Safety and efficacy of lower-dose unfractionated heparin for prophylaxis of deep vein thrombosis and pulmonary embolism in an Asian population

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The objective of this study is to analyze the tolerance and efficacy of the subcutaneous administration of a reduced 2500-unit low-dose unfractionated heparin given for an efficacious, yet Asian population-sensitive, prophylaxis for deep vein thrombosis and fatal pulmonary embolism. Eighty-seven Japanese patients were operated on either for abdominal or pelvic complications or both, as well as for gynecologic conditions including ovarian, cervical, and corpus cancers. Thirty-two of the patients were administered the experimental low dose of unfractionated calcium heparin for prophylaxis. The 2500 units of low-dose unfractionated heparin were given subcutaneously 2 h preoperatively and again 12 h postoperatively. Other standard methods of mechanical prophylaxis, including graduated compression stockings and intermittent pneumatic compression, were performed. Fifty-five of the patients were not administered heparin, but did receive the same standard mechanical graduated compression stockings and intermittent pneumatic compression prophylaxis. We compared the surgical and postsurgical complications noted for low-dose unfractionated heparin patients with the results of those who received no heparin prophylaxis and analyzed this data using the Mann–Whitney U-test. There was no significant difference in the mean of the blood loss volumes. There were also no significant differences found in the perioperative bleeding complications between the two groups. However, three (3/55; 6%) of the patients in the no-heparin group suffered a symptomatic pulmonary embolism, although none were fatal. There were no pulmonary embolism onsets in the heparin prophylaxis group. We feel that we have provided evidence that several serious complications, such as perisurgical hemorrhage, deep vein thrombosis, fatal pulmonary embolism, and increased postoperative recovery times, can be prevented by prophylaxis with 2500-unit low-dose unfractionated heparin. Blood Coagul Fibrinolysis 19:585–589 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction
Deep venous thrombosis (DVT) and thrombotic pulmonary embolism are significant complications in postoperative patients. The risk of perioperative development of DVT among patients with malignant gynecologic disease undergoing major operations ranges from 20 to 38% if no preventive measures are taken [1–3]. Approximately, 50% of these perioperative DVT form during the actual operation, an additional 25% occur within 72 h of surgery [4,5]. Only 5% of all perioperative DVT form later than 1 week postoperatively.

Forty percent of all deaths directly resulting from gynecologic surgery are estimated to be attributable to a thrombotic pulmonary embolism [6]. One study shows the risk factors for DVT to be the following: age more than 40 years, obesity, perioperative immobility, trauma, malignancy, previous radiation therapy, and medical conditions, including diabetes mellitus, cardiac disease such as heart failure, severe varicose veins, previous DVT (with or without embolization), chronic pulmonary disease, and antithrombin, protein C, or protein S deficiency [7]. Another study discusses the risk factors occurring during abdominal and pelvic surgery [2].

Intraoperative factors associated with postoperative DVT include an increased duration of anesthesia, increased blood loss, and the need for a transfusion in the operating room. Recognizing that these risk factors exist, it becomes imperative that we consider and implement more effective prophylactic measures for perisurgical DVT.

Prophylaxis of DVT is generally recommended for patients undergoing general surgical procedures [8–10]. There is good evidence that prophylaxis with low-dose unfractionated heparin (LDUH), with 5000 units being the dose, significantly reduces the incidences of both DVT

and fatal pulmonary embolism. In addition to its proven efficacy, LDUH is inexpensive and easily administered.

Several reports regarding native Asian populations have critically noted that they have a uniquely lower prevalence of DVT and pulmonary embolism [11–19]. One report suggests that prevalence of DVT and pulmonary embolism among Hong Kong Chinese was only 10% of that in Western countries, and that when pulmonary embolism occurred it was clinically milder [16]. Kobayashi et al. [20] suggest that this may be in part due to their diet and note that the incidence of pulmonary embolism is on the rise as diets change to a more Western style.

Perioperative and postoperative DVT and pulmonary embolism in Asian populations are, however, still problematic. Although levels of fatal pulmonary embolism dropped significantly when Japan instituted its insurance-reimbursed venous thromboembolism prophylactic measures in 2004, the rate has now become stabilized in the last 3 years, and is not improving further [21]. The question arises as to whether Asian surgical teams need to use as intensive a DVT prophylaxis dose as is performed in Western countries, since the appearance of DVT and pulmonary embolism in Asians is still currently significantly lower than it is in Westerners.

The objective of this study is to analyze the safety and efficacy of the subcutaneous administration of a reduced 2500-unit LDUH given for an efficacious, yet Asian population-sensitive, prophylaxis for DVT and fatal pulmonary embolism.

Materials and methods

This is a retrospective study conducted at the Osaka University Hospital of Japan. From November 2005 to February 2007, 87 Japanese patients were operated on either for abdominal or pelvic complications or both, as well as for gynecologic conditions including ovarian, cervical, and corpus cancers. The procedures were performed under general anesthesia. Patients were excluded from the study if they required presurgical anticoagulant or antiplatelet therapy. They were also excluded if they had hepatic or renal failure, or a history of systemic bleeding diathesis.

All patients who were scheduled to have an operation were required to have a preoperative DVT screening based upon comparative measurements of the diameter of their thighs and legs, as well as a clinical examination by palpation for Homan's sign. DVT was suspected if the difference in diameter measurement between the left and right thighs or legs was 2 cm or above [22]. A diagnosis of DVT was then confirmed using ultrasonography. Patients were excluded from the study if they were diagnosed with DVT prior to the operation.

All patients provided a written informed consent. The patients were operated on by the same two well respected surgeons to ensure the most accurate results for this study. The majority of patients who received the experimental 2500-unit LDUH for DVT prophylaxis did so after March 2006. They were compared with a similar cohort of patients, who had received no heparin prophylaxis, most of whose surgeries occurred prior to March 2006.

Thirty-two of the patients were administered the experimental low-dose of unfractionated calcium heparin for prophylaxis. The 2500 units of LDUH were given subcutaneously 2 h preoperatively and again 12 h postoperatively. Other standard methods of mechanical prophylaxis, including graduated compression stockings (GCS) and intermittent pneumatic compression, (IPC) were performed. Fifty-five of the patients were not administered heparin, but did receive the same standard mechanical GCS and IPC prophylaxis.

Perioperative blood loss was recorded from sponge weights and suction aspirates. We compared the surgical and postsurgical complications noted for LDUH patients with the results of those who received no heparin prophylaxis and analyzed the data using the Mann–Whitney U-test.

