DVT [16] and PE [17] is important to improve the possible outcomes. In this regard, the D-dimer levels have been reported to be a negative predictor for DVT; levels of less than 0.5 μg/ml of D-dimer are considered to exclude DVT/PE in Europe and North America [16].

DIC [18, 19] is often observed in patients with leukemia, solid cancers, infections, gynecological conditions, and aneurysms, and it is frequently associated with severe bleeding and organ failure. Since DIC is still a fatal condition [20], it is important to diagnose it early through the use of hemostatic molecular markers [21]. The International Society of Thrombosis and Haemostasis (ISTH) established diagnostic criteria for overt-DIC using fibrin-related markers such as D-dimer [22].

The present study was designed to evaluate the cut-off values of D-dimer as a negative predictor for DVT and PE. For this purpose, the plasma D-dimer levels were determined in 381 patients suspected of having thrombosis, and in 100 healthy volunteers.

2 Materials and methods

2.1 Subjects

From January 1, 2005 to December 31, 2005, 381 patients (median age 61.0 years, 25–75% range 50.0–72.0 years, and sex: 245 females and 136 males) were suspected of having some type of thrombosis in several hospitals affiliated with Mie University School of Medicine. The plasma concentrations of D-dimer were examined in these patients, and were then evaluated in order to identify any correlations with the diagnosis of thrombosis. The study protocol was approved by the Human Ethics Review Committees of all participating institutions, and a signed consent form was obtained from each subject. The underlying diseases in these patients included orthopedic conditions in 125 patients, cancer in 65, digestive diseases in 44, cardiovascular diseases in 46, autoimmune diseases in 18, infectious diseases in 15, hematological diseases in 13, diabetes mellitus in 10, trauma and burn in 7, obstetric diseases in 6, thrombophilia in 4, other diseases in 2, and no underlying disease in 26. Of these patients, 184 were diagnosed with thrombosis while 169 were not. Twenty-one of the patients were examined after undergoing liver transplantation, and seven were examined within 3 days after the operation, and these patients were excluded from analysis of the cut-off value. In the thrombotic patients, 76 patients had DVT, 37 had PE, 43 had DIC, 14 had cerebral vascular accident due to thrombosis (CVA), 8 had portal vein thrombosis (PVT), and 6 had AMI or arteriosclerosis obliterans (ASO). DVT was diagnosed with echo or venography. PE was diagnosed either by ventilation–perfusion lung scanning, computed tomography (CT), or pulmonary angiography. DIC was diagnosed by ISTH overt-DIC diagnostic criteria [10]. CVA was diagnosed by CT or magnetic resonance imaging (MRI) and AMI was diagnosed based on the electrocardiogram findings and laboratory data. ASO was diagnosed using the ankle brachial index. For receiver operating characteristic (ROC) analysis, the subjects consisted of DVT, other thrombotic diseases, and non-thrombotic diseases with high D-dimer levels.

The plasma concentrations of D-dimer were measured in patients with thrombosis at onset and those without thrombosis at the first consultation. The same parameters were also measured in 100 healthy volunteers (HV; mean age 41.5 years, range 20–58 years; 47 males and 53 females).

2.2 Measurement of plasma concentrations of D-dimer

The new D-dimer assay (VIDAS D-dimer EXCLUSION, bioMerieux, Marcy l'Etoile, France) is a quantitative ELISA method automated via a VIDAS immunoanalyzer. The new D-dimer assay combines the two-step sandwich immunoenzymatic method followed by final fluorescence detection. All values higher than 10,000 ng/ml were obtained after manual predilution (1/10) of the sample. All values are expressed in ng/ml of fibrinogen equivalent units (FEU).

The plasma D-dimer level was also measured by LPIA-D-dimer (Mitsubishi Chemical Medience, Tokyo, Japan) using a JIF23 monoclonal antibody, which recognizes the plasmin-digested N-terminus of the γ chain on the D region, was used for latex agglutination [23].

2.3 Statistical analysis

The data are expressed as the median (25–75% tile). Differences between groups were examined for significance using Mann–Whitney's U test, while correlations between two variables were evaluated by Pearson's correlation analysis. A P-value of less than 0.05 indicated a significant difference. The usefulness of the D-dimer levels in the diagnosis of thrombosis and DVT or PE was examined by ROC analysis [24]. 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

In healthy volunteers, the plasma D-dimer level was not normally distributed with a median value of 0.12 μg/ml using VIDAS-D-dimer and 0.28 μg/ml using LPIA-D-dimer. The 95% confidence interval (CI) of VIDAS-D-dimer and

LPIA-D-dimer ranged from 0.05 to 0.38 μg/ml and from 0.10 to 1.27 μg/ml, respectively. Plasma D-dimer levels as assessed by VIDAS-D-dimer (median 25–75% tile) were significantly higher in patients with thrombosis (5.04 μg/ml; 3.12–6.85 μg/ml), liver transplantation (3.02 μg/ml; 1.75–5.03 μg/ml), and after surgery (2.89 μg/ml; 2.45–5.30 μg/ml) than in those without thrombosis (0.95 μg/ml; 0.40–1.87 μg/ml; $P < 0.01$, each) (Fig. 1a). The plasma D-dimer level as assessed by LPIA-D-dimer was significantly higher in patients with thrombosis (14.42 μg/ml; 8.13–26.75 μg/ml), after liver transplantation (11.56 μg/ml; 6.45–21.88 μg/ml), and surgery (8.05 μg/ml; 4.86–15.98 μg/ml) than in those without thrombosis (1.27 μg/ml; 0.71–2.82 μg/ml) ($P < 0.01$, each) (Fig. 1b). The 95% CI in patients with thrombosis was 0.98–26.89 μg/ml for VIDAS-D-dimer and 3.02–44.59 μg/ml for LPIA-D-dimer.

