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Gender Is a Significant Factor Affecting Blood Coagulation Systems

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Abstract: The risk of cardiogenic cerebral infarction is quantified by the CHA_2DS_2-VASc score in patients with atrial fibrillation, with female gender shown to be one of the risk factors. However, the relationships between gender and blood coagulation markers have not been investigated. Thus, the aim of the present study was to investigate relationships between gender and the coagulation and fibrinolysis systems. In the present study, 1025 patients (517 females [F group], 508 males [M group]) who visited the outpatient clinic and had markers of the fibrinolytic and coagulation systems measured at the Division of Cardiology of Showa University Hospital from June 2011 to June 2014 were evaluated retrospectively. Thrombomodulin (TM), prothrombin fragment 1+2 (PTF 1+2), thrombin-antithrombin complex (TAT), plasmin-α_2-plasmin inhibitor complex (PIC), and D-dimer levels were analyzed. Furthermore, patients without diabetes mellitus and vascular disease were divided into two groups according to age: a younger (Y) group (< 75 years) and an elderly (E) group (≥ 75 years). In the Y group, TM levels were significantly lower in the F than M group ($P < 0.0001$), but in the E group there was no significant difference in TM levels between these two groups. PTF 1+2 levels were significantly higher in the F group for each age group (Y group, $P = 0.0426$; E group, $P = 0.0214$). In the Y group, PIC levels were significantly higher in the F than M group ($P = 0.0015$), but there was no difference in PIC levels between the F and M groups in the E group. Thus, in the F group, vascular endothelial dysfunction progressed in the E group. These observations suggest that the coagulation system is relatively accelerated, without any acceleration in the fibrinolytic system, in the F group with aging. The present study has shown that, in outpatients of a cardiovascular department, gender is a significant factor affecting blood coagulation systems.

Key words: females, endothelial function, coagulation system, fibrinolytic system, thrombosis

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Introduction

The $CHADS_2$ score is widely used to assess the risk of stroke from atrial fibrillation. One point is added for each of the factors of heart failure, hypertension, age ≥ 75 years, and diabetes mellitus. In addition, 2 points are added for a history of stroke or transient ischemic attack. An assessment is made based on the total score for all five items. The $CHADS_2$ score ranges from 0 to 6 points, and the incidence of stroke is known to increase with a higher score[1].

However, the risk of a stroke over 1 year is 1.9% even in patients with a $CHADS_2$ score of 0 and 2.8% in patients with a $CHADS_2$ score of 1[1]. Even though this risk is low, it exceeds the incidence of strokes in the general population[2]. To stratify low-risk patients with $CHADS_2$ scores of 0 or 1, the CHA_2DS_2-VASc score has been proposed[3].

The CHA_2DS_2-VASc score considers the history of vascular disease (VD), including a myocardial infarction, peripheral arterial disease, and aortic plaque, as well as age category (with 1 point added for age 65–74 years and 2 points added for age ≥ 75 years) and gender (1 point added for female gender), in addition to the $CHADS_2$ score. The CHA_2DS_2-VASc score assesses the risk of stroke with scores ranging from 0 to 9. With a CHA_2DS_2-VASc score of 0, the incidence of stroke over 1 year is 0%, but it rises to 1.3% with a score of 1 and to 2.2% with a score of 2. Therefore, the Japanese atrial fibrillation guidelines[4] recommend anticoagulant therapy for patients with $CHADS_2$ scores of 1 or higher. It has also been proposed that anticoagulant therapy be considered in patients with a history of VD or age 65–74 years, which corresponds to a CHA_2DS_2-VASc score of 1. Coagulation and fibrinolysis factors and vascular endothelial damage are also reported to be associated with the risk of stroke[5].

In the present study, the blood coagulation factors thrombomodulin (TM), prothrombin fragment 1+2 (PTF 1+2), thrombin-antithrombin complex (TAT), plasmi-α_2-plasmin inhibitor complex (PIC), and D-dimer were evaluated in cardiovascular department outpatients. The effects of gender on the coagulation system among the CHA_2DS_2-VASc score items were also evaluated.

Methods

The present cross-sectional study was performed on 1346 patients who visited the outpatient clinic and had markers of the fibrinolytic and coagulation systems measured at the Division of Cardiology of Showa University Hospital between June 2011 and June 2014. Blood samples were checked to evaluate the degree of arteriosclerosis, endothelial function, and the condition of the coagulation and fibrinolytic systems. In the present study, TM, TAT, PTF 1+2, PIC, and D-dimer were analyzed.