Results

Table 1 shows several important characteristics of the patients, including the patient's age, BMI, activated partial thromboplastin time (APTT), hemoglobin content, and platelet counts. The characteristics of the two groups had no significant differences. The mean of the operational duration was not significantly different between the two groups.

Table 1 Patient characteristics

	LDUH (n = 30)	No heparin prophylaxis (n = 57)	P
Mean age (years)	55.3 ± 11.6	54.8 ± 11.8	0.8509
BMI	22.9 ± 5.0	21.7 ± 3.9	0.2463
APTT (s)	30.2 ± 4.3	30.0 ± 3.5	0.857
Hemoglobin (g/dl)	12.7 ± 1.6	12.7 ± 1.3	0.594
Platelets (×10⁴/dl)	26.9 ± 9.7	28.7 ± 12.7	0.5123
Diagnosis			
Ovarian cancer	11	18	
Corpus cancer	16	26	
Cervical cancer	3	13	
Surgical procedure			
TAH BSO	3	7	
TAH BSO PLN	8	5	
TAH BSO PLN PAN	9	26	
RH BSO PLN	10	19	
Surgical data			
Mean operative time (min)	317.8 ± 144.4	363.0 ± 138.1	0.0969

APTT, activated partial thromboplastin time; BMI, body mass index; BSO, bilateral salpingo-oophorectomy; LDUH, low-dose unfractionated heparin; PAN, para aortic lymphadenectomy; PLN, pelvic lymphadenectomy; RH, radical hysterectomy; TAH, total abdominal hysterectomy.

Table 2 Bleeding during surgical procedure

	LDUH (n = 30)	No heparin prophylaxis (n = 57)	P
Mean operative blood loss (ml)	1054.0 ± 1221.0	912.0 ± 814.0	0.9602
Diagnosis			
Ovarian cancer	1773.0 ± 1864.2	1457.6 ± 1153.3	0.7958
Corpus cancer	704.3 ± 554.5	524.6 ± 373.6	0.4293
Cervical cancer	598.8 ± 123.9	975.9 ± 474.4	0.1464
Surgical procedure			
TAH BSO	693.3 ± 828.6	504.3 ± 864.6	0.6667
TAH BSO PLN	396.3 ± 343.3	286.0 ± 101.9	0.9433
TAH BSO PLN PAN	1538.0 ± 1835.8	1072.4 ± 972.4	0.7885
RH BSO PLN	1204.5 ± 799.6	1004.2 ± 554.9	0.5818

APTT, activated partial thromboplastin time; BMI, body mass index; BSO, bilateral salpingo-oophorectomy; LDUH, low-dose unfractionated heparin; PAN, para aortic lymphadenectomy; PLN, pelvic lymphadenectomy; RH, radical hysterectomy; TAH, total abdominal hysterectomy.

Table 2 shows the bleeding volume during the surgical procedure, comparing the blood loss volume in the surgical procedures where a prophylactic 2500 units of LDUH was administered with the group that received no prophylaxis. There was no significant difference in the means of the blood loss volumes. There were also no significant differences found in the perioperative bleeding complications between the two groups. However, three (3/55; 6%) of the patients in the no-heparin group suffered a symptomatic pulmonary embolism, although none were fatal. There were no deaths in this study attributable to thromboembolism. We did not observe that resurgery was required for postsurgical abdominal hematomas during this study.

Discussion

Gynecologic surgery patients undergoing pelvic surgical procedures are at a higher-than-average risk than for other surgical patients of developing perioperative and postoperative DVT and pulmonary embolism. According to the American College of Chest Physician's guidelines [23], patients undergoing extensive surgery for malignancy, and for patients with additional DVT/pulmonary embolism risk factors, are recommended to have routine prophylaxis with LDHU [5000 units three times daily (t.i.d.), grade 1A], or a higher dose of low-molecular-weight heparins (LMWH) (i.e., >3400 units/day, grade 1A). Alternative considerations include IPC alone continued until hospital discharge (grade 1A), or a combination of LDUH or LMWH plus mechanical prophylaxis with graduated compression stockings (GCS) or intermittent pneumatic compression (IPC) (all grade 1C).

Most prophylaxis trials of subcutaneous LDUH administered a dose of 5000 units 1–2 h before surgery, followed by administration of 5000 units b.i.d. (twice daily) or t.i.d. until patients were either ambulating or were discharged from the hospital. A meta-analysis of 46 randomized clinical trials in general surgery compared therapy with LDUH with no prophylaxis or a placebo [8]. The rate of DVT was significantly reduced [from 22 to 9%; odds ratio

(OR) 0.3; needed to be treated (NNT) 7], as were the rates of symptomatic pulmonary embolism (from 0.8 to 0.3%; OR 0.4; NNT 182), and the all cause mortality (from 4.2 to 3.2%; OR 0.8; NNT 97). Prophylaxis with LDUH was associated with a small increase in the rate of bleeding events (from 3.8 to 5.9%; OR 1.6; NNT 47). These findings were verified in another meta-analysis in which the rate of wound hematomas was increased with LDUH use (6.3 vs. 4.1% in control subjects; OR 1.6; NNT 45), although the rate of major bleeding was not [24]. Although both meta-analyses concluded that the administration of heparin, 5000-units t.i.d., was more efficacious than that of 5000-units b.i.d., without increasing bleeding, this was based on indirect comparisons, and we are not aware of any studies that directly compared these two regimens.

LMWH are fragments of unfractionated heparin produced by either chemical or enzymatic depolymerization. LMWH are as effective as LDUH in the prevention of perioperative DVT [25]. For general surgery patients, LDUH and LMWH have similar efficacy and bleeding rates. In high-risk general surgery patients, higher doses of LMWH provide greater protection of perioperative DVT than lower doses. Bergqvist *et al.* [26] reported that in cancer patients, prophylaxis with dalteparin, 5000 unit daily was significantly more efficacious than with 2500 unit daily, without an increased risk of bleeding in northern Europeans. However, according to our results, it could be sufficiently efficacious at a 2500 unit dose of LMWH for prophylaxis of DVT and pulmonary embolism in an Asian population. Some studies have reported significantly fewer wound hematomas and other bleeding complications with LMWH than with LDUH, whereas, other trials have shown the opposite effect [27–33]. Two meta-analyses that found similar efficacy for LDUH and LMWH, described differences in bleeding rates that were dependent on the dose of LMWH used. Lower doses of LMWH (i.e., ≤3400 U daily) were associated with less bleeding than LDUH (3.8 vs. 5.4%, respectively; OR 0.7), whereas, higher doses resulted in more bleeding events (7.9 vs. 5.3%, respectively; OR 1.5) [34].

