Effects of Aging on the Coagulation Fibrinolytic System in Outpatients of the Cardiovascular Department

Akinori Ochi, MD; Taro Adachi, MD, PhD; Koichiro Inokuchi, MD; Ko Ogawa, MD; Yuya Nakamura, MD; Yuta Chiba, MD, PhD; Shiro Kawasaki, MD, PhD; Yoshimi Onishi, MD, PhD; Yoshimasa Onuma, MD; Yumi Munetsugu, MD; Hiroyuki Ito, MD, PhD; Tatsuya Onuki, MD, PhD; Yoshino Minoura, MD, PhD; Norikazu Watanabe, MD, PhD; Mitsuharu Kawamura, MD, PhD; Taku Asano, MD, PhD; Youichi Kobayashi, MD, PhD

**Background:** Although clinical trials demonstrate that the elderly with atrial fibrillation have risks of thrombosis and bleeding, the relationship between aging and coagulation fibrinolytic system in "real-world" cardiology outpatients is uncertain.

**Methods and Results:** We retrospectively evaluated 773 patients (mean age: 58 years; 52% men; Asian ethnicity). To thoroughly investigate markers of coagulation and fibrinolysis, we simultaneously measured levels of D-dimer, prothrombin-fragment1+2 (F1+2), plasmin-α2 plasmin inhibitor complex (PIC), and thrombomodulin (TM). There were correlations between aging and levels of F1+2, D-dimer, PIC, and TM (R=0.61, 0.57, 0.49, and 0.30, respectively). We compared 3 age groups, which were defined as the Y group (<64 years), M group (65–74 years), and the O group (>75 years). Levels of markers were higher in older individuals (D-dimer: 1.0±0.8 vs. 0.8±0.8 vs. 0.6±0.4 μg/ml, F1+2: 281.8±151.3 vs. 224.6±107.1 vs. 155.5±90.0 pmol/L, PIC: 0.9±0.3 vs. 0.8±0.3 vs. 0.6±0.5 μg/ml, and TM: 2.9±0.8 vs. 2.7±0.7 vs. 2.5±0.7 FU/ml). We performed logistic regression analysis to determine F1+2 and PIC levels. Multivariate analysis revealed that aging was the most important determinant of high F1+2 and PIC levels.

**Conclusions:** Hypercoagulable states develop with advancing age in "real-world" cardiology outpatients. *(Circ J 2016; 80: 2133–2140)*

**Key Words:** Aging; Endothelial dysfunction; Fibrinolysis; Thrombosis

Thrombosis is an increasingly serious problem within a rapidly aging society. It is known that the prevalence of atrial fibrillation (AF) increases with aging. Aging is a risk factor for cardiogenic brain embolisms in patients with AF. AF increases the risk of ischemic stroke, systemic embolism, and death. It is also known that the elderly might be susceptible to hypercoagulable states. Some clinical trials have demonstrated that the elderly with AF have an increased risk of thrombosis and bleeding during anticoagulation therapy.

Vitamin K antagonists have been used for many years to prevent stroke. Warfarin has target values in anticoagulation therapy, which are measured by PT-INR. Furthermore, it has been reported that warfarin could have beneficial effects in very old patients with AF.

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Recently, direct oral anticoagulants (DOACs) have been used instead of warfarin to prevent stroke. Although the safety and efficacy of DOACs were confirmed in clinical trials, the biochemical indicators of coagulation are not defined. Although many studies of age-dependent changes in the markers of coagulation and fibrinolysis derived from healthy population-based settings have been reported, the relationship between aging and coagulation fibrinolytic system in "real-world" cardiology outpatients remains uncertain.

The aim of the present study was to evaluate the relationships between aging and biochemical markers of coagulation, fibrinolysis, and vascular endothelial function in "real-world" outpatients of a cardiovascular department and to consider the
appropriate use of anticoagulants in the elderly.

