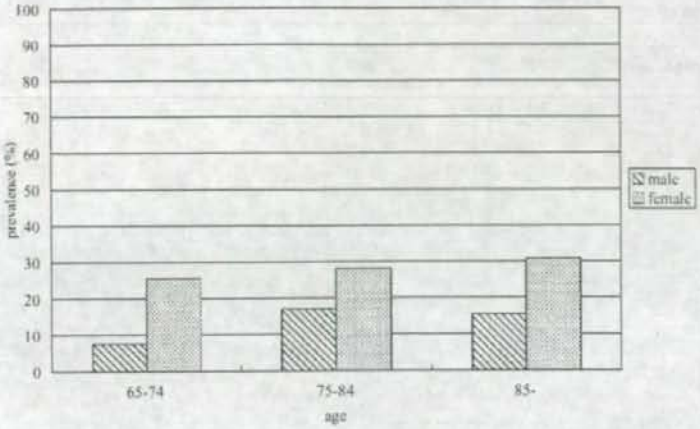

Fig. 2. Prevalence of symptomatic knee OA by age and sex group

Table 2. Comparison of the subjects with and without radiographic knee OA

Parameter	OA (n = 179)	Non-OA (n = 417)	P
Age	74.7 ± 6.6	73.1 ± 6.1	0.004
Menopause	48.8 ± 5.0	48.1 ± 5.3	NS
BMI	24.1 ± 3.2	22.4 ± 2.7	<0.001
BMD (g/m^2)	0.5 ± 0.1	0.6 ± 0.2	NS
Sex (male/female)	36/143	168/249	<0.001
Fracture (+)	73 (41.2%)	160 (39.0%)	NS
Operation (gastrectomy/overiectomy/others)	5/6/164	26/17/367	NS
Knee trauma (+)	14 (8.0%)	25 (6.1%)	NS
Osteoporosis (+)	31 (17.7%)	55 (13.4%)	NS
DM* (+)	14 (8.0%)	32 (7.8%)	NS
Gout (+)	4 (2.3%)	4 (1.0%)	NS
Sports activity — like	21 (12.2%)	83 (20.3%)	0.028
Working			
White-collar workers	89 (51.1%)	219 (53.0%)	NS
Blue-collar workers	85 (48.9%)	194 (47.0%)	
Smoking — yes	23 (12.8%)	90 (21.7%)	0.016
Alcohol ingestion (yes)	26 (14.5%)	89 (21.5%)	N.S.
Milk ingestion (yes)	140 (78.2%)	317 (76.2%)	N.S.

OA, osteoarthritis; BMI, body mass index; BMD, bone mineral density; DM, diabetes mellitus; NS, not significant
Age, Menopause, BMI, and BMD are represented as means ± SD

Cochran-Armitage test for trend). In all age groups, an investigation of the sex distribution revealed a greater prevalence of knee OA in women.

The prevalence of symptomatic knee OA were 21.2% overall: 10.7% in men and 26.7% in women. Therefore, the prevalence of symptomatic knee OA was higher in women than in men ($P < 0.001$). Of the cases of definite radiographic knee OA, 61.1% were symptomatic in men and 73.4% in women. Figure 2 shows the prevalence of symptomatic knee OA by age and sex grouping. In both sexes, the prevalence continued to increase with age.

Table 2 shows the comparison of the subjects with and without radiographic knee OA. There were significant differences between osteoarthritic and nonosteoarthritic subjects with regard to age ($P = 0.004$), BMI ($P < 0.001$), sex ($P < 0.001$), sports activity ($P = 0.028$), and cigarette smoking ($P = 0.016$).

We subsequently performed logistic regression analysis using the stepwise method. We found significant differences in the risk of radiographic knee OA with BMI, sex, age, and BMD. Odds ratios for BMI were 1.22 [95% confidence interval (CI) 1.13–1.31], suggesting that a 1 kg/m^2 increase in BMI raised the risk of knee OA by 22%. The risk of knee OA in women was 6.73-fold (95% CI 3.17–14.30) the risk in men. Odds ratios for age were 1.09 (95% CI 1.05–1.13), suggesting that a 1-year increase in age raised the risk of knee OA by 9%. Odds ratios for BMD were 1.53 (95% CI 1.20–1.95), suggesting that a 0.1 g/cm^2 increase in BMD raised the

Table 3. Logistic regression analysis (predictive factors determined by stepwise method)

Variables entered in the model	Odds ratio	95% CI	*P*
BMI	1.22	1.13–1.31	<0.001
Sex (female/male)	6.73	3.17–14.30	<0.001
Age	1.09	1.05–1.13	<0.001
BMD* (0.1 g/cm^2)	1.53	1.20–1.95	0.001
Variables not entered in the model			
Fracture			
Operation			
Knee trauma			
Osteoporosis			
DM			
Gout			
Sports activity			
Working			
Smoking			
Alcohol			
Milk			

risk of knee OA by 53% (Table 3). Therefore, high BMI, female sex, older age, and high BMD were significantly associated with an increased risk for radiographic knee OA.

Discussion

The present study showed that the prevalence of definite radiographic knee OA was 30.0% overall: 17.7% in men and 36.5% in women. Although population-based prevalence studies of knee OA have been reported worldwide, there are considerable discrepancies in population prevalence by reviewers.[12,13] These discrepancies were due to differences in race and lifestyle factors, different methodologies (e.g., inclusion or absence of men), or different definitions of symptomatic and radiographic OA. To minimize these discrepancies, radiographs in this study were evaluated by the Kellgren-Lawrence grading system, which has the most widely used radiographic criteria for OA. We used the same definition of radiographic OA (grade 2 or higher) as was used in well-known epidemiological studies, such as the Framingham OA Study and the Rotterdam Study, because we can then compare the prevalence of knee OA.

Thus, we compared the prevalence of knee OA in our study and other populations that were studied using the same criteria: radiographic OA with a Kellgren-Lawrence grade of 2 or higher in patients 65–74 years of age. The prevalences in our study were 32.3% in women and 13.5% in men, which was higher than in Bulgaria (9.6% in both men and women),[14] the United States (18.0% in women, 8.3% in men),[15] and Sweden (26.5% in women, 4.5% in men),[5] but lower than in Holland (35.2% in women, 20.9% in men)[16] or Northern England (56.3% in women, 42.3% in men).[17] These discrepancies may be due to differences in race, lifestyle, or socioeconomic background.

Many population-based studies have shown that obesity, aging, and female sex are risk factors for knee OA. Despite the strong association between aging and knee OA, the exact relations between aging and knee OA remain unclear. With age, joint use becomes more frequent, as does joint injury; cartilage degeneration progresses; and quadriceps muscle power becomes weak. Thus, OA is thought not to be simply the result of mechanical "wear and tear" from joint use with age but to have multiple causes.

Obesity has been shown to increase the risk for knee OA in various populations.[1,2] Obesity could increase stress on cartilage and induce breakdown simply on the basis of excess force across a weight-bearing joint. This is a mechanical effect by which obesity could cause OA. Another mechanism is metabolic concomitants of adiposity. Obese individuals (i.e., those with excess adipose tissue) may have abnormal levels of certain hormones or circulating factors, such as an insulin-like growth factor that may affect cartilage breakdown and lead to OA. Felson and Chaisson pointed out that weight loss is likely to alleviate symptoms and delay disease progression in patients with knee OA.[18] Therefore, obesity is an important modifiable risk factor for OA.

Results from many epidemiological studies show that the prevalence of OA is similar in men and women until age 50, after which its prevalence increases more rapidly in women, as recognized from our report.[4] These findings suggest that estrogen deficiency may promote the development of OA. However, there is an ongoing debate as to whether estrogen contributes to the patho-

genesis of OA, with some investigators suggesting a protective effect[3,19] and others identifying a negative role.[20] Other studies have examined sex-based genetic differences,[21,22] cartilage thickness,[23] and sex-related anatomical differences.[24] However, the exact reasons for sex differences in knee OA are not clear.

Another potential mechanism for estrogen in the pathogenesis of OA involves its indirect role in maintaining bone density. Several studies have shown that knee OA is associated with higher bone mass than in healthy controls.[7-9] Hypotheses for this association include one that higher bone mass increases subchondral bone stiffness and therefore increases joint loading and the risk for joint OA. Yokozeki et al. reported that the BMD in patients with severe knee OA was significantly greater than that in healthy individuals.[25] However, the BMD was measured at the lumbar spine, which might reflect the influence of facet joint OA or the presence of osteophytes or aortic calcification. In the Chingford study, Hart et al. reported that bone density at the lumbar spine and femoral neck is higher in a population of middle-aged women with osteoarthritis, and these results were not affected after adjustment for confounding variables including the presence of osteophytes in the lumbar spine.[26] In the Rotterdam study, Burger et al. also reported that radiographic knee OA is associated with higher BMD at the femoral neck.[6] Also in the Framingham study, Hannan et al. found that femoral BMD is higher in women with knee OA.[8] In a study of forearm BMD in postmenopausal women, Iwamoto et al. found significantly higher BMD in women who had knee OA than in the controls.[27] In the present study, we also showed that knee OA is associated with higher BMD measured at the distal radius with no influence of OA.