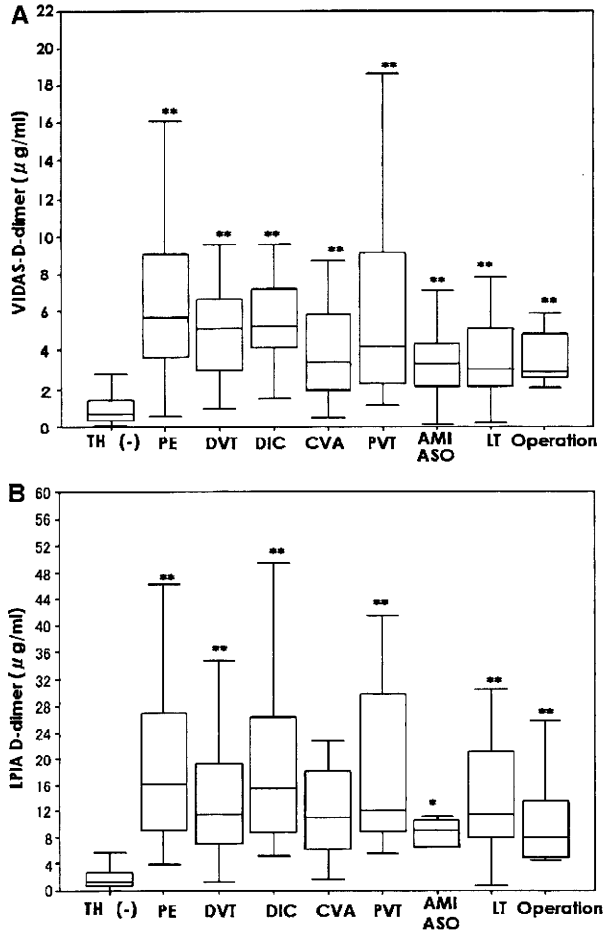

Fig. 1 The plasma level of D-dimer by VIDAS (**a**) and LPIA (**b**) in patients without thrombosis, in those with various thromboses, and in those after liver transplantation and surgery. *PE* pulmonary embolism, *DVT* deep vein thrombosis, *DIC* disseminated intravascular coagulation, *CVA* cerebral vascular accident due to thrombosis, *PVT* portal vein thrombosis, *AMI* acute myocardial infarction, *ASO* arteriosclerosis obliterans. ** $P < 0.01$; * $P < 0.05$

In each case of thrombosis, no significant difference was observed in the VIDAS-D-dimer levels among DVT (5.05 μg/ml; 2.92–6.63 μg/ml), PE (5.65 μg/ml; 3.63–9.08 μg/ml), DIC (5.18 μg/ml; 4.07–7.22 μg/ml), CVA (3.36 μg/ml; 1.93–5.79 μg/ml), PVT (4.12 μg/ml; 2.24–9.15 μg/ml), and AMI/ASO (3.27 μg/ml; 2.12–4.27 μg/ml)(Fig. 1a).

No significant difference was observed in the LPIA-D-dimer levels among DVT (11.55 μg/ml; 7.10–19.40 μg/ml), PE (16.13 μg/ml; 9.15–27.00 μg/ml), DIC (15.35 μg/ml; 8.81–26.40 μg/ml), CVA (11.05 μg/ml; 6.06–17.97 μg/ml), PVT (12.07 μg/ml; 8.95–29.73 μg/ml), and AMI/ASO (9.18 μg/ml; 4.91–10.70 μg/ml) (Fig. 1b).

ROC analysis showed the VIDAS-D-dimer levels to be useful in the diagnosis of all thromboses (Fig. 2a) and DVT/PE/PVT (Fig. 2b). The areas under the curves (AUC) in all thromboses (VIDAS-D-dimer: 0.957, and LPIA-D-dimer: 0.968) and DVT/PE/PVT (VIDAS-D-dimer: 0.958, and LPIA-D-dimer: 0.971) were significantly higher. ROC analysis provided adequate cut-off values of the D-dimer levels in the diagnosis of all thromboses and DVT/PE.

VIDAS-D-dimer levels were closely correlated with LPIA-D-dimer levels [$Y = 0.491736 + 3.006X$, $r = 0.8204$ ($P < 0.001$)] (Fig. 3).

The negative predictive value (NPV) for both VIDAS-D-dimer and LPIA-D-dimer for all thrombosis was not 100% at any plasma D-dimer level (Fig. 4a). In contrast, the NPV for DVT/PE/PVT was 100% at levels of less than 0.5 μg/ml using VIDAS-D-dimer and less than 1.2 μg/ml using LPIA-D-dimer (Fig. 4b).

4 Discussion

According to the current findings, the median value of the plasma D-dimer level by VIDAS-D-dimer in healthy volunteers was 0.12 μg/ml, and the 95% CI was from 0.05 to 1.27 μg/ml. In a European report [16] that used the same D-dimer kit, the mean D-dimer level was 0.21 μg/ml, and ranged from 0.07 to 0.49 μg/ml. The current findings are similar to those described in the European report.