### Methods

**Patients**

The design of this study was a single-center, cross-sectional study. Patients were recruited from Showa University Hospital, Tokyo, from June 2011 to June 2014. In total, 1,011 outpatients of the cardiovascular department were enrolled but emergency patients were not included. All patients had some indication for an internal medicine consultation. We defined them as “real-world” outpatients. The inclusion criterion of the study was that patients should be stringently evaluated for markers of coagulation, fibrinolysis, and vascular endothelial function. Reasons for exclusion were the presence of AF or deep venous thrombosis, a condition for which anticoagulants are likely to have been prescribed for some time, or renal dysfunction, which was defined as creatinine clearance (CCr) level <50 ml/min. The CCr was evaluated using the Cockcroft-Gault formula: CCr (ml/min)=[(140-age)× body weight (kg)×(0.85 if female)]/[72×serum creatinine (mg/dl)], which is the most widely used estimator of renal function. Anthropometric measurements such as height and weight were recorded at the time of visit. We did not exclude patients on antiplatelet medication in order to reflect “real-world” cardiology outpatients affected by cardiovascular disease. After application of the exclusion criteria, 773 patients were divided into 3 age groups based on CHA2DS2-VASc score: Y group (<64 years of age), M group (65–74 years), and O group (>75 years). CHADS2 and CHA2DS2-VASc scores were calculated to assess the potential state of hypercoagulation. CHADS2 score was constructed by assigning 1 point each for the presence of congestive heart failure, hypertension, age ≥75 years, and diabetes mellitus (DM); 2 points were assigned for history of stroke or transient ischemic attack (TIA). CHA2DS2-VASc score was constructed by assigning 1 point for age ≥65 years, female sex, and vascular diseases, while 2 points were assigned for history of stroke or TIA and age ≥75 years. Clinical data of the patients, including underlying diseases, medication, and laboratory data, were collected from their medical records by 2 cardiologists. Approval for the study was obtained from the Institutional Review Board of the University of Showa, Tokyo, Japan.

**Blood Sampling and Assays**

An experienced nurse or a cardiologist performed blood sampling from the antecubital veins using a 21–23-gauge needle after overnight fast. Blood sampling was conducted in the hospital laboratory and samples were analyzed as soon as possible.

To thoroughly investigate coagulation disorders, we simultaneously measured plasma levels of D-dimer, prothrombin fragment 1+2 (F1+2), thrombin–antithrombin complex (TAT), plasminogen-α2 plasmin inhibitor complex (PIC), total plasminogen activator inhibitor-1 (tPAI-1), and thrombomodulin (TM). These markers are generally measured in patients with disseminated intravascular coagulation syndrome to evaluate conditions. D-dimer originates from the formation and dissolution of cross-linked fibrin and reflects the activation of coagulation and fibrinolysis. F1+2 is known to be a reliable index of prothrombin conversion to thrombin by activated factor X. Thrombin reacts with its inhibitor, antithrombin, and generates TAT. F1+2 and TAT are thought to be markers of coagulation activity. Alpha 2 antiplasmin is known to be a direct inhibitor of plasmin, which results in the production of PIC, a benchmark of plasmin formation and neutralization.

Venous blood samples were collected in plastic tubes containing 3.2% sodium citrate. The levels of D-dimer, PIC, and tPAI-1 were measured using the latex agglutination test (JCA-BM9130, JEOL Ltd, Tokyo, Japan). The levels of F1+2 and TAT were measured using an enzyme-linked immunoassay (ELISA) kit (BEPIII, Siemens Healthcare, Tokyo, Japan). The levels of TM were also measured using an ELISA kit, AP-X (Kyuowa Medex Co Ltd, Tokyo, Japan). After collection of blood samples, all plasma specimens were centrifuged for 15 min at 1,500 g.

