INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Elevated Fibrin-related Markers in Patients with Malignant Diseases Frequently Associated with Disseminated Intravascular Coagulation and Venous Thromboembolism

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Abstract

Objective Many patients with malignant diseases are frequently complicated with some type of thrombosis, such as venous thromboembolism (VTE) or disseminated intravascular coagulation (DIC).

Methods This retrospective study was designed to examine the frequency of thrombosis in 478 patients with malignant diseases in comparison to that observed in 121 patients without malignant diseases and to evaluate the efficacy of fibrin-related markers (FRMs), such as soluble fibrin, fibrinogen and fibrin degradation products and D-dimer, in diagnosing thrombosis.

Results The frequency of thrombosis, including 62 cases of VTE, 63 cases of DIC and nine cases of cerebrovascular thrombosis, was significantly higher in the patients with malignant diseases (28.0%) than in the patients without malignant diseases (12.5%). DIC was frequently detected in the patients with hepatic cell cancer and hematopoietic malignancy, while VTE was frequently observed in the patients with colon cancer, breast cancer and urinary tract cancer. The FRMs levels were significantly higher in the patients with thrombosis than in the patients without thrombosis. A receiver operating characteristic analysis showed these markers to be useful for diagnosing thrombosis.

Conclusion Patients with malignant diseases have a high risk of thrombosis, and elevated FRMs levels are useful for diagnosing thrombosis in patients with malignant diseases.

Key words: malignant disease, DIC, VTE, D-dimer, SF

(Intern Med 53: 413-419, 2014)
(DOI: 10.2169/internalmedicine.53.1102)

Introduction

Patients with cancer or hematopoietic malignancy have a high risk of developing thrombotic diseases, such as venous thromboembolism (VTE) (1) or disseminated intravascular coagulation (DIC) (2). These risks vary according to the type of malignancy and stage of disease, and are steadily increased by concomitant patient-related thrombotic risk factors, such as an advanced age, infection, heart disease, respiratory disease, hospitalization and surgical or nonsurgical cancer treatment (3, 4). The association between thrombotic conditions and malignant disease is known as Trousseau's syndrome, which has multiple definitions and mechanisms (5). The hyperexpression or release of tissue factor (TF) is one of the most important factors for the develop-

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Received for publication June 2, 2013; Accepted for publication September 29, 2013
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Table 1. Subjects

	Malignant diseases	Benign diseases	p value
Age	65.0 (55.0-72.3)	49.0 (37.0-63.0)	p<0.001
Sex (F:M)	200 : 278	67 : 54	NS
Thrombosis	134	14	p<0.001
Cerebral vascular event	9	0	NS
Disseminated intravascular coagulation	63	4	p<0.01
Venous thromboembolism	62	10	NS

ment of hypercoagulability due to thrombosis in patients with malignant disease (6, 7).

The presence of thrombosis is sometimes fatal and can disturb the quality of life among patients with cancer and leukemia. Therefore, providing thromboprophylaxis is important for these patients (8). As there are many patients with cancer and leukemia, all patients cannot be treated with anticoagulants. Therefore, it is necessary to assess the presence of hypercoagulability in patients with malignant diseases, and only patients with hypercoagulability and those at high risk for thrombosis should be treated with anticoagulants. The plasma levels of fibrin-related markers (FRMs), such as D-dimer, fibrinogen and fibrin degradation products (FDPs) and soluble fibrin (SF), are useful for diagnosing thrombosis and have been reported to be elevated in patients with deep vein thrombosis (DVT)/pulmonary embolism [PE; (9, 10) and DIC (10, 11). An unlikely pretest probability in combination with a negative D-dimer test has been reported to be able to safely rule out DVT in Europe and North America (12). The cut-off level for D-dimer as a negative predictor of DVT is reported to be less than 0.5 μg/mL (13). The plasma levels of D-dimer and SF may increase in cancer patients without thrombosis. Increasing the cut-off value of D-dimer in cancer patients has thus been reported to possibly increase the test's clinical usefulness (14), while the combination of a low or unlikely Wells pretest probability with a negative D-dimer result can be used to rule out DVT in patients with cancer (15).

The present study was designed to examine the incidence of thrombotic diseases in patients with malignant diseases and to evaluate the cut-off values of FRMs for thrombotic diseases. For this purpose, the FRM levels were assessed in 478 patients suspected of having thrombosis in comparison to those observed in 121 patients without malignant diseases.

