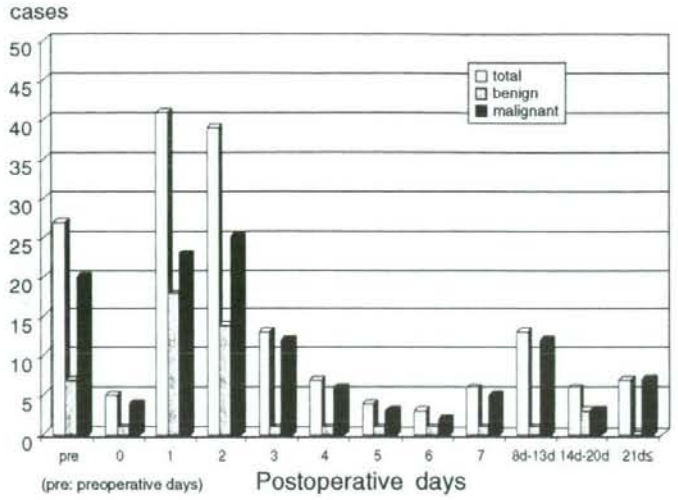

Fig 3. Number of pulmonary thromboembolism cases in gynecological patients by perioperative days.

of postpartum PTE occurred within 3 days postpartum, and all occurred within the first week. Univariate analysis showed that PTE onset was associated with BMI >25 (kg/m^2) (OR 1.89; 95% CI 1.01–3.55; p<0.05), >27 (OR 3.47; 95% CI 1.75–6.91; p<0.001), as well as C/S (OR 14.27; 95% CI 6.89–29.55; p<0.0001) (Table 2).

In gynecological PTE patients, 27 cases (15.2%) showed preoperative onsets: 7 cases (25.9%) in benign diseases and 20 cases (74.1%) in malignant diseases. Among malignant diseases, 15 cases (75%) had ovarian cancer. Postoperatively, PTE shows a large peak on day 1 and 2 (80 cases, 55.6%) and subsequently declines, although some cases were registered as late as 3 weeks postoperatively (Fig 3). PTE was reported among endometrial cancer (53 cases) and ovarian cancer (47 cases) (Fig 4). Univariate analysis showed that the onset of PTE was associated with BMI >25 in both benign (OR 4.8; 95% CI 2.2–10.4; p<0.001) and in malignant diseases (OR 2.4; 95% CI 1.4–4.2; p<0.01) (Table 2).

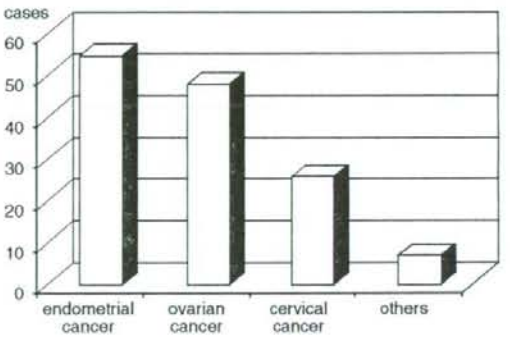

Fig 4. Number of pulmonary thromboembolism cases by malignant diseases.

Discussion

Venous thromboembolism (VTE), which had been considered a relatively rare disease in Japan, has been on the increase in recent years possibly as eating habits have become more similar to those of the West. Patients with VTE have a clinical problem in which there is deep venous thrombosis (DVT) or PTE caused by DVT. In Western nations, the incidence of symptomatic DVT in obstetric patients is reported to be 0.5 to 7 per 1,000 deliveries, and the number has been decreasing slightly in recent years as a result of improved prophylaxis[1–4] Previously, more than 2/3 of DVT was thought to occur in the puerperal period, particularly in the first week postpartum or the last gestational week, but recent reports describe that DVT can occur at any stage of pregnancy. DVT is 5 times more prevalent in pregnancy than in non-pregnant periods, and occurs 7- to 10-fold more frequently in C/S compared with vaginal deliveries[4,8,9] Approximately 4% to 5% of obstetrical DVT could lead to PTE, and, conversely, more than 90% of PTE cases are thought to be caused by DVT of the lower extremities[10] Once PTE occurs it is extremely serious, with a mortality rate reported to be 18–30% if left untreated[11] and has long been the leading cause of maternal mortalities[12,13] Likewise, obstetrical PTE in Japan has been increasing, making it the leading cause of direct obstetrical deaths[7,14,15] Furthermore, most PTE cases are observed in the puerperal period, the majority of them after C/S[4,16] Pregnancy is conducive to VTE for the following reasons: 1) enhanced coagulation, platelet activation, reduced fibrinolysis, and reduced protein S activity; 2) the venous smooth muscle relaxation effect of estrogen/progesterone; 3) compression of the iliac vein and inferior vena cava by the enlarged gravid uterus; and 4) vascular (particularly endothelial) disorders of the iliac vein region caused by surgical interventions, such as cesarean section and retention of blood caused by postoperative immobilization. In the field of obstetrics, the diseases and conditions that are risk factors are middle-aged pregnancy, prolonged immobilization due to hyperemesis, threatened abortion or threatened premature labor, severe preeclampsia, placenta previa, placental abruption, delivery by cesarean section and marked varix of the lower limbs[1–4]

There are few reports regarding the incidence of DVT in the gynecology field in Japan. According to research using the ^{125}I-fibrinogen uptake test, Matsumoto et al found that the incidence of postoperative DVT was 10.8% (7/65)

among all gynecological surgeries and 19.4% (6/31) in radical hysterectomy or modified radical hysterectomy[17] which might be a lower rate compared to the West, although by no means a small figure. The risk factors in the field of gynecology are surgeries for giant uterine myoma, giant ovarian tumor, ovarian cancer, uterine cancer and severe pelvic adhesion. Other patients at risk are those with ovarian hyperstimulation syndrome, patients taking oral contraceptives, and postmenopausal patients receiving hormone replacement therapy. Many cases that require long operations with lymphatic resection, massive bleeding or transfusions are also at risk[1-4] According to Nicolaides et al, the incidence of postoperative DVT, including asymptomatic cases, is less than 1–10% in small operations of short duration, 10–40% in moderate risk patients over 40 years of age, and is believed to be 40–80% in high-risk patients with a history of VTE or malignant tumor patients requiring extended surgery. Furthermore, the incidence of fatal PTE is reported to be less than 0.01%, 0.1–0.8% and 1–5%, respectively[18] Japanese data of this kind are currently not available.

In the West, particularly among Caucasians, there is a prevalence of thrombosis caused by genetic structural abnormalities of clotting factors, which is further aggravated by environmental factors to produce a high rate of PTE[19] The Japanese ethnically have fewer structural abnormalities of clotting factors; however, environmental factors, pregnancy and labor, and invasive surgery play a major role in the occurrence of PTE in Japanese[20] As our present survey has shown, PTE has been on the rise in Japan in recent years. In the field of obstetrics and gynecology, PTE has increased by 6.5-fold over the past decade. PTE occurred in 0.02% of total births. The incidence of PTE is 1 per 5,000 births, which is approaching the level of Western countries. The risk of PTE was 22 times higher in C/S compared with vaginal deliveries. The onset period appeared as 3 peaks: early pregnancy, midterm to late pregnancy, and postpartum. PTE onset at early pregnancy could possibly be caused by dehydration and immobilization due to hyperemesis, enhanced coagulation and the formation of thrombophilia. PTE onset in midterm to late pregnancy is believed to be the result of prolonged immobilization due to complications of severe preeclampsia, threatened premature labor, and multiple pregnancies. The postpartum largest peak with the highest incidence on day 1 is attributed to C/S. From the analysis of the onset of PTE in cases with C/S, cesarean delivery itself is thought to be an increased risk of such event. Furthermore, a close relationship between obesity (BMI >27, OR 3.47; BMI >25, OR 1.89) and PTE was observed as well as Western countries. BMI >30 is a risk factor for PTE in Westerners; however, BMI >27 might be characteristic in Japanese pregnant women.

The incidence among gynecological cases was 0.08% of total operations, the risk of PTE, however, was 16 times higher in malignant diseases compared with benign diseases and 7 times higher compared with C/S. In gynecological PTE patients, 15.2% of the cases showed preoperative onsets, and among malignant diseases, 75% of the cases had ovarian cancer. Postoperatively, PTE showed a large peak on day 1 and 2 and subsequently declines; however, some cases were registered as late as 3 weeks postoperatively. Sakuma et al reported that the incidence of PTE was high in patients with ovarian cancer and uterine cancer according to postmortem examination[21] Therefore, careful observation preoperatively and postoperatively is required in gynecological patients, particularly those with malignant diseases. A close relationship between obesity (BMI >25) and PTE was observed in both benign (OR 4.8) and in malignant diseases (OR 2.4) as well as obstetrical patients.