**Statistical Analysis**

All values are expressed as the mean±standard deviation (SD), median value, 25–75th percentile, and number (n, %). As values and groups were not normally distributed, correlation analysis was performed by Spearman’s rank correlation. We used the Kruskal-Wallis test to compare the average of continuous variables and chi-square tests to compare categorical data between groups. Furthermore, we used the Steel-Dwass test to compare the 3 age groups with blood markers.
Results

Clinical Characteristics of Patients

The clinical characteristics of the patients who were enrolled in this study are shown in Table 1. The mean age of the patients was 58.6±16.8 years (median, 62 years; range, 10–98 years) and 52% were men. The mean height and body weight were 161.8 cm and 61.9 kg, respectively. Physique was quite normal for Japanese ethnicity according to the National Health and Nutrition Survey in Japan 2013. More than half of patients had hypertension (52%). An angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker (n=251, 33%) was most often prescribed for patients with hypertension followed by a calcium-channel blocker (n=206, 27%). Several patients (16%) had a history of DM. As the outpatients were from a cardiovascular department, the proportion of patients who had a history of cardiovascular events was relatively high at 19%. A total of 20% of patients were taking antiplatelet medication. As we excluded patients with AF or those taking anticoagulants, there were few patients who had a history of stroke or TIA (1.0%). The average CHADS2 and CHA2DS2-VASc scores were 0.90±0.95 points (median, 1; range, 0–5 points) and 1.84±1.65 points (median, 2; range, 0–6), respectively. The average HAS-BLED score was 0.85±0.96 points (median, 1; range, 0–4).

The clinical characteristics of the patients divided into the 3 age groups are shown in Table 2. The mean age of the Y, M, and O groups was 47.0±13.2 years (median age, 50 years), 69.3±2.8 years (median age, 70 years), and 79.6±4.0 years (median age, 79 years), respectively. With respect to creatinine clearance, the O group was lower than the M and Y groups (65.4±12.1 ml/min; 76.3±17.8 ml/min; 109.6±31.4 ml/min, P<0.0001). With respect to a history of hypertension, it was significantly higher in the O and M groups than in the Y group (78%; 66%; 38%, respectively). The prevalences of DM and vascular disease were similar. As aging is a component of the CHADS2, CHA2DS2-VASc, and HAS-BLED scores, the scores were higher in those who were older.

Comparison Between Blood Markers and Aging

Figure 1 is a scatter diagram of aging and blood markers. Logistic regression analysis revealed a positive correlation between aging and levels of D-dimer, F1+2, PIC, and fibrinogen by Spearman’s rank correlation (R=0.57, R=0.61, R=0.49, R=0.45, P<0.001, respectively). Furthermore, there was a positive correlation between aging and the levels of TM, which is thought to be a benchmark of vascular endothelial dysfunction (R=0.31, P<0.001). In contrast, there was no correlation between aging and levels of TAT and tPAI-1 (R=0.17, R=0.16, R=−0.02, respectively). Comparisons between the 3 age groups and markers are shown in Table 3 and Figure 2. D-dimer levels were signifi-
In the O group, the F1+2 levels of 75 patients (58.1%) were above the normal range (69–229 pmol/L). No F1+2 levels in the O group were below the normal range.

For PIC levels, the O and M groups were significantly higher than the Y group (0.9±0.3 μg/ml; 0.8±0.3 μg/ml; 0.6±0.5 μg/ml, P<0.001, respectively).

In the O group, the F1+2 levels of 75 patients (58.1%) were above the normal range (69–229 pmol/L). No F1+2 levels in the O group were below the normal range. For PIC levels, the O and M groups were significantly higher than the Y group (0.9±0.3 μg/ml; 0.8±0.3 μg/ml; 0.6±0.5 μg/ml, P<0.001, respectively).