However, the association of BMD with knee OA also remains controversial. Several studies concluded that some cases of severe OA may be associated with low BMD. Previous reports from Japan have suggested that varus OA of the knee may be associated with high or low BMD in which trabecular microfractures of the proximal tibia may create the varus deformity with secondary OA.[10] More recent studies showed that OA progression may be associated with increased bone resorption and even low BMD.[28] It seems likely that high BMD contributes only to the initial occurrence of disease and not necessarily to its progression. More studies are needed to clarify this.

There are some limitations of this study. Although our subjects were drawn from a population-based study, they were healthy enough to visit the hospital. This selection introduces a health-selection bias. On the other hand, we recruited community inhabitants for the medical checkup on musculoskeletal conditions. This selection might introduce symptomatic knee patients. It is difficult to judge which more strongly influences this result or if both are offset. Another limitation is that this study was performed in a limited geographic region, which may not be representative of Japan as a whole. Miyagawa is a typical mountain village, suggesting that our data must be compared with the data of other villages or cities in Japan. Finally, our analysis did not include patellofemoral joint radiographs, which would likely increase the prevalence of radiographic outcomes and perhaps increase the concordance between radiographic outcomes and symptoms.

Conclusions

The prevalence of definite radiographic knee OA was 30.0% and that of symptomatic knee OA was 21.2%. Moreover, high BMI, female sex, older age, and high BMD were significantly associated with an increased risk for radiographic knee OA.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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Elevated Levels of Soluble Fibrin in Patients with Thrombosis or a Pre-Thrombotic State

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Abstract: *Background*: Soluble fibrin (SF) is considered to be useful for the diagnosis of thrombosis, however, evidence for the diagnosis of pre-thrombosis by SF is still not well established.

Objective: The present study was designed to evaluate the usefulness of new SF assay (New SF) in the diagnosis of thrombosis and a pre-thrombotic state.

Patients/Methods: The plasma concentrations of New SF were measured in 748 inpatients suspected to have thrombosis and they correlated with thrombosis.

Results and Conclusions: The plasma concentrations of New SF were significantly higher in patients with disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and cerebral thrombosis, in comparison to those of patients without thrombosis, but there was no significant difference of the New SF assay between patients with thrombosis and those after an operation. The New SF assay was moderately correlated with the other two SF assays. The New SF levels were significantly higher in patients before the onset of thrombosis than in those without thrombosis but other hemostatic molecular markers were not significantly elevated. Our findings suggest that the New SF assay is useful for the diagnosis of not only thrombosis but also of a pre-thrombotic state.

Keywords: Pre-thrombotic state, deep vein thrombosis (DVT), soluble fibrin (SF), disseminated intravascular coagulation (DIC).

INTRODUCTION

The presence of soluble fibrin (SF) [1] in plasma is an indicator of thrombin activation in the blood as are the thrombin-antithrombin complex (TAT) (2) and prothrombin fragment F1+2 [2]. SF, D-dimer and fibrinogen degradation products (FDP) are fibrin-related markers which are also considered useful for the diagnosis of thrombosis, and 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, 11] and thrombotic thrombocytopenic purpura (TTP) [11]. The International Society of Thrombosis and Haemostasis (ISTH) established the diagnostic criteria for overt-DIC using fibrin-related markers [12]. 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 standardization of D-dimer assays remains to be resolved, several studies [13, 14] reported basic data for the standardization of D-dimer.

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Thrombin cleaves fibrinopeptide A and B from the Aαand Bβchains of fibrinogen, respectively. These are called desAA-fibrin monomer (FM) and desAABB-FM, which polymerize with each other and form fibrin clots. These molecules in soluble form circulate in the blood and are termed as SF. SF mainly consists of desAA-FM or desAABB-FM, which forms a complex with fibrinogen or its derivates [15-17]. Recently, the monoclonal antibody J2-23 which recognizes the epitope within the Aα502-521 region of fibrinogen, was developed for measuring the SF level [18].

The present study was designed to evaluate the usefulness of the New SF assay in the diagnosis of thrombosis and the pre-thrombotic states associated with DVT, DIC, and cerebral thrombosis. For this purpose, we determined the plasma concentration of these molecules in 748 patients suspected to have thrombosis and 91 healthy volunteers.

MATERIALS AND METHODS

Subjects

From January 1, 2004 to December 31, 2006, 748 patients (median; 25% percentile - 75% percentile) (63 years of age; 49-72 years of age; 490 females and 258 males) were suspected of thrombosis (DVT, DIC or CT) in the hospitals

affiliated with Mie University Graduate School of Medicine. The plasma concentrations of hemostatic molecular markers 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. Fourty-nine patients who were 3 days of having undergone an operation (OPE) and 31 patients who had already undergone liver transplantation (LT) were excluded from the analysis for thrombosis or pre-thrombotic state. Among the remaining 668 patients, 435 patients (62 years of age; 45-71 years of age; 267 females and 168 males) did not have any thrombosis, 208 patients had thrombotic diseases and 25 patients had thrombosis within a week after blood sampling. There are 149 with DVT (67 years of age; 53-74 years of age; 113 females and 36 males), 33 with DIC (65 years of age; 51-75 years of age; 21 females and 12 males), 15 with cerebral thrombosis (68 years of age; 67-75 years of age; 7 females and 8 males) and 11 other thromboses (69 years of age; 54-74 years of age; 7 females and 4 male). DVT was diagnosed with echo or venography. DIC was diagnosed by the ISTH overt-DIC diagnostic criteria (10). Cerebral thrombosis was diagnosed by computed tomography (CT) or magnetic resonance imaging (MRI) and AMI was diagnosed by the electrocardiogram and laboratory data. Among the underlying diseases in these patients, cancer was identified in 111 patients, orthopaedic conditions in 257, haematological diseases in 44, autoimmune diseases in 39, thrombophilia in 28, infectious diseases in 20, digestive diseases in 71, cardiovascular diseases in 86, diabetes mellitus in 14, obstetric disease in 11, trauma and burn in 9, no underlying disease in 30, and other diseases in 33 (Table **1**).

Citrated blood samples were obtained from the peripheral veins of healthy subjects (see below) and patients under fasting condition and then centrifuged for 20 minutes at 3,000 rpm. The supernatants (plasma) were analyzed within 4 hours. 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 91 healthy subjects (mean age, 21.5 years; range, 20 - 28 years; 57 males and 34 females), who were free of any diseases including thrombotic disease or hyperlipidemia as confirmed by an annual medical checkup.

Measurement of Plasma Concentrations of Hemostatic Molecular Markers

The plasma levels of SF were determined by the latex agglutination method using three different kits; IATRO SF (Mitsubishi Chemical Medience Corporation. Inc., Tokyo, Japan) containing the monoclonal antibody IF-43(17), Auto LIA FMC (Rosch Diagnostics, Tokyo, Japan) containing monoclonal antibody F405 [19] and Nanopia SF (Daiichi Pure Chemical, Tokyo, Japan) containing monoclonal antibody J2-23 [18]. IF-43 recognizes a segment of the fibrin Aα chain [(Aα-17-78) residue segment] exposed in the E region of the fibrin monomer (FM) when the FM molecule binds the D region of another FM or fibrinogen [17]. F405 recognizes a segment in the N terminal region of the fibrin Aα chain (Aα-17-25). J2-23 recognizes an epitope in the C-terminal region of the fibrin Aα chain (Aα-1502-521) [18]. The plasma D-dimer and FDP levels were measured by the latex agglutination method using the Nanopia D-dimer and Nanopia FDP (Daiichi Pure Chemical, Tokyo, Japan). The plasma thrombin antithrombin complex (TAT) and plasmin plasmin inhibiter complex (PPIC) were measured by ELISA using a TAT test Kokusai F (Sysmex, Kobe, Japan) and PIC test Kokusai F (Sysmex, Kobe, Japan), respectively.

Statistical Analysis

The data are expressed as median (25% -75% percentile). Differences between the groups were examined for statistical significance using the Mann-Whitney's U test while correlations between the two variables were tested by Pearson's correlation analysis. A P value less than 0.05 denoted the presence of a statistically significant difference. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

RESULTS

In healthy subjects, the plasma concentrations of New SF were not distributed normally, with a maximum value of 9.8 μg/ml, minimum value 0μg/ml, and median value of 0.3 μg/ml. In healthy volunteers, the 95% confidence interval (CI) of New SF was from 0 μg/ml to 5.47 μg/ml.

