Soluble Thrombomodulin and Brain Infarction
Case–Control and Prospective Study

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Background and Purpose—Increased soluble thrombomodulin (sTM) concentration has been associated with recurrent coronary events, whereas in one prospective study it predicted fewer first-ever coronary events. One study found no relationship between brain infarction (BI) and sTM levels. Among all subjects of the Étude du Profil Génétique de l’Infarctus Cérébral (GENIC) cohort and those free of previous vascular history, we investigated the relationship between sTM level and BI risk, and among cases, its relationship with BI prognosis.

Methods—Patients with BI (n=492) were consecutively recruited from 12 centers. Hospital controls without a history of stroke (n=492) were individually matched for age, sex, and center. Blood samples were collected after hospitalization. Determination of sTM levels was centralized in a single laboratory.

Results—Soluble TM concentration significantly increased with age and hypertensive status, but was similar in cases and controls. With analyses restricted to 278 pairs of subjects with no previous vascular history, sTM concentration >59.6 μg/L (second and third tertiles compared with the first) was associated with fewer first-ever BI (adjusted odds ratio of 0.56 (95% CI, 0.35 to 0.89; P=0.014). Among the cases, increased sTM concentration was associated with a higher death rate after a median follow-up of 5.2 (1.4 to 6.4) years. The adjusted hazard ratio per 1 SD of sTM concentration increase (34.2 μg/L) was 1.19 (95% CI, 1.02 to 1.39; P=0.028).

Conclusions—Increased sTM concentration may be protective against BI in subjects with no previous vascular disease, whereas it may predict a fatal outcome in patients who have already had a BI. Consequently, sTM levels should be interpreted according to vascular history. (Stroke. 2004;35:1946-1951.)

Key Words: brain infarction ■ thrombomodulin ■ epidemiology ■ prognosis ■ risk factors

Thrombomodulin (TM) is an integral membrane-bound glycoprotein expressed at the surface of endothelial cells (EC). Thrombin binding to TM promotes protein C activation, which in turn results in decreased thrombin generation.1 TM also modulates thrombin-induced platelet activation and acts as a cell receptor that controls cell proliferation induced by thrombin.2,3 Thrombin-TM complex also activates the thrombin-activatable fibrinolysis inhibitor (TAFI), which inhibits tissue plasminogen activator (tPA)-induced fibrinolysis.4 TM expression was observed in brain vessels and protein C activation was observed in human brain after carotid artery occlusion.5,6 The positive effects of activated protein C observed in animal models suggest that it could be a promising new drug for brain infarction (BI).7

TM exists in a soluble form (sTM) in plasma and urine. Increased sTM may reflect (1) increased membrane TM expression or (2) increased proteolytic cleavage and subsequent release in the circulation. Loss of TM from the surface of EC may favor thrombosis.8,9 Concurring with this hypothesis, increased sTM concentration has been associated with recurrent myocardial infarction and peripheral artery disease.10,11 An increased sTM level is therefore usually regarded as a marker for endothelial damage. However, a large study found, paradoxically, increased sTM levels to be associated with a reduced risk of first-ever coronary events.12 This suggests that nondamaged EC may, constitutively, have a higher expression of TM with an anticoagulant effect that protects patients against thrombotic events. As regards cerebrovascular disease, one study found no relationship between increased sTM concentration and the risk of BI,13 although sTM was associated with lacunar stroke and asymptomatic carotid stenosis progression in other studies.12,14 The relationship between sTM concentration and the prognosis of BI has never been investigated.

Because increased sTM levels appear to be protective in subjects with no previous vascular event and deleterious in...
those who had already experienced such an event, our objective was to study the relationship between sTM levels and the risk of BI in the Étude du Profil Génétique de l’Infarctus Cérébral (GENIC) cohort and in the subset of patients who were free of previous vascular disease. We also investigated the relationship between sTM levels and the death rate 5 years after the qualifying BI.

Subjects and Methods
The Ethics Committee of Cochin Hospital approved the research protocol, and all subjects signed the informed consent form.

Cases
Cases (n=510) were recruited consecutively among all patients admitted to 12 French neurological centers if they fulfilled the following criteria: (1) clinical symptoms suggesting stroke, (2) no brain hemorrhage on CT scan, (3) infarct proven by MRI, (4) 18 to 85 years of age, and (5) both parents of white origin. Cases were included in the week-interval following the event. Patients reporting a previous cardio- or cerebrovascular history were eligible.