TM is expressed on the surface of endothelial cells and serves as a cofactor for thrombin. TM binds to thrombin reversibly, deactivating clotting activity. It reduces blood coagulation by converting thrombin to an anticoagulant enzyme from a procoagulant enzyme. Because TM is expressed primarily on the surface of endothelial cells, when a patient has an endothelial disorder TM on the membrane goes into the blood. Thus, serum TM (sTM) concentrations are used as markers of endothelial function.

TAT and PTF 1+2 were used as markers of the coagulation system. Thrombin is a key enzyme of the coagulation cascade that binds antithrombin, and TAT is affected not only by the amount of thrombin, but also by TM located on the endothelium. When thrombin is generated from prothrombin by Factor Xa, a PTF 1+2 peptide is released from prothrombin. Thus, PTF 1+2 more accurately reflects the amount of thrombin than TAT, and PTF 1+2 is also a marker of the coagulation system.

PIC is a marker of the fibrinolytic system. Plasmin is a key enzyme of the fibrinolytic system. The α_2-plasmin inhibitor is a neutralizing agent for plasmin. Plasmin combines with the α_2-plasmin inhibitor and converts it to PIC. Thus, PIC is a marker of the fibrinolytic system.

Venous blood samples were collected in plastic tubes with 3.2% sodium citrate. The blood samples were obtained in the hospital laboratory, and the samples were analyzed as soon as possible. Levels of TM were measured using a commercially available ELISA kit (AP-X; Kyowa Medex, Tokyo, Japan). Levels of PTF 1+2 and TAT were also measured using a commercially available ELISA kit (BEP Ⅲ; Siemens Health Care, Tokyo, Japan). Levels of PIC and D-dimer were measured using a latex agglutination test (JCA-BM9130; JEOL, Tokyo, Japan).

A medical history, including internal medical details about medical treatment history and physical examination, was obtained for each subject from the medical records and medical interviews. All clinical data, including laboratory data, underlying diseases, and medications, were collected from the patients' medical records by two cardiologists. The patients were then divided into different groups. All patients were evaluated using the $CHADS_2$, CHA_2DS_2-VASc, and HAS-BLED scores, whether or not they had atrial fibrillation.

Patients were defined as having congestive heart failure (CHF) if they had been hospitalized for it. Patients were defined as having hypertension if they were taking antihypertensive drugs. Patients with diabetes mellitus were defined as those taking antidiabetic drugs or whose serum HbA1c levels were greater than 6.4 % [NGSP; if patient HbA1c values were given according to the Japan Diabetes Society (JDS), values were converted to NGSP values using the formula NGSP HbA1c = JDS HbA1c × 1.02 + 0.25]. Patients with VD were defined as those who had a prior myocardial infarction, arteriosclerosis obliterans, or aortic plaque. Patients were defined as having a stroke or transient ischemic attack (TIA) if they had been treated for a stroke or TIA in the past.

Patients were divided into two groups according to gender, an M group (male patients) and an F group (female patients). Levels of TM, TAT, PTF 1+2, PIC, and D-dimer were compared between the groups.

Exclusion criteria

Patients with atrial fibrillation, on anticoagulants, with kidney disease (serum creatinine ≥ 1.3 mg / dl, because the estimated glomerular filtration rate of most patients with serum creatinine ≥ 1.3 mg / dl is less than 30 ml / min per 1.73 m^2), severe infections (e.g. disseminated intravascular coagulation), and those recovering from acute diseases that could affect the fibrinolytic and coagulation systems after resuscitation were excluded from the study. This study was approved

by the Institutional Review Board of Showa University, Tokyo, Japan.

Statistical analysis

Continuous variables are presented as the mean ± SD, whereas qualitative variables are presented as percentages. The Shapiro-Wilk W-test was used to evaluate the distributions of TM, TAT, PTF 1+2, PIC, and D-dimer, and none of these exhibited a normal distribution. Because of their skewed distribution, TM, TAT, PTF 1+2, PIC, and D-dimer values were analyzed by the Wilcoxon rank-sum test. $P < 0.05$ was considered significant. Statistical analyses were performed using JMP software (Version pro 12 ; SAS Institute, Cary, NC, USA).

Results

All patients

The clinical characteristics of all patients at baseline are given in Table 1. In all, 321 patients were excluded from the study based on the exclusion criteria ; thus, 1025 of 1346 patients were included in the analysis. The mean age of the study cohort overall and for the F and M groups was 62.9 ± 17.6, 64.6 ± 18.6, and 61.2 ± 16.4 years, respectively. Compared with the M group, the F group was older ($P < 0.0001$) and had a lower prevalence of diabetes mellitus ($P < 0.0001$) and vascular disease ($P < 0.0001$).