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ORIGINAL ARTICLE

Elevated levels of soluble fibrin in patients with venous thromboembolism

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Abstract The fibrin-related markers (FRMs), including soluble fibrin (SF), D-dimer and fibrin and fibrinogen degradation products (FDP) are considered to be useful for the diagnosis of thrombosis; however, evidence for the diagnosis of thrombosis by SF is still not well established. The present study was designed to evaluate the usefulness of SF in the diagnosis of venous thromboembolism (VTE). The plasma concentrations of FRMs were measured in 551 inpatients suspected to have a VTE. The plasma levels of SF, D-dimer and FDP were significantly higher in patients with VTE than patients without VTE and those were significantly higher in patients without VTE than in healthy volunteers. In a receiver operating characteristic analysis for the diagnosis of VTE, the area under the curve was 0.950 for SF, 0.933 for FDP and 0.805 for D-dimer. The appropriate cut-off values for the diagnosis were as follows SF 5.9 μg/ml, FDP 2.1 μg/ml and D-dimer 4.8 μg/ml. To obtain a 100% negative predictive value for the diagnosis of VTE, the SF was less than 5.2 μg/ml, FDP was less than 1.3 μg/ml, and D-dimer was less than 0.5 μg/ml. Our findings suggest that the SF assay is useful for the diagnosis and exclusion of VTE.

Keywords SF · D-dimer · FDP · VTE · Diagnosis

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1 Introduction

The fibrin-related markers (FRMs) which include fibrin and fibrinogen degradation products (FDP), soluble fibrin (SF) and D-dimer, are sensitive markers for thrombotic diseases [1, 2]. The FRMs are reported to be elevated in deep vein thrombosis (DVT)/pulmonary embolism (PE) [3–5], disseminated intravascular coagulation (DIC) [6–8], acute myocardial infarction (AMI) [9, 10] and thrombotic thrombocytopenic purpura (TTP) [11]. The International Society of Thrombosis and Haemostasis (ISTH) established the diagnostic criteria for overt-DIC using FRM [12]. PE is a common, frequently undiagnosed, and potentially fatal event. Because the symptoms of PE are common, including dyspnoea and chest pain [13–15], the early recognition of DVT [16] and PE [17] by FRM is important clinically.

FDP is the most classical and basic marker of FRM, but the use of FDP is less common than that of D-dimer. D-

dimer is widely used to diagnose thrombosis as DVT but many of the commercially available D-dimer assay kits contain different monoclonal antibodies and standard substances, and are based on different assay systems. Since the issue of the standardization of D-dimer assays remains to be resolved, several studies [18, 19] have reported the basic data for the standardization of D-dimer.

The presence of soluble fibrin (SF) [20] in plasma is an indicator of thrombin activation in the blood, as are the thrombin–antithrombin complex [21] and prothrombin fragment F1 + 2 [21]. Thrombin cleaves fibrinopeptide A and B from the Aα and Bβ chains of fibrinogen, respectively. These are called desAA-fibrin monomer (FM) and desAABB-FM, which polymerize with each other and forms fibrin clots. These molecules in soluble form circulate in the blood are termed as SF. SF mainly consists of desAA-FM or desAABB-FM, which forms a complex with fibrinogen or its derivates [22–24]. Recently, the monoclonal antibody J2–23, which recognizes the epitope within the Aα502–521 region of fibrinogen, was developed for measuring the SF level[25].

The present study was designed to evaluate the usefulness of the SF assay in the diagnosis of thrombosis, such as DVT and PE. For this purpose, we determined the plasma concentration of these molecules in 551 patients suspected of a having venous thromboembolism and 99 healthy volunteers (HV).

2 Materials and methods

2.1 Subjects

From 1 January 2004 to 31 December 2007, 551 patients (median 25–75%) (63, 48–72 years of age; 325 females and 226 males) were suspected of having thrombosis in the hospitals affiliated with Mie University Graduate School of Medicine. The plasma concentrations of fibrin and fibrinogen degradation products (FDP), SF and D-dimer and were examined in these patients and correlated with thrombosis. The study protocol was approved by the Human Ethics Review Committees of the participating institutions and a signed consent form was obtained from each subject. Among these patients, 484 patients (62, 47–71 of age; 278 females and 206 males) did not have any thrombosis, 67 patients had a VTE (DVT or PE) (67, 54–74 years of age; 47 females and 20 males). DVT was diagnosed by either echo or venography and PE was diagnosed by computed tomography, angiography or ventilation-perfusion lung scan.

Among the underlying diseases in these patients, orthopaedic conditions were identified in 117 patients, cancer in 102, cardiovascular diseases in 83, haematological diseases in 55, digestive diseases in 31, autoimmune diseases in 28, respiratory diseases in 21, thrombophilia in 15, no underlying disease in 14, infectious diseases in 10, trauma and burn in 8, and other diseases in 7 (Table 1).

Citrated blood samples were obtained from the peripheral veins of healthy subjects (see below) and patients under fasting conditions and then centrifuged for 20 min at 3,000 rpm. The supernatants (plasma) were analyzed within 4 h. The plasma concentrations of SF and D-dimer were measured in patients with thrombosis at the onset and those without thrombosis at the first consultation. The same parameters were also measured in 99 healthy subjects (mean age 22 years, range 21–30 years; 41 females and 58 males), who were free of any diseases including thrombotic disease or hyperlipidemia as confirmed by an annual medical check-up.

Table 1 Underlying diseases of the subjects

Diseases	Age; median (25th–75th percentile)	Sex (F:M)	DVT (%)
Orthopaedic diseases	61 (34–73)	121:56	24 (13.6)
Cancer	65 (53–74)	42:60	6 (5.9)
Cardiovascular diseases	66 (50–72)	49:34	11 (13.3)
Hematological diseases	59 (36–68)	29:26	1 (1.8)
Digestive diseases	61 (34–73)	15:16	4 (12.9)
Autoimmune diseases	57 (52–63)	23:5	3 (10.7)
Respiratory diseases	62 (43–72)	12:9	0
Thrombophilia	42 (30–60)	12:3	4 (26.7)
No underlying disease	67 (53–76)	10:4	14 (100)
Infectious diseases	65 (49–72)	4:6	0
Trauma/burn	36 (18–60)	3:5	0
Other diseases	36 (32–55)	5:2	0

2.2 Measurement of plasma concentrations of SF, D-dimer and FDP

The plasma levels of SF were determined by the latex agglutination method using Nanopia SF (SEKISUI MEDICAL CO, LTD, Tokyo, Japan) containing monoclonal antibody J2–23 [25]. J2–23 recognizes an epitope in the C-terminal region of the fibrin Aα chain (Aα502–521). The plasma D-dimer and FDP levels were measured by the latex agglutination method using the Nanopia D-dimer and Nanopia P-FDP kits (SEKISUI MEDICAL CO, LTD).

2.3 Statistical analysis

The data are expressed as the median (25–75th% percentile). Differences between the groups were examined for statistical significance using the Mann–Whitney U test while correlations between two variables were tested by Pearson's correlation analysis. P value less than 0.05 denoted a significant difference. The usefulness of D-dimer levels in the diagnosis of thrombosis and VTE was examined by receiver operating characteristic (ROC) analysis [26]. The cut-off values were determined by ROC analysis. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

3 Results

The plasma concentrations of SF were not distributed normally among healthy volunteers; the 95% confidence interval (CI) of SF was from 0 to 5.47 μg/ml. The 95% CIs of D-dimer and FDP in healthy volunteers were from 0.4 to 1.2 μg/ml and from 0.3 to 2.1 μg/ml, respectively. The plasma levels of SF tended to be high in all subjects, especially in those with infectious diseases, those with trauma and burn and those without underlying disease. The plasma levels of D-dimer tended to be high in those with orthopaedic conditions and those without underlying disease, and those of FDP tended to be high in those with infectious diseases and those without underlying disease (Table 2).

The plasma levels of SF were significantly higher in patients with VTE (22.1, 11.4–38.3 μg/ml) than patients without VTE (3.4, 1.9–5.5 μg/ml) and those were significantly higher in those without VTE than in HV ($P < 0.001$, respectively; Fig. 1). The plasma levels of D-dimer were significantly higher in patients with VTE (1.8, 1.0–5.3 μg/ml) than patients without VTE (0.8, 0.5–1.4 μg/l) and those were significantly higher in those without VTE than in HV (0.5, 0.5–0.6 μg/ml) ($P < 0.001$, respectively; Fig. 2). The plasma levels of FDP were significantly higher in patients with VTE (12.2, 7.2–20.8 μg/ml) than patients without VTE (1.4, 0.8–3.5 μg/ml) and those were significantly higher in those without VTE than in HV (0.7, 0.5–1.0 μg/ml) ($P < 0.001$, respectively; Fig. 3).

The relationship between SF and FDP ($Y = 3.804 + 0.911X$, $r = 0.553$) and that between SF and D-dimer ($Y = 5.599 + 0.542X$, $r = 0.543$) were moderately close, and the relationship between FDP and D-dimer ($Y = 2.204 + 0.549X$, $r = 0.905$) was markedly close.

