship between 21 plasma markers (16 for coagulation or anticoagulation, 4 for blood lipids, and 1 for fasting plasma glucose) and patients with ACI. We also assessed the contribution of other factors, such as hypertension, smoking, diabetes, alcohol use, and obesity.

Materials and Methods

Cases

Between July 2005 and June 2006, 95 patients with suspected ACI were admitted to Union Hospital, Wuhan, China. All had (or would have) a computed tomography scan or magnetic resonance imaging on the first (or second) day of hospitalization. Of 95 patients, 63 patients were confirmed as having an ACI, but only 50 patients were included in the study. (We excluded 13 patients from the study: 4 for sampling failure, 6 for disagreement with blood sampling for different reasons [2 for age ≥80 years, 2 for poor mental status, 1 for fear of drawing blood, and 1 for emotional instability], and 3 for insufficiency of the blood sample.) All patients included in the study fulfilled the following criteria: (1) clinical symptoms and signs suggesting acute stroke, (2) magnetic resonance imaging or computed tomography scan indicated infarct instead of brain hemorrhage or occupying lesion, (3) from Chinese Han
population and unrelated, and (4) a duration from onset of ACI symptoms to blood sampling of 48 hours or less. Patients with a previous cerebrovascular history were eligible.

Control Subjects

Control subjects (n = 54) were recruited from patients hospitalized in the Department of Otorhinolaryngology during the same period. None had any of the following conditions within 3 months before admission: surgery, trauma, fracture, malignancy, hematologic disorder, acute infection, organ dysfunction, or use of drugs or comorbidities (such as epistaxis, nasopharyngeal carcinoma, and sudden deafness) known to have great effects on the coagulation and anticoagulation systems. Control subjects with a history of stroke or venous thromboembolism were excluded.

Data Collection and Risk Factor Definition

Information about demographic characteristics and risk factors was collected in a case report form. Hypertension was defined as a history of receiving antihypertensive agents or a systolic blood pressure of 140 mm Hg or more and/or a diastolic blood pressure of 90 mm Hg or more on repeated measurements within a few days. Diabetes was considered when the fasting plasma glucose (FPG) level was 126 mg/dL (7.0 mmol/L) or more, a random blood glucose level was 200 mg/dL (11.1 mmol/L) or more with relevant symptoms, a 2-hour plasma glucose oral glucose tolerance test result was 200 mg/dL (11.1 mmol/L) or more, and/or the patient was using glucose-lowering agents. Status of smoking and alcohol use was coded as none or current use. Presence of obesity was considered when the body mass index was 30 kg/m² or more for men and 28.6 kg/m² or more for women.

Blood Samples

Every blood sample was drawn from a fasting subject early in the morning of the second day during hospitalization in 2 Vacutainer tubes, one with 3.2% sodium citrate (anticoagulant/blood ratio, 9:1, Gongdong, Taizhou, China) and the other with separation gel (Becton Dickinson, Franklin Lakes, NJ). The former was centrifuged within 30 to 60 minutes at 2,500g for 6 minutes at room temperature, and plasma was subdivided and frozen at –80°C until analysis. The latter was quickly sent to the Department of Laboratory Medicine and analyzed for blood lipid and FPG levels in an automatic biochemical analyzer (Hitachi 7170A, Tokyo, Japan).

Reagents for Assay

Enzyme-linked immunosorbent assay kits for protein C (PC), free protein S, and total protein S were purchased from HYPHEN BioMed, Andrésy, France; kits for thrombomodulin and activated factor VII from American Diagnostica, Greenwich, CT; kits for factor VII antigen, tissue-type plasminogen activator, plasminogen activator inhibitor-1, and activity of tissue factor (aTF) from AssayPro, Winfield, MO; kits for P selectin from GeneMay, San Diego, CA; kits for fibrinogen, D dimer, activated partial thromboplastin time, and prothrombin time from Dade Behring, Marburg, Germany; kits for activated protein C (APC) ratio were purchased from Chromogenix, Milan, Italy; and kits for thrombin time were from the Biochemical Drug Plant of Special Economic Zone, Zhuhai, China. All markers were detected according to the corresponding instructions with the reagents, and all analysts had no information about the samples grouping until results were calculated.

The Union Hospital Ethics Committee approved the study protocol, and all participants gave their oral informed consent. This study was completed as a retrospective case-control study with a limited sample size.