Fig. 1 shows levels of TM, PTF 1+2, TAT, PIC, and D-dimer in the M and F groups. TM levels were significantly lower in the F than M group ($P < 0.0001$). PTF 1+2 levels (Fig. 1c) were significantly higher in the F than M group ($P = 0.0151$), whereas there was no significant difference in TAT (Fig. 1b) or D-dimer (Fig. 1e) levels between the two groups. PIC levels were significantly lower in the F than M group ($P = 0.0038$; Fig. 1d).

Table 1. All patient's Characteristics

	Total (n = 1025)	M (n = 508)	F (n = 517)	*P*-value
Age (years)	62.9 ± 17.60	61.2 ± 16.4	64.6 ± 18.6	< 0.0001
Age > 75	295 (28.8%)	115 (22.6%)	180 (34.8%)	< 0.0001
CHF	94 (9.2%)	52 (10.2%)	42 (8.1%)	NS
Hypertension	588 (57.4%)	306 (60.2%)	282 (54.5%)	NS
DM	171 (16.7%)	110 (21.7%)	61 (11.8%)	< 0.0001
Stroke / TIA	20 (2.0%)	10 (2.0%)	10 (1.9%)	NS
VD	202 (19.7%)	150 (29.5%)	52 (10.1%)	< 0.0001
$CHADS_2$ score	1.2 ± 1.131	1.2 ± 1.15	1.1 ± 1.10	NS
CHA_2DS_2-VASc score	2.2 ± 1.848	2.0 ± 1.72	2.5 ± 1.94	< 0.0001
HAS-BLED score	1.0 ± 1.059	1.0 ± 1.04	1.1 ± 1.07	NS

Continuous variables are presented as the mean ± SD, whereas qualitative variables are presented as percentages. The Kruskal-Wallis test was used to compare continuous variables and the Chi-squared test was used to compare categorical variables between females (F) and males (M).

CHF, congestive heart failure ; DM, diabetes mellitus ; TIA, transient ischemic attack ; VD, vascular disease.

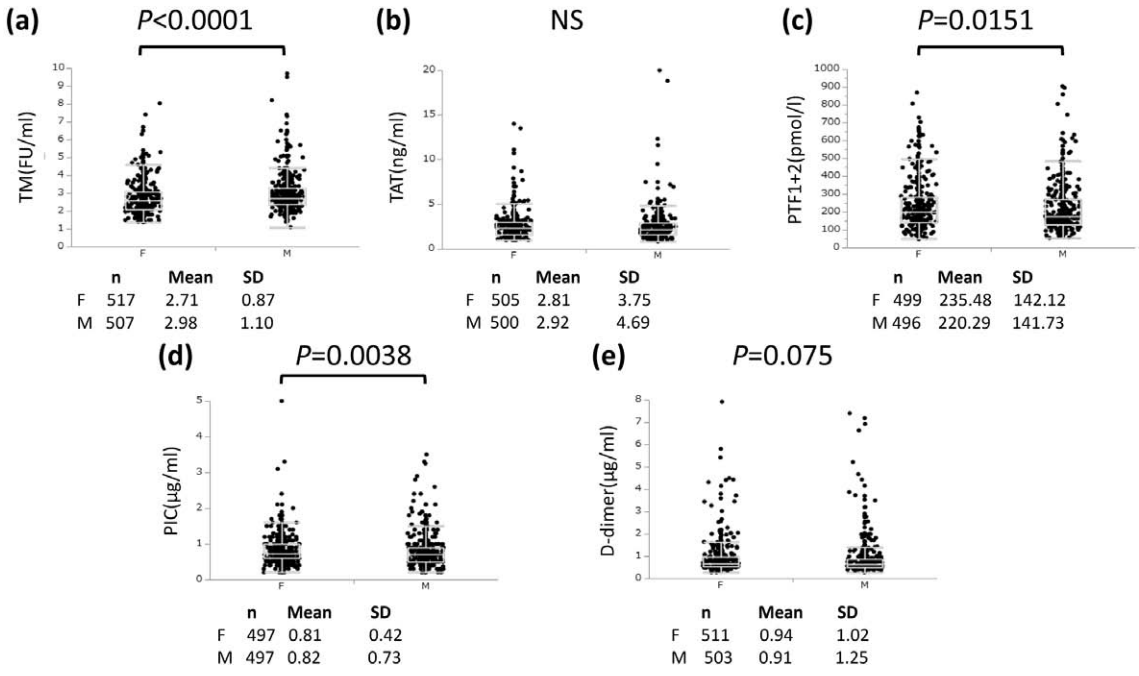


Fig. 1. Relationships between gender and blood markers in all patients
(a) Thrombomodulin (TM), (b) thrombin-antithrombin complex (TAT), (c) prothrombin fragment 1+2 (PTF 1+2), (d) plasmin-α_2-plasmin inhibitor complex (PIC), and (e) D-dimer levels in male (M) and female (F) subjects. The normal range for concentrations of the markers are as follows: TM, 1.8–4.1 FU / ml; PTF 1+2, 69–229 pmol / l; PIC, ≤ 0.8 μg / ml; TAT, 1.0–4.1 ng / ml; D-dimer, ≤ 1.0 (μg / ml). Comparisons were made using the Steel-Dwass test.