The frequency of thrombosis was high in obstetric disease, infectious disease and thrombophilia and the absolute number of thromboses was high in cancer and cardiovascular disease (Table **1**). The plasma levels of New SF tended to be high in all subjects with underlying disease, especially those with obstetric or infectious disease. The plasma levels of IATRO SF, LIA AUTO FMC, D-dimer, FDP and TAT were also tended to be high in all patients with underlying diseases, but those of PPIC were not markedly high in those (Table **2**). The plasma levels of New SF were significantly higher in patients with thrombosis (22.3μg/ml; 10.9-40.9μg/ml) than patients without thrombosis (5.9μg/ml; 2.6-15.9μg/ml) or liver transplantation (9.6μg/ml; 5.2-28.3μg/ml) ($p< 0.01$, respectively) but there was no significant difference in the New SF level between those with thrombosis and those after operation (22.1μg/ml; 10.7-31.1μg/ml) (Fig. **1**). The plasma levels of IATRO SF and AUTO LIA FMC were also significantly higher in patients with thrombosis (8.9μg/ml; 3.9-23.7μg/ml and 11.5μg/ml; 5.0-50.8μg/ml, respectively) than in those without thrombosis (2.5μg/ml; 0.7-4.7μg/ml and 1.6μg/ml; 0.7-3.6μg/ml, respectively) ($p< 0.01$, respectively) (Fig. **1**). The plasma levels of New SF were significantly elevated in patients with DVT/PE, DIC, cerebral thrombosis or other types of thrombosis and IATRO SF, AUTO LIA FMC, D-dimer, FDP or TAT were markedly elevated in patients with DVT/PE, DIC or cerebral thrombosis, and the plasma PPIC levels were markedly elevated in patients with DVT/PE or DIC (Table **3**). There was no significant difference in the New SF levels among DVT/PE, DIC, cerebral thrombosis and other thromboses (Fig. **2**).

The plasma levels of New SF were closely correlated with those of IATRO SF ($Y = 9.93 + 0.99 X$, $r = 0.669$, $p< 0.001$) (Fig. **3A**) or AUTO LIA FMC ($Y= 14.15 + 0.27 X$, $r = 0.527$, $p< 0.001$) (Fig. **3B**). The correlation coefficient of New SF with the other hemostatic molecular markers is shown in Table **4**.

Table 1. Underlying Diseases of the Subjects

Diseases	Age; Median (25%-75% percentile)	Sex (F:M)	TH (%)	DVT (%)	DIC (%)	CT (%)	Others (%)
Cancer	66 (55 - 72)	52 : 59	23 (20.7)	13 (11.7)	8 (0.9)	0 (0)	2 (1.8)
Orthopaedic Diseases	65 (54 - 74)	204 : 53	69 (28.5)	66 (27.3)	0	2 (0.8)	1 (0.4)
Hematological Diseases	58 (33 - 66)	27 : 17	5 (11.4)	2 (4.5)	2 (4.5)	0	1 (2.3)
Autoimmune Diseases	56 (36 - 62)	25 : 4	7 (24.1)	6 (20.7)	0	1 (3.4)	0
Thrombophilia	49 (33 - 67)	24 : 4	12 (42.9)	5 (17.9)	1 (3.6)	4 (14.3)	2 (7.1)
Infectious Diseases	67 (56 – 63)	7 : 13	9 (45.0)	0	8 (40.0)	0	1 (5.0)
Digestive Diseases	57 (45 - 65)	34 : 37	9 (12.5)	8 (11.1)	1 (1.4)	0	0
Cardiovascular Diseases	64 (47 - 72)	58 : 28	31 (36.0)	18 (20.9)	7 (8.1)	1 (1.2)	5 (5.8)
Diabetes mellitus	68 (61 - 77)	8 : 6	3 (35.7)	2 (14.2)	0	1 (7.1)	2 (14.2)
Obstetric Diseases	36 (30 - 40)	11 : 0	6 (54.5)	4 (36.4)	2 (18.2)	0	0
Trauma / burn	61 (43 - 79)	4 : 5	3 (33.3)	0	2 (22.2)	1 (11.1)	0
Other Diseases	63 (43 - 72)	15 : 18	3 (9.1)	1 (3.0)	2 (6.1)	0	0
No underlying disease	65 (49 – 74)	18 : 12	30 (100)	26 (86.7)	1 (3.3)	3 (10)	0

TH: thrombosis, DVT: deep vein thrombosis, DIC: disseminated intravascular coagulation, CT: cerebral thrombosis.

Table 2. Hemostatic Molecular Markers in Underlying Diseases of the Subjects

Diseases	New SF (μg/ml)	IATRO SF(A) (μg/ml)	LIA AUTO FMC (μg/ml)	D-Dimer (μg/ml)	FDP (μg/ml)	PPIC (μg/ml)	TAT (ng/ml)
Cancer	8.9 (3.4 - 21.7)	3.2 (1.2 - 7.5)	2.1 (0.7 - 7.2)	1.4 (0.9 - 5.1)	3.3 (1.6 - 8.6)	0.9 (0.6 - 1.2)	3.4 (1.8 - 9.0)
Orthopaedic Disease	10.8 (4.6 - 24.5)	3.2 (0.8 - 7.0)	3.8 (1.6 - 8.1)	3.4 (1.1 - 8.7)	8.3 (2.6 - 16.6)	0.8 (0.6 - 1.1)	7.6 (2.7 – 15.6)
Hematological Disease	9.3 (2.9 - 22.4)	1.7 (0.4 - 5.2)	1.0 (0.6 - 2.5)	0.8 (0.7 - 1.1)	1.6 (1.2 - 2.4)	0.5 (0.4 - 0.7)	1.4 (1.1 - 2.0)
Autoimmune Disease	9.2 (3.1 - 25.6)	2.9 (1.1 - 4.9)	1.6 (0.6 - 4.1)	1.0 (0.7 - 4.3)	2.2 (1.2 - 8.4)	0.6 (0.4 - 0.8)	1.9 (1.1 - 6.7)
Thrombophilia	13.2 (6.7 - 24.0)	2.2 (0 - 5.4)	2.5 (1.0 - 5.3)	1.5 (0.9 - 3.2)	2.8 (1.6 - 6.5)	0.6 (0.4 - 0.8)	2.5 (1.6 - 7.6)
Infectious Disease	19.4 (10.6 - 39.9)	6.6 (3.5 - 12.0)	9.2 (4.2 - 17.7)	5.1 (2.4 - 12.0)	13.2 (3.5 - 21.3)	1.2 (0.9 - 1.6)	9.1 (4.2 - 22.4)
Digestive Disease	7.9 (3.1 - 18.8)	4.7 (2.9 - 16.4)	8.1 (2.2 - 20.6)	3.2 (1.1 - 7.9)	6.3 (2.6 - 11.4)	0.8 (0.5 - 1.2)	4.9 (2.0 - 21.2)
Cardiovascular Disease	9.3 (2.9 - 24.5)	3.5 (1.0 – 10.5)	2.7 (0.9 - 9.5)	2.0 (0.8 - 7.5)	2.9 (1.2 - 10.8)	0.7 (0.5 - 1.4)	3.4 (1.2 - 12.3)
Diabetes mellitus	12.3 (3.1 - 23.4)	4.2 (3.1 - 6.2)	2.0 (1.2 - 4.6)	1.7 (0.8 - 6.6)	3.6 (1.9 - 13.7)	0.7 (0.5 - 0.9)	3.1 (1.5 - 10.9)
Obstetric Disease	19.3 (14.4 - 32.2)	6.9 (3.3 - 10.3)	3.4 (1.6 - 11.5)	3.7 (1.5 - 5.1)	6.4 (5.2 - 11.5)	0.5 (0.4 - 3.6)	7.0 (2.6 - 24.3)
Trauma / burn	22.1 (12.3- 37.6)	10.8 (7.7 - 43.7)	14.8 (4.6 - 78.7)	10.1 (3.8 - 29.2)	27.9 (12.8 - 46.0)	1.5 (0.9 - 3.0)	18.4 (6.2 - 50.3)
Other Diseases	4.6 (2.2 - 13.1)	2.1 (0.6 - 4.5)	1.2 (0.5 - 2.6)	0.8 (0.7 - 1.3)	1.4 (1.0 - 2.8)	0.6 (0.5 - 0.9)	1.7 (1.1 - 2.3)
No underlying disease	18.4 (7.4 - 37.8)	9.1 (4.4 - 30.2)	13.6 (4.9 - 111)	8.1 (3.9 - 18.0)	15.8 (8.1 - 32.5)	1.7 (0.8 - 5.1)	11.9 (6.4 - 21.6)

Data show the median (25% - 75%) percentile.
SF: soluble fibrin, FMC: fibrin monomer complex, FDP: fibrin and fibrinogen degradation products, PPIC: plasmin plasmin inhibitor complex, TAT: thrombin antithrombin complex.