Furthermore, the clinical advantage of LMWH over LDUH include its once-daily administration and the lower risk of heparin-induced thrombocytopenia (HIT) [35]. Therefore, LMWHs are becoming more commonly used.

The reasoning for using LDUH administered as a reduced 2500 units in this study, as opposed to the more traditional 5000 units, is that in Japan no other form of chemoprophylaxis against venous thromboembolism except for LDUH is permitted. Additionally, there are significantly lowered risk factors for venous thrombosis when comparing Asian with Western patient populations,

so by lowering the dosage we might lower some of the undesired side effects of LDUH.

To minimize risk, we have developed criteria to screen out patients who have had a DVT history before surgery. Criado and Burnham [22] evaluated the clinical presentation of DVT and found that a difference in calf circumference of less than 2 cm demonstrated a negative predictive value of 85% among outpatients, and of 93% in inpatients. Therefore, we were able to eliminate patients with a high risk of DVT and pulmonary embolism before surgery. We can find no other reports of 2500-unit LDUH administration preoperatively and postoperatively, such as in our current study.

In conclusion, we feel that we have provided good evidence that complications such as perisurgical hemorrhage, DVT, fatal pulmonary embolism and increased postoperative recovery times can be prevented by prophylaxis with 2500-unit LDUH. We expect that the tolerance and efficacy of prophylaxis with 2500-unit LUDH is not significantly different from 5000-unit LDUH or LMWH. However, because this study is small in patient number, we hope to receive future funding to extend this study by conducting an initial randomized control study for corroborative evidence.

A low-dose of LMWH for prophylaxis of DVT and pulmonary embolism could be just as efficacious as 2500 unit of LDUH in our Asian populations; however, we can find no reports concerning such a low dose of LMWH for prophylaxis for Asian populations, so further study would be necessary to show that efficacy and unit.

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REGULAR ARTICLE

ADAMTS13 related markers and von Willebrand factor in plasma from patients with thrombotic microangiopathy (TMA)

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Abstract

The ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type I domain 13) related markers were measured in the plasma of healthy volunteers and thrombotic microangiopathy (TMA) patients including thrombotic thrombocytopenic purpura (TTP) to examine their efficacy in the diagnosis of TTP.
The plasma levels of the ADAMTS13 antigen and ADAMTS13-factor XI complex were significantly lower in TMA patients with a significant decreased ADAMTS13 activity (and these patients were considered to have TTP) than in the healthy volunteers. The plasma levels of ADAMTS13 antigens closely correlated with those of ADAMTS13-factor XI complex. Autoantibody for ADAMTS 13 was also positive in almost all TTP patients. In addition, the ratio of von Willebrand factor (VWF)/ADAMTS13 activity was significantly high in TTP suggesting that this ratio might be more useful for the

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differential diagnosis of TTP than the ADAMTS13 assay alone.

These findings suggest that ADAMTS13 related markers are useful for the diagnosis and analysis of TTP.

Introduction

ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type I domain 13), which was identified in 2001 [1–5], is a zinc metalloprotease, that specifically cleaves the ultra large Von Willebrand factor (VWF) multimer at the Tyr (1605)–Met (1606) bond located in the A2 region of VWF [6,7]. The unusually large Von Willebrand factor (VWF) multimer, produced in and then quickly released from vascular endothelial cells, has often been found in the plasma of patients with familial and non-familial thrombotic thrombocytopenic purpura (TTP) [8,9]. These unusually large VWF multimers have been thought to interact with circulating platelets, thus resulting in platelet clumping due to an elevated shear stress [8]. VWF is a large glycoprotein which is essential for high-shear stress associated platelet adhesion and aggregation [10].

Many studies [6,7,11] have shown low plasma levels of ADAMTS13 activity to be associated with TTP; a life-threatening syndrome characterized by thrombocytopenia and microangiopathic hemolytic

Table 1 Subjects with TMA

ADAMTS13	Age	Sex	Outcome	Activity (%)	Antigen (%)	Activity/ antigen ratio	Complex with FXI (%)	Antibody	VWF (%)	VWF/activity ratio
SL	59	M	Survive	3>	30	NC	27	Positive	272	>90
SL	44	M	Death	3>	108	NC	219	Positive	163	>54
SL	55	M	Survive	3>	63.4	NC	25	Positive	276	>92
SL	34	F	Survive	3>	10	NC	95	Positive	145	>48
SL	46	F	Survive	3>	27.8	NC	41	Positive	199	>65
SL	45	F	Survive	3>	5.9	NC	51	Positive	116	>38
SL	43	F	Survive	3>	15.9	NC	67	Positive	145	>48
SL	45	F	Survive	3>	9.2	NC	13	Negative	47.2	>15
SL	34	F	Survive	3>	90.5	NC	206	Positive	58.3	>19
SL	14	F	Survive	3>	37.6	NC	38	Positive	122	>40
SL	41	M	Death	3>	7.9	NC	31	Positive	145	>48
SL	17	F	Survive	3>	8.2	NC	23	Positive	135	>44
SL	16	F	Survive	3>	7.9	NC	95	Positive	132	>44
SL	61	M	Survive	3>	5.5	NC	18	Positive	153	>51
SL	75	M	Survive	3>	16.6	NC	33	Positive	191	>63
SL	64	F	Survive	3>	57.5	NC	17	Positive	32	>32
SL	79	M	Survive	3>	8.0	NC	13	Positive	178	>59
SL	67	M	Death	12.5	6.2	2.02	26	Positive	188	15.0
SL	50	F	Survive	13.8	8.3	1.66	12	Positive	332	24.2
ML	71	F	Survive	27.5	78.6	0.34	24	Negative	342	12.4
ML	72	F	Death	33.8	25.8	2.13	37	Negative	295	8.7
ML	51	F	Death	38.8	57.9	0.67	23	Negative	222	5.7
ML	38	M	Survive	43.8	55.1	0.79	61	Negative	200	4.6
ML	27	M	Survive	43.8	115	0.38	45	Negative	213	4.9
ML	68	F	Survive	47.5	34.2	1.39	54	Positive	716	15.1
ML	17	M	Survive	48.8	64.5	0.76	74	Positive	38.4	0.8
ML	53	M	Survive	50.0	84.8	0.60	94	Negative	135	2.7
ML	41	M	Death	67.5	81.1	0.83	37	Negative	314	4.7
Normal	28	M	Death	78.8	195	0.4	130	Negative	238	3
Normal	84	F	Survive	80.0	61.4	1.93	96	Negative	312	3.9
Normal	24	M	Survive	96.3	175	0.55	154	Negative	180	1.9
Normal	44	F	Death	98.8	106	0.93	44	Negative	228	2.3
Normal	51	F	Survive	128.8	168	0.76	97	Negative	202	1.6

SL; significantly low, ML; moderate low, NC; not calculated.