Patients without diabetes mellitus and cardiovascular disease

Next, we evaluated patients without diabetes mellitus and vascular disease, because there were significant differences between the F and M groups in the prevalence of diabetes mellitus and vascular disease (Table 2). The mean age of the F group was significantly older than that of the M group ($P < 0.0001$). There were no significant differences in the prevalence of congestive heart failure, hypertension, and stroke or TIA between the F and M groups.

Patients were further divided into groups according to age, namely a younger (Y) group (< 75 years) and an elderly (E) group (≥ 75 years), and according to the $CHADS_2$ score (Table 3). In Y group, although there were no difference between F and M group, F group were older than M group in E group ($P = 0.0394$). And levels of serum creatinine in M group were higher than F group in both age groups ($P < 0.0001$).

Levels of TM, PTF 1+2, TAT, PIC, and D-dimer in each gender and age group are shown in Fig. 2a–e. In the Y group, TM levels were significantly lower in the F than M group (2.36 ± 0.53 vs 2.72 ± 0.87 μg / ml, respectively; $P < 0.0001$), whereas there were no differences in TM levels between the F and M groups in the E group (Fig. 2a). In both the Y and E groups, PTF 1+2 levels were significantly higher in the F group (178.45 ± 87.50 and 323.43 ± 162.14 pmol / l, respectively) than in the M group (165.56 ± 86.94 and 264.95 ± 100.39 pmol / l, respectively; $P = 0.0214$ and $P = 0.0426$; Fig. 2c). Furthermore, PTF 1+2 scores were signifi-

Table 2. Patient's characteristics without diabetes mellitus and cardiovascular disease

	Total (n = 734)	M (n = 312)	F (n = 422)	*P*-value
Age (years)	60.0 ± 18.7	57.0 ± 17.4	62.3 ± 19.3	< 0.0001
Age > 75	295 (28.8%)	115 (22.6%)	180 (34.8%)	< 0.0001
CHF	39 (3.8%)	17 (3.3%)	22 (4.3%)	NS
Hypertension	347 (33.9%)	146 (28.7%)	201 (38.9%)	NS
Stroke / TIA	10 (1.0%)	3 (0.6%)	7 (1.4%)	NS
$CHADS_2$ score	0.81 ± 0.90	0.73 ± 1.15	0.87 ± 0.93	0.0375
CHA_2DS_2-VASc score	1.61 ± 1.56	1.09 ± 1.19	2.00 ± 1.69	< 0.0001
HAS-BLED score	0.74 ± 0.84	0.57 ± 0.74	0.86 ± 0.90	< 0.0001

Continuous variables are presented as the mean ± SD, whereas qualitative variables are presented as percentages. The Kruskal-Wallis test was used to compare continuous variables and the Chi-squared test was used to compare categorical variables between females (F) and males (M).
CHF, congestive heart failure ; DM, diabetes mellitus ; TIA, transient ischemic attack ; VD, vascular disease.

Table 3. Patient's characteristics without diabetes mellitus and cardiovascular disease in each age groups

	Y		*P*-value	E		*P*-value
	M (n = 256)	F (n = 290)		M (n = 115)	F (n = 180)	
Age (years)	51.9 ± 14.7	53.3 ± 16.52	NS	80.9 ± 6.2	82.0 ± 5.2	0.0394
CHF	13 (2.3%)	9 (1.6%)	NS	4 (2.1%)	12 (6.6%)	NS
Hypertension	103 (18.9%)	102 (18.9%)	NS	96 (83.5%)	143 (79.9%)	NS
Stroke / TIA	1 (0.2%)	2 (0.4%)	-	2 (1.1%)	5 (2.7%)	0.0154
Serum creatinine	0.84 ± 0.18	0.62 ± 0.13	< 0.0001	0.92 ± 0.27	0.71 ± 0.20	< 0.0001
$CHADS_2$ score	0.46 ± 0.57	0.40 ± 0.55	NS	1.98 ± 0.94	1.91 ± 0.70	NS
CHA_2DS_2-VASc score	0.69 ± 0.78	1.13 ± 1.21	0.0004	2.98 ± 0.94	3.91 ± 0.70	< 0.0001
HAS-BLED score	0.38 ± 0.60	0.52 ± 0.75	0.0535	1.44 ± 0.70	0.67 ± 1.57	NS

Continuous variables are presented as the mean ± SD, whereas qualitative variables are presented as percentages. The Kruskal-Wallis test was used to compare continuous variables and the Chi-squared test was used to compare categorical variables between females and males.
CHF, congestive heart failure ; TIA, transient ischemic attack.

cantly higher in the E than Y groups for both M and F groups. There were no significant differences in TAT levels (Fig. 2b) between the F and M groups in either the Y or E groups.