Statistical Analysis

Continuous data are presented as mean (SD) or median (interquartile range). Differences between groups were analyzed by an unpaired samples t test for quantitative variables with normal distribution, the Mann-Whitney U test for quantitative variables with skewed distribution, and the χ² test for qualitative variables. Covariates with a P value of .05 or less in univariate analysis were included in a multivariate logistic regression analysis to assess risk factors for ACI. Statistical testing was conducted at a 2-tailed α level of .05. The data were analyzed by using the SPSS software package (version 13.0 for Windows, SPSS, Chicago, IL).

Results

The baseline characteristics of all subjects are given in Table 1. There was significant difference in prevalence of hypertension between case and control groups (68% vs 2%; P < .001).
Plasma levels of coagulation and anticoagulation parameters, FPG, and blood lipids in the 2 groups are given in Table 2. Levels of thrombomodulin, aTF, and fibrinogen in cases were significantly higher than in controls (P < .001, P < .05, and P < .01, respectively). The APC ratio and concentration of high-density lipoprotein cholesterol (HDL-C) were significantly lower in cases compared with controls (P < .001 and P < .01, respectively). No significant differences were found in the levels of PC, free protein S, total protein S, activated factor VII, factor VII antigen, P selectin, tissue-type plasminogen activator, plasminogen activator inhibitor-1, FPG, low-density lipoprotein cholesterol, total cholesterol, triglycerides, prothrombin time, activated partial thromboplastin time, thrombin time, or D dimer between the 2 groups.

According to multivariate analysis Table 3, hypertension, thrombomodulin, fibrinogen, aTF, and the APC ratio had significant associations with ACI (P < .001, P < .05, P < .05, P < .05, and P < .01, respectively).

We also compared the general characteristics and hematologic markers in patients with ACI who had a history of stroke with patients who did not. Age and plasma concentration of fibrinogen were higher in patients with a history of stroke (P = .029 and P = .032, respectively). The plasma level of PC was lower in patients with a history of stroke (P = .01). There were no significant differences in the prevalence of hypertension, diabetes, smoking, alcohol use, and obesity in the 2 subgroups (all P > .5) Table 4. There were also no significant differences in the remaining hematologic markers and blood lipids (data not shown).

**Discussion**

Earlier studies had confirmed that hypertension was one of the most important risk factors for cerebrovascular disease. A recent study also indicated that hypertension was the most prevalent (87.5%) risk factor followed by ischemic heart disease (35%) and diabetes. Hypertension is strongly associated with atheromatous deposits blocking or narrowing brain arteries, predisposing to local clot formation. Atheroma and its ischemic consequences may damage cerebral arterioles and the brain tissue they supply. Results of a large cohort study indicated that improvement of hypertension control might be responsible for a steady decline in incidence of lacunar infarction in a Japanese population. Our findings also showed that hypertension contributed to the development of acute cerebral infarction (multivariate analysis: odds ratio [OR], 143.74; 95% confidence interval [CI], 12.92-1,599.67). So, lowering blood pressure has an important role in protecting against ischemic stroke.

Thrombomodulin is an integral membrane-bound glycoprotein, predominantly expressed at the surface of vascular endothelial cells. As a cofactor for thrombin-mediated cleavage of PC at the N-terminus, it amplifies the event of activation...
of PC more than 1,000-fold. It is also a cofactor for thrombin-mediated activation of the thrombin-activatable fibrinolysis inhibitor. In this study, we detected the concentrations of soluble thrombomodulin (sTM) in plasma and found that the levels in cases were significantly higher than in controls (Table 2). Previous studies also indicated that increased sTM levels had been associated with recurrent myocardial and cerebral infarction. Univariate and multivariate analyses indicated that sTM was a risk factor with a lower 95% CI bound of the association between high levels of sTM and recurrent cerebral infarction. Univariate and multivariate analyses found a relationship between high plasma levels of fibrinogen and cerebral infarction (OR, 1.59; 95% CI, 1.12-2.25; and OR, 2.09; 95% CI, 1.02-4.27, respectively). Therefore, we can conclude that the plasma fibrinogen level is an independent risk factor for cerebral infarction.

Although the prevalence of hypertension in the ACI subgroups had no contrast, a significant difference was found in the plasma levels of fibrinogen between the 2 subgroups (Table 4). Bivariate correlation analysis found no relationship between plasma fibrinogen levels and ages in patients with a history of stroke (P = .084; Spearman rank correlation coefficient = 0.543). So the difference of plasma levels of fibrinogen between the 2 subgroups may be mainly due to the contribution of a history of cerebral infarction rather than old age.