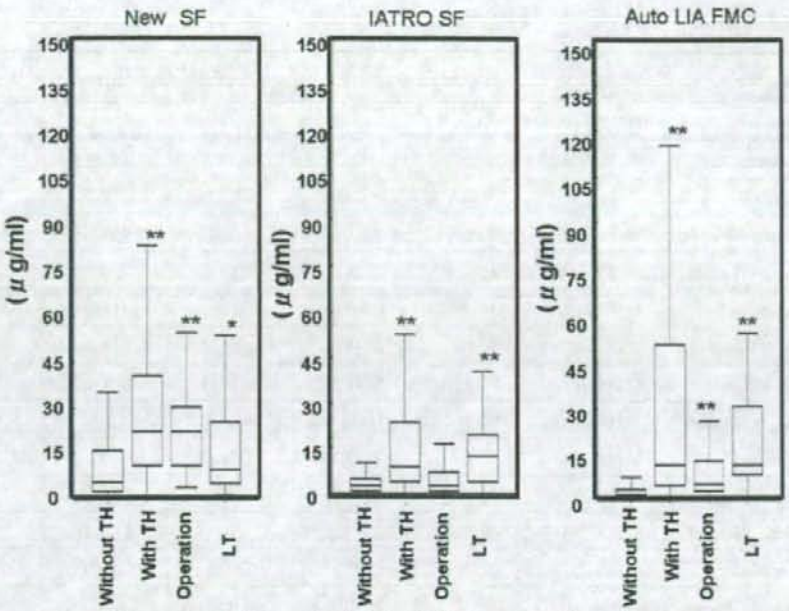

Fig. (1). Plasma concentrations of New SF, IATRO SF and AUTO LIA FMC in patients without thrombosis, those with thrombosis, those after operation and those with livert transplantation.
TH: thrombosis, operation: patients within 3 days after operation, LT: patients after liver transplantation. **: $p < 0.01$ compared to patients without thrombosis.

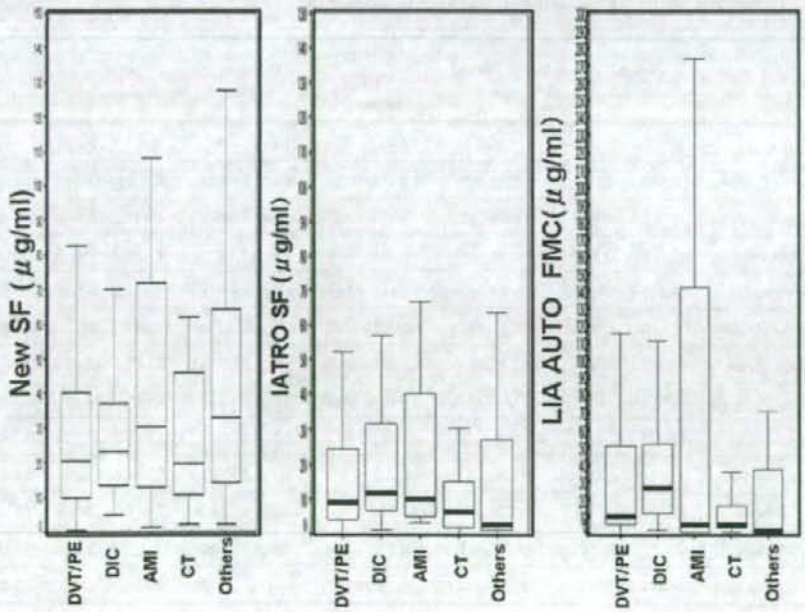

Fig. (2). The plasma concentrations of New SF in patients with DVT/PE, DIC, cerebral thrombosis and other diseases.
CT: cerebral thrombosis.

Table 3. Hemostatic Molecular Markers in DVT/PE, DIC, CT or Other Thrombosis

Thrombosis	New SF (μg/ml)	IATRO SF (μg/ml)	LIA AUTO FMC (μg/ml)	D-dimer (μg/ml)	FDP (μg/ml)	PPIC (μg/ml)	TAT (ng/ml)
DVT/PE	20.4 (9.9 - 40.3)	8.9 (4.0 - 25.0)	10.0 (5.2 - 51.9)	8.8 (4.6 - 15.9)	16.0 (8.9 - 34.5)	1.2 (0.8 - 2.1)	13.3 (7.0 - 31.1)
DIC	23.3 (13.6 - 41.3)	11.6 (6.5 - 34.7)	26.6 (10.8 - 52.0)	10.3 (7.0 - 25.3)	16.8 (9.4 - 33.9)	1.4 (0.8 - 2.6)	22.4 (11.8 - 39.2)
CT	19.7 (9.8 - 53.6)	6.3 (1.2 - 16.3)	5.2 (2.7 - 16.9)	5.1 (2.6 - 8.4)	10.0 (6.9 - 17.5)	0.8 (0.7 - 1.1)	8.8 (3.8 - 13.5)
Others	33.0 (10.7 - 70.0)	5.5 (1.7 - 40.1)	1.4 (0.7 - 55.4)	1.8 (0.7 - 10.2)	2.7 (1.8 - 15.4)	0.7 (0.4 - 1.9)	2.6 (1.5 - 16.9)

Data show the median (25%s - 75%s) percentile

TH: thrombosis, DVT: deep vein thrombosis, DIC: disseminated intravascular coagulation, CT: cerebral thrombosis

SF: soluble fibrin, FMC: fibrin monomer complex, FDP: fibrin and fibrinogen degradation products, PPIC: plasmin plasmin inhibitor complex, TAT: thrombin antithrombin complex

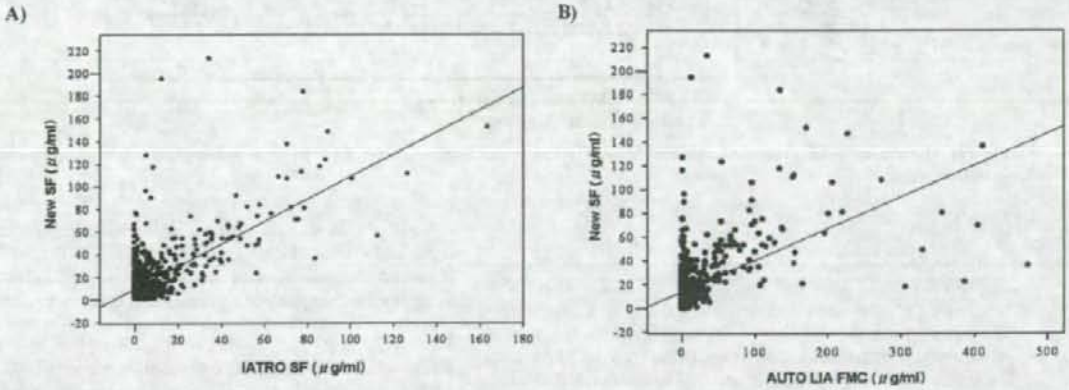

Fig. (3). The relationship between New SF and IATRO SF (A) and AUTO LIA FMC (B).

Table 4. Correlation Coefficient of New SF with the Hemostatic Molecular Markers

	r	p
IATRO SF	0.669	p< 0.001
AUTO LIA FMC	0.528	p< 0.001
D-dimer	0.360	p< 0.001
FDP	0.434	p< 0.001
PPIC	0.219	p< 0.001
TAT	0.392	p< 0.001

SF: soluble fibrin, FMC: fibrin monomer complex, FDP: fibrin and fibrinogen degradation products, PPIC: plasmin plasmin inhibitor complex, TAT: thrombin antithrombin complex

There were 25 patients in which blood samples were collected within a week before the onset of thrombosis (19 with DVT/PE, 3 with DIC and 3 with cerebral thrombosis). The plasma levels of New SF within a week before the onset of thrombosis (8.0μg/ml; 4.9-20.8μg/ml) were significantly higher than those in patients without thrombosis ($p< 0.05$) but there was no significant difference of the plasma levels of IATRO SF (0.8μg/ml; 0.4-1.65μg/ml), AUTO LIA FMC (1.5μg/ml; 0.8-4.3μg/ml), FDP (2.1μg/ml; 1.5-3.8μg/ml), D-dimer (1.4μg/ml; 1.1-2.6μg/ml), PPIC (0.6μg/ml; 0.5-0.8μg/ml) and TAT(3.9μg/ml; 3.2-9.2μg/ml) between those within a week before onset of thrombosis and those without thrombosis (Fig. **4**).