Table 2 Familial TMA

Family	Age	TMA	Sex	Activity (%)	Antigen (%)	Activity/antigen ratio	Complex with FXI (%)	Antibody	VWF (%)	VWF/activity ratio
1	21	+	F	143.8	200	0.72	88	Negative	102	0.7
2	1	+	M	112.5	102	1.1	64	Negative	54.8	0.5
2	24	+	F	121.3	120	1	77	Negative	57.1	0.5
2	26	+	M	82.5	119	0.69	59	Negative	72.7	0.9
3	54	+	F	118.8	62.2	1.9	103	Negative	108	0.9
3	30	+	F	200	71.7	2.79	97	Negative	185	0.9
4	73	−	M	36.3	182	0.20	84	Negative	77.2	2.1
4	44	+	M	3>	4.1	NC	12	Negative	227	>79
4	69	−	F	37.1	155	0.24	29	Negative	58.5	1.60

SL; significantly low, ML; moderate low, NC; not calculated.

anemia, which is often associated with neurological dysfunction, renal failure, and fever [12,13]. Thrombotic microangiopathy (TMA) including TTP is often observed after bone marrow transplantation [14] or liver transplantation [15].

A severely deficient ADAMTS13 activity (less than 5% of that in normal plasma) is caused by either a mutation of the ADAMTS13 gene [2,16] or by inhibitory antibodies against ADAMTS13 [17–19]. Since measuring the ADAMTS13 activity is important in the diagnosis of TTP, Kokame developed a fluorescence resonance energy transfer (FRET) assay to determine the ADAMTS13 activity [20]. An assay of an inhibitor for ADAMTS13 which measures of the activity requires a high level of skill and quite a long time to diagnose acquired TTP. There are several patients with TTP who possess normal levels of ADAMTS13 activity [19], thus suggesting that other biological factors in addition to ADAMTS13 may therefore play a role in TTP. A recent report demonstrated that ADAMTSA13 is able to form a stable complex with FXI and FXIa and these complexes are found in plasma [21].

In this study, the ADAMTS13 related markers were measured in the plasma of 50 healthy volunteers and 40 patients with TMA and thus examined the usefulness of a diagnosis of TMA, especially TTP.

Materials and methods

The activity and antigen of ADAMTS13, ADAMTS13–FXI complex and VWD antigen were measured in 50 healthy volunteers (19 females and 31 males; median age, 31 years; range, 19–51 years) and in 40 patients with TMA (22 females and 18 males; median age, 44 years; range, 1–84 years) (Table 1). In the 40 patients with TMA, who were admitted to either Mie University Hospital or the affiliated hospital from 1996 to 2006, 4 families including 7 patients demonstrated congenital TMA (Table 2). Regarding the underlying diseases, there were 15 autoimmune diseases, but there was no post bone marrow transplantation, post liver transplantation nor patients with verotoxin producing *Escherichia coli* infection. The diagnosis of TMA was made based on the presence of thrombocytopenia due to the consumption, microangiopathic hemolytic anemia, neurologi-

cal abnormalities, renal function impairment and high fever [19]. In TMA, the patients who had less than 20% of ADAMTS13 are considered to have TTP.

Although these patients were treated with plasma exchange, plasma transfusion, anti-platelet drug or steroid, 8 patients with TMA died.

The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine and a signed consent form was obtained from each subject.

Blood sampling was done in on admission or in the diagnosis of TMA. Human plasma was obtained by centrifugation at 3000 g at 4 °C for 15 min from whole blood that was treated with a 1/10 volume of 3.8% sodium citrate as an anti-coagulant.

The ADAMTS13 activity was measured by a fluorescent assay and the fluorogenic substrate, FRETS-VWF73, was chemically synthesized by the Peptide Institute, Inc. (Osaka, Japan) [20]. The assay was performed according to the method of Kokame et al. [20]. In healthy volunteers, the ADAMTS13 activity (median value; 25% tile–75% tile) (113.0%; 61.1%–261.5%) was not being normally distributed (Table 3). ADAMTS13 antigen, ADAMTS13–FXI complex and the inhibitor of ADAMTS13 were measured by ELISA using an IMUBIND®ADAMTS13 ELISA (American Diagnostica Inc.; ADI, CT, USA), IMUBIND® ADAMTS13/FXI Complex ELISA (ADI) and IMUBIND®ADAMTS13 Autoantibody ELISA (ADI), respectively. In healthy volunteers, ADAMTS13 antigen (95.4%; 60.4%–177.0%) and ADAMTS13–FXI complex (101.0%; 37.0%–264.0%) were not being distributed normally (Table 3). In an assay of autoantibody for ADAMTS13, the 95% CI of the healthy volunteers ranged from 2.75 to 19.55 AU/ml, thus suggesting that more than 20 AU/ml of autoantibody is considered to be positive for inhibitor. The VWD antigen was measured using a VIDAS VWF (BIOMÍRIEUX, Marcy l'Etoile, France). In healthy volunteers, VWF antigen (113%; 61.1%–261.5%) were not being distributed normally, and VWF antigen/ADAMTS13 activity ratio was 1.05; 0.55–3.64 (Table 3).