Table 4:

<table>
<thead>
<tr>
<th>Variable</th>
<th>No History of Stroke (n = 39)</th>
<th>History of Stroke (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age (y)</td>
<td>60.6 (13.5)</td>
<td>69.4 (8.6) *</td>
</tr>
<tr>
<td>Male sex</td>
<td>23 (59)</td>
<td>8 (73) *</td>
</tr>
<tr>
<td>Hypertension</td>
<td>26 (67)</td>
<td>8 (73) *</td>
</tr>
<tr>
<td>Diabetes</td>
<td>15 (38)</td>
<td>6 (55) *</td>
</tr>
<tr>
<td>Smoking</td>
<td>7 (18)</td>
<td>3 (27) *</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>4 (10)</td>
<td>2 (18) *</td>
</tr>
<tr>
<td>Obesity</td>
<td>9 (23)</td>
<td>2 (19) *</td>
</tr>
<tr>
<td>Mean (SD) protein C level (%)</td>
<td>112.2 (26.7)</td>
<td>85.0 (28.8) *</td>
</tr>
<tr>
<td>Mean (SD) fibrinogen level (g/L)</td>
<td>4.30 (1.66)</td>
<td>5.28 (1.07) *</td>
</tr>
</tbody>
</table>

\* Data are given as number (percentage) unless otherwise indicated. The \( \chi^2 \) and Mann-Whitney U tests were used to compare proportions and continuous variables, respectively. To convert fibrinogen values from Système International units to conventional units (mg/dL), divide by 0.01. \( \* P < .05 \). \( \dag P > .5 \).
level of serum HDL-C was an independent risk factor for ACI (OR, 0.15; 95% CI, 0.02-1.55). Limited samples and a high prevalence of hypertension in the cases may account for this finding.

The APC ratio gives an overall estimation of the anticoagulant effect of APC and, thus, provides information on the thrombotic risk associated with inherited and acquired APC resistance. Interestingly, the APC ratio is not a simple, 1-variable reflection of the APC response in vivo. Instead, it seems to reflect an anticoagulant system response that may decrease under a hypercoagulable state.33 van der Bom et al34 showed that a low APC ratio was associated with an increased risk of stroke, independent of the factor V mutation. Our study also indicated that a decreased APC ratio was associated with an increased risk of cerebral infarction according to univariate and multivariate analyses (OR, 0.01; 95% CI, 0.001-0.165; and OR, 0.01; 95% CI, 0.00-0.32, respectively), but no significant difference was found in the ACI subgroups in the present study. This finding needs to be ascertained in a larger case-control study.

There were no significant differences in other variables except mean age, protein C, and fibrinogen (Table 4) in the subgroups. Bivariate correlation analysis did not find a relationship between any 2 of the 3 parameters (data not shown). Naturally, small samples could not verify this finding.

To explain the aberrations in thrombomodulin, fibrinogen, aTF, HDL-C, and APC ratio between cases and controls, we divided ACI cases into 2 subgroups by hypertension status: hypertensive ACI and normotensive ACI. By using the Mann-Whitney U test, we found no differences in thrombomodulin, fibrinogen, aTF, HDL-C, and APC ratio between subgroups except mean age, protein C, and fibrinogen (Table 4). The values for cholesterol and fibrinogen from Système International units to conventional units are as follows: cholesterol levels (mg/dL), divide by 0.02586; fibrinogen (mg/dL), divide by 0.01.

The Mann-Whitney U test was used to compare means of continuous variables.

### Table 5
Markers in Hypertensive and Normotensive Acute Cerebral Infarction Subgroups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive (n = 34)</th>
<th>Normotensive (n = 16)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombomodulin (ng/mL)</td>
<td>4.91 (1.92)</td>
<td>4.11 (1.42)</td>
<td>.069</td>
</tr>
<tr>
<td>Activated protein C ratio</td>
<td>2.54 (0.28)</td>
<td>2.39 (0.37)</td>
<td>.154</td>
</tr>
<tr>
<td>Activity of tissue factor (pg/mL)</td>
<td>185.6 (79.6)</td>
<td>209.9 (89.5)</td>
<td>.417</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol (mmol/L)</td>
<td>1.360 (1.013)</td>
<td>1.236 (0.521)</td>
<td>.082</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>4.4 (1.6)</td>
<td>4.7 (1.6)</td>
<td>.625</td>
</tr>
</tbody>
</table>

† The Mann-Whitney U test was used to compare means of continuous variables.

Confirm our findings and investigate other risk factors for acute ischemic stroke.

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### References