![Figure 1. Scatter diagrams of aging and 4 blood markers. Correlation analysis was performed by Spearman's rank correlation according to all patients, male, and female. F1+2, prothrombin-fragment 1+2; PIC, plasmin-α2 plasmin inhibitor complex.](image)

**Table 3. Comparison Between Age and Levels of Blood Markers**

<table>
<thead>
<tr>
<th></th>
<th>Y group (≤64 years)</th>
<th>M group (65–74 years)</th>
<th>O group (≥75 years)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Number (n=773)</td>
<td>432 (56%)</td>
<td>212 (27%)</td>
<td>129 (17%)</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>285.9±60.6</td>
<td>277 (244.8–315.3)</td>
<td>325.7±63.4</td>
<td>311 (284–360)</td>
</tr>
<tr>
<td>D-dimer (μg/ml)</td>
<td>0.6±0.4</td>
<td>0.5 (0.4–0.6)</td>
<td>0.8±0.8</td>
<td>0.7 (0.6–0.9)</td>
</tr>
<tr>
<td>TAT (ng/ml)</td>
<td>2.3±2.7</td>
<td>1.9 (1.4–2.6)</td>
<td>2.7±3.8</td>
<td>2.2 (1.6–2.9)</td>
</tr>
<tr>
<td>F1+2 (pmol/L)</td>
<td>155.5±90.0</td>
<td>141 (109–177)</td>
<td>224.6±107.1</td>
<td>200 (156–262)</td>
</tr>
<tr>
<td>PIC (μg/ml)</td>
<td>0.6±0.5</td>
<td>0.6 (0.4–0.7)</td>
<td>0.8±0.3</td>
<td>0.7 (0.6–0.9)</td>
</tr>
<tr>
<td>TM (FU/ml)</td>
<td>2.5±0.7</td>
<td>2.4 (2.1–2.8)</td>
<td>2.7±0.7</td>
<td>2.7 (2.3–3.1)</td>
</tr>
<tr>
<td>tPAI-1 (ng/ml)</td>
<td>23.8±24.3</td>
<td>17 (10–28)</td>
<td>23.3±19.7</td>
<td>17 (10–25.5)</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test was used to compare continuous variables between groups. Data are presented as mean±SD or median (range from 25th to 75th percentile). F1+2, prothrombin-fragment 1+2; PIC, plasmin-α2 plasmin inhibitor complex; TAT, thrombin antithrombin complex; TM, thrombomodulin; tPAI-1, plasminogen activator inhibitor-1. Normal range: D-dimer ≤1.0 (μg/ml); F1+2 69–229 (pmol/L), fibrinogen 200–400 (mg/dl); PIC ≤0.8 (μg/ml); TAT 1.0–4.1 (ng/ml); TM (male) 2.1–4.1, (female) 1.8–3.9 (FU/ml); tPAI-1 ≤50 (ng/ml).
Aging Activates Hypercoagulable States

There were no significant differences in the markers of coagulation and fibrinolysis between male and female patients. Next, we investigated the correlation between blood markers and TM levels. There was correlation between D-dimer, F1+2, and PIC levels (R=0.28, R=0.37, P<0.001, respectively). There was no correlation between the levels of TAT and PIC and those of TM.

In the O group, the PIC levels of 71 patients (55.0%) were above the normal range (≤0.8 μg/ml). There was a correlation between F1+2 and D-dimer levels, and between F1+2 and PIC levels according to Spearman’s rank correlation (R=0.60, R=0.38, P<0.001, respectively). Furthermore, for TM levels, the O and M groups were significantly higher than the Y group (2.9±0.8 FU/ml; 2.7±0.7 FU/ml; 2.5±0.7 FU/ml, P<0.001, respectively). As shown in Figure 2, there were no significant differences in the markers of coagulation and fibrinolysis between male and female patients.

Next, we investigated the correlation between blood markers and TM levels. There was correlation between D-dimer, F1+2, and TM levels (R=0.28, R=0.37, P<0.001, respectively). There was no correlation between the levels of TAT and PIC and those of TM.

Figure 2. Comparison among the 3 age groups and 4 blood markers. Steel-Dwass test was used to compare each group. F1+2, prothrombin-fragment1+2; PIC, plasmin-α2 plasmin inhibitor complex; TAT, thrombin-antithrombin complex.