Table 3 ADAMTS13 related markers in healthy volunteers

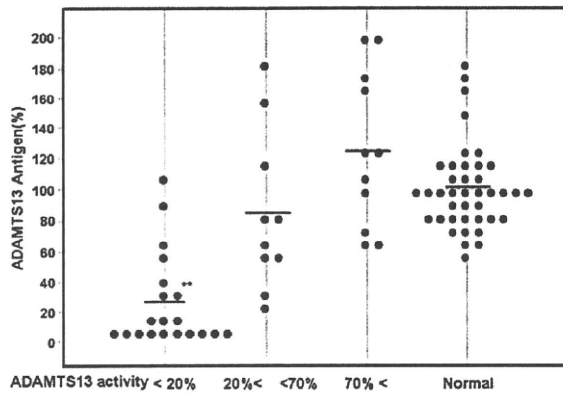|	Median	95% CI
VWF antigen (%)	113	61.1–261.5
ADAMTS13 activity (%)	106.6	65.8–153.5
ADAMTS13 antigen (%)	95.4	60.4–177.0
ADAMTS13–FXI complex (%)	101.0	37.0–264.0
Autoantibody (AU/ml)	7.8	2.8–19.5
ADAMTS13 activity/antigen ratio	1.11	0.55–3.64
VWF antigen/ADAMTS13 activity ratio	1.05	0.55–3.64

Figure 1 Plasma levels of ADAMTS13 antigen in patients with TMA.

Statistical analysis

The data are expressed as the mean±SD. The differences between the groups were examined for statistical significance using the Mann–Whitney's U test. The correlation between the groups was examined for statistical significance using Pearson's correlation analysis. A p value of less than 0.05 was considered to indicate the presence of a statistically significant difference.

Results

The plasma levels of VWF antigen (median value; 25% tile–75% tile), were significantly higher in patients with TMA (180.0%; 29.4% –538.4%) than in the healthy volunteers ($p<0.01$). While the plasma levels of ADAMTS13 antigen (20.6%; 0%–171.9%) and ADAMTS13–FXI complex (32.0%; 8.0%–212.0%) were significantly lower in those with TMA than in the healthy volunteers ($p<0.01$). The ADAMTS13 activity of 20 patients with TMA (19 acquired and 1 familial patients) was less than 20% (significant low group) and that of 10 patients with TMA was from 20% to 70% (moderate low group) and that of 11 patients with TMA was higher than 70% (normal group). The plasma levels of ADAMTS13 antigen were also significantly lower in the patients with significant low ADMTS13 activity (9.6%; 7.9%–33.8%) than those with moderate
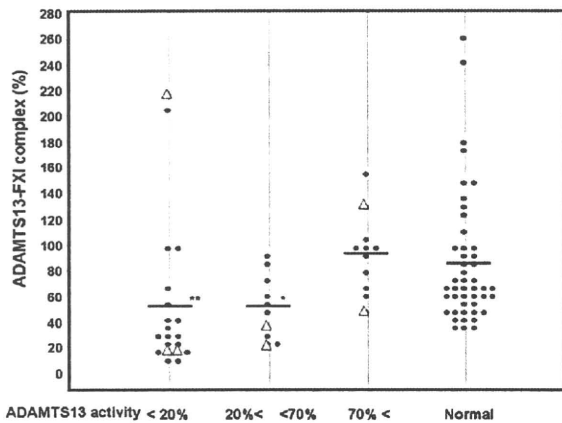

Figure 2 Plasma levels of ADAMTS13–FXI complex in patients with TMA △; patients who died during clinical course of TMA.
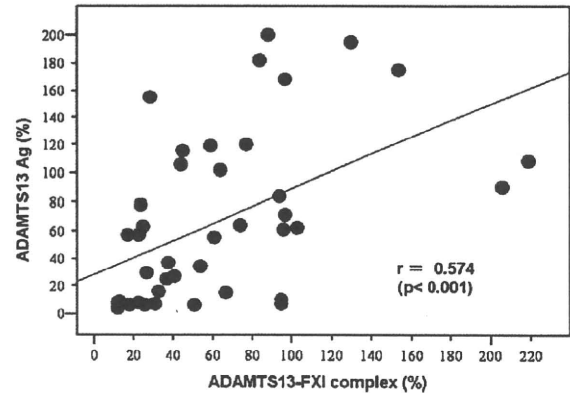

Figure 3 Relationship between ADAMTS13 antigen and ADAMTS13–FXI complex in patients with TMA. $Y=28.724+0.606X$, $r=0.574$, $p<0.001$.

low (71.6%; 55.1%–115.0%) or with normal (119.0%; 79.3%–173.3%) ADAMTS13 activity, and healthy volunteers ($p<0.01$, respectively) (Fig. 1). In patients with less than 3% of ADAMTS13 activity, ADAMTS13 antigen was able to be detected and ADAMTS13 antigen was not well correlated with ADAMTS13 activity. Autoantibody for ADAMTS13 was positive in 18/19 of significantly low ADAMTS13 group and in 2/9 of moderate low ADAMTS13 group but negative in all of normal ADAMTS13 group and familial TMA group, showing that the results from autoantibody ELISA were similar to those from the inhibitor assay by FRETS-VWF73.

The plasma levels of ADAMTS13–FXI complex were widely distributed and significantly lower in the patients with significant low AAMTS13 activity (29.0%; 17.5%–59.0%) than in normal ADAMTS13 group (96.0%; 67.3%–101.5%) and healthy volunteers ($p<0.001$, respectively, Fig. 2). Two patients with low ADAMTS 13 activity had high ADAMTS13–FXI complex and high ADAMTS13 antigen. The plasma levels of ADAMTS13–FXI complex also tend to be low in the patients who died during the clinical course of TMA. The plasma levels of ADAMTS13–FXI complex were also correlated with ADAMTS 13 antigen ($Y=28.724+0.606X$, $r=0.574$, $p<0.001$, Fig. 3).

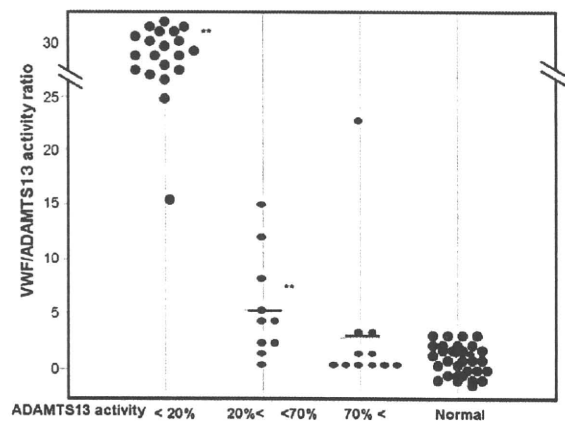Although there was no significant difference in the VWF antigen levels among the significant low (149%; 127%–195%),

Figure 4 Ratio of VWF/ADAMTS13 activity in patients with TMA.

moderate low (206%; 77%–295%) and normal ADAMTS 13 (180%; 80%–222%) groups, the ratio of VWF/ADAMTS13 activity was significantly higher in the patients with TMA than in the healthy volunteers ($p < 0.001$). The ratio of VWF/ADAMTS 13 activity was significantly higher in the significantly low ADAMTS 13 group than the moderate low (4.7; 2.1–8.7) and normal ADAMTS13 (0.9; 0.7–2.7) group (Fig. 4). The cutoff levels of VWF/ADAMTS 13 activity ratio between TTP and other TMA appear to be 15.0. The patients with familial TTP had no detectable inhibitor, and only one family showed a low ADAMTS13 activity and a high VWF/ADAMTS13 ratio (Table 3).