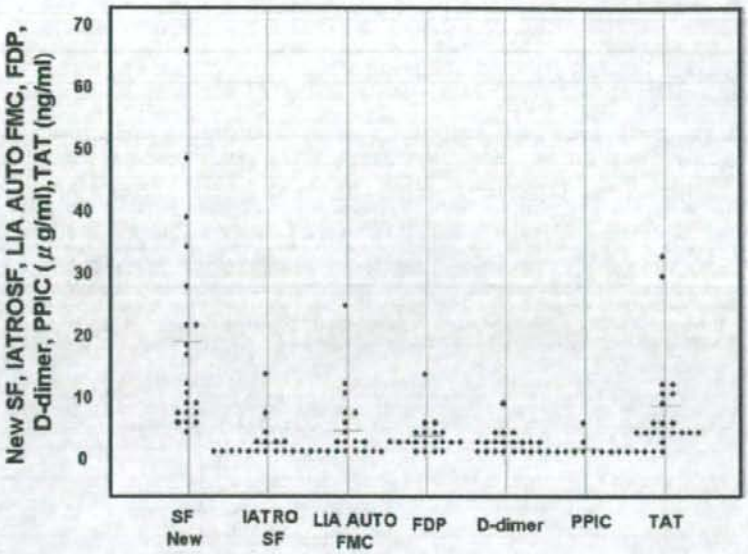

Fig. (4). The plasma levels of New SF, IATRO SF, AUTOLIA FMC, D-dimer, FDP, TAT, PPIC in patients within a week before the onset of thrombosis.

DISCUSSION

In healthy subjects, the normal range of New SF levels was less than 6.0 μg/ml, and that was similar to the other two SF levels [17, 19]. The monoclonal antibodies in all three assays recognize the α-chain of fibrinogen, which is an important site for the activation from fibrinogen to fibrin by thrombin. With regard to the underlying diseases that are frequently associated with thrombosis, such as DVT and DIC, the risk for thrombosis should be evaluated by a simple test and then treated with adequate agents immediately. In the present study, the plasma levels of all three SFs were tended to be high in all underlying diseases associated with thrombosis, and those were significantly elevated in patients with thrombosis, with liver transplantation or after an operation, but there was no significant difference among those with thrombosis, with liver transplantation, or following an operation. The concentrations of the three SFs were significantly elevated in patients with thrombosis such as DIC, DVT and CVA. Therefore, the high concentrations of SF could be considered as markers of thrombosis, since both parameters were also reported to be elevated in DVT [20, 21], DIC [20, 22] and diabetes mellitus [23]. There was no significant difference in the New SF levels among DVT/PE, DIC, cerebral thrombosis and other thromboses, similar to the other SFs [20]. Indeed, the plasma levels of New SF were closely correlated with those of IATRO SF or AUTO LIA FMC and moderately correlated with those of TAT or D-dimer.

The early detection of the thrombotic state is very important; since PE is a common, frequently undiagnosed, and potentially fatal event. Because the symptoms of PE are common, including dyspnoea and chest pain [24-26], the early recognition of DVT [27] and PE [28] is important clinically. Cerebral thrombosis sometimes has a fatal outcome and often reduces the quality of life. DIC [29] is often observed in patients with leukaemia, solid cancers, infections, gynaecological conditions and aneurysms, and it is frequently associated with severe bleeding and organ failure. Since DIC is a fatal condition, it is important to promptly diagnose DIC by hemostatic molecular markers [30]. Fibrin related marker such as D-dimer 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 [20].

The plasma levels of new SF were significantly elevated in patients with thrombosis within a week before the onset of thrombosis, but the plasma levels of IATRO SF, AUTO LIA FMC, FDP, D-dimer, PPIC and TAT were not significantly increased within a week before the onset of thrombosis. These findings indicate that the New SF assay may be useful for the detection of the pre-thrombotic state. The diagnosis of the pre-thrombotic state which is a hypercoagulable state before the onset of thrombosis, is considered to be important in preventing the progression of pre-DIC to overt-DIC [6]. By an early diagnosis of the pre-thrombotic state (hypercoagulable state), we might prevent the onset of thromboses such as DVT, PE, DIC or cerebral thrombosis and thereby improve the outcome in various underlying diseases which carry a risk for the development of thrombosis.

ACKNOWLEDGEMENTS

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Cutoff Values of D-Dimer and FDP in Plasma for the Diagnosis of Thrombosis

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Abstract: Fibrin-related markers, such as fibrin and fibrinogen degradation products (FDP) and D-dimer, are considered useful for the diagnosis of thrombosis. However, the evidence for making a diagnosis of thrombosis based on fibrin-related markers is still not yet well established.

The plasma concentrations of soluble fibrin and D-dimer were prospectively measured in 680 inpatients suspected of having thrombosis between October 1, 2003 and January 31, 2005, and correlated with thrombosis.

The normal ranges of D-dimer and FDP were within 0.76 μg/ml and 1.50 μg/ml, respectively. Out of 680 patients, 129 patients showed plasma concentrations associated with thrombosis, including 73 with deep venous thrombosis (DVT)/ pulmonary embolism (PE). The plasma D-dimer and FDP concentrations were significantly higher in the patients with thrombosis than in the patients without thrombosis, but there were no significant differences in the D-dimer and FDP levels among the patients with thrombosis. The plasma D-dimer levels were significantly correlated with the plasma FDP levels in all the patients and there was no significant difference in the ratio of FDP/ D-dimer among the various diseases. A ROC analysis showed that both FDP and D-dimer were useful for the diagnosis of all types of thrombosis and DVT. The cutoff values of D-dimer (3.8 μg/ml) and FDP (7.7 μg/ml) had high sensitivity, specificity and negative predictive values (NPV) but low positive predictive value.

Our findings suggest that FDP showed a close correlation with D-dimer, which is known to be a marker for a hypercoagulable state, and it is also reflects a high risk for thrombosis.

Keywords: Hypercoagulable state, deep vein thrombosis, fibrin and fibrinogen degradation products, D-dimer.

INTRODUCTION

Fibrin and fibrinogen degradation products (FDP) including D-dimer, are considered to be useful for detecting the state of thrombosis, and they have been reported to be elevated in deep vein thrombosis (DVT)/pulmonary embolism (PE) [1-3], disseminated intravascular coagulation (DIC) [4, 5], acute myocardial infarction (AMI) [6, 7] and thrombotic thrombocytopenic purpura (TTP) [8]. However, a serum assay of FDP can be rather time consuming since it is necessary to wait for clot formation and clot lysis. Recently, the FDP levels in plasma were reported to be successfully measured using a specific monoclonal antibody [9], thus making the plasma FDP assay as fast and easy perform as the D-dimer assay. The International Society of Thrombosis and Haemostasis (ISTH) has established the diagnostic criteria for overt-DIC using fibrin-related markers; FDP, D-dimer and soluble fibrin [10]. D-dimer is widely used to diagnose thrombosis as DVT but many of the commercially available D-dimer assay kits contain different monoclonal antibodies, standard substances and they are based on different assay systems. Several studies [11, 12] have reported basic data for the standardization of D-dimer assays; however, this issue remains to be resolved.

PE is a common, frequently undiagnosed, and potentially fatal cause of several common symptoms, including dyspnoea and chest pain [13-15]. Since PE is often a fatal disease caused by DVT, an early clinical evaluation of DVT [16] and PE [17] is therefore crucial. In this regard, D-dimer has been reported to be a negative predictor for DVT and a D-dimer level of less than 0.5 μg/ml is considered to exclude DVT/PE with the most commonly used D-dimer assays in Europe and North America [16]. DIC [18, 19] is often observed in patients with leukaemia, solid cancers, infections, gynaecological conditions and aneurysms, and it is also frequently associated with severe bleeding and organ failure. Since DIC is a fatal condition [20], it is important to diagnose it early using hemostatic molecular markers [21].

The present study was designed to evaluate the cutoff values of FDP, including D-dimer, in the diagnosis of several types of thrombosis, including DVT, DIC, cerebral thrombosis, and AMI, prospectively. For this purpose, we determined the plasma concentrations of these molecules in 680 patients suspected of having thrombosis, as well as in 100 healthy volunteers.

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MATHERIALS AND METHODS

Subjects

From October 1, 2003 to January 31, 2005, 680 patients (age, 60.2 ±17.3 years, mean ± SD; 411 females and 269 males) were suspected of having thrombosis (DVT or DIC) in the hospitals affiliated with Mie University School of Medicine. Plasma concentrations of FDP and D-dimer 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. Thirty-five patients within 7 days after operation (OPE) and 28 patients who had undergone liver transplantation (LT) were excluded from analysis of the cutoff value. Among the remaining 617 patients, 488 patients (56.8 ±17.9 years; 279 females and 209 males) did not have any thrombosis, while 129 patients had thrombotic diseases [73 with DVT (62.7 ±17.8 years; 60 females and 13 males) including 30 with DIC (64.7 ±15.1 years; 10 females and 20 males), 12 with cerebral thrombosis (71.0 ±2.9 years; 5 females and 7 males); 6 with acute myocardial infarction (AMI) (68.1 ±11.5 years; 2 females and 4 males), 4 with arteriosclerosis obliterans (ASO) (71.2 ±7.0 years; 2 females and 2 males) and 4 with portal vein thrombosis (63.1 ±11.3 years; 2 females and 2 males)] (Table **1**). DVT was diagnosed with Doppler ultrasonographic examination or venography. DIC was diagnosed using the ISTH overt-DIC diagnostic criteria [10]. Cerebral thrombosis was diagnosed by either computed tomography (CT) or magnetic resonance imaging (MRI) and AMI was diagnosed by the electrocardiogram and laboratory data. Portal vein thrombosis PVT was diagnosed by Doppler ultrasonographic examination or CT. Among the underlying diseases in these patients, cancer was identified in 184 patients, orthopaedic conditions in 152, cardiovascular diseases in 66, digestive diseases in 39, infectious diseases in 33, autoimmune diseases in 31, hematological diseases in 23, diabetes mellitus in 15, obstetrics in 15, thrombophilia in 10, trauma and burns in 9, and no underlying diseases in 31 (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. Plasma concentrations of FDP 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 100 healthy subjects (mean age, 41.5 years; range, 20 - 58 years; 47 males and 53 females), who were free of any diseases including thrombotic disease or hyperlipidemia as confirmed by an annual medical check-up.