Table 4. Determinants of F1+2 Level (≥230 pmol/L) by Logistic Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>Univariate logistic regression</th>
<th>Multivariate logistic regression</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>CHF (n=47)</td>
<td>2.300 (1.252–4.180)</td>
<td>0.0077</td>
</tr>
<tr>
<td>Hypertension (n=404)</td>
<td>3.019 (2.143–4.297)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age ≥75 years (n=128)</td>
<td>7.182 (4.769–10.940)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age 65–74 years (n=212)</td>
<td>2.005 (1.421–2.824)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM (n=121)</td>
<td>2.668 (1.778–3.997)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke/TIA (n=8)</td>
<td>4.558 (1.108–22.381)</td>
<td>0.036</td>
</tr>
<tr>
<td>Vascular disease (n=143)</td>
<td>3.007 (2.053–4.404)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex category (female) (n=374)</td>
<td>1.050 (0.762–1.449)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Data are presented as OR (95% CI). Logistic regression models were applied to assess the determinants of F1+2 level. CI, confidence interval; OR, odds ratio. Other abbreviations as in Tables 1,3.
Determinants of F1+2 and PIC Levels

It seems natural that the prevalence of underlying disease would increase with age among outpatients of a cardiovascular department. Therefore, because of the differences among the 3 age groups for underlying diseases, we speculated that factors other than aging might have an influence on coagulation markers. We attempted to identify the determinants of coagulant and fibrinolytic markers from among the components of the CHA2DS2-VASc score. As there were strong correlations between aging and the levels of F1+2 and PIC, we selected the F1+2 levels as a coagulation activation marker and those of PIC as a fibrinolytic marker. F1+2 is a highly sensitive marker of thrombin generation and directly reflects formation of thrombin while PIC reflects fibrinolysis activity.

We performed logistic regression analysis to investigate the determinants of high F1+2 and PIC levels. As previous studies have not reported cut-off values for F1+2 and PIC levels, we set these at ≥230 (pmol/L) and ≥0.8 (pmol/L), respectively, according to the value of the upper limit of their normal ranges.

Table 4 shows the determinants of high F1+2 levels by logistic regression analysis. Univariate analysis revealed that CHF, hypertension, age ≥75 years and age 65–74 years, DM, stroke/TIA and vascular disease were significant determinants of high F1+2 levels. Hypertension, age ≥75 years, age 65–74 years, stroke/TIA, and vascular disease were selected as target variables for the multivariate analysis using the stepwise method. Multivariate analysis including these components revealed that age ≥75 years, age 65–74 years, vascular disease, and hypertension were independent determinants of high F1+2 levels. Table 5 shows the determinants of high PIC levels by logistic regression analysis. Univariate analysis revealed that CHF, hypertension, age ≥75 years, age 65–74 years, DM, stroke/TIA, and vascular disease were significant determinants of high PIC levels. We selected CHF, age ≥75 years, age 65–74 years, DM, stroke/TIA, and sex (female) for the multivariate analysis using the stepwise method. Multivariate analysis including these components revealed that age ≥75 years, age 65–74 years, and DM were independent determinants of high PIC levels.

Discussion

We believe that “real-world” studies of age-dependent changes in the number of markers of coagulation and fibrinolysis have not been reported. The major finding of the present study was the close relationship between aging and markers of coagulation, fibrinolysis, and vascular endothelial function in “real-world” outpatients of a cardiovascular department. Furthermore, vascular endothelial dysfunction might affect these results. In this study, there was a correlation between aging and the levels of F1+2, D-dimer, and PIC. In contrast, there was no correlation between aging and the levels of TAT. In addition, there were no sex-associated differences in the markers of coagulation and fibrinolysis. There was also a correlation between the levels of TM and those of F1+2 and D-dimer. When examining the determinants of high F1+2 and PIC levels, being older than 75 years was the most important factor among the components of the CHA2DS2-VASc score.