Materials and Methods

Subjects

Between August 1, 2003 and July 31, 2010, 478 consecutive patients with advanced malignant diseases (median age: 65.0 years, 25-75% range: 55.0-72.3 years old, and sex: 200 women and 278 men) and 121 consecutive patients without malignant diseases (49.0 years; 37.0-63.0 years, 67 women and 54 men) were suspected of having some type of thrombosis due to symptoms or abnormalities on laboratory tests (Table 1). The malignant diseases consisted of 48 cases of hematopoietic malignancy, 52 cases of hepatic cell carcinoma, 99 cases of lung cancer, 26 cases of stomach cancer, 21 cases of uterine cancer, 98 cases of colon cancer, 24 cases of ovarian cancer, 13 cases of breast cancer, 16 cases of urinary tract cancer,18 cases of cancer of unknown origin, 18 cases of sarcoma, 14 cases of pancreatic cancer and 31 others. The cases without malignant diseases consisted of 42 cases of soft tissue tumors (STTs), 18 cases of bone tumors, 13 cases of thymoma, eight cases of fibroid tumors, seven cases of lipoma, seven cases of essential thrombocythemia (ET), five cases of neck tumors, five cases of schwannoma, three cases of angioma, two cases of ovarian tumors, two cases of meningioma and nine others. ET sometimes increases the levels of marked blast cells, resulting in a poor outcome; however, the seven ET patients had few blast cells in the peripheral blood, suggesting that they were in the chronic phase of the disease. None of the patients with ET had VTE and were treated with warfarin.

The plasma concentrations of D-dimer and soluble fibrin (SF) and the serum levels of fibrinogen and fibrin degradation products (FDP) were retrospectively examined in all patients and analysed in order to identify any correlations with the diagnosis of thrombosis. The study protocol was approved by the Human Ethics Review Committee of Mie University Hospital, and informed consent was obtained from all subjects. A total of 148 patients were diagnosed to have thrombosis, while 451 were not. No patients were examined after undergoing liver transplantation or within three days after an operation. DVT was diagnosed using echo or venography. PE was diagnosed using either ventilation-perfusion lung scanning, computed tomography (CT) or pulmonary angiography. DIC was diagnosed according to the International Society on Thrombosis and Haemostasis (ISTH) overt-DIC diagnostic criteria (16). Cerebral thrombosis was diagnosed on CT or magnetic resonance imaging (MRI).

The concentrations of SF, FDP and D-dimer were measured in the patients with thrombosis at the onset of disease and in those without thrombosis at the first consultation. The data were usually obtained before the administration of chemotherapy or anticoagulation therapy. In each case, the consultation was conducted within two days after the appearance of either symptoms or laboratory abnormalities.

Table 2. **Frequency of Thrombosis**

	n	DIC	DVT	Thrombosis
Hematopoietic tumor	48	10 (20.8%)	4 (8.3%)	16 (33.3%)
Hepatic cell carcinoma	52	22 (42.3%)	4 (7.7%)	29 (55.8%)
Lung cancer	99	5 (5.1%)	9 (9.1%)	15 (9.1%)
Stomach cancer	26	5 (19.2%)	2 (7.7%)	8 (30.8%)
Uterus cancer	21	0 (0%)	9 (42.9%)	9 (42.9%)
Colon cancer	98	2 (2.0%)	10 (19.4%)	13 (13.3%)
Ovarian cancer	24	2 (8.3%)	9 (37.5%)	11 (45.8%)
Breast cancer	13	3 (23.1%)	2 (15.4%)	5 (38.5%)
Urinary tract cancer	16	4 (25.0%)	3 (18.8%)	8 (50.0%)
Unknown origin cancer	18	1 (5.6%)	1 (5.6%)	2 (11.1%)
Sarcoma	18	3 (16.7%)	0 (0 %)	3 (16.7%)
Pancreas cancer	14	2 (14.3%)	3 (21.4%)	5 (35.7%)
Others	31	4 (12.9%)	6 (19.49%)	10 (32.3%)
Total	478	63(13.2%)	62 (13.0%)	134 (28.0%)

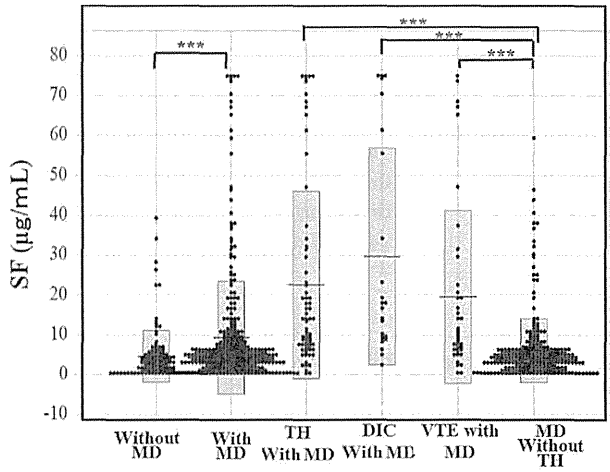

Figure 1. Plasma levels of SF in the patients with and without malignant diseases (MD), MD patients with and without thrombosis, MD patients with disseminated intravascular coagulation (DIC) and MD patients with venous thromboembolism (VTE). SF: soluble fibrin, MD: malignant diseases, TH: thrombosis, DIC: disseminated intravascular coagulation, VTE: venous thromboembolism, ***: p< 0.001

