The present study was designed to test the hypothesis that circulating levels of thrombomodulin are elevated in patients with hypertension in proportion to the severity of the vascular damage. A cross-sectional study was carried out using a population consisting of 96 patients with essential hypertension without clinically evident cardiovascular disease (mean age: 65 ± 10 years) and 99 healthy normotensive control subjects (64 ± 9 years). Blood was sampled and serum concentrations of soluble thrombomodulin were measured using an enzyme immunoassay method. We calculated the ratio of the concentration of thrombomodulin to that of creatinine, because soluble thrombomodulin is excreted by the kidney and the serum level of thrombomodulin was correlated with that of creatinine ($p < 0.05$). The association between the ratio and other clinical variables was investigated. The ratio of the thrombomodulin to creatinine concentrations was higher in hypertensive (29.3 ± 10.9) than in control subjects (24.4 ± 5.9; $p < 0.0001$). Systolic blood pressure was correlated with the ratio but the ratio showed no correlation with serum lipid levels when analyzed using data from all subjects. In hypertensive patients, the ratio correlated with the grade of sclerotic, but not hypertensive, changes in the fundus oculi (Scheie’s classification, $p < 0.001$). Furthermore, the ratio correlated with brachial-ankle pulse wave velocity ($p < 0.001$). However, no correlation was detected between the ratio and blood pressure. These results suggest that circulating levels of thrombomodulin are elevated in hypertensive patients as compared to normotensive subjects and that the thrombomodulin level may be a molecular marker of the latent progression of atherosclerosis in hypertensive patients.

(Hypertens Res 2003; 26: 479–483)

Key Words: atherosclerosis, essential hypertension, fundus oculi, pulse wave velocity, thrombomodulin


text

**Introduction**

Thrombomodulin (TM), an integral membrane glycoprotein, is a high-affinity receptor for thrombin on the endothelial cell surface and has been implicated in the endothelial regulation of fibrinolysis and coagulation (1). TM is widely distributed on the endothelium of human arteries, veins, capillaries and lymphatics in all organs and tissues except the brain (2, 3).

After proteolytic cleavage from the endothelial surface, soluble TM can be detected in the circulation (3–5). The physiological role of soluble TM is unknown, but its concentration has been considered to reflect endothelial damage (6, 7). Thus, a positive correlation between the concentration of soluble TM and the risk of atherosclerotic disease is widely assumed. However, the results of studies on soluble TM in atherosclerotic diseases are conflicting (5). Some studies have reported a positive association between soluble TM and
coronary or peripheral artery disease (8–10), while others have failed to find any association (11, 12).

Hypertension is one of the most important risk factors of atherosclerosis. Endothelial function is impaired in hypertension (13–15), and this plays a crucial role in the initial development of atherosclerosis (16). This raises the question of whether soluble TM is elevated in patients with hypertension in proportion to the progression of atherosclerosis. However, few studies have reported the blood concentration of soluble TM level in patients with hypertension.

The diagnosis of essential hypertension was based on clinical history, physical examination, and laboratory and radiological investigations. Patients with diabetes mellitus, severe pulmonary disease, acute or chronic liver disease, renal dysfunction (serum creatinine $\geq 2.0$ mg/dl), or inflammatory illness were excluded from the study. Twenty-four patients exhibited high serum cholesterol levels ( $> 240$ mg/dl) and/or were being treated for hypercholesterolaemia. No patients exhibited cardiovascular disease including coronary or peripheral artery disease or stroke. The medications used to treat the hypertensive patients were as follows: diuretics (n = 12), calcium channel blockers (n = 61), angiotensin converting enzyme inhibitors (n = 20), $\beta$-blockers (n = 27), angiotensin II receptor antagonists (n = 4), and hydroxymethylglutaryl coenzyme reductase inhibitors (n = 20). The control group consisted of 99 healthy volunteers (Table 1). None of the control subjects had any disease or received any medication. Blood samples were collected in the morning after overnight fasting for determination of serum TM levels and for biochemical analysis.