In the ROC analysis for the diagnosis of VTE, the 3 curves of SF, D-dimer and FDP showed convexity at the top. The area under the curve (AUC) was 0.950 in SF, 0.933 in FDP and 0.805 in D-dimer (Fig. 4). The appropriate cut-off values for the diagnosis were as follow: SF 5.9 μg/ml [sensitivity 98.5%, specificity 80.1%, positive predictive value (PPV) 36.3%, negative predictive value (NPV) 99.8% and odds ratio 265.7], FDP 2.1 μg/ml (sensitivity 98.6%, specificity 68.1%, PPV 26.2%, NPV 99.7% and odds ratio 140.9), D-dimer 4.8 μg/ml (sensitivity 28.4%, specificity 96.6%, PPV 48.7%, NPV 92.1% and odds ratio 11.1) (Table 3). In 100% of NPV for the diagnosis of VTE, SF was less than 5.2 μg/ml, FDP was less

Table 2 Plasma levels of SF, D-dimer and FDP in the underlying diseases of the subjects

Diseases	SF (μg/ml)	D-Dimer (μg/ml)	FDP (μg/ml)
Orthopaedic diseases	3.8 (2.4–8.0)	4.9 (1.9–12.9)	0.9 (0.6–1.7)
Cancer	3.4 (1.8–6.2)	0.9 (0.7–1.4)	1.5 (0.9–3.2)
Cardiovascular diseases	3.9 (2.2–10.6)	1.1 (0.5–2.0)	2.2 (0.8–7.5)
Hematological diseases	2.6 (1.2–5.5)	0.7 (0.4–1.2)	1.0 (0.7–2.0)
Digestive diseases	4.4 (2.0–8.4)	0.9 (0.6–1.6)	1.3 (0.8–3.9)
Autoimmune diseases	3.1 (1.7–4.8)	0.6 (0.4–0.9)	1.2 (0.7–3.1)
Respiratory diseases	2.5 (1.0–4.8)	0.6 (0.5–0.9)	1.1 (0.7–1.3)
Thrombophilia	3.8 (1.7–9.4)	0.5 (0.4–1.0)	1.4 (0.7–3.2)
No underlying disease	23.6 (7.0–32.0)	2.1 (1.0–5.3)	10.2 (5.0–19.9)
Infectious diseases	5.8 (1.5–18.3)	1.2 (0.9–2.7)	4.3 (1.8–6.9)
Trauma/burn	10.4 (1.8–12.0)	0.9 (0.7–1.4)	3.4 (1.4–5.6)
Other diseases	2.1 (0.0–4.7)	0.9 (0.6–1.9)	1.5 (1.4–4.1)

Data show the median (25–75%) percentile

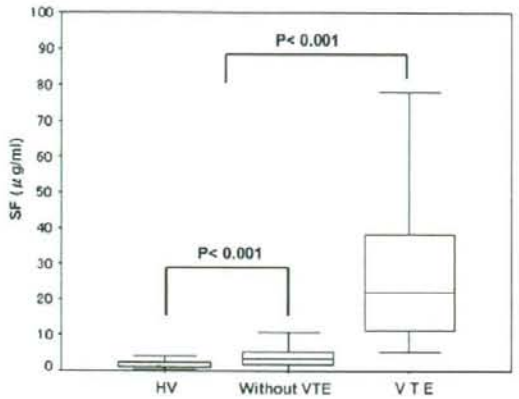

Fig. 1 Plasma concentrations of SF in patients without VTE, those with VTE and healthy volunteers. *VTE* venous thromboembolism, *HV* healthy volunteer

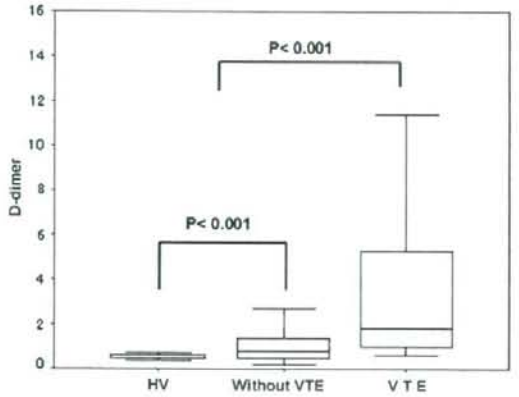

Fig. 2 Plasma concentrations of D-dimer in patients without VTE, those with VTE and healthy volunteers *VTE* venous thromboembolism, *HV* healthy volunteer

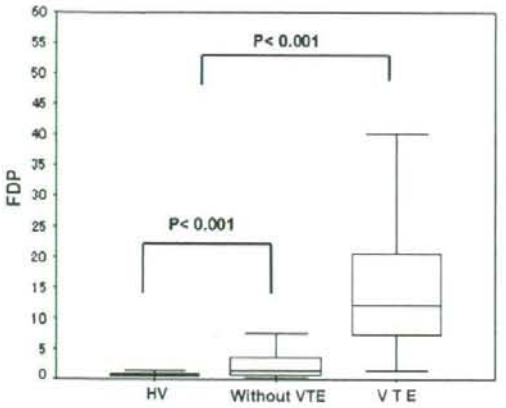

Fig. 3 Plasma concentrations of SF in patients without VTE, those with VTE and healthy volunteers *VTE* venous thromboembolism, *HV* healthy volunteer

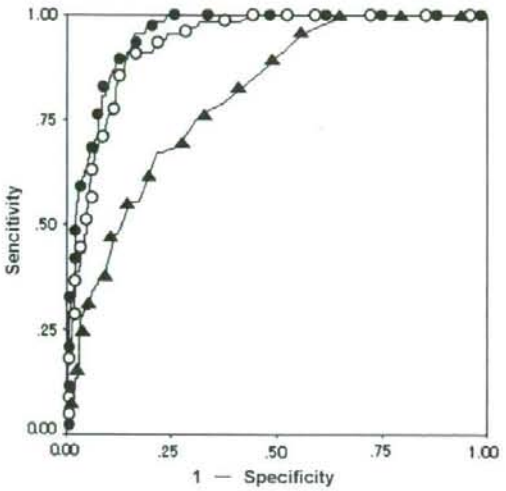

Fig. 4 ROC analysis for diagnosis of VTE. *Closed circle* SF, *open circle* FDP, *closed triangle* D-dimer AUC SF 0.950, FDP 0.933, D-dimer 0.805

than 1.3 μg/ml, and D-dimer was less than 0.5 μg/ml (Fig. 5).

4 Discussion

In the present study, the normal SF level was less than 6.0 μg/ml, and that was similar to the previous reports for other kinds of SF determination [22, 24]. The monoclonal antibodies in the Nanopia SF [25], Iatro SF [24] and Auto LIA FMC [27] assays recognize the α-chain of fibrinogen, which is an important site for the activation of fibrinogen to fibrin by thrombin. The normal range of D-dimer and FDP were from 0.4 to 1.2 μg/ml and from 0.3 to 2.1 μg/ml, respectively. These findings are in agreement with those of previous reports [2, 28].

The plasma levels of SF, D-dimer and FDP were significantly higher in patients with VTE than patients without VTE, suggesting that these FRMs were useful for the diagnosis of VTE. In previous reports [2, 28, 29], the high concentrations of SF and D-dimer could be considered as markers of thrombosis, including VTE. However, no significant difference was observed among those with thrombosis, those with liver transplantation or those with a post operative status [2].

Table 3 Appopriate cut-off value of SF, D-dimer and FDP for the diagnosis of VTE

Marker	Cut off value (μg/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Odds ratio
Highest odds ratio						
SF	5.9	98.5	80.1	36.3	99.8	265.7
D-Dimer	4.8	28.4	96.6	48.7	92.1	11.1
FDP	2.1	98.5	68.1	26.2	99.7	140.9
Highest NPV (100%)						
SF	5.2	100	76.0	32.4	100	
D-Dimer	0.5	100	34.3	14.9	100	
FDP	1.3	100	56.1	20.7	100	

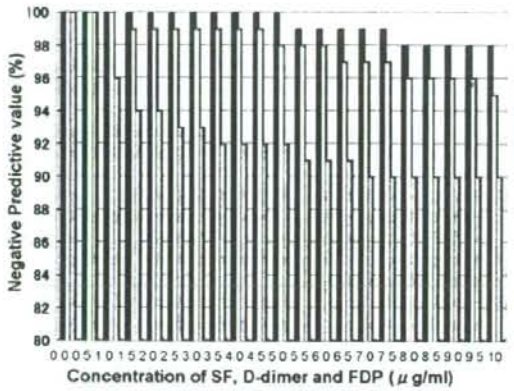

Fig. 5 Negative predictive value for the diagnosis of VTE. *Closed bar* SF, *shaded bar* D-dimer, *open bar* FDP

The plasma levels of SF tended to be high in all subjects, especially those with infectious diseases, those with trauma and burn and those without underlying disease, suggesting that these diseases have a hypercoagulable state or thrombosis. The plasma levels of D-dimer also tended to be high in those with orthopaedic conditions and those without underlying disease, indicating that D-dimer levels might be high in orthopaedic conditions without thrombosis, and that D-dimer may therefore not be useful for the diagnosis of thrombosis under those conditions.