The plasma D-dimer level was significantly higher in patients with thrombosis and after liver transplantation and surgery, but there was no significant difference in D-dimer levels among patients with thrombosis, liver transplantation, and after surgery. These findings suggest that the high plasma level of D-dimer is not just a marker of thrombosis but also a high risk for thrombosis referred to as a hypercoagulable state [25], as it was reported to be elevated in DVT [26, 27], DIC [4, 28], and hyperlipidemia [29]. However, the plasma LPIA-D-dimer level was not markedly higher in patients with AMI or ASO. AMI- or ASO-related thrombosis occurred either in small vessels or developed slowly.

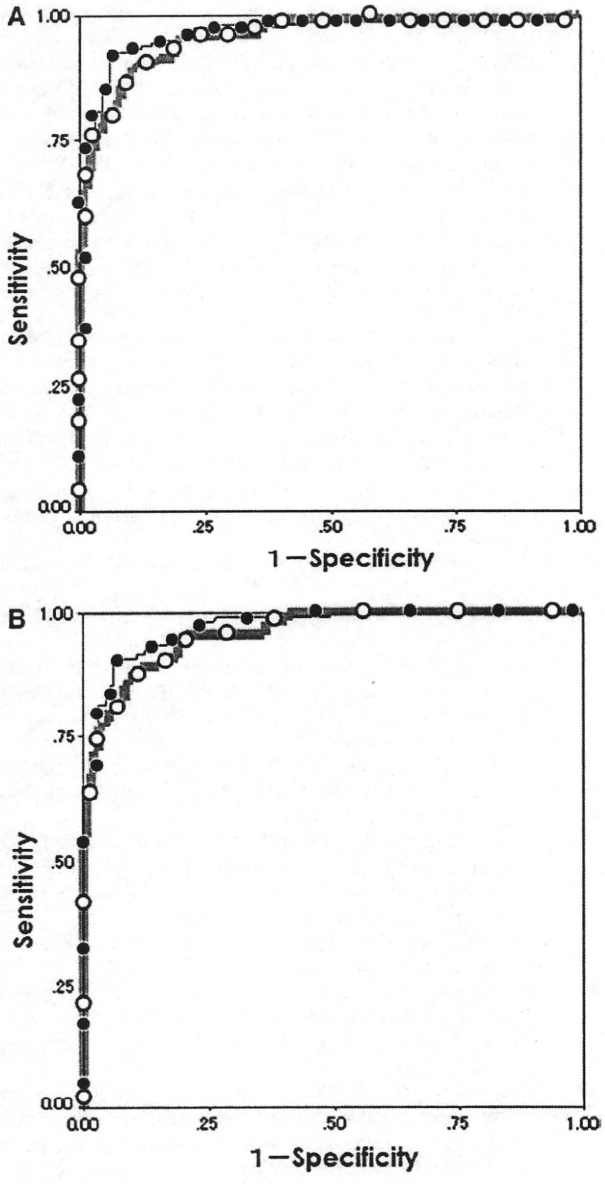

Fig. 2 ROC analysis of D-dimer levels for all thrombosis (**a**) or DVT/PE (**b**). *Open circle* VIDAS-D-dimer, *filled circle* LPIA-D-dimer. **a** AUC was 0.957 in VIDAS-D-dimer and 0.968 in LPIA-D-dimer. **b** AUC was 0.958 in VIDAS-D-dimer and 0.971 in LPIA-D-dimer

VIDAS-D-dimer levels were different from LPIA-D-dimer levels, which is frequently used in Japan; thus, LPIA-D-dimer levels were 2- or 3-fold higher than VIDAS-D-dimer levels. However, both D-dimer levels were significantly higher in patients with thrombosis. ROC analysis including the AUC also showed that both D-dimer results were useful for the diagnosis of various thromboses such as DVT, PE, and PVT. Both the AUC were similar. The VIDAS-D-dimer levels were closely correlated with the LPIA-D-dimer levels, with the latter being about 3× higher than the former.