Measurement of Plasma Concentrations of D-Dimer, FDP, Antithrombin, Plasmin Inhibitor and Plasminogen

The plasma D-dimer and FDP levels were measured by the latex agglutination method using the Nanopia D-dimer and Nanopia FDP (Daiichi Kagakuyakuhin, Tokyo, Japan). The activities of antithrombin, plasmin inhibitor and plasminogen were measured by the chromogenic substrate method using a testchyme ATIII 2 kit, testchyme APL 2 kit and testchyme PLG 2 kit (Daiichi Kagakuyakuhin), respectively.

Table 1. Underlying Diseases of Subjects

	DVT/PE	DIC	CT	AMI	ASO	PVT	TH(-)	Total
Orthopaedic C	24	1		1			126	152
Solid organ cancer	11	7	2			3	161	184
Digestive D	3					1	35	39
Cardiovascular D	3	5	1	4	1		52	66
Infectious D		12					21	33
Without underlying D	22	2	6	1			0	31
Autoimmune D	1						30	31
Hematological D	2	2					19	23
Diabetes mellitus	1		1		3		10	15
Obstetrics	2						13	15
Thrombophilia	3		2				5	10
Trauma/burn	1	1					7	9
Others							9	9
Total	73	30	12	6	4	4	488	617

C: conditions, D: diseases, DVT: deep vein thrombosis, PE: pulmonary embolism, DIC: disseminated intravascular coagulation, CT: cerebral thrombosis, AMI: acute myocardial infarction, ASO: arteriosclerosis obliterans, PVT: portal vein thrombosis, TH(-): without thrombosis.

Statistical Analysis

All data are expressed as the mean ± SD. The differences between the groups were examined for statistical significance using Mann-Whitney's U test while correlation between 2 variables was tested by Pearson's correlation analysis. A *P* value less than 0.05 denoted a significant difference. The usefulness of D-dimer and soluble fibrin (SF) for the diagnosis of thrombosis and DVT was examined based on a receiver operating characteristic (ROC) analysis [22]. The cutoff values were determined by the ROC analysis. All statistical analyses were performed using SPSS II software package (SPSS Japan, Tokyo).

RESULTS

DVT or PE was observed in various diseases, although the frequency of DVT or PE was markedly high in the patients with orthopaedic diseases and solid cancer. Meanwhile, the frequency of DIC was high in the patients with infectious diseases and solid cancers (Table **1**). In the healthy subjects, the plasma concentrations of D-dimer and FDP were not distributed normally, with maximum values of 1.16 μg/ml and 2.40 μg/ml, minimum values of 0.25 μg/ml and 0.50 μg/ml, and median values of 0.48 μg/ml and 0.90 μg/ml, respectively. In the healthy volunteers, the 95% confidence intervals (CI) of D-dimer and FDP were 0.76 μg/ml and 1.50 μg/ml, respectively.

The plasma D-dimer and FDP concentrations (median; 25 % - 75% tile) were significantly higher in the patients with thrombosis (9.45 μg/ml; 4.15 – 14.94 μg/ml and 14.77μg/ml; 7.99 – 23.34 μg/ml), OPE (7.13 μg/ml; 2.89 – 11.94 μg/ml and 11.10 μg/ml; 6.01 – 18.14 μg/ml) or LT (6.88 μg/ml; 2.37 – 10.77 μg/ml and 11.28 μg/ml; 4.58 – 16.53 μg/ml) than in the patients without thrombosis (1.09 μg/ml; 0.74 – 2.29 μg/ml and 2.17 μg/ml; 1.46 – 4.64 μg/ml)($p< 0.001$, respectively). The plasma D-dimer and FDP concentrations were also significantly higher in the patients without thrombosis than in the healthy volunteers ($p< 0.001$) (Fig. (**1**)). However, there were no significant difference in D-dimer and FDP levels among the patients with thrombosis, those with OPE and those with LT, and among various underlying diseases. The plasma levels of antithrombin (AT), plasminogen and plasmin inhibitor activity were significantly lower in the patients with thrombosis than in the patients without thrombosis, and the plasma levels of plasminogen and plasmin inhibitor activity were significantly lower in the patients with LT than in those without thrombosis ($p< 0.01$, respectively) (Table **2**).

The plasma D-dimer and FDP concentrations were significantly higher in the patients with DIC (9.90 μg/ml; 5.30 – 17.50 μg/ml and 15.00 μg/ml; 8.60 – 36.40 μg/ml) and DVT (10.1 μg/ml; 4.95 – 16.35 μg/ml and 17.30 μg/ml; 8.30 – 26.73 μg/ml) than in the patients without thrombosis ($p< 0.01$, respectively) (Fig. (**2**)). The plasma D-dimer and FDP concentrations were higher in the patients with cerebral thrombosis (CT) (4.70 μg/ml; 1.55 – 9.65 μg/ml and 8.00 μg/ml; 4.00 – 15.90 μg/ml) and ASO (9.80 μg/ml; 7.80 – 16.40 μg/ml and 15.20 μg/ml; 8.60 – 18.70 μg/ml) than in the patients without thrombosis ($p< 0.05$, respectively).

The plasma D-dimer levels were significantly correlated with the plasma FDP levels in all patients ($Y = 0.489 X + 0.525$, $R= 0.962$, $p< 0.001$) (Fig. (**3**)). There was no significant difference in the ratio of FDP/DD among the various diseases. In the patients with more than 10.0 μg/ml, 97 patients had more than 70% of plasminogen levels and 78 patients had less than 70% of plasminogen levels. The former was considered the normal fibrinolysis group and the latter was the hyper fibrinolysis group. There was no significant difference in the ratio of FDP/D-dimer between the normal fibrinolysis group (1.64; 1.51 - 1.97) and the hyper fibrinolysis group (1.55; 1.46 - 1.79).

Fig. (**4**) shows the positive predictive values (PPV) for several cutoff values of D-dimer and FDP in the patients with thrombosis. When a D-dimer value of >3.0 μg/ml and an FDP value of >6.0 μg/ml was used, more than 50% of patients, excluding those with liver transplantation or post-operation, had some thrombosis.

An ROC analysis showed that both FDP and D-dimer were useful for the diagnosis of all types of thrombosis and, in particular, DVT (Fig. (**5**)). The areas under the curve (AUC) of D-dimer were similar to that of FDP in all types of thrombosis and DVT/PE. The ROC analysis provided adequate cutoff values of D-dimer and FDP for the diagnosis of all types of thrombosis and DVT/PE (Table **3**). The cutoff values of D-dimer (3.8 and 3.4 μg/ml) for the diagnosis of all types of thrombosis and DVT/PE were similar, while those of FDP (7.6 and 7.7 μg/ml) were also similar. Both D-dimer (3.8 μg/ml) and FDP (7.7 μg/ml) had high sensitivity, specificity and negative predictive value (NPV) but low positive predictive value.

DISCUSSION

In our study, the frequency of DVT or PE was the highest among the various types of thrombosis and DVT or PE was frequently observed in orthopaedic diseases and solid organ cancer, while the frequency of DIC was high in infectious diseases and solid organ cancers. These findings are similar to previous reports [13, 14, 19, 23]. The frequency of thrombosis depends on the underlying disease. Regarding the underlying diseases that are frequently associated with thrombosis, the risk for thrombosis should be evaluated by a simple test such as D-dimer and FDP.

In the present study, the normal ranges of D-dimer and FDP were within 0.76 μg/ml and 1.50 μg/ml, respectively. There are many D-dimer assay kits and the cut off value depends on the kit used. In the most commonly used D-dimer assay in Europe and North America, D-dimer concentrations of less than 0.5 μg/ml are considered to exclude DVT/PE [6]. However, in Japan, the D-dimer concentration is more than 0.5 μg/ml in many patients without thrombosis and this cut-off value is therefore not useful as a NPV for DVT/PE in Japan [24], especially because the D-dimer kits that are frequently used in Japan have a wide normal range (about 0.3 – 2.5 μg/ml). The plasma D-dimer and FDP concentrations were also significantly higher in the patients without thrombosis than in the healthy volunteers, suggesting that some underlying diseases may increase in FDP and D-dimer levels without causing thrombosis.