Discussion

The plasma levels of the VWF antigen were significantly high in patients with TMA. VWF is released from vascular endothelial cells as is thrombomodulin and plasminogen activator inhibitor 1 (PAI-I) and they are referred to as injured vascular endothelial cell injured markers [22]. These markers are significantly elevated in patients with a poor outcome [23], thus suggesting that these markers reflect the outcome. Furthermore, the ratio of VWF/ADAMTS13 activity was significantly higher in TMA, thus suggesting that the ultra large multimers of VWF accelerate microthrombus formation by activated platelets. According to our findings, the ratio might therefore be more useful for the diagnosis of TTP than ADAMTS13 alone.

ADAMTS13 was recently identified to be a new hemostatic factor, which was previously called VWF cleaving protease [24]. Either congenital or acquired defects of the enzymatic activity of this protein lead to TTP [19,25]. ADAMTS13 specifically cleaves a peptidyl bond between Y1605 and M1606 in the A2 domain of VWF which defines the minimal region that can be recognized as a specific substrate by ADAMTS13 [26].

More than half of TMA patients who have significantly low ADAMTS 13 activity are considered to have TTP and these patients also had significant low ADAMTS13 antigen levels. The activity of ADAMTS13 did not show a close correlation with the presence of the ADAMTS13 antigen. Even in patients with less than 3% of ADAMTS13 activity, they had detectable levels of ADAMTS13 and the activity/antigen ratio of ADAMTS13 was also significantly low in patients with TMA. These findings suggest that the inhibitor not only induces the clearance of ADAMTS13 protein in the circulation but it also inhibits the activity of ADAMTS13. In an assay for the inhibitor of ADAMTS13, 18 of 19 TMA patients with significant low ADAMTS13 activity were positive for the autoantibody. These findings show that the results from autoantibody ELISA were similar to those from the inhibitor assay using FRETS-VWF73.

The plasma levels of ADAMTS13–FXI complex tend to be widely distributed and significantly low in TMA patients with significant low ADAMTS13 activ-

ity. This presence of this complex is well correlated to the presence of the ADAMTS 13 antigen. ADAMTSA13 forms a stable complex with FXI and FXIa and these complexes are found in the plasma [21]. Although the role of ADAMTS13–FXI complex remains unclear, the measurement of ADAMTS13–FXI complex and ADAMTS 13 antigen may be useful for the diagnosis of TTP.

The ADAMTS13 activity was significantly low in both the patients with TTP and in those belonging to the TTP family, thus indicating that ADAMTS13 plays an important role in the onset of TTP. However, 6 patients had an ADAMTS13 activity of more than 60%, thus suggesting that the TTP in these patients may have been caused by abnormalities in other factor such as Factor H [27] and CD46 [28].

In 40 patients with TMA, 8 patients died within 3 months, while 32 patients had a complete remission. There was no significant difference in the VWF antigen, ADAMTS13 activity, ADAMTS13 antigen, ADAMTS13–FXI complex, inhibitor, activity/antigen ratio of ADAMTS13 or the ratio of VWF/ADAMTS13 activity between survivors and non-survivors. Then, the predictive marker for the outcome of TTP may be required.

In summary, the measurement of the ADAMTS13 activity, as well as the presence of ADAMTS13 antigen and the complex associated with the FXI of ADAMTS13 might therefore be useful for the diagnosis of TTP.

Acknowledgments

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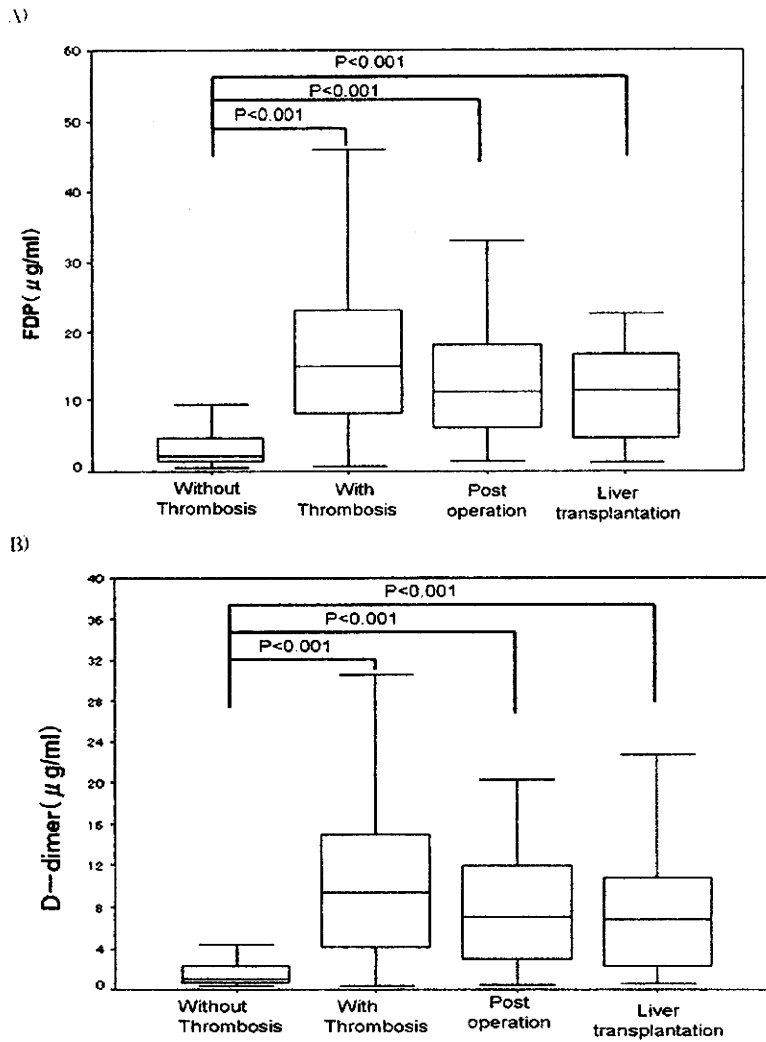
Fig. (1). The plasma levels of FDP and D-dimer in patients with or without thrombosis, either post-operation or after a liver transplantation. A) FDP, B) D-dimer.