In the Y group, PIC levels were significantly higher in the F than M group (0.68 ± 0.27 vs 0.64 ± 0.53 μg / ml, respectively ; $P = 0.0015$), but there were no significant differences in PIC levels between the F and M groups in the E group (Fig. 2d). Similarly, D-dimer levels were significantly higher in the F than M group (0.71 ± 0.93 vs 0.58 ± 0.26 μg / ml, respectively ; $P = 0.0009$), with no significant differences in D-dimer levels between the F and M groups in the E group (Fig. 2e).

Patients were also divided into three age groups (so younger [SY], < 65 years ; little younger [LY], between 65 and < 75 years ; and elderly [E], ≥ 75 years) to evaluate the effects of age

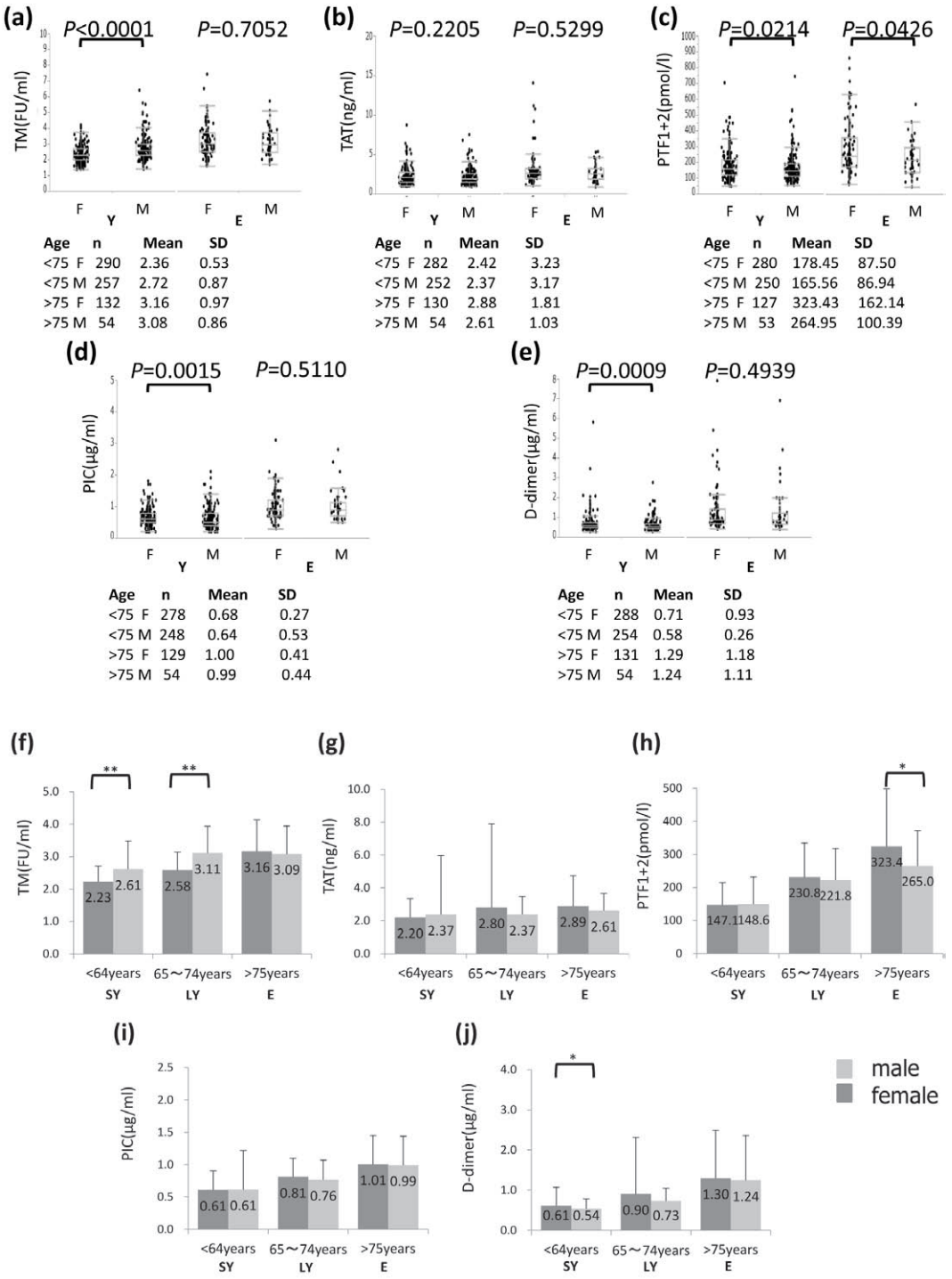
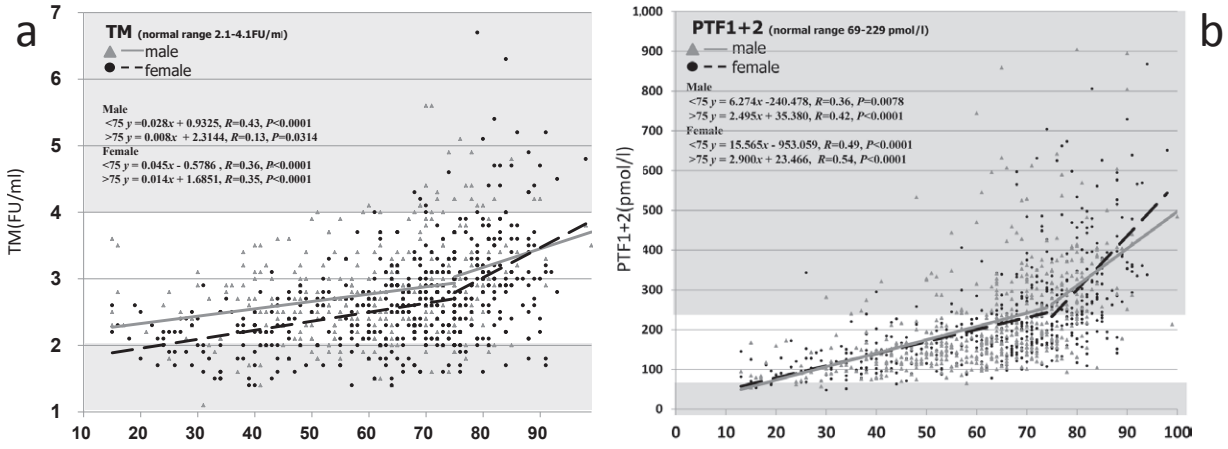