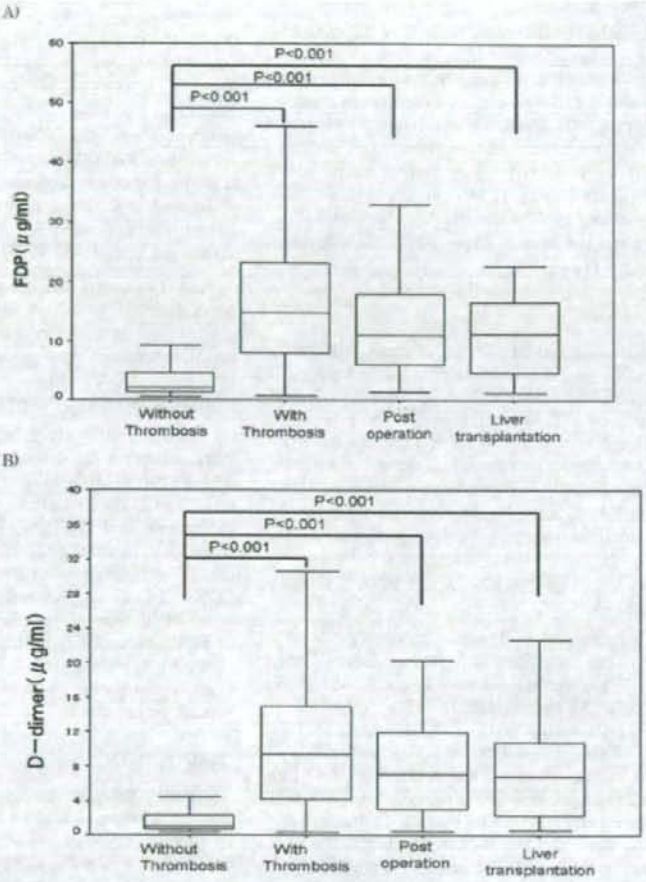

Fig. (1). The plasma levels of FDP and D-dimer in patients with or without thrombosis, either post-operation or after a liver transplantation. A) FDP, B) D-dimer.

Table 2. Plasma Levels of Antithrombin, Plasminogen and Plasmin Inhibitor

	Without TH	With TH	Post Operation	LT
Antithrombin (%)	96.8 (86.5 - 107)	85.3** (73.8 - 98.2)	90.5* (80.3 - 97.2)	88.1* (62.1 - 104.8)
Plasminogen (%)	99.0 (88.0 – 109.6)	90.7** (72.9 – 105.2)	88.8* (79.4 - 103.9)	52.4** (41.4 - 87.0)
Plasmin inhibitor (%)	104.0 (95.0 – 113.3)	96.9** (85.0 – 108.2)	97.3** (90.3 - 105.2)	76.3** (61.3 - 92.6)

Data are shown as median (25 % tile – 75 % tile).
**; p< 0.01, *; p< 0.05 in comparison to without TH.
TH: thrombosis, LT: liver transplantation.

Although the plasma D-dimer and FDP concentrations were significantly higher in the patients with thrombosis than in the patients without thrombosis, there were no significant differences in the D-dimer and FDP levels among the patients with thrombosis, those who were post-operation and those with LT, thus suggesting that these assays may be useful for patients on medication alone or for pre-operative patients. In the present study, we demonstrated that the concen-

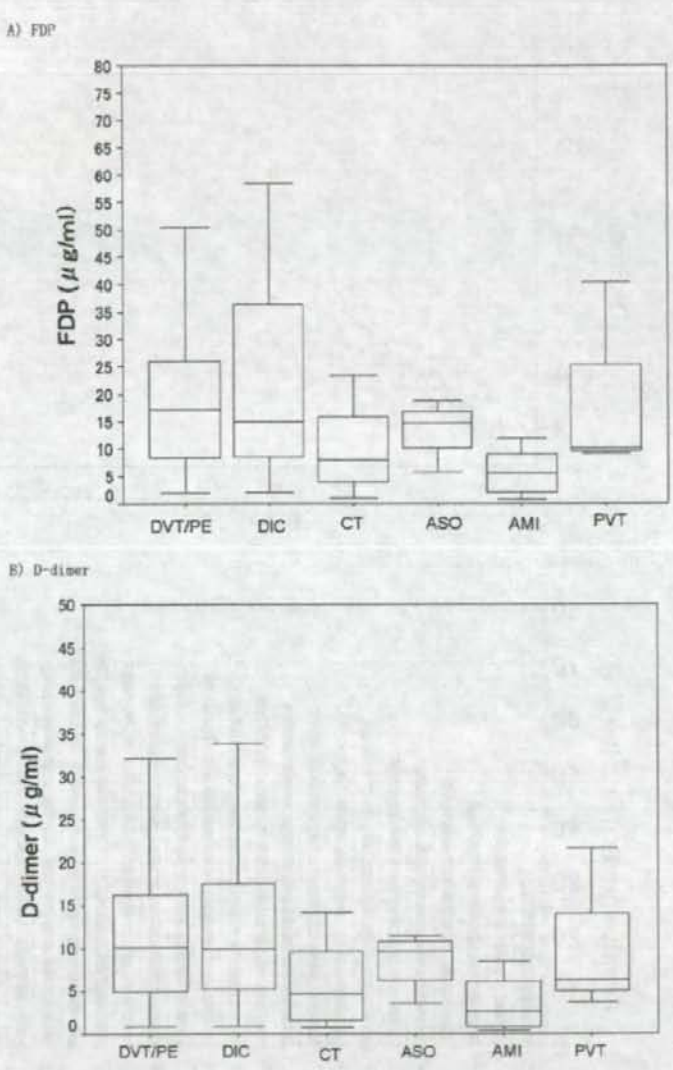

Fig. (2). The plasma levels of FDP and D-dimer in patients with various types of thrombosis.
A) FDP, B) D-dimer.
DVT: deep vein thrombosis, PE: pulmonary embolism, DIC: disseminated intravascular coagulation, CT: cerebral thrombosis, ASO: AMI: acute myocardial infarction, PVT: portal vein thrombosis, TH: thrombosis, **: $p < 0.01$, *: $p < 0.05$.

trations of both D-dimer and FDP were significantly high in the patients with thrombosis, such as DVT/PE, DIC, CVA and AMI. Therefore, high concentrations of D-dimer and FDP could be considered as markers of thrombosis, since both parameters have also been reported to be elevated in DVT [25, 26], DIC [4, 27] and hyperlipidemia [28]. We previously reported prospective studies that evaluated the soluble fibrin (SF) and D-dimer assay and the cutoff value of the diagnosis for thrombosis [23]. These findings were similar to those of previous reports [23].

The plasma D-dimer levels were significantly correlated with the plasma FDP levels in all patients. It has been reported that the positive rate of FDP for thrombosis depends on the plasma levels of D-dimer [29]. There was no significant difference in the ratio of FDP/DD among the various diseases and between the normal fibrinolysis group and the hyper fibrinolysis group. It has also been reported that there is a hyperfibrinolyitc type and a hypofibrinolytic type of DIC [30, 31], thus suggesting that the ratio of FDP/DD might be higher in the hyper fibrinolysis group than in the normal fi-

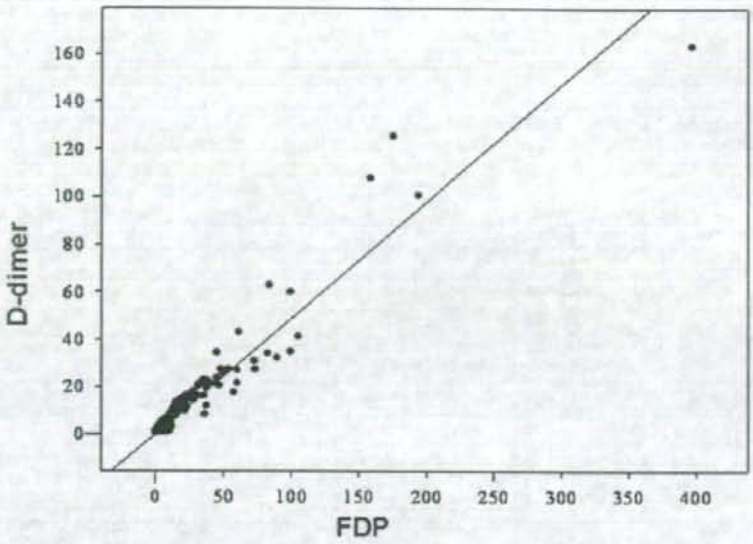

Fig. (3). Relationship between the plasma FDP and plasma D-dimer levels.