The ROC analysis showed that SF, FDP and D-dimer are useful markers for the diagnosis of VTE; in particular, SF was the best marker of the FRMs. An appropriate cut-off value for the diagnosis of VTE was 5.9 μg/ml in SF, 2.1 μg/ml in FDP and 4.8 μg/ml in D-dimer. Except in the D-dimer, these cut-off values were close to the normal range and a slight increase of the SF and FDP from the normal range shows a high risk of thrombosis. At a value of 5.9 μg/ml for SF, both the sensitivity and specificity were sufficiently high, thus suggesting that SF is the best marker for the diagnosis of thrombosis at the onset. At the value of 4.8 μg/ml for D-dimer, the specificity was highest, suggesting that the diagnosis of VTE might be confirmed by high D-dimer levels.

In 100% of NPV for the diagnosis of VTE, SF was less than 5.2 μg/ml, FDP was less than 1.3 μg/ml and D-dimer was less than 0.5 μg/ml. In Europe and North America, D-dimer concentrations of less than 0.5 μg/ml are considered to exclude DVT/PE [17]. However, some D-dimer kits, which are frequently used in Japan have different cut-off values for the exclusion of DVT/PE [28]. These findings for D-dimer were similar to previous reports [28]. However, this study is the first to show that the SF level is a valuable indicator for the exclusion of DVT/PE.

Finally, the FRMs such as D-dimer, FDP and SF are considered to be useful for the diagnosis of thrombosis, and the SF level reflects the early phase of DVT/PE while D-dimer reflects the secondary fibrinolysis after clot formation [2]. By establishing an early diagnosis of thrombosis by FRM, we might improve the outcome in various underlying diseases, which carry a risk for the development of thrombosis.

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Elevated Levels of Prothrombin Fragment 1 + 2 Indicate High Risk of Thrombosis

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Prothrombin fragment 1 + 2 (F1 + 2) is considered to be useful for diagnosis of thrombosis. However, the evidence for a diagnosis of thrombosis by F1 + 2 is still not well established. The plasma concentrations of F1 + 2, soluble fibrin, D-dimer, and thrombin-antithrombin complex were measured in 694 patients suspected of having thrombosis and then were correlated with thrombosis. Plasma concentrations of F1 + 2, soluble fibrin, D-dimer, and thrombin-antithrombin complex were significantly higher in patients with thrombosis, compared with patients without thrombosis. When cutoff values of more than 300 pmol/L for F1 + 2 were used for the diagnosis, more than 50% of the patients were thus found to have thrombosis. The findings showed that F1 + 2, soluble fibrin, D-dimer, and thrombin-antithrombin complex have similar diagnostic ability. The plasma concentration of F1 + 2 closely was well correlated with thrombin-antithrombin complex, soluble fibrin, and D-dimer. Finally, F1 + 2 is one of the most useful parameters for the diagnosis of thrombosis.

Keywords: thrombosis; F1 + 2; SF, D-dimer; TAT

The prothrombin fragment 1 + 2 (F1 + 2) is cleaved from the aminoterminal end of human prothrombin when this zymogen is activated by factor Xa to yield thrombin.[1] The determination of human F1 + 2 in plasma with an antibody against a synthetic peptide has been reported.[2] Monitoring of F1 + 2 in patients treated with oral anticoagulants[3,4] and elevated plasma levels of F1 + 2 in patients with disseminated intravascular coagulation (DIC)[5,6] have been reported. Increased plasma level of thrombin-antithrombin complex (TAT)[7] also reflected with thrombin generation such as enhanced F1 + 2 levels.

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Disseminated intravascular coagulation[8,9] is often observed in patients with leukemia, solid cancers, infections, gynecological conditions, and aneurysms, and it is also frequently associated with severe bleeding and organ failure. Because DIC is frequently a fatal condition,[10] it is important to diagnose DIC at an early stage using hemostatic molecular markers.[11] Pulmonary embolism (PE) is a common, frequently undiagnosed, and potentially fatal cause of several common symptoms, for example, dyspnoea and chest pain.[12-14] Because PE is often a fatal disease caused by deep-vein thrombosis (DVT), the early evaluation of DVT[15] and PE[16] is therefore considered to be clinically important.

Fibrin-related markers such as D-dimer, fibrin and fibrinogen degradation products, and soluble fibrin (SF) are considered to be useful for the diagnosis of thrombosis. These markers are reported to be elevated in DVT/PE,[17-19] DIC,[20-22] acute myocardial infarction (AMI),[23,24] and thrombotic thrombocytopenic purpura.[25] In this regard, D-dimer has been

Table 1. Clinical Characteristics of the Patients Included in the Study

Underlying Diseases	Total	Without Thrombosis	With Thrombosis	Rate (%)
Solid cancer	191	167	24	12.5
Orthopedic diseases	178	150	28	16.0
Cardiovascular diseases	60	53	7	12.0
Collagen diseases	36	33	3	8.0
Digestive diseases	36	34	2	6.0
Infectious diseases	29	18	11	38.0
Hematological diseases	26	20	6	23.1
Diabetes mellitus	16	11	5	31.3
Without underlying diseases	15	0	15	100
Aneurysm and varicose	12	5	7	58.3
Trauma and burn	11	5	6	54.5
Obstetric diseases	6	6	0	0
Thrombophilia	4	2	2	50.0
Other diseases	11	11	0	0
Total	633	517	116	18.3

reported to be a negative predictor for DVT and less than 0.5 μg/mL of D-dimer is considered to exclude DVT in the most commonly used D-dimer assays in Europe and North America.[15] The International Society of Thrombosis and Haemostasis (ISTH) established the diagnostic criteria for overt-DIC using fibrin-related markers.[26] D-dimer is widely used to diagnose thrombosis as DVT, but many of the commercially available D-dimer assay kits contain different monoclonal antibodies and standard substances, and are based on different assay systems. The issue of standardization of D-dimer assays remains to be resolved, and several studies[27,28] have reported the basic data for standardization of D-dimer.

The present study was designed to evaluate the cutoff values of F1 + 2 in the diagnosis of thrombosis such as DVT, DIC, cerebral thrombosis, and AMI prospectively and to compare the findings to those for TAT, D-dimer, and SF. For this purpose, we determined the plasma concentrations of these molecules in 694 patients suspected of having thrombosis and also in 67 healthy volunteers.

Materials and Methods

Subjects

From June 1, 2003 to September 31, 2004, 694 patients (age range = 57.7 ± 17.8 years; mean = ±SD 398 females and 296 males) were suspected to have thrombosis (DVT, DIC, cerebral thrombosis or acute myocardial infarction) at hospitals affiliated with Mie University Graduate School of Medicine. The plasma concentrations of F1 + 2, SF, TAT, and D-dimer were examined in these patients and then were correlated with thrombosis. The study protocol was approved by the Human Ethics Review Committees of participating institutions, and a signed informed consent form was obtained from each subject. Thirty-four patients within 3 days after operation (OPE) and 29 patients who had undergone liver transplantation (LT) were excluded from analysis of the cutoff value. However, of the remaining 631 patients, 515 patients did not have any thrombosis, whereas 116 patients had thrombotic diseases, 66 with DVT, 27 with DIC, 10 with cerebral vascular accidents due to thrombosis (CVA), 5 with AMI, 4 with portal vein thrombosis, and 4 with arteriosclerosis obliterans. Deep-vein thrombosis was diagnosed by either ultrasonography or venography. Disseminated intravascular coagulation was diagnosed based on the ISTH overt-DIC diagnostic criteria.[26] Cerebral vascular accidents due to thrombosis were diagnosed either by computed tomography or by magnetic resonance imaging, and AMI was diagnosed by electrocardiograms and clinicolaboratory data. The underlying diseases of these patients are shown in Table 1.

Citrated blood samples were obtained from the peripheral veins of healthy subjects (see below) and from patients under fasting conditions. The blood samples were then centrifuged for 20 minutes at 3000 rpm. The supernatants (plasma) were analyzed within 4 hours. The plasma concentrations of F1 + 2, SF, TAT, and D-dimer were measured in patients with thrombosis at the onset and those without thrombosis at first consultation. The same parameters were also measured in 67 healthy subjects (age range = 38.6 ±

17.7-years-old; 58 males and 9 females) who were free of any diseases including thrombotic disease or hyperlipidemia, as confirmed by annual medical checkup.