It has been previously reported that the levels of several coagulation factors, namely fibrinogen, factor VII, factor VIII and factor IX, increase with age. Furthermore, it has also been reported in studies of healthy populations that levels of coagulation activation markers, such as factor VIIa, factor Xa, factor IXa, F1+2, and TAT, increase with age. With respect to markers of fibrinolysis, D-dimer and PIC have also been shown to be elevated in an aging healthy population. In the present study, markers of coagulation and fibrinolysis increased with age in “real-world” cardiovascular outpatients. There was a positive correlation between the coagulation marker (F1+2) and fibrinolytic markers (D-dimer and PIC). Therefore, coagulation markers and fibrinolytic markers are activated in the elderly. Using these biochemical markers, we could corroborate clinical trial data that the risks of thrombosis and bleeding increase with advancing age. Moreover, we would like to emphasize the fact that the mean levels of D-dimer, F1+2, and PIC exceeded the upper limit of the normal range in those aged over 75 years. We believe these findings are novel. Although we often measure the levels of D-dimer to assess the prothrombotic state, the levels of F1+2 and PIC often exceed the normal range, particularly in those aged >75 years, while the levels of D-dimer are in the normal range.

In the present study, the levels of coagulant and fibrinolytic markers were also elevated with aging, as supported by findings from a previous study. Contrary to our expectations, the levels of TAT were not consistent with those in a previous report, which showed that the levels of TAT did not correlate with aging. These discrepant findings indicate that the levels of TAT are often unstable, which is biologically plausible because TAT levels are affected by several factors. We think there are 2 main reasons why TAT levels are unstable. The first is that there are 2 pathways that metabolize thrombin. The first pathway involves antithrombin combining with thrombin and generating TAT, while the other is a TM-protein C-protein S pathway. If the vascular endothelial function is impaired, the complexes of thrombin–antithrombin and TAT–TM are unstable, and this instability might affect TAT levels.

Table 5. Determinants of PIC Level (≥0.8 pmol/L) by Logistic Regression Analysis

<table>
<thead>
<tr>
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<th>Multivariate logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>CHF (n=47)</td>
<td>2.092 (1.149–3.844)</td>
<td>0.016</td>
</tr>
<tr>
<td>Hypertension (n=404)</td>
<td>1.858 (1.376–2.517)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age ≥75 years (n=128)</td>
<td>4.174 (2.799–6.304)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age 65–74 years (n=212)</td>
<td>1.993 (1.440–2.761)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM (n=121)</td>
<td>2.326 (1.562–3.477)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke/TIA (n=8)</td>
<td>5.113 (1.169–35.052)</td>
<td>0.030</td>
</tr>
<tr>
<td>Vascular disease (n=143)</td>
<td>1.769 (1.220–2.564)</td>
<td>0.0027</td>
</tr>
<tr>
<td>Sex category (female) (n=374)</td>
<td>1.139 (0.790–1.640)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Data are presented as OR (95% CI). Logistic regression models were applied to assess the determinants of PIC level. Abbreviations as in Tables 1, 3, 4.
normal, TM plays a significant role in anticoagulation. On the other hand, if vascular endothelial function is damaged and the expression of TM in the vascular endothelium will be down-regulated, and antithrombin plays an important role in natural anticoagulation. The second reason for TAT levels being unstable is that levels of coagulation markers are strongly influenced by sampling method, particularly TAT. 16,17

In the present study, there were no significant sex-associated differences in the markers of coagulation and fibrinolysis among this group of outpatients of a cardiovascular department. This result might support the subanalysis of the J-RHYTHM registry indicating that female sex is not a risk factor for thromboembolic events among Japanese patients with AF. 18