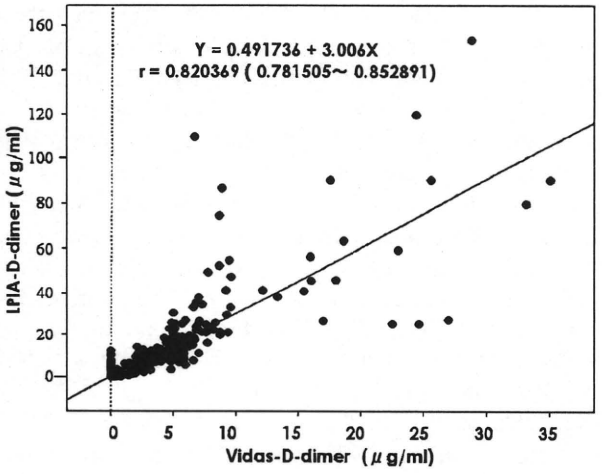

Fig. 3 The relationship between VIDAS-D-dimer and LPIA-D-dimer

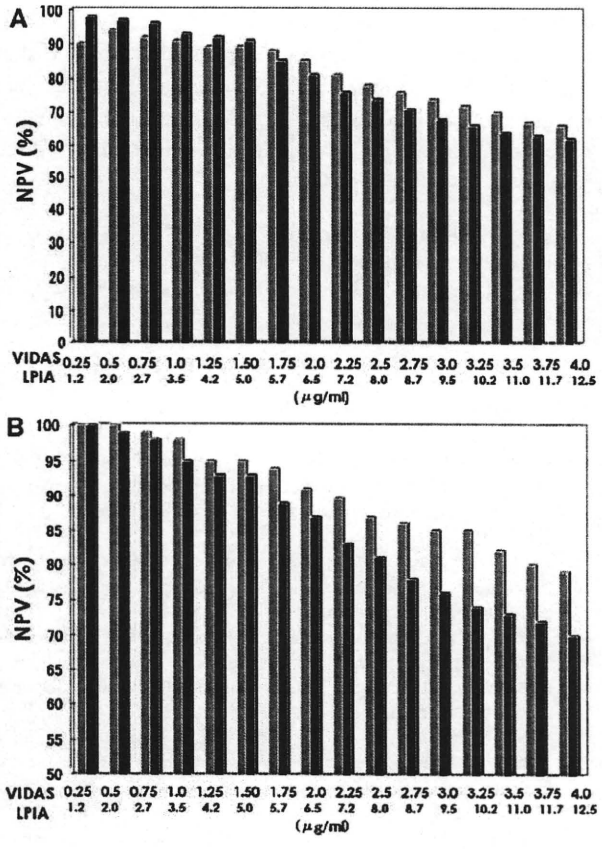

Fig. 4 The NPV in VIDAS-D-dimer and LPIA-D-dimer for all cases of thrombosis (**a**) and DVT/PE/PVT (**b**). *Gray bar* VIDAS-D-dimer, *black bar* LPIA-D-dimer

To maintain the 100% NPV, the cut-off value of VIDAS-D-dimer was 0.5 μg/ml for DVT/PE, and that of LPIA-D-dimer was 1.2 μg/ml. In Europe and North America, plasma D-dimer levels of less than 0.5 μg/ml are

thought to exclude DVT/PE [16]. However, in Japan, the D-dimer level is more than 0.5 μg/ml in many patients, and this cut-off value is not useful as an NPV for DVT/PE in Japan [30], especially because D-dimer kits, which are frequently used in Japan, tend to have a wide normal range (about 0.3–2.5 μg/ml; [4]).

A high false positive rate for D-dimer can potentially result in an increase in pulmonary vascular imaging, increased length of stay in overcrowded emergency departments, and an increased rate of false-positive diagnoses of DVT or PE [31]. Therefore, the cut-off values of LPIA-D-dimer for thrombosis should be more than the 95% CI of healthy volunteers (1.27 μg/ml). However, the cut-off value of VIDAS for NPV was the same as that in European countries (0.5 μg/ml). Although, Japanese physicians trust the 0.5 μg/ml cut-off value as the NPV to exclude DVT or PE, when they are using LPIA-D-dimer, an adequate cut-off value still needs to be established for the D-dimer assay.

In conclusion, these findings suggest that a high plasma level of D-dimer, known as a reliable marker for a hypercoagulable state, indicates a high risk of thrombosis, and is especially useful as an NPV; however, each D-dimer requires a specific cut-off value.

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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

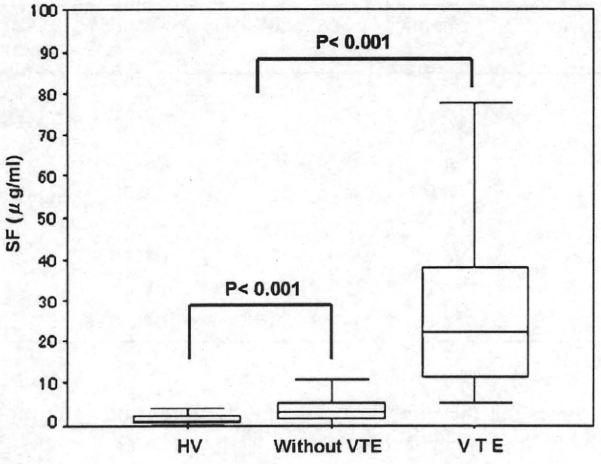

Fig. 1 Plasma concentrations of SF in patients without VTE, those with VTE and healthy volunteers. *VTE* venous thromboembolism, *HV* healthy volunteer

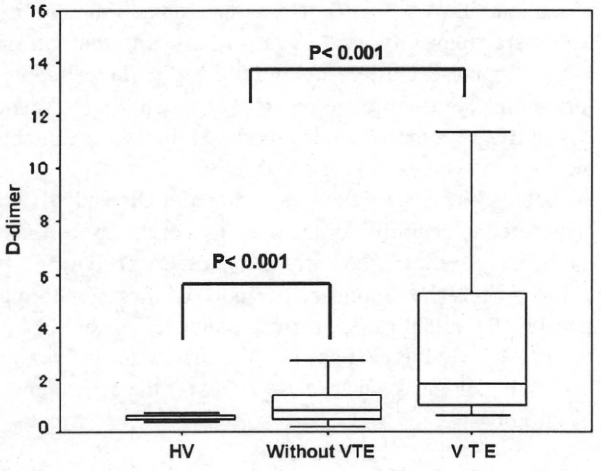

Fig. 2 Plasma concentrations of D-dimer in patients without VTE, those with VTE and healthy volunteers *VTE* venous thromboembolism, *HV* healthy volunteer