Measurement of Plasma Concentrations of F1 + 2, TAT, D-Dimer, and SF

The plasma levels of F1 + 2 were measured by a new enzyme-linked immunosorbent assay (ELISA) for the determination of F1 + 2 (Dade Behring Marburg GmbH, Marburg, Germany). Two different monoclonal antibodies in this kit recognize the terminal end of N fragment 2. The plasma levels of TAT were measured using the TAT test (Sysmex, Kobe, Japan) by ELISA. The plasma D-dimer levels were measured by LPIA-ACE D-dimer (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan) using JIF23 monoclonal antibody. The JIF23 monoclonal antibody, which recognizes plasmin-digested N-terminus of the γ chain on the D region, was used for latex agglutination.[29] Soluble fibrin was also determined by the latex agglutination method using IATRO SF (Mitsubishi Kagaku Iatron Inc) containing monoclonal antibody IF-43, which recognizes a segment of the fibrin Aa chain (Aα-17-78) residue segment exposed in the E region of fibrin monomer (FM) when the FM molecule binds to the D region of another FM or fibrinogen. The antibody is coated for the SF assay.[30]

Statistical Analysis

The data are expressed as mean ± SD. Differences between groups were examined for statistical significance using the Mann-Whitney *U* test, whereas the correlation between the 2 variables was evaluated by Pearson's correlation analysis. A *P* value of less than .05 denoted the presence of a statistically significant difference. The usefulness of D-dimer and SF for the diagnosis of thrombosis, DVT, and DIC were examined by a receiver operating characteristic (ROC) analysis.[31] The cutoff values were determined by an ROC analysis. All statistical analyses were performed using the SPSS II software package (SPSS, Tokyo, Japan).

Results

The frequency of thrombotic diseases was high in patients with solid cancer, orthopedic diseases, hematological diseases, infectious diseases, cardiovascular diseases, diabetes mellitus, and aneurysm (Table 1). In healthy subjects, plasma concentrations of F1 + 2 were not distributed normally, with a maximum value of 214 pmol/L, minimum value 42 pmol/L, and median value of 121 pmol/L. The 95% confidence interval (95% CI) of F1 + 2 was from 56 to 213 pmol/L.

The plasma levels of F1 + 2, SF, D-dimer, and TAT (median; 25-75 percentile) were significantly higher in the patients with thrombosis (516 pmol/L; 349-709 pmol/L, 18.49 μg/mL; 8.68-35.24 μg/mL, 11.38 μg/mL; 6.58-19.06 μg/mL, and 15.87 ng/mL; 9.04-36.98 ng/mL, respectively), OPE (431 pmol/L; 331-520 pmol/L, 13.32 μg/mL; 8.17-22.10 μg/mL, 7.32 μg/mL; 3.04-12.78 μg/mL, and 17.38 ng/mL; 13.04-32.87ng/mL, respectively), and LT (590 pmol/L; 249-985 pmol/L, 16.03 μg/mL; 5.60-24.08 μg/mL, 6.54 μg/mL; 2.67-12.68 μg/mL, and 19.36 ng/mL; 12.69-32.51 ng/mL, respectively) than in those without thrombosis (192 pmol/L; 138-274 pmol/L, 2.79 μg/mL; 0.76-5.29 μg/mL, 0.88 μg/mL; 0.45-2.27 μg/mL, and 2.43 ng/mL; 1.58-5.50 ng/mL; $P < .01$, each; Figure 1). On other hand, the plasma concentrations of F1 + 2, SF, D-dimer, and TAT were significantly higher in patients without thrombosis than in healthy subjects ($P < .01$, each).

Figure 2 shows the positive predictive values (PPV) and negative predictive values (NPV) for several cutoff values of F1 + 2 in patients with thrombosis. When F1 + 2 levels of >300 pmol/L were used, more than 50% of the patients, excluding either those with LT or those who had undergone an operation, had some thrombosis and NPV for thrombosis was >95%. When the cutoff values were set at <100 pmol/L for F1 + 2, NPV for thrombosis was 100%, but PPV was <25%.

An ROC analysis showed the curves of F1 + 2, SF, D-dimer, and TAT to be similar and useful for the diagnosis of all thromboses (Figure 3). The area under the curve (AUC) of those markers was markedly high (Table 2). An ROC analysis provided adequate cutoff values of F1 + 2 (300 pmol/L), SF 6.8 (μg/mL), D-dimer (3.0 μg/mL), and TAT (7.8 ng/mL) for the diagnosis of all thromboses (Table 3). The sensitivity and specificity of F1 + 2 were 86.2% and 80.6%, respectively. These statistical values of F1 + 2 were similar to those of other molecular markers.

Table 4 shows the correlation between F1 + 2 and other hemostatic molecular markers. The plasma levels of F1 + 2 were closely correlated with SF, D-dimer, and TAT, and the correlation between F1 + 2 and TAT was the best.

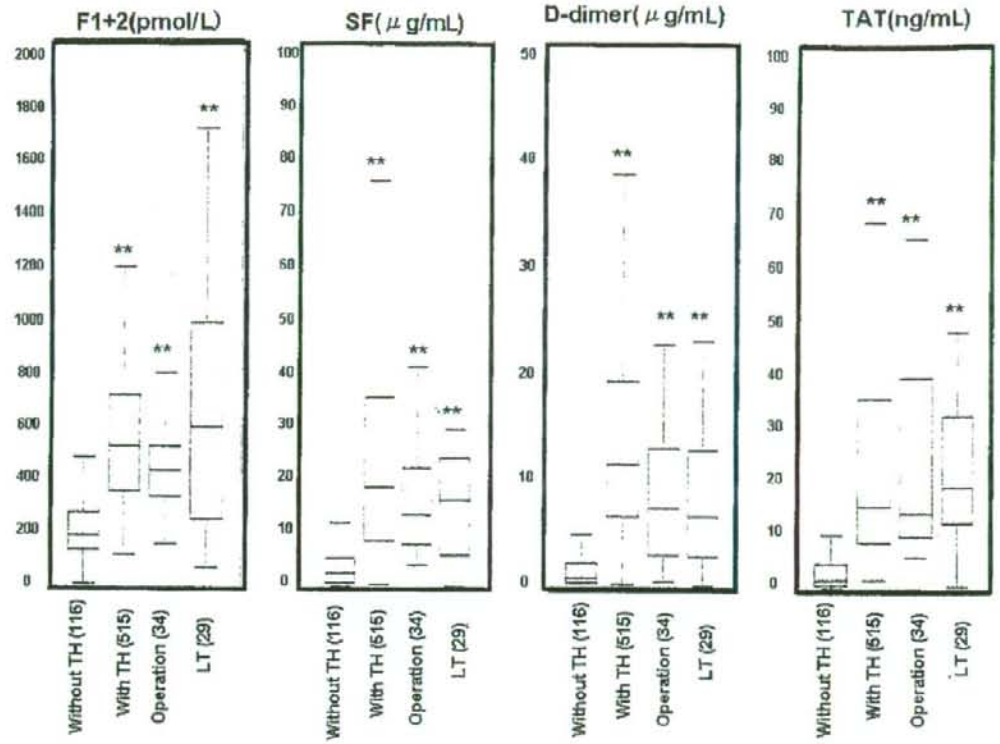

Figure 1. Plasma levels of F1 + 2, SF, D-dimer, and TAT in patients with or without thrombosis, those after the operation and those after LT. SF indicates soluble fibrin; TAT, thrombin-antithrombin complex; LT, liver transplantation. ** indicates $P < .01$ (comparison to without thrombosis). The box shows 25 percentile, median, and 75 percentile. Operation; patients within 3 days after operation, LT; patients after liver transplantation.

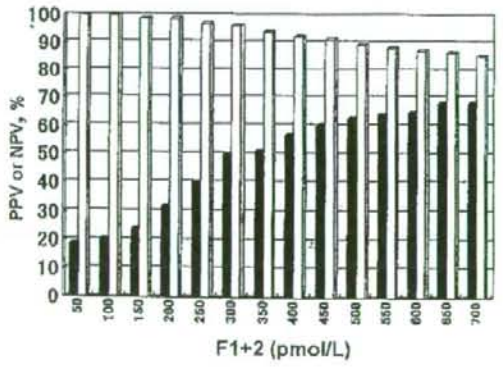

Figure 2. The PPV and NPV for plasma concentrations of F1 + 2 in thrombosis. PPV indicates positive predictive values; NPV, negative predictive values. Solid bar represents PPV and white bar represents NPV.

Discussion

The frequency of thrombotic diseases was high in patients with solid cancer, orthopedic diseases, hematological diseases, infectious diseases, cardiovascular diseases, diabetes mellitus, and aneurysm, suggesting that prevention of thrombosis will be important in these underlying diseases. Although the sample number was not ideal in this study, DVT was frequently associated with cancer and orthopaedic diseases, whereas DIC was frequently associated with cancer, infectious diseases, and aneurysm. Such frequencies were similar to those reported in previous studies.[9,12,13] Regarding the underlying diseases frequently associated with thrombosis (eg, DVT and DIC), the risk for thrombosis should be evaluated by a simple test. In the present study, we demonstrated the concentrations

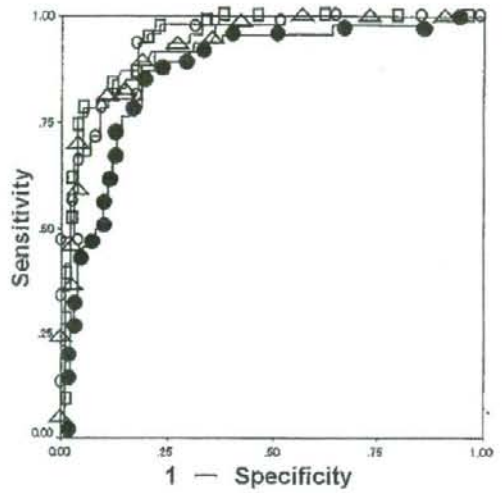

Figure 3. ROC analysis of F1 + 2, SF, D-dimer, and TAT for thrombosis. ROC indicates receiver operating characteristic; SF, soluble fibrin; TAT, thrombin-antithrombin complex. (□) TAT; (○) F1+2; (□) SF; (•) D-dimer.