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 pa-tients with thrombosis, those who were post-operation and those with LT, thus suggesting that these assays may be use-ful for patients on medication alone or for pre-operative pa-tients. In the present study, we demonstrated that the concen-
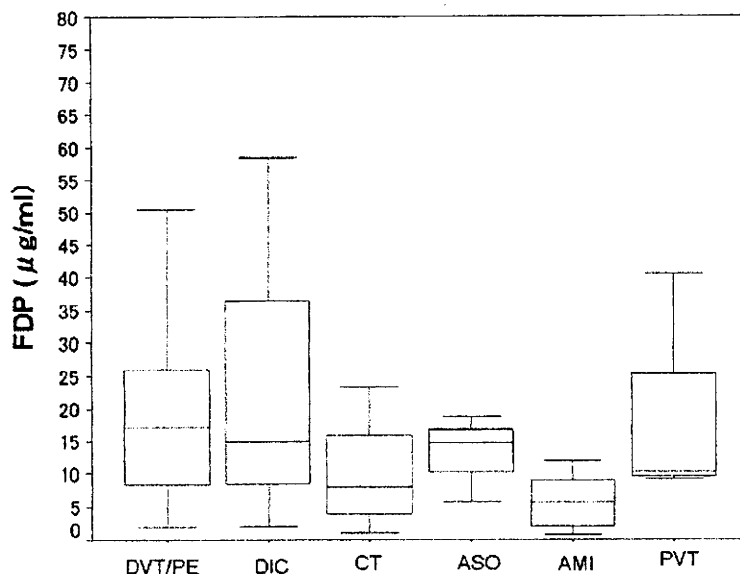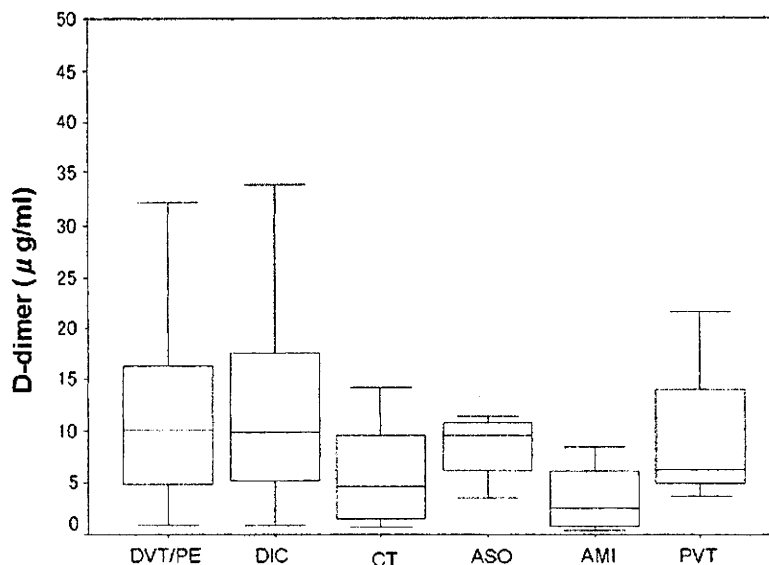
Fig. (2). The plasma levels of FDP and D-dimer in patients with various types of thrombosis.
A) FDP, B) D-dimer.
DVT: deep vein thrombosis, PE: pulmonary embolism, DIC: disseminated intravascular coagulation, CT: cerebral thrombosis, ASO:
AMI: acute myocardial infarction, PVT: portal vein thrombosis, 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-
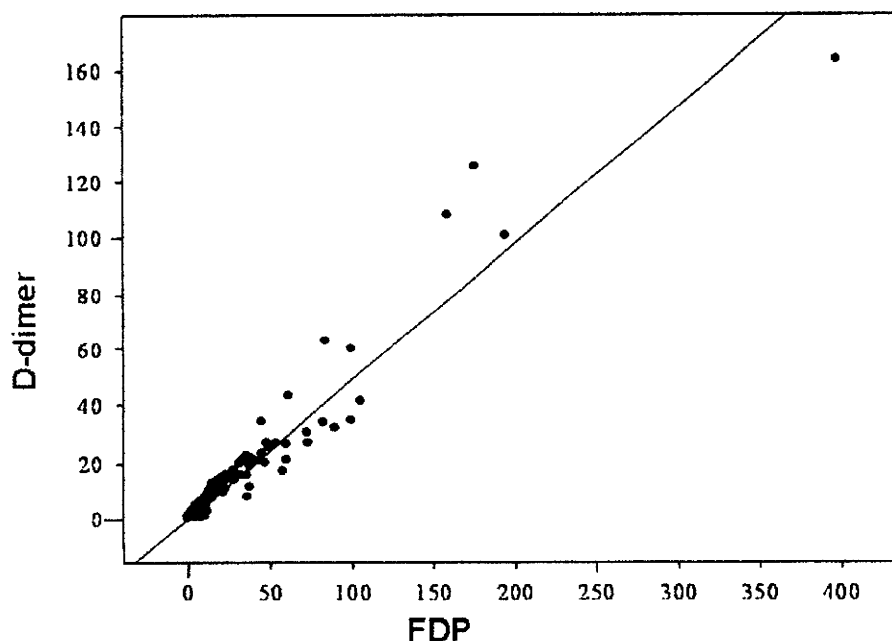
Fig. (3). Relationship between the plasma FDP and plasma D-dimer levels.
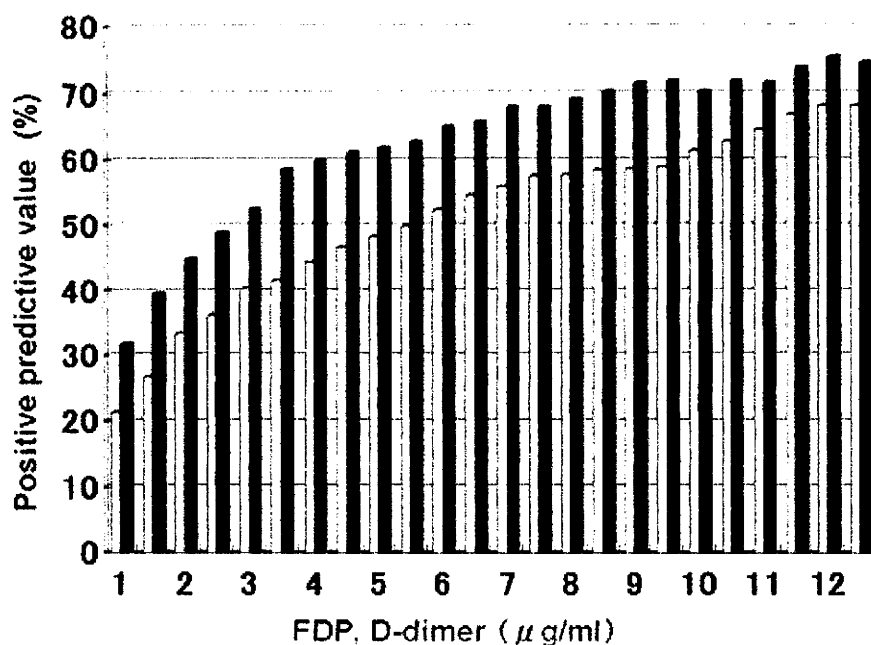