The mechanism for elevated coagulation markers in the elderly has not been elucidated. We demonstrated that levels of circulating TM also increased with aging, which suggests decreased TM levels in the vascular endothelium. 19 In terms of endothelial dysfunction, a previous study reported a relationship between TM and a number of cardiac diseases. For example, it has been reported that gene expression of TM decreased in the endocardium of rapidly paced rat atria 20 and levels of TM increased in patients with persistent AF. 21 In addition, it has been reported that levels of TM are associated with an increased risk of myocardial infarction and cerebral infarction. 22,23 Thus, the level of TM may be regarded as a biomarker of endothelial function. In the present study, there was a positive correlation between the levels of TM and those of F1+2 and D-dimer, which means that activation of coagulation might originate from vascular endothelial disorders. Research of the present study into the determinants of the levels of high F1+2 and PIC among the components of the CHA2DS2-VASC score demonstrated that both coagulation and fibrinolysis were activated in individuals aged over 65 years. The odds ratio of age 75 years and age 65–74 years was predominantly higher compared with other factors. From these results, it may be that aging was exaggerated as a significant variable based on the CHADS2 and CHA2DS2-VASC scores with respect to the aforementioned biochemical markers.

Many clinical studies have reported that serological analysis of coagulation markers is effective for the assessment of thrombosis. For example, D-dimer measurement is a recognized useful tool for the prediction of venous thromboembolism (VTE) and VTE recurrence. 24 In addition, it has been reported that elevation of D-dimer levels can predict thromboembolic and cardiovascular events in patients with AF during anticoagulation therapy. 25 On the other hand, a study of healthy centenarians reported that high plasma levels of coagulation activation markers in this older population did not necessarily mirror a high risk of thrombosis. 26 Although markers of coagulation, fibrinolysis, and vascular endothelial function were activated with aging in the present cohort of “real-world” cardiology outpatients, we should evaluate the results of serological analysis of markers in aged patients with caution. Therefore, it might be effective to evaluate markers of coagulation, fibrinolysis, and endothelial function simultaneously for appropriate use of DOACs in the elderly.

Study Limitations

Although this study provided important insights into the correlation between aging and biochemical markers of coagulation and fibrinolysis in cardiovascular outpatients, we have to emphasize the shortcomings of our study. It was a single-center, cross-sectional study and selection bias cannot be ignored. Furthermore, the size of the study population was not large enough. With regards to blood collection method, we could not integrate the gauge of needle or take seasonal variation into consideration. It has previously been reported that high plasma fibrinogen concentrations are observed during the coldest months. 27 As for the selection of patients, we intended to investigate “real-world” cardiovascular outpatients as naturally as possible. Consequently, we did not exclude concomitant use of antiplatelet medication, which may have significantly influenced the aforementioned markers of coagulation and fibrinolysis. One of the important limitations of the study is the underlying diseases of the patients. In order to evaluate the factors influencing the markers of coagulation and fibrinolysis, we performed logistic regression analyses using models for the components of CHA2DS2-VASC score because this type of scoring is used globally. Although renal insufficiency is not included in the CHA2DS2-VASC score, it has been previously reported that markers of coagulation can be affected by renal dysfunction. 22 With respect to research into the mechanism behind elevation of coagulation markers, endothelial dysfunction might be a factor. However, we could not directly evaluate endothelial function in individual subjects based on our fundamental experiment. For example, previous studies have reported that reduction in endothelial nitric oxide synthase expression might be the main mechanism of left atrial thrombus formation in a unique rat model. 28 Further in an attempt to explore the mechanism, we chose TM as a marker of endothelial dysfunction. Though many studies have reported that elevated levels of TM might reflect vascular endothelial dysfunction, physiological analysis, such as flow-mediated dilation or ankle-brachial index, and carotid ultrasonography might be more effective in understanding the correlation between a prothrombotic state and vascular endothelial dysfunction. 29 Furthermore, we could not take into account activation of protein C, which binds to TM. 13 Further investigations such as prospective studies are needed to reveal the mechanism behind the elevation of coagulation markers in aged patients.

In conclusion, our study showed a close relationship between aging and blood markers of coagulation and fibrinolysis systems in “real-world” cardiovascular outpatients. Markers of coagulation and fibrinolysis significantly correlated with aging in this study. It is thought that vascular endothelial dysfunction might increase coagulation activity.

Acknowledgments

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