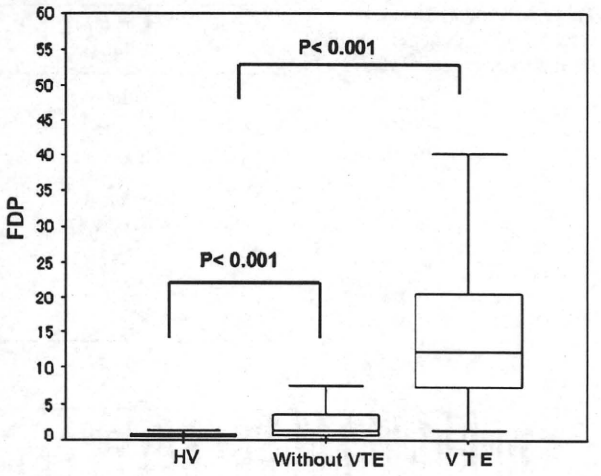

Fig. 3 Plasma concentrations of SF in patients without VTE, those with VTE and healthy volunteers *VTE* venous thromboembolism, *HV* healthy volunteer

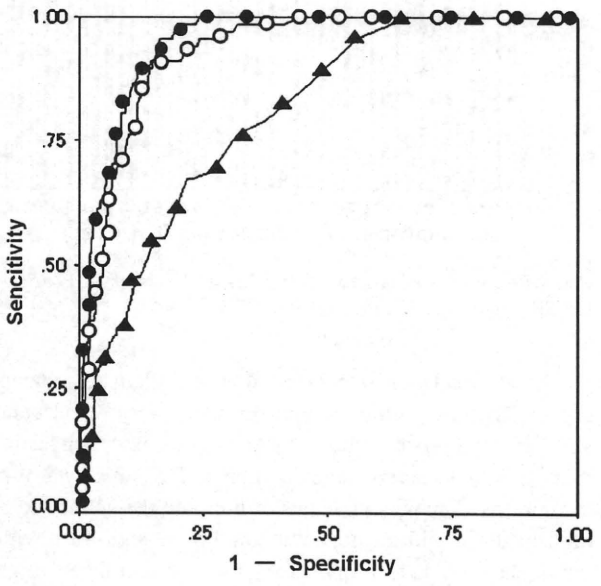

Fig. 4 ROC analysis for diagnosis of VTE. *Closed circle* SF, *open circle* FDP, *closed triangle* D-dimer AUC SF 0.950, FDP 0.933, D-dimer 0.805

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	

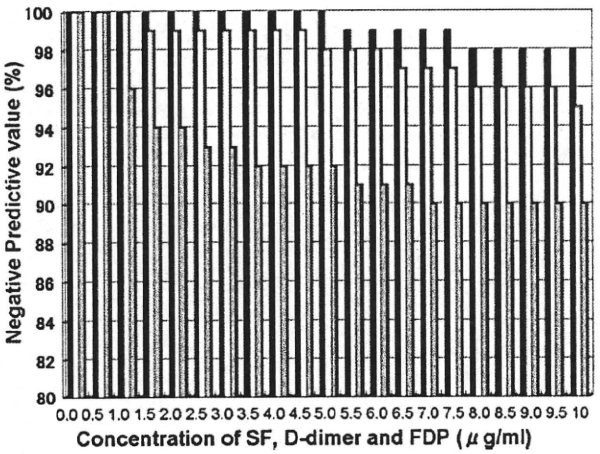

Fig. 5 Negative predictive value for the diagnosis of VTE. *Closed bar* SF, *shaded bar* D-dimer, *open bar* FDP

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 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

1567-2700/08 $55.00+.00

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.