Measurement of the plasma concentrations of D-dimer and SF and the serum levels of FDP

The plasma and serum samples were obtained and analyzed within four hours. The plasma D-dimer levels were measured with LPIA-ACE D-dimer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) using JIF-23 monoclonal antibodies. JIF-23 monoclonal antibodies, which recognize the plasmin-digested N-terminus of the γ chain on the D region, were used for latex aggregation (17). The SF was also determined according to the latex agglutination method using IATRO SF (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) containing the monoclonal antibody IF-43, which 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. The antibody was coated for the SF assay (10). The serum FDP concentrations were measured according to the latex agglutination method using IATRO FDP (Mitsubishi Chemical Medience Corporation, Tokyo, Japan).

Statistical analysis

The data are expressed as the median (25% tile-75% tile). Differences between groups were examined for significance using the Mann-Whitney U test. A p-value of less than 0.05 was considered to indicate a significant difference. The usefulness of the D-dimer and SF levels in diagnosing thrombosis and DVT or PE was examined using a receiver operating characteristic (ROC) analysis (18). The cut-off values were determined according to a ROC analysis. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo, Japan).

Results

The frequency of thrombosis, including 62 cases of VTE, 63 cases of DIC and nine cases of cerebral thrombosis, was significantly higher in the patients with malignant diseases (134; 28.0%) than in the patients without malignant disease (14; 12.5%, p<0.001). No patients with VTE were complicated with DIC, and no patients with DIC were complicated with symptomatic VTE. In particular, the rate of complication with DIC was significantly higher in the patients with malignant diseases (63; 13.2%) than in the patients without malignant diseases (4; 3.6%, p<0.01) (Table 1). DIC was frequently detected in the patients with hepatic cell carcinoma, hematopoietic malignancy, stomach cancer, breast cancer and urinary tract cancer, while VTE was frequently observed in the patients with colon cancer, breast cancer, urinary tract cancer, uterine cancer, pancreatic cancer and ovarian cancer (Table 2).

The levels of SF (4.9 μg/mL; 2.6-8.2 μg/mL vs. 2.9 μg/mL; 0.8-5.1 μg/mL, p<0.001), FDP (11.6 μg/mL; 6.0-23.5 μg/mL vs. 4.1 μg/mL; 1.0-10.2 μg/mL, p<0.05), and D-dimer (4.7 μg/mL; 0.6-10.6 μg/mL vs. 0.6 μg/mL; 0.3-1.8 μg/mL, p<0.001) were significantly higher in the patients with malignant diseases than in those with benign diseases (Fig. 1-3). Among the patients with malignant diseases, the levels of SF (12.5 μg/mL; 7.1-29.9 μg/mL vs. 4.1 μg/mL; 1.9-6.2 μg/mL, p<0.001), FDP (22.0 μg/mL; 10.5-52.8 μg/mL vs. 6.0 μg/mL; 1.7-11.2 μg/mL, p<0.001) and D-dimer (14.1 μg/mL; 8.1-23.2 μg/mL vs. 1.1 μg/mL; 0.5-6.6 μg/mL, p<0.001) were significantly higher in the patients with thrombosis than in those without thrombosis (Fig. 1-3). These markers were also significantly higher in the patients with DIC or VTE than in those without thrombosis, although only the D-dimer levels were significantly higher in the patients with DIC than in those with VTE (17.0 μg/mL; 9.0-30.2 μg/mL vs. 12.0 μg/mL; 7.1-17.4 μg/mL, p<0.05). The levels of SF were significantly higher in the patients with hepatic cell cancer, the levels of FDP were significantly higher in the patients with hepatic cell cancer, hematopoietic malignancy, ovarian cancer, breast cancer, urinary