Fig. 2. Comparison blood markers of patients without diabetes mellitus and cardiovascular disease between females and males in each age groups

(a, f) Thrombomodulin (TM), (b, g) thrombin-antithrombin complex (TAT), (c, h) prothrombin fragment 1+2 (PTF 1+2), (d, i) plasmin-α_2-plasmin inhibitor complex (PIC), and (e, j) D-dimer levels in male (M) and female (F) subjects without diabetes mellitus and vascular disease according to age. (a-e) Subjects were divided into two age groups, a younger (Y) group (< 75 years) and an elderly (E) group (≥ 75 years). Subjects were divided into three age groups: so younger [SY], < 65 years; little younger [LY], between 65 and < 75 years; and elderly [E], ≥ 75 years. Data are given as the mean ± SD. * $P < 0.005$; ** $P < 0.0001$. The Steel-Dwass test was used for comparisons between females and males.



(a) Scatter diagram of aging and TM (b) Scatter diagram of aging and PTF 1+2

Fig. 3. Scatter diagrams of (a) thrombomodulin (TM) and (b) prothrombin fragment 1+2 (PTF 1+2) in patients without diabetes mellitus and vascular disease. Correlations were analyzed by Spearman's rank correlation according to gender.

on CHA_2DS_2-VASc scores in detail (Fig. 2f–j). In the SY group, TM levels were significantly lower in the F than M group (2.23 ± 0.47 vs 2.61 ± 0.85 μg / ml, respectively ; $P < 0.0001$) ; similarly, in the LY group, TM scores were significantly lower in the F than M group (2.58 ± 0.56 vs 3.11 ± 0.84 μg / ml, respectively ; $P < 0.0001$; Fig. 2f).

There were no significant differences between the M and F groups in terms of PTF 1+2 (Fig. 2g), TAT (Fig. 2h) or PIC (Fig. 2i) levels in either the SY or LY groups. However, D-dimer levels in the SY group were significantly higher in the F than M group (0.61 ± 0.46 vs 0.54 ± 0.24 μg / ml, respectively ; $P < 0.005$), although there were no differences between the F and M groups in the LY group (Fig. 2j).