Controls
Controls without a history of stroke (n=510) were recruited among individuals hospitalized at the same institutions for any reason other than neurological diseases; these reasons consisted of orthopedic (46%), ophthalmologic (12%), rheumatologic (11%), surgical (6%), and other (25%) causes. One control was matched by sex, age (±5 years), and center to each case. Subjects reporting a positive cardiovascular history other than stroke were eligible. Their parents had to be of white origin.

Data Collection and Risk Factor Definition
Information about demographic characteristics and risk factors was collected using a structured questionnaire. Hypertension was defined as a history of treated hypertension. Smoking history was coded as never, previous, or current smoker. Subjects were classified as diabetic if they were treated for insulin-dependent or noninsulin-dependent diabetes. Use of lipid-lowering drugs was assessed. A history of MI, angioplasty, coronary artery bypass surgery, or stroke was considered as a history of treated hypertension. Smoking history was coded as never, previous, or current smoker.

Investigations
ECG, extracranial duplex, and transcranial Doppler were performed on all cases and controls. The presence of plaques, arterial stenoses, and occlusions was assessed. Transthoracic echocardiography was performed in 464 patients (70%); nevertheless, these patients were included in the study because the CT scan clearly showed a recent BI. Two neurologists (P.A. and François Chédru) reviewed the MRI scans. They determined the anatomical location and extent of the BI and its size in millimeters (the largest diameter was chosen). Conventional or magnetic resonance cerebral angiogram was available for 41% of the patients.

Magnetic resonance cerebral angiography could not be performed, or was of poor quality, in only 38 cases (7.5%); nevertheless, these patients were included in the study because the CT scan clearly showed a recent BI. Two neurologists (P.A. and François Chédru) reviewed the MRI scans. They determined the anatomical location and extent of the BI and its size in millimeters (the largest diameter was chosen). Conventional or magnetic resonance cerebral angiogram was available for 41% of the patients.

Cases and controls were blood-sampled during their hospitalization. Blood was drawn in the morning from fasting subjects. The median (range) delay for blood sample after admission was 5 days (0 to 61) in the cases and 6 days (0 to 87) in the controls (Wilcoxon test, P=0.001).

Soluble TM Concentration
Soluble TM concentration was measured in a centralized laboratory by an ELISA assay according to the manufacturer’s instructions (Diagnostica Stago Inc).

Brain Infarction Subtype Classification
Patients were classified into etiologic subtypes by 2 neurologists (P.A. and François Chédru) according to prespecified criteria as previously reported, after a review of clinical files, discharge summaries, follow-up visit reports, and results of investigations (for more details, see our Web site at http://www.ccr.jussieu.fr/GENIC/Welcome.html).

Follow-Up
Patients with BI were followed-up after inclusion in the study. The first examination was performed during hospitalization on day 10 or on discharge (median delay of 11 [range, 0 to 40] days). The second examination was performed after 6 months during a consultation with their treating neurologist (median delay of 193 [range, 29 to 587] days). The third examination was performed by a research nurse who visited the patients at home (median delay of 2.7 [range, 1.4 to 4.1] years). Finally, a last contact to assess mortality was scheduled after 5 years (median delay of 5.3 [range, 4.0 to 6.4] years) (end of follow-up) by phone call to the patients or their family; if no answer was received, mortality information was obtained from the general practitioner or from the Registry Office of their place of birth (where the births and deaths of each individual are recorded in France, without exception) with a “dead” or “alive” answer.

At the end of the follow-up of the 510 cases, 163 cases had died and 44 cases were lost after the third examination.

Data Analysis
Data analysis was based on 492 pairs of cases and controls for whom an sTM concentration was available. We studied the association between sTM and several risk factors separately in the cases and controls. We used analysis of covariance adjusted for age and sex (with an additional adjustment for the blood sampling delay; use of lipid-lowering drugs for the lipid parameters; and additional adjustment for antihypertensive treatment for blood pressure). The variables that were associated with sTM among the controls were subsequently used to adjust the analyses of the relationship between sTM and BI.

Conditional logistic regression for matched sets was used to compare the sTM distribution. With categorization of sTM according to tertiles (in the whole population), the relative risk of BI for the upper 2 tertiles relative to the lowest tertile was estimated by calculating the odds ratio (OR) and 95% CI. As the ORs for BI associated with the second and upper tertile were very similar, we also computed the ORs for the sTM concentration of both the second and third tertile compared with the first. The same analyses were performed in matched pairs of cases and controls with no previous cardio- or cerebrovascular history.