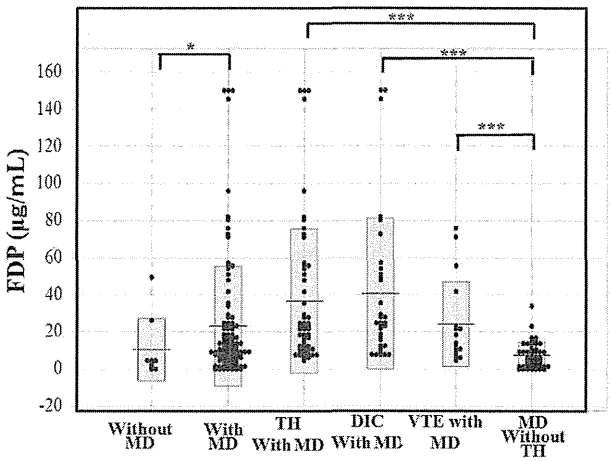

Figure 2. **Plasma levels of FDP in the patients with and without malignant diseases (MD), MD patients with and without thrombosis, MD patients with disseminated intravascular coagulation (DIC) and MD patients with venous thromboembolism (VTE). FDP: fibrinogen and fibrin degradation products, MD: malignant diseases, TH: thrombosis, DIC: disseminated intravascular coagulation, VTE: venous thromboembolism, ***: p< 0.001, *: p< 0.05**

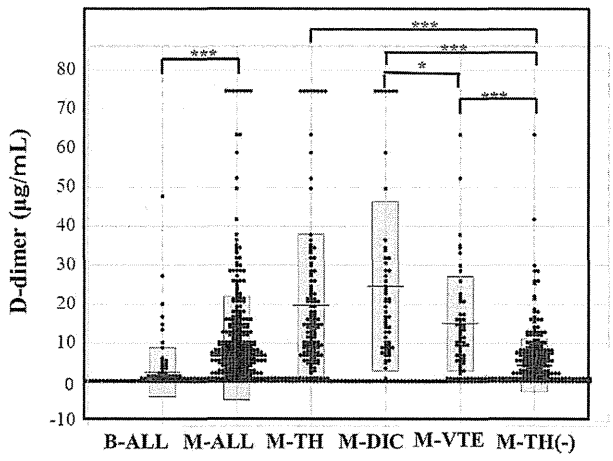

Figure 3. **Plasma levels of D-dimer in the patients with and without malignant diseases (MD), MD patients with and without thrombosis, MD patients with disseminated intravascular coagulation (DIC) and MD patients with venous thromboembolism (VTE). MD: malignant diseases, TH: thrombosis, DIC: disseminated intravascular coagulation, VTE: venous thromboembolism, ***: p<0.001, *: p<0.05**

Table 3. **Plasma Levels of SF, FDP and D-dimer in the Patients with Various Malignant Diseases**

	SF (μg/mL)	FDP (μg/mL)	D-dimer (μg/mL)
Hematopoietic tumor	8.6 (0.9-20.5)	12.4 (6.4-22.9)**	7.3 (3.2-11.0)***
Hepatic cell carcinoma	10.4 (6.0-23.6)***	17.2 (9.5-24.8)***	14.9 (7.3-22.6)***
Lung cancer	4.8 (3.2-6.3)	9.6 (0.1-13.2)	0.9 (0.4-5.9)
Stomach cancer	3.7 (0.7-12.8)	8.2 (0.4-15.1)	1.1 (0.5-8.1)
Uterus cancer	5.6 (3.0-6.2)	7.3 (1.8-15.0)	6.5 (4.4-13.1)***
Colon cancer	4.0 (1.5-8.0)	9.8 (3.1-22.2)	0.8 (0.4-3.5)
Ovarian cancer	5.1 (2.6-10.1)	13.7 (7.7-26.2)*	7.5 (3.0-11.4)***
Breast cancer	5.6 (4.2-10.9)	17.7 (6.2-140.8)*	7.8 (4.3-19.3)*
Urinary tract cancer	5.4 (3.8-7.6)	27.8 (14.3-58.4)***	7.2 (3.4-27.3)***
Unknown origin cancer	6.1 (3.2-8.9)	11.0 (5.3-20.4)	4.5 (0.5-15.5)
Sarcoma	2.9 (1.7-5.6)	32.0 (14.3-58.2)*	3.8 (0.5-15.5)
Pancreas cancer	5.8 (3.6-9.3)	22.7 (7.5-36.7)*	10.4 (4.8-13.8)***
Others	2.7 (1.2-6.2)	8.6 (2.4-14.9)	8.2 (1.0-12.7)**
Malignant diseases without thrombosis	4.1 (1.9-6.2)	6.0 (1.7-11.2)	1.1 (0.5-6.6)