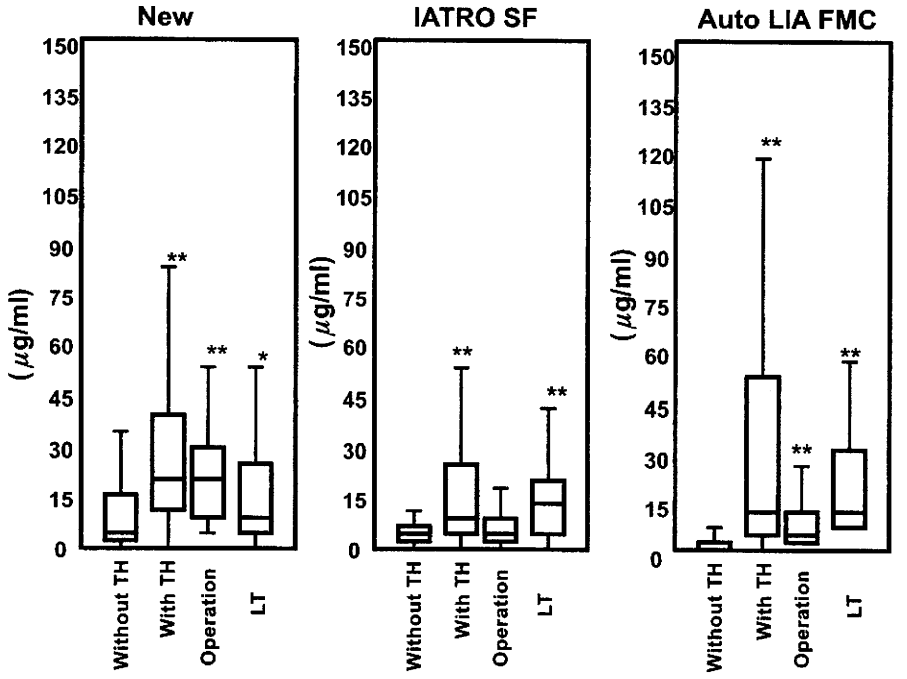

Fig. (1). Plasma concentrations of New SF, IATRO SF and AUTO LIA FMC in patients without thrombosis, those with thrombosis, those after operation and those with livert transplantation.
TH: thrombosis, operation: patients within 3 days after operation, LT: patients after liver transplantation. **: p<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

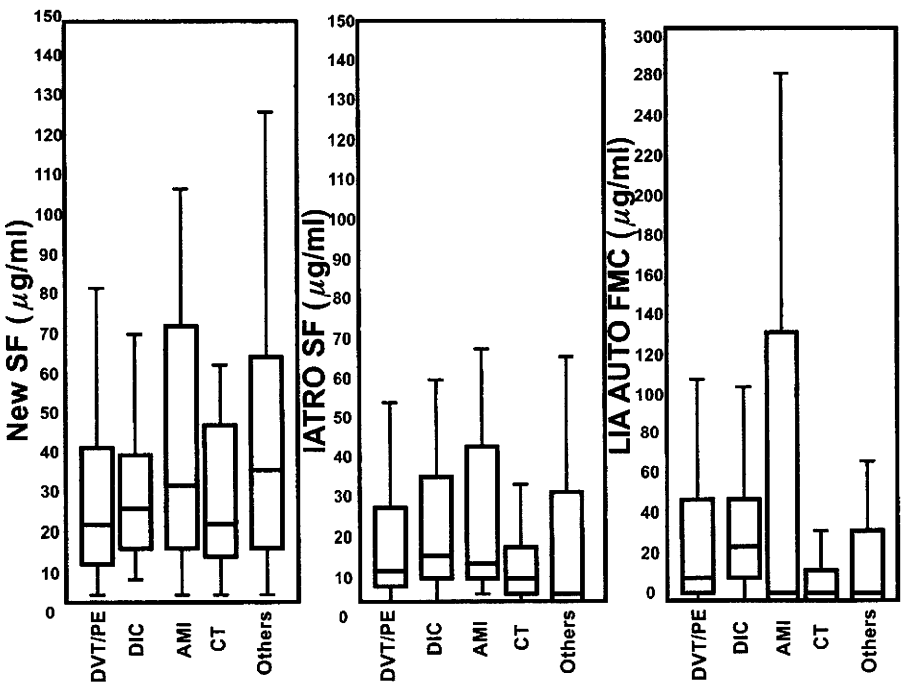

Fig. (2). The plasma concentrations of New SF in patients with DVT/PE, DIC, cerebral thrombosis and other diseases.
CT: cerebral thrombosis.

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% - 75%) 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.

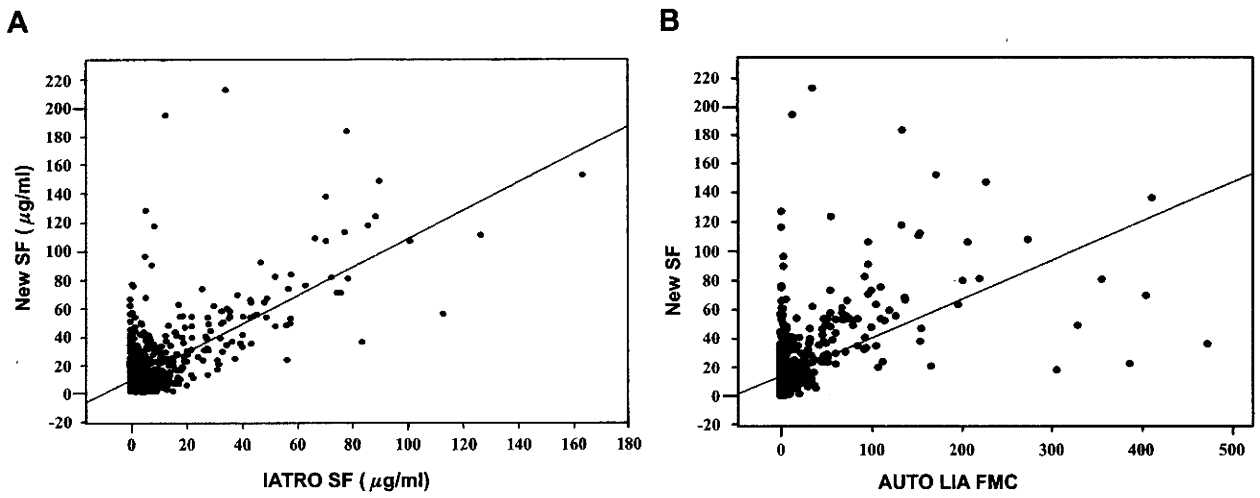

Fig. (3). The relationship between New SF and IATRO SF (**A**) and AUTO LIA FMC (**B**).

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**).