Table 2. Area Under the Curve of Receiver Operating Characteristic in F1 + 2, Soluble Fibrin, D-Dimer, and Thrombin-Antithrombin Complex

	AUC
F1 + 2	0.938
D-dimer	0.910
SF	0.901
TAT	0.940

NOTES: AUC = area under the curve; SF = soluble fibrin; TAT = thrombin-antithrombin complex.

of F1 + 2 to be significantly high in patients with thrombosis such as DIC, DVT, CVA, and AMI, and these findings were similar to those for SF, D-dimer, and TAT.

In healthy subjects, the plasma concentrations of F1 + 2 were not distributed normally, and the 95% CI of F1 + 2 ranged from 56 pmol/L to 213 pmol/L, thus indicating that the patients with more than 220 pmol/L of F1 + 2 may have a hypercoagulable state. In contrast, the plasma levels of F1 + 2 were significantly high in patients with thrombosis such as the plasma levels of SF, D-dimer, and TAT. As a result, high concentrations of SF, D-dimer, and TAT could thus be considered as markers of thrombosis, because these parameters were also reported to be elevated in DVT,[32,33] DIC,[20,34] and hyperlipidemia.[35] It should be noted, however, no prospective studies have previously evaluated the F1 + 2 assay including the cutoff value for the diagnosis of thrombosis. The plasma levels of F1 + 2 were significantly high in patients with all types of thrombosis although the levels were also high in some patients who were not found to have any thrombosis.

More than 50% of patients who had more than 300 pmol/L of F1 + 2 had some thrombosis, suggesting that these patients need anticoagulant therapy such as aspirin for atherosclerotic thrombosis or warfarin for venous thrombosis. It is considered that these patients with a high value of F1 + 2 have hypercoagulable state. D-dimer is also useful for the diagnosis of DVT, but the cutoff values of D-dimer should be mentioned in each measurement kit.[28]

An ROC analysis showed that the curves of F1 + 2, SF, D-dimer, and TAT to be similar. Because both AUC of these markers, especially F1 + 2 and TAT, were high in ROC analysis, we believe that these markers are useful for the diagnosis of either thrombosis or a hypercoagulable state. In particular, both F1 + 2 and TAT may be more useful than D-dimer and SF for the diagnosis of thrombosis by AUC. An ROC analysis provided adequate cutoff values of F1 + 2 (300 pmol/L), SF (6.8 μg/mL), D-dimer (3.0 μg/mL), and TAT (7.8 ng/mL) for the diagnosis of all types of thromboses. The sensitivity and specificity of F1 + 2 were 86.2% and 80.6%, respectively. These statistical values of F1 + 2 were similar to those observed for other molecular markers, thus suggesting that the ability to diagnose thrombosis is similar among F1 + 2, D-dimer, SF, and TAT. Soluble fibrin has also been reported to reflect the early phase of DVT/PE, whereas D-dimer reflects secondary fibrinolysis after clot formation and the measurements of both D-dimer and SF may be recommended.[36,37] Both F1 + 2 and TAT reflect an earlier phase of thrombosis. The plasma levels of F1 + 2 were closely correlated with those of SF, D-dimer, and TAT and the correlation between F1 + 2 and TAT was best, thus suggesting that both markers reflect thrombin formation. Specificity for thrombosis was better in F1 + 2 than in TAT, thus suggesting that F1 + 2 may be the most useful marker for the earlier phase of thrombosis.

In conclusion, our findings suggest that high concentrations of hemostatic molecular markers,

Table 3. Cutoff Values of F1 + 2, Soluble Fibrin, D-Dimer, and Thrombin-Antithrombin Complex

	Cutoff Values	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Odds Ratio
F1 + 2	300 pmol/L	86.2	80.6	50.0	96.3	26.07
SF	6.8 μg/mL	89.7	88.0	62.7	97.4	63.01
D-dimer	3.0 μg/mL	82.8	82.4	51.3	95.5	22.47
TAT	7.8 ng/mL	82.4	82.4	72.4	90.4	24.79

NOTE: PPV = positive predictive values; NPV = negative predictive values; SF = soluble fibrin; TAT = thrombin-antithrombin complex.

Table 4. Correlation Between F1 + 2 and Other Hemostatic Molecular Markers[a]

	F1 + 2	SF	D-Dimer	TAT
F1+2	1.0	0.543 ($P < .001$)	0.681 ($P < .001$)	0.760 ($P < .001$)
SF	0.543 ($P < .001$)	1.0	0.588 ($P < .001$)	0.691 ($P < .001$)
D-dimer	0.681 ($P < .001$)	0.588 ($P < .001$)	1.0	0.710 ($P < .001$)
TAT	0.760 ($P < .001$)	0.691 ($P < .001$)	0.710 ($P < .001$)	1.0

NOTE: SF = soluble fibrin; TAT = thrombin-antithrombin complex.
[a]The numbers show the correlation coefficients.

especially F1 + 2 which is also known as a marker for a hypercoagulable state, reflect a high risk for thrombosis.

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Original article

Prevention of postoperative venous thromboembolism in Japanese patients undergoing total hip or knee arthroplasty: two randomized, double-blind, placebo-controlled studies with three dosage regimens of enoxaparin

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Abstract

Background. Enoxaparin is a low-molecular-weight heparin indicated in Europe and North America for the prevention of venous thromboembolism (VTE) in patients undergoing major orthopedic surgery. Registration trials of enoxaparin have been conducted primarily in Caucasian populations, and the efficacy and safety of enoxaparin in Japanese patients have not been demonstrated. We evaluated three dosage regimens of postoperative enoxaparin in Japanese patients undergoing elective total hip or knee arthroplasty.

Methods. Two multicenter, randomized, double-blind studies enrolled 436 and 396 Japanese adults undergoing total hip or knee arthroplasty, respectively. The dosage regimens of enoxaparin were 20 mg once daily (qd), 40 mg qd, 20 mg twice daily (bid), or placebo for 14 consecutive days. The primary efficacy endpoint was the incidence of VTE in the modified intention-to-treat (mITT) population up to 15 days after surgery. VTE was defined as a composite of deep vein thrombosis (determined by venography) and symptomatic pulmonary embolism (confirmed by appropriate objective methods). Patients were also followed up at 90 days for VTE events. The primary safety outcome was the incidence of any bleeding during treatment and the follow-up period.

Results. In the mITT populations, the incidence of VTE was 41.9% and 60.8% in the placebo groups after hip or knee arthroplasty, respectively. 25.9% and 44.9% in the enoxaparin 20 mg qd groups. 33.8% and 35.1% in the enoxaparin 40 mg qd groups, and 20.0% and 29.8% in the enoxaparin 20 mg bid groups. Only enoxaparin 20 mg bid significantly lowered the risk of VTE relative to placebo (by 52.2% and 51.0% after hip and knee arthroplasty, respectively). At the 90-day follow-up, no further cases of VTE were reported. In both the hip and knee studies, the four treatment groups did not differ significantly regarding the incidence of patients with any bleeding.

Conclusions. Our findings support the use of enoxaparin (20 mg bid daily, commencing 24–36 h postoperatively) in Japanese patients undergoing total hip or knee arthroplasty.