### Methods

#### Subjects and Protocol

The procedures followed were in accordance with institutional guidelines. The respective ethics committees of the hospitals approved the study protocol and each subject gave informed consent prior to the start of the study. We studied 96 patients with essential hypertension (Table 1), either receiving (81 patients) or not receiving medical treatment (15 patients), recruited from the hypertensive outpatients clinics of Nagoya City University Hospital, Johoku Hospital and Enshu General Hospital, Japan. Hypertension was defined as systolic blood pressure of $\geq 140$ mmHg and/or diastolic blood pressure of $\geq 90$ mmHg on at least three occasions and/or the taking of antihypertensive medication. Blood pressure was measured in the right arm after a 5-min rest period, following the recommendation of the American Heart Association (20). The diagnosis of essential hypertension was based on clinical history, physical examination, and laboratory and radiological investigations. Patients with dia-

### Statistical Analysis

All data are expressed as the means $\pm$ SD. Statistical analysis was performed using StatView 5.0 software (SAS Institute Inc., Cary, USA). Because the distribution of TM is skewed toward higher values, the significance of any difference in distribution was assessed by nonparametric tests. For intergroup comparisons, the two-tailed unpaired Student’s $t$-test, Mann-Whitney $U$-test, or Fisher’s test was used. Bivariate associations between serum soluble TM concentrations or
the ratio of serum soluble TM to creatinine concentrations (TM/Cr ratio) and other variables were assessed by Spearman’s rank correlation analysis. Values of \( p < 0.05 \) were considered to indicate statistical significance.

### Results

Serum soluble TM levels were higher in patients with hypertension than in healthy normotensive control subjects (Table 1). We calculated the TM/Cr ratio (4) because TM is eliminated solely by renal metabolism (3, 4). Indeed, the levels of TM were correlated with those of creatinine (Table 2). The TM/Cr ratio was found to be higher in hypertensive patients than in healthy normotensive control subjects (Table 1), but the TM/Cr ratio showed no correlation with serum lipid levels (Table 2). Systolic, but not diastolic, blood pressure correlated with the TM/Cr ratio when analyzed using data from all subjects (Table 2).

In contrast to the analysis using the data from all subjects, in hypertensive patients, a correlation was not detected between the TM/Cr ratio and blood pressure (Table 3). Interestingly, Spearman rank correlation analysis revealed that the TM/Cr ratio correlated with the grade of sclerotic changes, but not with hypertensive changes, in the fundus oculi (Fig. 1). Furthermore, significant correlation was observed between the TM/Cr ratio and baPWV in hypertensive patients (Fig. 2). The TM/Cr ratio was not affected by the different medications used to treat the hypertensive patients (data not shown).

### Table 2. Results of Spearman Rank Correlation Analysis between the Levels of TM or the TM/Creatinine Ratio and the Clinical Variables for All Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>TM</th>
<th>TM/creatinine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \rho ) value</td>
<td>( p ) value</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.109</td>
<td>0.1306</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>-0.031</td>
<td>0.7661</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.158</td>
<td>0.0280</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.076</td>
<td>0.2913</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.052</td>
<td>0.4667</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>-0.099</td>
<td>0.1696</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>0.079</td>
<td>0.2750</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-0.061</td>
<td>0.3959</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.161</td>
<td>0.0252</td>
</tr>
</tbody>
</table>

TM, thrombomodulin; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

### Table 3. Results of Spearman Rank Correlation Analysis between the Thrombomodulin/Creatinine Ratio and Clinical Variables for Hypertensive Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>( \rho ) value</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.028</td>
<td>0.7861</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>-0.096</td>
<td>0.3510</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>-0.001</td>
<td>0.9971</td>
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<tr>
<td>DBP (mmHg)</td>
<td>0.044</td>
<td>0.6672</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.032</td>
<td>0.7580</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>0.072</td>
<td>0.4834</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>0.138</td>
<td>0.1822</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-0.155</td>
<td>0.1297</td>
</tr>
<tr>
<td>ECG voltage (mV)</td>
<td>0.193</td>
<td>0.0606</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ECG voltage, SV\(_1\) + RV\(_5\) in electrocardiogram.

Fig. 1. Correlation between the serum thrombomodulin/creatinine (TM/Cr) ratio and the grade of Scheie’s classification in the fundus oculi (left: hypertensive changes; right: sclerotic changes) of patients with essential hypertension. Spearman rank correlation analysis revealed a significant correlation between the TM/Cr ratio and the grade of sclerotic (\( \rho = 0.449, \ p < 0.0001 \)), but not hypertensive (\( \rho = 0.173, \ p = 0.0911 \)), changes.
Discussion

The present study demonstrated that the circulating level of soluble TM, expressed as the TM/Cr ratio, was higher in hypertensive patients than in healthy normotensive control subjects. This implies a positive correlation between the serum TM level and the risk of atherosclerotic disease. Since the TM/Cr ratio was positively correlated with the grade of hypertensive retinopathy (sclerotic changes) and baPWV in hypertensive patients, the TM/Cr ratio may be useful as a sensitive molecular marker of the progression of atherosclerosis.