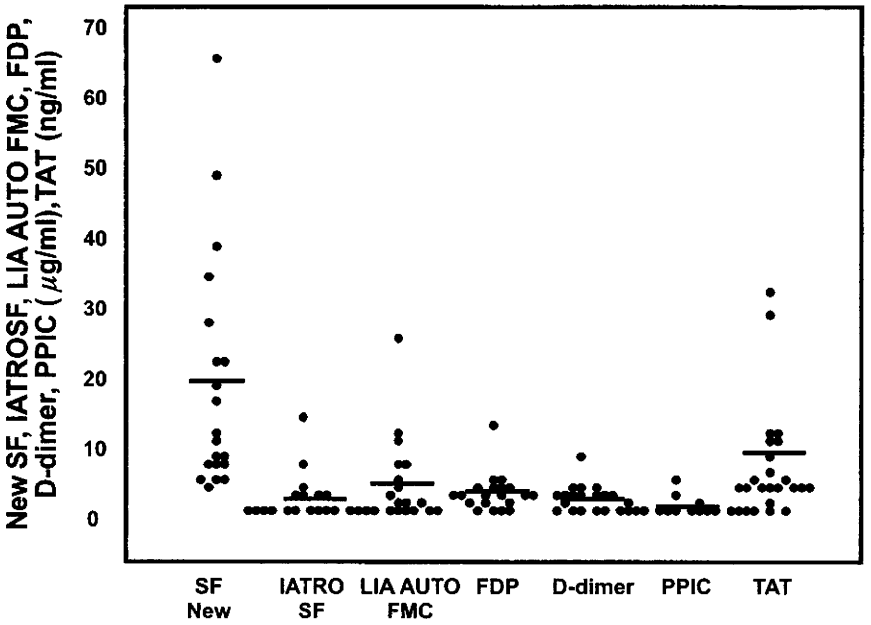

Fig. (4). The plasma levels of New SF, IATRO SF, AUTOLIA FMC, D-dimer, FDP, TAT, PPIC in patients within a week before the onset of thrombosis.

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.

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Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis

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Summary

Decreased plasma ADAMTS13 activity (ADAMTS13:AC) results in the accumulation of unusually large von Willebrand factor multimer (UL-VWFM) and the formation of platelet thrombi. It remains controversial whether or not plasma ADAMTS13:AC decreases in patients with liver cirrhosis (LC), and its relationship to clinical features has not been fully investigated. We measured ADAMTS13:AC and its related parameters in plasma in 33 patients with chronic hepatitis (CH) and in 109 patients with LC. ADAMTS13:AC decreased with increasing severity of liver disease (controls means 100%, CH 87%, Child A-LC 79%, Child B-LC 63%, and Child C-LC 31%), and showed severe deficiency (<3% of controls) in five end-stage LC. Activities measured by act-ELISA strongly correlated with those determined by the VWFM assay and ADAMTS13 antigen. Multivariate analysis showed Child-Pugh score and spleen volume independent factors contributing to ADAMTS13:AC. VWFM patterns were normal in 53% of cases, degraded in 31%, and unusually large in 16%. Patients with unusually large VWFM had the lowest ADAMTS13:AC as well as the highest Child-Pugh score, serum creatinine and blood ammonia levels. Plasma inhibitor against ADAMTS13 detected in 83% of patients with severe to moderate ADAMTS13:AC deficiency mostly showed marginal zone between 0.5 and 1.0 BU/ml. The IgG-type autoantibodies specific to plasma derived-ADAMTS13 was detected by Western blot in only five end-stage LC with severe ADAMTS13:AC deficiency. In conclusion, both plasma ADAMTS13 activity and antigen levels decreased with increasing severity of cirrhosis. An imbalance between the decreased ADAMTS13:AC and its increased substrate may reflect the predisposing state for platelet thrombi formation in patients with advanced LC.

Keywords

ADAMTS13 activity, liver cirrhosis, von Willebrand factor, thrombocytopenia, inhibitor

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Introduction

ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) is a metalloproteinase that specifically cleaves the Tyr1605-Met1606 bond of von Willebrand factor (VWF) A2 domain (1, 2). VWF is exclusively synthesized in vascular endothelial cells and is secreted into the circulation as unusually large VWF multimers (UL-VWFMs), which are most biologically active to aggregate platelets and form platelet thrombi under high shear stress (3). ADAMTS13 rapidly cleaves UL-VWFMs into smaller VWF multimers, which are less biologically active, and thereby prevents platelet hyperaggregation and thrombi formation (3). Congenital deficiency of ADAMTS13 activity (ADAMTS13:AC) caused by its gene mutations (1, 4), and acquired deficiency due to the development of the neutralizing autoantibodies, ADAMTS13 inhibitors (ADAMTS13:INH) (5, 6), result in thrombotic thrombocytopenic purpura (TTP), a life-threatening generalized disease (7). The *ADAMTS13* gene was originally cloned using liver cell libraries (2), and it was later shown that the enzyme is produced exclusively in hepatic stellate cells (HSCs) (8).