Scatter diagrams showing the relationship between aging and the blood markers TM and PTF 1+2 in the F and M groups within the Y and E groups are shown in Fig. 3. Linear regression analysis by Spearman's rank correlation revealed a positive correlation in E group of F and M groups for both TM ($R = 0.36$ and $R = 0.43$, $P < 0.001$, respectively) and PTF 1+2 ($R = 0.49$ and $R = 0.36$, $P < 0.001$, respectively).

Discussion

The $CHADS_2$ scoring system is used to evaluate the risk of stroke in patients with non-valvular atrial fibrillation. In this method of scoring, evaluations are made based on whether patients have congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, and a history of stroke. One point is added for each of the four categories of congestive heart failure, hypertension, age ≥ 75 years, and diabetes mellitus, and two points are added for a history of stroke. Thus, patients with all risk factors have a score of 6. Patients with higher $CHADS_2$ scores have a higher risk of stroke. In patients with scores of 2 or more, the risk of stroke over 1 year is ≥ 4% [1]. Thus, anticoagulant therapy is recommended in patients with $CHADS_2$ scores of 2 or

greater. However, if patients with a $CHADS_2$ score of ≤ 1 have a history of stroke, it has been shown that severe strokes occur at a rate similar to that in patients with high $CHADS_2$ scores[6].

The CHA_2DS_2-VASc score was created for the purpose of obtaining a more detailed assessment of the risk of stroke in patients with a $CHADS_2$ score ≤ 1. Considering that the risk of stroke is greater in patients with a history of VD, such as a myocardial infarction, in patients between 65 and 74 years of age, and in females, this score adds 1 point for each of these characteristics to the $CHADS_2$ score. The score for patients who satisfy all items is 9 points[3]. Among female patients, those under 65 years of age who do not have organic heart disease have no increased risk of stroke[7], and no points are added.

In the present study, the items in the CHA_2DS_2-VASc score that are also included in the $CHADS_2$ score were examined, focusing on gender. Impaired endothelial function decreases the production of nitrogen monoxide derived from vascular endothelial cells, and thrombi are thought to increase because of poor relaxation of vascular smooth muscle and decreased platelet aggregation.

The present study investigated how gender affects markers of coagulation, fibrinolysis, and endothelial function in "real world" outpatients of a cardiovascular department. It has been reported that the levels of coagulant and fibrinolytic markers are elevated with aging[8]. Thus, coagulation, fibrinolysis, and endothelial function were assessed by age group.

Vascular endothelial function

In the present study, sTM was used as an indicator of vascular endothelial function. TM itself is expressed in vascular endothelial cells and is a cofactor that binds with thrombin. Structurally, TM consists of five domains: a lectin-like domain (Domain 1) from the N-terminal; an epidermal growth factor-like domain (Domain 2); an O-linked sugar domain (Domain 3); a transmembrane domain (Domain 4); and a cytoplasmic domain (Domain 5) from the C-terminal. Domains 4 and 5 are present in the cell membrane and cytoplasm, whereas Domains 1, 2, and 3 appear intravascularly. The site of thrombin binding is Domain 2. In the coagulation cascade, thrombin activates fibrinogen and facilitates the reaction that produces fibrin. Thrombin also activates coagulation Factors Ⅴ, Ⅷ, Ⅺ, and ⅩⅢ, as well as the platelets, stimulating the reactions of the coagulation cascade. TM has a high affinity for thrombin and forms a complex. By binding with thrombin, TM inhibits the property by which thrombin promotes activation of the coagulation reaction in response to coagulation Factors Ⅴ, Ⅷ, Ⅺ, and ⅩⅢ. In addition, the TM-thrombin complex activates protein C. This activated protein C (APC) inactivates coagulation Factors Va and Ⅷa. As a result, the reaction that produces thrombin from prothrombin is inhibited. Thus, TM has anticoagulant activity through two pathways: direct inactivation of the coagulation response by binding with thrombin and a reaction via APC that indirectly inactivates the coagulation response[10].

The TM measured in the present study was not only the TM expressed in the vascular endothelium, but also soluble TM detected in the blood. When the vascular endothelium is damaged, the TM on endothelial membranes is broken down by neutrophil elastase that appears as a

result of the inflammatory response, and is then detected in the blood. The part broken down by neutrophil elastase at this time is Domain 2. Domain 2 is subdivided into six functional domains, among which the fourth, fifth, and sixth functional domains bind to thrombin. The neutrophil elastase that appears as a result of vascular endothelial damage breaks down a section between the fifth and sixth functional domains in Domain 2. That is, the part of TM that binds with thrombin is destroyed. In this way, TM appears in the blood following the destruction of the part that binds with thrombin. Therefore, the TM that appears in the blood has a weakened action against thrombin[11, 12]. It is thought that the presence of vascular endothelial damage leads to breakdown of the TM that is present in the endothelium so that sTM levels increase[13], reducing anticoagulation ability. The TM that can be measured in the serum is this released soluble TM.