In a previous study using cultured human umbilical vein endothelial cells, it was indicated that soluble TM released into the culture medium was not due to secretion from endothelial cells but was derived from injured cells, and that the increase in the TM level reflected an increase in cell damage (6). Therefore, the circulating TM levels in healthy adults may reflect TM released during the physiological turnover of endothelial cells in the vascular bed and an increase in the TM level may reflect an increase in pathological damage to the cells (6). Thus, it is possible that the increased TM/Cr ratio reflects endothelial damage in hypertensive patients. In fact, endothelium-dependent relaxations have shown to be impaired in hypertensive animals and in humans with essential hypertension (13–15). However, it has been reported that circulating TM levels were not related to an endothelium-dependent increase in forearm blood flow induced by acetylcholine in patients with hypercholesterolaemia (24). Impaired endothelium-dependent vasodilation is an early sign of developing atherosclerosis (16) and in such early stages, changes in the TM level may be minimal. Indeed, in the above-mentioned study, the TM level was the same in patients with hypercholesterolaemia and control subjects (24). Thus, the increased TM/Cr ratio in the hypertensive patients in the current study may indicate progressive endothelial damage or latent atherosclerosis. This concept is reinforced by the positive correlation between the TM/Cr ratio and the grade of sclerotic changes in the fundus oculi. To confirm this concept, we assessed arterial stiffness using baPWV in hypertensive patients. PWV is an indicator of arterial stiffness that is an important marker of vascular damage (25) and independent predictor of cardiovascular mortality (26, 27). A recent study has shown that arterial stiffness assessed by PWV is strongly associated with atherosclerosis at various sites in the vascular tree (28). Thus, in the present study, the correlation between the TM/Cr ratio and baPWV suggests that the TM/Cr ratio is associated with the latent progression of atherosclerosis in hypertensive patients. The increase in the TM/Cr ratio in hypertensive patients may not indicate a direct and causative relationship between high blood pressure and a high TM/Cr ratio, although the TM/Cr ratio was correlated with systolic blood pressure when data from all subjects were used for analysis. In fact, in hypertensive patients, the ratio did not correlate either with blood pressure or with the grade of hypertensive changes in the fundus oculi (Scheie’s classification). Furthermore, higher serum lipid levels may not contribute to the increase in the TM/Cr ratio in hypertensive patients, because the lipid levels did not correlate with the ratio.

The present results are in agreement with those of some previous reports (8–10), but conflict with those of a study investigating serum TM levels in patients with peripheral artery disease (12). The patients enrolled in this study did not exhibit any cardiovascular diseases associated with hypertension, such as coronary, cerebral or peripheral artery diseases. Thus, atherosclerosis was not clinically evident in our patients, although it might have been present and latent. This may explain, in part, the apparent disagreement between the results of the present study and those of the report mentioned above. The findings of genetic studies indicate that impaired expression of TM or decreased TM function could contribute to the risk of myocardial infarction (29, 30). Furthermore, it has recently been reported that a high concentration of soluble TM may be associated with a decreased risk of coronary heart disease in subjects who do not have cardiovascular disease, including hypertension (31). In healthy people, the concentration of soluble TM, which is determined by TM gene polymorphisms, reflects the quantity of TM expressed on the endothelial surface and increased expression of TM may raise the concentrations of soluble TM in the circulation. Thus, a high concentration of soluble TM may indicate a low prothrombotic state and low risk of coronary heart disease (31). On the other hand, the concentration of soluble TM is also affected by increased proteolytic activity that results in the cleavage of soluble TM from endothelial TM. The proteolytic enzymes are thought to be released from activated white blood cells (4, 31). Thus, in some pathological states, the soluble TM concentration may reflect the degree of vas-
cular damage and associated inflammation rather than the expression level of TM. Indeed, in hypertensive patients, TM levels were higher (19) or tended to be higher (18) than in normotensive subjects.

In conclusion, the results of the present study demonstrated that the TM/Cr ratio is higher in patients with hypertension than in healthy normotensive control subjects. Since the TM/Cr ratio was positively correlated with the grade of sclerotic changes in the fundus oculi and baPWV of hypertensive patients, it may serve as a useful sensitive molecular marker of the latent progression of atherosclerosis in hypertensive patients.

References