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Introduction

Patients undergoing hip or knee arthroplasty are at particularly high risk of postoperative venous thromboembolism (VTE).[1] If no antithrombotic measures are taken, the prevalence of total deep vein thrombosis (DVT) is in the range of 42%–57% for total hip arthroplasty (THA) and 41%–85% for total knee arthroplasty (TKA).[1,2] Thrombi involving the proximal deep veins, which are considered as the most likely to become symptomatic and to result in pulmonary embolism (PE), also occur in up to 36% of patients after joint arthroplasty surgery.[1] In Western countries, nearly all arthroplasty patients are treated with anticoagulants, leading to a paucity of recent data on PE in the absence of prophylaxis. The rate of PE in early trial s was 3%–28% in arthroplasty patients, and 0.1%–2.0% of these events were fatal.[1] The absolute risk for VTE in Japanese patients is comparable to that observed in European and North American studies: The risk indicated 23% and 49% in a prospective epidemiological study,[3] 33.8% and 65.3% after THA and TKA, respectively, in the placebo arm of a recent randomized clinical trial.[4]

In North American trials, enoxaparin 30 mg twice daily (bid) reduced the risk of total DVT by more than 70% compared with placebo after both THA and TKA, without increasing the bleeding risk.[5,6] Findings from a systematic review of 19 randomized controlled trials involving approximately 7000 patients undergoing elective hip arthroplasty showed that treatment with a low-molecular-weight heparin (LMWH) resulted in a 24% reduction in the incidence of both DVT and minor bleeding (both $P < 0.05$) compared with treatment with unfractionated heparin.[7] Routine use of such thromboprophylactic measures in the management of hip or knee arthroplasty has greatly reduced the likelihood of PE[8] without increasing the risk of

major bleeding.[7] Use of LMWH in patients undergoing THA or TKA has become standard practice in Western countries.[1]

The improved efficacy and safety of LMWH relative to unfractionated heparin for major surgery have been established, but the optimum dose and duration of therapy have not yet been determined for all patient populations.[9,10] Usual practice in Europe is to administer LMWH preoperatively, whereas in the United States these agents are administered postoperatively.[8,11] The approved enoxaparin dosage regimen in Europe is 40 mg once daily (qd) (administered 12 h preoperatively), whereas in North America a regimen of 30 mg bid (administered 12–24 h postoperatively) is recommended.[9]

Among Japanese patients, less information is available regarding the dosage and timing of initiation of enoxaparin treatment to maximize efficacy while minimizing hemorrhagic risk. To address this issue, we conducted two randomized, double-blind, Phase IIB/III trials that compared the clinical efficacy and safety versus placebo of three dosage regimens of enoxaparin initiated postoperatively in Japanese patients undergoing primary THA or TKA.

Patients and methods

Two randomized, double-blind, placebo-controlled, parallel-group, multicenter, Phase IIb/III clinical studies were conducted between 1999 and 2002 among patients undergoing primary THA and TKA at 51 medical institutions in Japan. Both studies were approved by the appropriate institutional review board and were undertaken in full compliance with the principles of Article 14-3 and Article 80-2 of the Pharmaceutical Affairs Law, the Ministry of Health and Welfare Ordinance on Good Clinical Practice, and the Declaration of Helsinki. Written informed consent was obtained from all patients before their participation in the study according to the guidelines at each institution.

Patient selection

Patients aged ≥20 years (no upper age limit was applied) undergoing elective primary THA or TKA were eligible for enrollment in the studies. Patients requiring revision THA or TKA were excluded. Other exclusion criteria were contraindication to heparin therapy; positive clinical evidence of chronic (postphlebitic syndrome) or acute DVT within 12 months of the study drug treatment; documented allergy to iodine or contrast medium; impaired renal function (creatinine clearance <30 ml/min or plasma creatinine level >1.5 mg/dl); severe hepatic disease; uncontrolled hypertension; illicit drug use or alcohol abuse; treatment with other investigational agents within 3 months of surgery; failure to achieve postoperative hemostasis; and female subjects if pregnant or breast-feeding. Use of the following medications or interventions was prohibited from 2 days before surgery until the end of the study drug treatment: low-dose aspirin and other antiplatelet agents, dextran, anticoagulants, thrombolytics, and nonsteroidal antiinflammatory drugs (within 72 h of surgery). In addition, use of intermittent pneumatic compression devices was not allowed from the end of surgery to the completion of venography; and postoperative epidural or spinal analgesia was prohibited from 2 h before the first dose of the study drug until completion of venography. The use of compression bandages and stockings was allowed.

Treatment regimens

Patients were randomized in a 1:1:1:1 ratio to receive a subcutaneous injection of enoxaparin 20 mg qd, enoxaparin 40 mg qd, enoxaparin 20 mg bid, or placebo (saline) into the anterolateral or posterolateral abdominal wall for 14 consecutive days (Fig. 1). The enoxaparin dosages used in this study were determined based on the approved dosage regimen in the United States (i.e., 30 mg bid)[12] and scaled to account for the difference in mean body weight between Japanese and Caucasian patients. Specifically, the mean body weight of Japanese patients who participated in a prospective epidemiological study of enoxaparin for DVT prophylaxis was approximately two-thirds that of their Caucasian counterparts (54 kg vs, 80 kg, respectively).[3] Therefore, in a postoperative regimen in Japanese patients, an enoxaparin dosage of 20 mg bid was thought to be equivalent to that prescribed in the United States. Once-daily regimens of enoxaparin 40 mg and 20 mg were also evaluated, as this protocol may be more acceptable to patients and medical staff.

Treatment was started 24–36 h after surgery. A postoperative regimen was used in all patients (including those with an epidural tube) because of published reports describing spinal hematomas in association with concurrent use of spinal or epidural anesthesia and heparin or LMWH in Western countries.[13,14] In Japan, spinal or epidural anesthesia is frequently used for patients undergoing surgery to the lower extremities. Treatment was scheduled to last for 14 days. Patients were followed up at 90 days after surgery to determine whether VTE events had occurred.

Analysis of populations

Patients were included in the safety population if they had provided written informed consent, had received

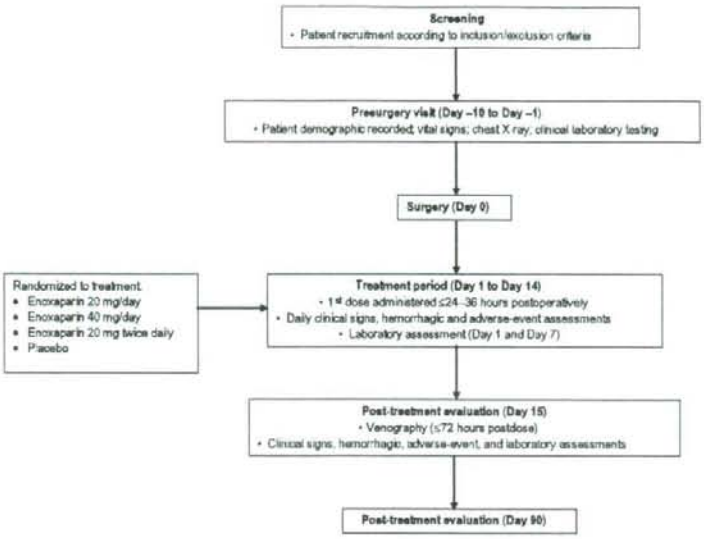

Fig. 1. Study time line, portraying scheduled observations, tests, evaluations, and interventions

at least one dose of the study medication, and there were no violations of good clinical practice in their management. Those in the safety populations who had clearly interpretable VTE imaging tests were included in the modified intention-to-treat (mITT) populations.

Assessments and outcome definitions

Patients underwent routine laboratory tests, physical examinations, vital signs, and chest radiography at preoperative screening, during surgery, and throughout the postoperative period (Fig. 1).

The primary efficacy endpoint was the incidence of VTE (DVT or PE) in the mITT populations objectively confirmed within 72 h after completion or discontinuation of treatment. Mandatory venography (or ultrasonography when venography was difficult to perform due to extensive swelling of the lower extremity caused by thrombi in the deep veins) was performed in all patients to detect any DVTs at the end of the study. Suspicion of a symptomatic PE was objectively confirmed by ventilation perfusion lung scans or pulmonary angiography. All objective tests, including venography, ventilation perfusion lung scans, and pulmonary angiography, were centrally adjudicated by an independent expert panel blinded to the treatment group of the patient.

The primary safety endpoint was the incidence of any bleeding — a composite of the incidence of major and minor bleeding. Bleeding was assessed daily during the 14-day treatment period, within 24 h after completion or discontinuation of treatment, and at day 15 at a follow-up consultation (Fig. 1). A bleeding episode was classified as major if it was retroperitoneal, intracranial, or intraocular or if it was associated with: death; transfusion of ≥2 units of packed red blood cells or whole blood (except autologous); a reduction in the hemoglobin level of ≥2 g/dl; or a serious or life-threatening clinical event that required medical intervention. Suspicion of intraabdominal or intracranial bleeding was confirmed by ultrasonography, computed tomography, or magnetic resonance imaging. Minor bleeding episodes were defined as having at least one of the following features: epistaxis lasting >5 min or requiring intervention; ecchymosis or hematoma with a maximum size of >5 cm; hematuria not associated with urinary catheter trauma; gastrointestinal hemorrhage not related to intubation or a nasogastric tube; wound hematoma or hemorrhagic wound complications not associated with major hemorrhage; or subconjunctival hemorrhage requiring cessation of medication.

Secondary safety endpoints were assessed over a similar time frame and included the incidence, type, and severity of generalized adverse events (AEs) based on clinical and biochemical evaluations.

Statistical analyses

For the THA study, VTE event rates were expected to be 30% in the placebo group, 20% in the enoxaparin 20 mg qd group, and 10% in the enoxaparin 40 mg qd group. With a one-sided type I error rate of 0.05 and a type II error of 0.1, a total of 72 patients were required

per treatment arm to a show a dose–response relation by the Cochran–Armitage trend test. To demonstrate a statistically significant difference between treatment with placebo and enoxaparin 40 mg qd with a one-sided type I error of 0.025 and a type II error of 0.1, a total of 92 patients per arm were needed. With the anticipated rate of patients who could not be evaluated at approximately 15%, the study plan was to enroll 110 patients per treatment arm.