This sTM was measured in the present study, and it was found to be significantly higher in males in the SY and LY groups (Fig. 2f). Conversely, in the E group, there were no significant differences in TM levels. In females over 75 years of age, the TM levels were equal to those in males. Focusing on liner regression analysis in the E group, the regression coefficient of the F group was higher than that of the M group (Fig. 3). That is, the vascular endothelial dysfunction that had been milder in females than males no longer differed between males and females. It is thought that vascular endothelial dysfunction progresses in females over 75 years of age.

The presence of vascular endothelial damage, or high sTM levels, has been reported to be an independent risk factor for acute cerebral infarction[13, 14]. Vascular endothelial damage has also been shown to be a risk factor for stroke[15].

Coagulation system

PTF 1+2 and TAT were investigated as factors that reflect clotting ability. Thrombin is a key enzyme of the coagulation cascade that binds antithrombin, which is a neutralizing agent for thrombin. Because TAT reflects the amount of thrombin, it is a marker of the coagulation system.

When thrombin is generated from prothrombin by Factor Xa, the peptide PTF 1+2 is released from prothrombin. Thus, PTF 1+2 reflects the amount of thrombin more accurately than TAT, and PTF 1+2 is also a marker of the coagulation system.

PTF 1+2 levels were significantly higher in the F than M group (Fig. 2c). Furthermore, when subjects were analyzed on the basis of three age groups (Fig. 2h), serum PTF 1+2 levels increased significantly with age. Although there was no significant difference in PTF 1+2 levels between the F and M groups in the SY and LY groups, in the E group PTF 1+2 levels were significantly higher in the F than M group. Similar to TM, in the E group, the regression coefficient for the F group was higher than that for the M group (Fig. 3b). It was thought that stimulation of the coagulation system is greater in females over 75 years of age. Past reports have indicated that PTF 1+2 is an independent predictor of ischemic events in the brain[4, 9]. Based on the PTF 1+2 results in the present study, it is thought that the coagulation system is stimulated in females. Thus, it is suggested that female gender is a risk factor for stroke.

TAT levels were higher in the F than M group, suggesting that clotting ability was stimulated, but a consistent trend was not seen in the comparisons of each age group. This was thought to be because relatively large standard deviations were seen in TAT values. One reason why a consistent trend was not seen in TAT may be that the values fluctuated depending on the blood collection technique[15] (TAT tends to be higher with stronger avascularization). To eliminate this factor, it would have been preferable to have the same person collect blood from all patients, but this was difficult in the actual clinical setting and is considered to be a limitation of the present study.

Fibrinolytic system

PIC was investigated as a marker of the fibrinolytic system. Plasmin is a key enzyme of the fibrinolytic system that binds to the α_2-plasmin inhibitor, which is a neutralizing agent for plasmin. Because the plasmin and α_2-plasmin inhibitor combine in a 1:1 ratio to form PIC, PIC is a marker of the fibrinolytic system. Evaluation of PIC levels (Fig. 2d) revealed higher levels in the F than M group within the Y group, suggesting that the fibrinolytic capacity was greater in females than males less than 75 years of age. In contrast, there was no significant difference in PIC levels between the F and M groups in the E group (over 75 years of age). Thus, stimulation of the fibrinolytic system is greater in females than males under 75 years of age, but there is no difference in the fibrinolytic state between males and females over 75 years of age.

TM levels suggest progression of vascular endothelial dysfunction in females over 75 years of age

Although the results regarding PTF 1+2 levels suggest that the coagulation system is stimulated in females over 75 years of age, the PIC results suggest no enhancement of the fibrinolytic system in this group. It is conceivable that the coagulation system is relatively accelerated in females over 75 years of age. For this reason, female gender is a risk factor for ischemic cerebral infarction, especially in individuals older than 75 years of age.

Study limitations

The present study was a cross-sectional study, and patient outcomes were not evaluated. For this reason, it was difficult to evaluate how often strokes occurred over the long term. In addition, of the subjects in the present study, the focus was on females. Females become menopausal with aging. There is a possibility that vascular endothelial function is affected by estrogen[17], but this was not evaluated in the present study. In addition, we did not identify menopausal patients accurately. Thus, we could not evaluate coagulation and fibrinolysis activities and the extent of vascular endothelial damage in menopausal subjects.

Conclusion

The present study has demonstrated that, in outpatients of a cardiovascular department, gender is a significant factor affecting blood coagulation systems.

Statement of conflict of interest

The authors have no financial conflicts of interest to disclose concerning the paper.

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