Fig. (4). Positive predictive value for thrombosis according to each FDP or D-dimer level.

brinolysis group. In our study, the number of DIC patients was not sufficient and hyperfibrinolysis 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 anti-thrombotic therapy, such as aspirin for atherosclerotic thrombosis or warfarin for venous thrombosis. It is consid-

ered 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

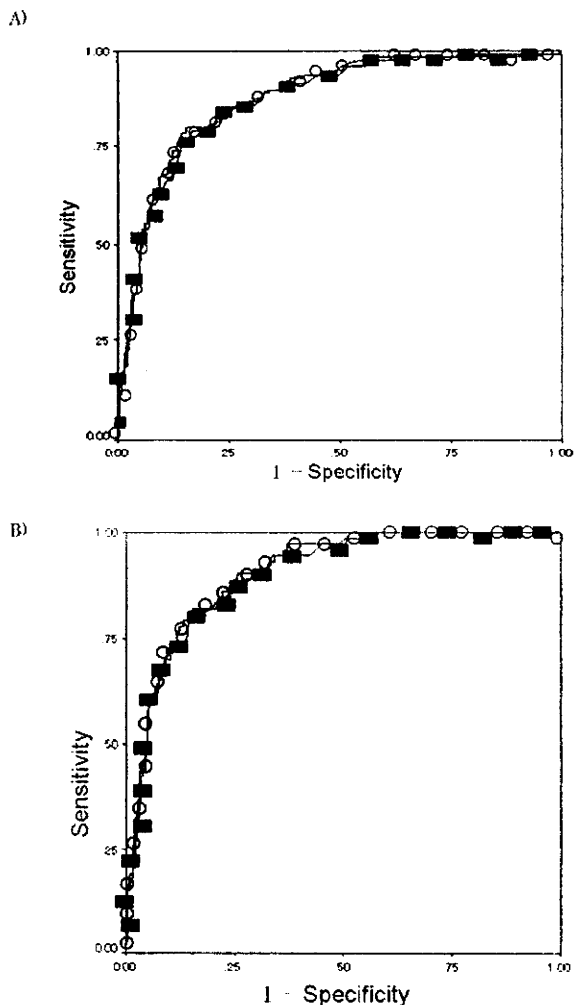| Cutoff value for	FDP		D-Dimer	
	DVT/PE	Thrombosis	DVT/PE	Thrombosis
Cutoff value	7.7 µg/ml	7.6 µg/ml	3.8 µg/ml	3.4 µg/ml
Sensitivity	80.8 %	76.9 %	80.8 %	80.0 %
Specificity	85.3 %	85.2 %	86.1 %	84.7 %
PPV	44.7 %	57.5 %	46.1 %	57.8 %
NPV	96.8 %	93.4 %	96.8 %	94.2 %
Odds ratio	24.42	19.05	26.08	22.16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

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

Negative predictive value of D-dimer for diagnosis of venous thromboembolism

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Abstract The D-dimer levels are considered to be useful for the diagnosis of thrombosis, and they can be clinically used as a negative predictive value (NPV). However, evidence for the efficacy of diagnosing thrombosis based on the D-dimer levels is still not well established. The present study was designed to evaluate the cut-off values of D-dimer levels as a negative predictor for thrombosis. The plasma concentrations of D-dimer were measured in inpatients suspected of having thrombosis, and then the findings were evaluated to assess the correlation with the diagnosis of thrombosis. In healthy volunteers, the median value of VIDAS-D-dimer was 0.12 µg/ml, and the 95% confidence interval was from 0.05 to 0.38 µg/ml. However, the plasma D-dimer levels were significantly higher in patients with thrombosis than in those without thrombosis; there was no significant difference in D-dimer levels among various thromboses such as pulmonary embolism (PE), deep vein thrombosis (DVT), and disseminated intravascular coagulation (DIC). The NPV for venous thromboembolism was 100% in patients with 0.5 µg/ml VIDAS-D-dimer and 1.2 µg/ml LPIA-D-dimer levels. Elevated D-dimer levels might indicate a high risk of thrombosis, especially DVT/PE, and they are thus considered to be useful as a negative predictor for thrombosis.

Keywords Hypercoagulable state · DVT · D-dimer · DIC · PE

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1 Introduction

The D-dimer levels are considered to be useful for the diagnosis of thrombosis, and they have been reported to be elevated in deep vein thrombosis (DVT)/pulmonary embolism (PE; 1–4), disseminated intravascular coagulation (DIC; 5–7), acute myocardial infarction (AMI; 8, 9), and thrombotic thrombocytopenic purpura (TTP; 10). The D-dimer levels are widely used to diagnose thrombosis such as DVT/PE, but many of the commercially available D-dimer assay kits contain different monoclonal antibodies, standard substances, and are based on different assay systems. Since these problems regarding the standardization of D-dimer assays remain to be resolved, several studies [11, 12] were designed to generate basic data for the standardization of D-dimer evaluation procedures. PE is a common, frequently undiagnosed, and potentially fatal cause of several symptoms, including dyspnea and chest pain [13–15]. Since PE is often a fatal disease caused by DVT, the early diagnosis of