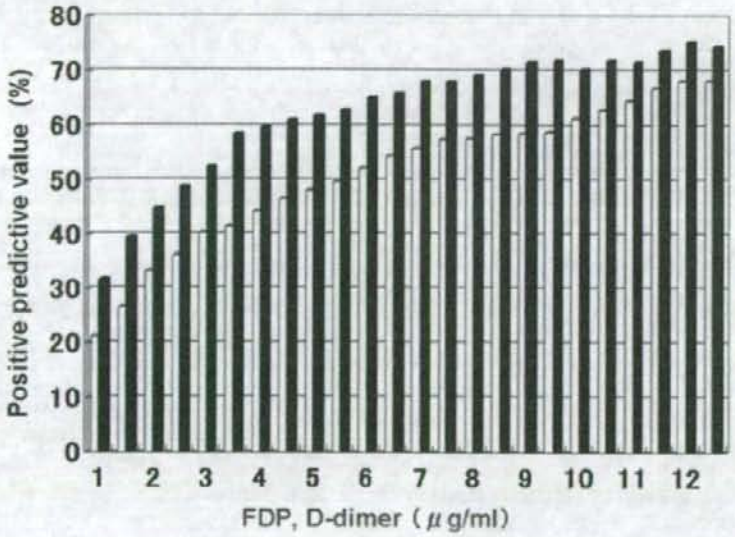

Fig. (4). Positive predictive value for thrombosis according to each FDP or D-dimer level.

brinolysis group. In our study, the number of DIC patients was not sufficient and hyperfibinolysis was not severe. A reduction in the plasminogen activity is also caused by organ failure.

When a D-dimer value of >3.0 μg/ml and a FDP value of >6.0 μg/ml was used, more than 50% of patients had some thrombosis, thus suggesting that these patients need antithrombotic therapy, such as aspirin for atherosclerotic thrombosis or warfarin for venous thrombosis. It is considered that these patients with a high value of FDP or D-dimer were in a hypercoagulable state. D-dimer is useful for the diagnosis of DVT but the cutoff values of D-dimer should be mentioned in each measurement kit. The ROC analysis showed that both FDP and D-dimer were useful for the diagnosis of all types of thrombosis and, in particular, DVT. Since both AUC of D-dimer and FDP were high in the ROC analysis, we believe that both markers are useful for the diagnosis of thrombosis or a hypercoagulable state. The ROC

Table 3. Cutoff Values of Plasma FDP and D-Dimer for DVT/PE

Cutoff value for	FDP		D-Dimer	
	DVT/PE	Thrombosis	DVT/PE	Thrombosis
Cutoff value	7.7 μg/ml	7.6 μg/ml	3.8 μg/ml	3.4 μg/ml
Sensitivity	80.8 %	76.9 %	80.8 %	80.0 %
Specificity	85.3 %	85.2 %	86.1 %	84.7 %
PPV	44.7 %	57.5 %	46.1 %	57.8 %
NPV	96.8 %	93.4 %	96.8 %	94.2 %
Odds ratio	24.42	19.05	26.08	22.16
AUC	0.902	0.875	0.907	0.879

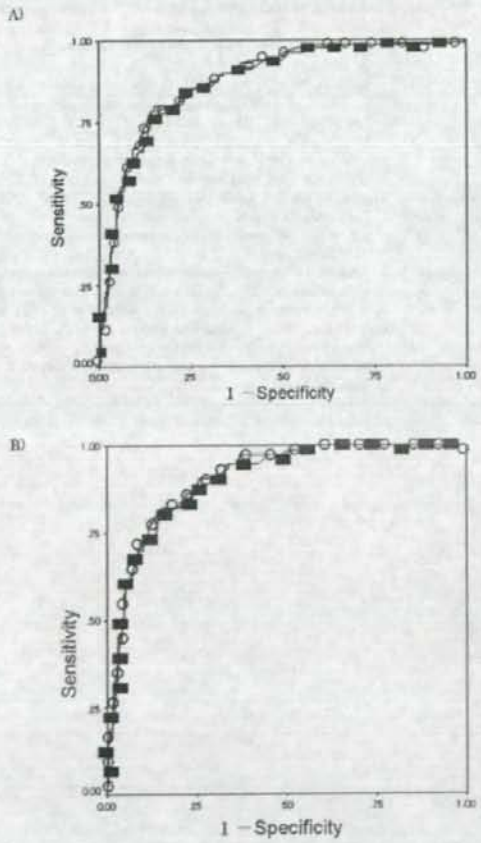

Fig. (5). An ROC analysis for thrombosis or DVT/PE. A) Thrombosis, B) DVT/PE.

analysis also provided adequate cutoff values of D-dimer and FDP for the diagnosis of all types of thrombosis and DVT/PE. The cutoff values of D-dimer for the diagnosis of all types of thrombosis and DVT/PE were similar, while that of FDP were also similar. Both D-dimer (3.8 μg/ml) and FDP (7.7 μg/ml) had high sensitivity, specificity and NPV but a low positive predictive value. In a previous study [23], soluble fibrin (SF) was reported to be more useful than D-dimer for the diagnosis of thrombosis. The odds ratios of SF for thrombosis, DVT and DIC were markedly high. The cut-off value of soluble fibrin (SF) (7.05 μg/ml) was similar for all types of thrombosis and DVT. A high false positive rate for the D-dimer can potentially result in an increase in pulmonary vascular imaging, an increased length of hospital stay, and increased false positive diagnosis of DVT or PE [32]. Therefore, we strongly consider that the cutoff values of SF and D-dimer for thrombosis should be higher than these values.

In conclusion, our findings suggest that high concentrations of plasma FDP including D-dimer also known as markers for a hypercoagulable state, reflect a high risk for thrombosis. However, a differential diagnosis of various types of thrombosis is difficult if it relies on a fibrin-related marker alone.

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Case report

Unilateral stress fracture of the femoral shaft combined with contralateral insufficiency fracture of the femoral shaft after bilateral total knee arthroplasty

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Introduction

Various complications following total knee arthroplasty (TKA) are becoming more widely recognized as the number of patients and the length of follow-up increase. Recognized complications include infection, implant loosening, implant setting, dislocation, implant failure, and stress fracture. Although stress fractures of the femoral neck after TKA have been reported by several authors, there is minimum information on the causes and treatment of this complication.[1–8] Moreover, stress fractures of the femoral shaft associated with TKA are rare. To the authors' knowledge, only one case [9] has been published thus far, and the etiology of stress fractures of the femoral shaft is unknown.

A stress fracture occurs in a healthy young individual because of repetitive overloading during training or exercise. The reported proportions of femoral shaft fatigue fractures, including undisplaced and displaced, of all stress fractures varies from 3% to 43% in military recruits and from 3% to 21% in athletes.[10]

Considering the whole femur, bilateral stress fractures account for 9%, but they rarely present in the femoral diaphysis.[11] Two of bilateral femoral diaphysis fractures during an early phase in a runner[12] and in a military recruit[11] have been reported, each of which was an undisplaced fracture.

We report a rare case of unilateral stress fracture of the femoral shaft combined with a contralateral fracture of the femoral shaft of a 73-year-old woman, secondary to TKA. The geometry of the bilateral femoral shafts was characterized by strong lordosis and bowing, which likely played an important role in these rare fractures. Our patient and her family were informed that data concerning the case would be submitted for publication, and they consented.

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Case report

A 73-year-old woman (height 142 cm, weight 68 kg, body mass index 33.7) presented with a 23-year history of bilateral osteoarthrosis. She complained of pain with severe limitation of walking and used canes. The only metabolic disease was diabetes. Preoperative radiographic evaluation demonstrated varus deformity of both knees with massive osteoarthritic changes (a varus deformity 25° of the right knee and 19° of the left knee) (Fig. 1). The femorotibial angle (FTA) of the right knee was 199° and of the left knee 193°. The bilateral femoral shaft was characterized by strong lordosis and bowing at the middle third. Clinical examination revealed a range of motion (ROM) of 80° flexion/–20° extension of the right knee and 80° flexion/–30° extension of the left knee. Bilateral femoral neck-shaft angles were normal (right femur 129°, left femur 126°) Bone mineral density (BMD) measurements were obtained in both hips; the BMD in the right and left femoral neck were 91% and 72% of the young adult mean, respectively. She seemed unable to put her weight on her left leg for long periods because she had severe left knee pain. The BMD of the lumbar vertebral body was 97%, suggesting normal BMD.

She was treated with a left cemented TKA (Fig. 2A), and a right cemented TKA was performed 3 months later (Fig. 2B). The postoperative anatomical axis was in the normal range of the tibiofemoral valgus angulation (6°). FTA was improved to 174° in both knees. The immediate postoperative course was uneventful. Three months postoperatively, she was pain-free and was able to walk with total weight bearing using a cane.

At 16 months after the left TKA she fell, resulting in severe right thigh pain. The radiograph showed a displaced transverse fracture of the right femoral shaft in the middle third, where lordosis and bowing of the femoral shaft was prominent (Fig. 3). Open reduction and internal fixation with an intramedullary nail was