We investigated the relationship between sTM and the survival status of the cases after a median follow-up of 5 years. We estimated

<table>
<thead>
<tr>
<th>TABLE 1.  General Characteristics of BI Cases and Controls</th>
</tr>
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<tbody>
<tr>
<td>Cases n=492</td>
</tr>
<tr>
<td>Age, median (range)</td>
</tr>
<tr>
<td>Male sex, % (n)</td>
</tr>
<tr>
<td>BMI, kg/m², mean (SD)</td>
</tr>
<tr>
<td>History of hypertension, % (n)</td>
</tr>
<tr>
<td>History of diabetes, % (n)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL, mean (SD)</td>
</tr>
<tr>
<td>Current smokers, % (n)</td>
</tr>
<tr>
<td>Cardiovascular history, % (n)</td>
</tr>
<tr>
<td>Stroke history, % (n)</td>
</tr>
</tbody>
</table>

χ² analysis and Student t test were used to compare proportions and continuous variables, respectively. Medians of age were compared with the Wilcoxon test.

*P<0.001; †P<0.01.
a survivor function according to the tertile of sTM concentration using the nonparametric Kaplan–Meier method. As we found a significant difference in the survival of the cases between the tertiles of sTM concentration, the Cox proportional hazard model was used to calculate the relative risks (RRs) of death (after checking the assumption of the proportional hazards). As the relative risk of death increased regularly with the tertiles of sTM concentration, we computed the RRs associated with the increase of 1 SD of sTM concentration. The Cox proportional hazard model was performed for adjustment on concomitant factors. For power calculations, we dichotomized the sTM level using the median of the distribution as a cutoff. We computed the smallest significant (at a 2-tailed level of 5%) OR or RR that our study sample size allowed us to detect with a power of 80%. Based on these calculations, 492 and 278 pairs of cases and controls ensured an 80% power to detect significant ORs of at least 0.69 and 0.61, respectively. In addition, 492 cases followed for a fixed length of 5 years yielded an 80% power to detect a RR of at least 1.53 (we assumed that the 5-year survival rate in the nonexposed group was 0.7).

Statistical testing was conducted at the 2-tailed level of 0.05. The data were analyzed using the SAS package.

### Results

#### Case–Control Study

The baseline characteristics of the study subjects are presented in Table 1. The frequencies (n) of the BI subtypes were: atherothrombotic, 22% (108); lacunar, 21% (105); cardioembolic, 16% (78); undetermined cause, 13% (63); unknown cause, 24% (117); dissections, 2% (11); and rare causes of BI, 2% (10).

Table 2 shows the relationship between sTM concentration and risk factors among BI cases and controls. We found significant relationships between sTM concentration and age, history of hypertension, high-density lipoprotein (HDL) cholesterol, triglyceride levels, and apolipoprotein A and B in both cases and controls. Significant relationships between sTM and sex, body mass index, systolic blood pressure, and history of diabetes, cardiovascular disease, or stroke were found only among cases. We found no significant association
between sTM concentration and blood sampling delay. After adjustment of blood sampling delay and the use of lipid-lowering drugs and other potential confounding factors (HDL cholesterol; triglyceride level; apolipoprotein A and B, history of hypertension, and history of diabetes), the mean ± SD sTM concentration was similar in the cases (79.3 ± 34.2 μg/L) and controls (81.4 ± 37.6 μg/L). There was no significant difference in sTM concentration among the BI subtypes (data not shown) and no relationship with markers of atherosclerosis such as intima media thickness, presence of carotid plaque, and carotid stenosis ≥30% as assessed by carotid duplex ultrasonography (Table 3).

After categorization of the sTM concentration into tertiles, we found no significant difference between the cases and controls (both first-ever and recurrent cases). However, when the analyses were restricted to the 278 pairs of cases and controls with no previous cardiovascular or cerebrovascular disease (first-ever BI), there was a significant association between BI and sTM concentration in the second and third tertiles. The OR associated with an sTM concentration >59.6 μg/L (second and third tertiles grouped) was 0.64 (95% CI, 0.43 to 0.94; P = 0.024). This relationship remained significant with an OR=0.56 (95% CI, 0.35 to 0.89; P=0.014) after adjustment for blood sampling delay, use of lipid-lowering drugs and other potential confounding factors (Figure 1).

**Case Follow-Up**

After a median follow-up of 5.2 (range, 1.4 to 6.4) years of the 492 cases, 156 cases died and 42 were lost after the third examination. Figure 2 shows the Kaplan–Meier estimates of survival for the cases according to the tertiles of sTM concentration. The curves show a significantly higher death rate with increasing sTM concentration (log-rank test: P<0.0001). The relative risk of death increased gradually with the tertiles of sTM concentration (P for trend = 0.001) (Table 4). The hazard ratio (adjusted for age and sex) per 1 SD increase in sTM concentration (34.2 μg/L) was 1.28 (95% CI, 1.12 to 1.47; P<0.001). These ratios remained significant after adjustment for confounding factors; hazard ratio = 1.19 (95% CI, 1.02 to 1.39; P=0.028).