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Variable	Chronic hepatitis (n=33)	Liver cirrhosis		
		Child A (n=35)	Child B (n=33)	Child C (n=41)
Age (years)	56.6 ± 12.4	66.4 ± 7.8[b]	63.6 ± 8.3[a]	64.9 ± 15.8[a]
Sex (male/female)	17/16	25/10	17/16	23/18
Cause of liver disease				
HCV/HBV/Alcohol/PBC/Cryptogenic	29/3/0/0/1	24/4/4/0/3	20/7/2/0/4	23/5/5/4/4
Child-Pugh score	—	5.5 ± 0.5	7.9 ± 1.0[c]	11.6 ± 1.5[e]
Spleen volume (mm^3)	219 ± 123	323 ± 181[a]	399 ± 250[b]	559 ± 248[b, c, d]
Ascites (-/easily mobilized/refractory)	0	35/0/0	21/10/2	8/6/27
Hepatorenal syndrome (+)	0	0	0	10
Encephalopathy (+)	0	0	9	33
Esophageal varices (-/mild/severe)[f]	0	10/12/13	3/7/23	3/6/32
Hepatocellular carcinoma (+)	2	22	16	19
JIS score[g]	0, 0	1.4 ± 0.9	2.8 ± 1.0[c]	3.7 ± 1.1[c, d]

The data are expressed as mean ± SD. HCV, hepatitis C virus; HBV, hepatitis B virus; PBC, primary biliary cirrhosis.
[a]$p<0.01$ and [b]$p<0.001$ vs. patients with chronic hepatitis, respectively. [c]$p<0.001$ vs. cirrhotics with Child A. [d]$p<0.01$ and [e]$p<0.001$ vs. cirrhotics with Child B, respectively. [f]Mild or severe esophageal varices indicate lesions without or with endoscopic signs of impending variceal rupture, respectively. [g]The Japan Integrated Staging score obtained via the summation of Child-Pugh score and tumor stage score (32).

Table 1: Clinical data of patients with chronic liver diseases.

Alternatively, increased plasma levels of VWF antigen (VWF:Ag)(9, 10), and thrombocytopenia are commonly seen in patients with advanced LC (11–13). Moreover, cases of advanced LC are often complicated by thrombosis in one or more organs in addition to the portal and hepatic veins (14–16), suggesting a predisposition toward thrombogenesis in advanced LC.

Regarding the possibility of a relationship of ADAMTS13 and various liver diseases, Mannucci et al. (17) originally reported a significant reduction of the ADAMTS13:AC in advanced LC. Subsequently, we have shown that this activity is significantly reduced in patients with hepatic veno-occlusive disease (18), alcoholic hepatitis (19), and those undergoing living-donor related liver transplantation (20). Furthermore, HCV-related cirrhosis patients typically develop TTP with ADAMTS13:INH (21). In cases of advanced LC, however, the recent two studies reported apparently opposite results on plasma levels of ADAMTS13:AC: in one study (22), activity was unchanged, whereas in the other it was reduced (17, 23). The authors of the former study further reported that plasma levels of VWF:Ag were increased, but the higher molecular weight multimer was more degraded than normal controls, thus maintaining normal levels of enzyme-to-substrate (ADAMTS13/UL-VWFMs) ratio to maintain blood fluidity. The study employed the collagen binding assay for ADAMTS13:AC, and the assay has been established, but it is possible that compounds in the tested samples affected the assays in a manner that is not well-understood. In the classic VWFM assay, a gold standard method for ADAMTS13:AC (24), a high concentration of free hemoglobin is well-known to inhibit the enzyme activity (25); likewise, in fluorogenic FRETS-VWF73 assays (26), both bilirubin and chylomicron in tested samples block the activity (27). More recently, however, a chromogenic enzyme-linked immunoassay for ADAMTS13:AC (ADAMTS13-act-ELISA) was shown to be totally insensitive to the presence of such compounds (28).

We have performed comprehensive studies in a large population of patients with chronic liver diseases (n=142), paying particular attention to ADAMTS13 and VWF; plasma levels of ADAMTS13:AC, ADAMTS13 antigen (ADAMTS13:AG), ADAMTS13:INH, VWF:Ag, VWF ristocetin cofactor activity (VWF:RCo), and analysis of plasma UL-VWFMs, in order to explore the relationship between ADAMTS13:AC, the clinical features, and laboratory findings of patients with liver cirrhosis. Furthermore, we examined the presence or absence of immunoglobulin G (IgG)-type autoantibodies specific to plasma derived-ADAMTS13 by Western blot in patients with ADAMTS13:INH in plasma.

Materials and methods

Patients

A total of 142 patients with chronic liver diseases were included in this study, of whom 33 had biopsy-proven chronic hepatitis and 109 had LC, including a case with TTP (21) (Table 1). Patients with a known history of coagulopathies, sepsis, or platelet disorders were excluded. The origin of liver disease was hepatitis C virus (HCV) in 96 cases; hepatitis B virus (HBV) in 19; alcohol abuse in 11; primary biliary cirrhosis (PBC) in four; and cryptogenic in 12. The diagnosis of cirrhosis was based on physical findings, laboratory tests, and in many cases had been confirmed by histological criteria. Of the LC patients, 35 were Child A, 33 were Child B, and 41 were Child C, according to Child-Pugh's criteria (29). Spleen volume, determined by computed tomography scans (30), increased as liver disease progressed. Ascites was easily mobilized in 16 patients and refractory in 29, 10 of whom finally progressed to hepatorenal syndrome according to the criteria described previously (31). Spontaneous bacterial peritonitis (SBP) occurred in 10 patients with refractory ascites, and in seven patients this was complicated by hepatorenal syn-