For the TKA study, it was assumed that the type I and type II error rates would be the same as for the THA study; VTE event rates were expected to be 50% in the placebo group, 35% in the enoxaparin 20 mg qd group, and 25% in the enoxaparin 40 mg qd group. Therefore, 62 patients per treatment arm were required to show a dose–response relation (Cochran–Armitage trend test), and 85 patients per arm were required for pairwise comparison. With the anticipated rate of patients who could not be evaluated at approximately 15%, the study plan was to enroll 100 patients per treatment arm. If these assumptions were met, two-sided tests at the 0.025 level of statistical significance would have 90% power.

The χ^2 test was used to determine if there was a difference between groups for all baseline characteristics except age and the body mass index (BMI), for which an analysis of variance test was used. The Cochran–Armitage test was used to examine whether the incidence of thromboembolism was reduced in a dose-dependent fashion and to explore the relation between the incidence of VTE across groups. A Dunnett test was performed to test differences in the incidence of thromboembolism between the placebo and enoxaparin 20 mg qd/enoxaparin 40 mg qd groups. Fisher's exact test was performed to confirm statistically significant differences between the placebo and enoxaparin 20 mg bid groups.

Major and minor bleeding categories were analyzed combined, as any bleeding event, and separately; pairwise comparisons of the incidence of bleeding events were made using the χ^2 test. Each bleeding event was identified as bleeding from surgical or other sites.

Results

Patient disposition and analysis populations

A total of 407 THA and 364 TKA patients were randomized and received one or more doses of the study medication (safety population), among whom 337 and 315 patients, respectively, were included in the mITT population for each study (Fig. 2). The most common reason for withdrawal from the mITT population was a missing or defective evaluation of VTE imaging test data (due to protocol violations during their assessment).

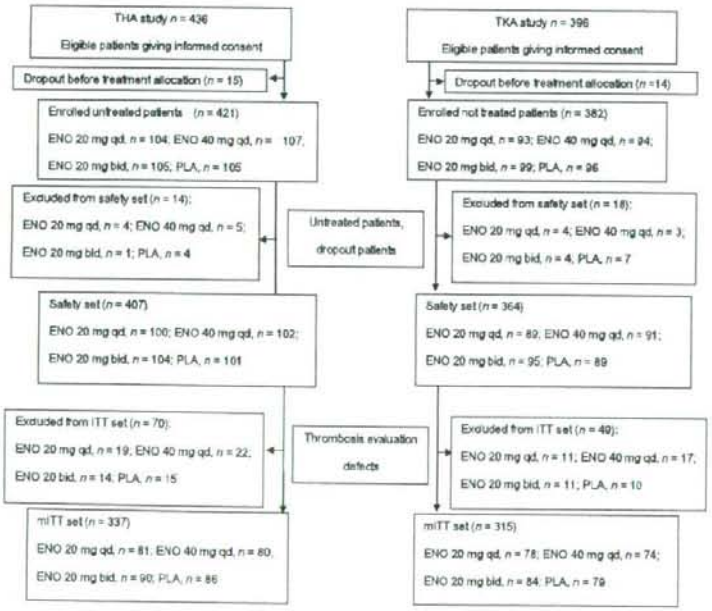

Fig. 2. Disposition of Japanese patients in two studies comparing three enoxaparin dosage regimens versus placebo as thromboprophylaxis for total hip or knee arthroplasty. *bid*, twice daily; *ENO*, enoxaparin; *ITT*, intention to treat; *mITT*, modified intention to treat; *PLA*, placebo; *qd*, once daily; *THA*, total hip arthroplasty; *TKA*, total knee arthroplasty

Table 1. Baseline demographics and clinical characteristics of the intention-to-treat population who underwent primary total hip arthroplasty

Characteristic	Placebo ($n = 86$)	Enoxaparin 20 mg qd ($n = 81$)	Enoxaparin 40 mg qd ($n = 80$)	Enoxaparin 20 mg bid ($n = 90$)
Female (no.)	75 (87.2%)	71 (87.7%)	74 (92.5%)	75 (83.3%)
Age (years), mean (SD)	62.0 (10.3)	63.3 (10.4)	60.6 (9.9)	63.0 (9.3)
Height (cm), mean (SD)	152.5 (7.9)	150.9 (7.6)	151.8 (6.6)	151.3 (7.3)
Weight (kg), mean (SD)	56.0 (10.0)	53.6 (9.3)	54.2 (9.8)	54.3 (9.4)
BMI (kg/m^2), mean (SD)	24.0 (3.4)	23.5 (3.4)	23.5 (3.7)	23.7 (3.6)
BMI > 25 kg/m^2 (no.)	34 (39.5%)	23 (28.4%)	26 (32.5%)	31 (34.4%)
Systolic BP (mmHg), mean (SD)	127.6 (17.2)	126.1 (16.4)	125.9 (17.5)	128.1 (16.2)
Orthopedic disease (no.)				
Osteoarthritis	76 (88.4%)	67 (82.7%)	66 (82.5%)	75 (83.3%)
Rheumatoid arthritis	2 (2.3%)	6 (7.4%)	5 (6.3%)	7 (7.8%)
Osteonecrosis	3 (3.5%)	3 (3.7%)	4 (5.0%)	2 (2.2%)
Other	5 (5.8%)	5 (6.2%)	5 (6.3%)	6 (6.7%)
History of smoking (no.)	14 (16.3%)	12 (14.8%)	10 (12.5%)	16 (17.8%)
Previous major disease/complications/surgery (no.)	7 (8.1%)	1 (1.2%)	3 (3.8%)	12 (13.3%)
Previous minor disease/complications/surgery (no.)	85 (98.8%)	81 (100%)	77 (96.3%)	86 (95.6%)
Use of cement (no.)	35 (40.7%)	32 (39.5%)	35 (43.8%)	42 (46.7%)
Type of anesthesia (no.)				
Regional	0	0	0	0
General	14 (16.3%)	14 (17.3%)	13 (16.3%)	19 (21.1%)
Both	72 (83.7%)	67 (82.7%)	67 (83.8%)	71 (78.9%)
Duration of surgery (h), mean (SD)	2.15 (0.78)	2.17 (0.64)	2.06 (0.65)	2.05 (0.66)

BP, blood pressure; bid, twice daily; BMI, body mass index; qd, once daily

Patient population: demographics and baseline medical characteristics

Patients in all treatment arms in each study had similar demographic characteristics, clinical diagnoses, disease etiologies, and disease histories except with regard to body weight in the TKA study (Tables 1, 2). The only exception was the TKA study in which patients in the enoxaparin 20 mg bid group had the highest mean weight ($P = 0.010$) (Table 1). Although osteoarthritis was the most common reason for joint arthroplasty in both studies, it affected more patients requiring THA than TKA (84% vs. 68%, respectively). Rheumatoid arthritis was the second most common reason for joint arthroplasty, affecting approximately one-third of patients in the TKA group. Fewer than 15% of patients in both studies had a history of tobacco use. Physical DVT prophylaxis with compression stockings or bandages was used by 53.7% of patients in THA study and 64.4% in the TKA study.

Clinical outcomes

THA study

The incidence of the primary efficacy endpoint (VTE objectively confirmed within 72 h of completing or discontinuing treatment) in the mITT population was 41.9% in the placebo group, 25.9% in the enoxaparin 20 mg qd group ($P = 0.022$, Dunnett test), 33.8% in the enoxaparin 40 mg qd group ($P = 0.188$, Dunnett test), and 20.0% in the enoxaparin 20 mg bid group ($P = 0.001$, Fisher's exact test) (Fig. 3, Table 3). There was no enoxaparin dose–response relation for the incidence of VTE ($P = 0.112$, Cochran–Armitage test).

One patient in the THA study treated with enoxaparin 40 mg qd had both DVT (confirmed by venography) and symptomatic PE (incidence 1.2%). At the 90-day follow-up visit, no additional episodes of VTE were reported. No episodes of PE occurred in the placebo, enoxaparin 20 qd, or enoxaparin 20 mg bid groups. Proximal DVT occurred in 10.4% of patients treated with placebo versus 3.7%, 7.5%, and 3.3% in the enoxaparin 20 mg qd, 40 mg qd, and 20 mg bid groups, respectively.

TKA study

The incidence of VTE was 60.8% in the placebo group, 44.9% in the enoxaparin 20 mg qd group, 35.1% in the enoxaparin 40 mg qd group, and 29.8% in the enoxaparin 20 mg bid group (Table 3, Fig. 3). The incidence of VTE was significantly lower, compared with the placebo group, in the enoxaparin 40 mg qd ($P = 0.001$, Dunnett test) and 20 mg bid groups ($P = 0.001$, Fisher's exact test) but not in the enoxaparin 20 mg qd ($P = 0.039$, Dunnett test; $P < 0.025$ was required for significance). Enoxaparin 20 mg bid was not inferior to enoxaparin 40 mg qd based on the 95% confidence interval (CI) of