**Discussion**

Our results suggest that increased sTM concentration may be associated with a reduced risk of BI in subjects with no previous vascular disease, whereas after BI it may predict a higher death rate. This concurs with the findings of previous observations made on coronary artery disease.10–12 The underlying mechanism of this relationship remains obscure. Considering the protective effect observed in their study, Salomaa et al12 suggested that increased sTM concen-

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**TABLE 3. IMT, Carotid Plaque, and Carotid Stenosis ≥30% by Tertiles of sTM Concentration Among BI Cases and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>sTM μg/L</td>
<td>N</td>
<td>IMT, M (SD)</td>
<td>Plaques, % (n)</td>
<td>Stenosis ≥30% or Occlusion (n)</td>
</tr>
<tr>
<td>&lt;61.5</td>
<td>164</td>
<td>0.77 (0.16)</td>
<td>56.8 (89/152)</td>
<td>22.8 (36/158)</td>
</tr>
<tr>
<td>61.5–87.7</td>
<td>165</td>
<td>0.81 (0.14)</td>
<td>70.4 (112/159)</td>
<td>28.2 (46/163)</td>
</tr>
<tr>
<td>&gt;87.7</td>
<td>163</td>
<td>0.83 (0.15)</td>
<td>69.4 (111/160)</td>
<td>36.0 (58/161)</td>
</tr>
<tr>
<td>P for trend*</td>
<td>0.39</td>
<td>0.65</td>
<td>0.12</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*Adjustment for age and sex (logistic regression or ANCOVA).
IMT indicates intima-media thickness.

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![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Adjusted ORs* of BI by tertiles of sTM concentration in whole population and in matched pairs with no previous cardiovascular or cerebrovascular history. (*conditional logistic regression for matched sets, adjusted for blood sampling delay, use of lipid-lowering drugs, HDL-C, triglyceride level, apolipoprotein A and B, history of hypertension, and history of diabetes.) Note: For clarity we do not present the subjects with vascular history (24 matched pairs), but interaction was not significant (P>0.20).

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Overall survival of BI cases according to tertiles of sTM concentration.
TABLE 4. Relative Risk of Death of BI Cases After 5 Years Follow-Up, According to sTM Concentration

<table>
<thead>
<tr>
<th>sTM, μg/L</th>
<th>Death Rate*</th>
<th>Hazard Ratio† (95% CI)</th>
<th>Hazard Ratio‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;61.5</td>
<td>20.7</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>61.5–87.7</td>
<td>35.8</td>
<td>1.60 (1.03–2.49)</td>
<td>1.37 (0.84–2.20)</td>
</tr>
<tr>
<td>&gt;87.7</td>
<td>46.6</td>
<td>2.04 (1.34–3.11)</td>
<td>1.68 (1.06–2.65)</td>
</tr>
<tr>
<td>P for trend:</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TM/SD (34.2)</td>
<td>—</td>
<td>1.28 (1.12–1.47)</td>
<td>1.19 (1.02–1.39)</td>
</tr>
</tbody>
</table>

* Mortality rates were estimated by the Kaplan-Meier method.
† Adjustment for age and sex.
‡ Additional adjustment for BMI >25, HDL-C <0.45 g/L, LDL-C >1.22 g/L, history of diabetes, Rankin >3 at inclusion, and cardiovascular history.

third examinations. Hence, our results partially concur with the findings of this previous study.

Our study suffers from several limitations. First, the case–control design used in the first part of our study makes our results weak until they are confirmed by a prospective study. Indeed, one previous prospective study failed to demonstrate a significant correlation between sTM concentration and BI. However, the number of incident cases was smaller than in our study, with 87 ischemic and 18 hemorrhagic strokes matched with 216 controls from a prospective cohort of 40,000 cases. Subjects were of a younger age (55.1 years of age), and had lower exposure to major risk factors (hypertension, diabetes, smoking) compared with the subjects of the GENIC study (Table 1). This observation may account for the rather low incidence of BI in this large prospective cohort and the lack of a relationship between sTM and BI observed. Our results should therefore be confirmed by a prospective study done before sTM is accepted as a definite risk factor for BI among subjects exposed to a higher risk of BI. Second, we did not evaluate factor VIII coagulant activity, sICAM1, and possibly other unknown confounding factors that may interact with the effect of sTM on BI incidence and prognosis.

In conclusion, our study suggests that the impact of increased sTM level should be interpreted according to the patient’s vascular history because it reduces the primary risk of BI among patients free of previous vascular history and increases the risk of death in patients with BI.

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References


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