*; p<0.05, **; p<0.01, ***; p<0.001, in comparison to the FDP, SF and D-dimer levels in the non-thrombotic patients with malignant diseases

tract cancer, sarcoma or pancreatic cancer and the levels of D-dimer were significantly higher in the patients with hepatic cell cancer, hematopoietic malignancy, uterine cancer, ovarian cancer, breast cancer, urinary tract cancer, sarcoma, pancreatic cancer or others in comparison to those observed in the non-thrombotic patients with malignant diseases (Table 3). In the ROC analysis, the areas under the curve (AUCs) for SF, FDP and D-dimer with respect to thrombosis, DIC and VTE were high (Table 4). The adequate cut-off values of SF, FDP and D-dimer for thrombosis were 6.3 μg/mL, 10.6 μg/mL and 7.4 μg/mL, respectively, which were slightly different from those observed for VTE and DIC. The odds ratios for SF and D-dimer were highest in the patients with DIC, while the odds ratio for VTE was higher in the patients with an elevated D-dimer level than in those with an elevated SF value. The 30- and 90-day mortality rates were significantly higher in the patients with thrombosis than in those without thrombosis (Table 5). The mortality rate was markedly high in the patients with DIC and tended to be high in the patients with VTE or cerebral thrombosis. The plasma levels of SF and FDP were significantly higher in the 90-day non-survivors than in the 90-day survivors; however, the levels of D-dimer were not significantly different (Table 6).

Discussion

This study demonstrated a high frequency of thrombosis in patients with malignant diseases in comparison to those without malignant diseases, suggesting that a hypercoagulable state exists in patients with malignant diseases. An elevated TF expression has been reported in tumor cells and

Table 4. ROC Analysis of the SF, FDP and D-dimer Levels for Thrombosis, DIC and VTE

		SF	FDP	D-dimer
AUC	thrombosis	0.819	0.855	0.884
	DIC	0.888	0.884	0.909
	VTE	78.5	0.792	0.861
Cutoff value	thrombosis	6.3 μg/mL	10.6 μg/mL	7.4 μg/mL
	DIC	8.1 μg/mL	12.1 μg/mL	8.0 μg/mL
	VTE	6.1 μg/mL	9.9 μg/mL	6.8 μg/mL
Sensitivity	thrombosis	77.1%	73.5%	79.5%
	DIC	83.6%	75.8%	81.5%
	VTE	72.1%	70.6%	75.9%
Specificity	thrombosis	77.1%	73.5%	79.5%
	DIC	83.6%	75.8%	81.5%
	VTE	72.1%	70.6%	75.9%
Odds ratio	thrombosis	10.9	7.6	14.9
	DIC	25.5	9.6	19.6
	VTE	6.3	6.0	9.9

Table 5. Outcome of Patients with Thrombosis

	30 days mortality	90 days mortality
Patients with thrombosis	26/134 (19.4%)***	37/134 (27.6%)###
DIC	20/63 (31.7%)	29/63 (46.0%)
VTE	5/62 (8.1%)	7/62 (11.3%)
Cerebral thrombosis	1/9 (11.1%)	1/9 (11.1%)
Patients without thrombosis	12/344 (3.5%)***	27/344 (8.5%)###

***; p<0.001 in difference between patients with and without thrombosis
###; p<0.001 in difference between patients with and without thrombosis

Table 6. Plasma Levels of SF, FDP and D-dimer in 90 Days Survivor and Non-survivor

	SF (μg/mL)	FDP (μg/mL)	D-dimer (μg/mL)
90 days survivor	2.92 (0.53-8.87)***	4.60 (2.45-7.41)***	10.15 (4.50-10.15)
90 days non-survivor	13.00 (7.13-24.34)***	14.10 (5.12-39.56)***	16.00 (9.80-28.60)

***; p<0.001

leukocytes obtained from patients with malignant diseases (2, 7). Elevated TF activates coagulation factor VII thereby causing thrombosis. The difference in the frequency of DIC was significant between the patients with malignant diseases and those without malignant diseases; however, that of VTE was not. The patients with malignant diseases were significantly older than those without malignant diseases, indicating that the age of patients with malignant diseases may affect the onset of thrombosis (19). However, the pathogenic mechanisms of DIC depend on the underlying disease, such as leukemia, solid cancer and sepsis (2, 7). These findings suggest that DIC is primarily caused by a high expression of TF and plasminogen activator (20, 21), while VTE is usually caused by hemostatic and several physical factors. Enhancement of both the coagulation and fibrinolysis systems may therefore play an important role in the onset of DIC in patients with malignant diseases (22). Although no patients had both VTE and DIC, VTE and DIC have similar pathogenic mechanisms, such as an increased expression of TF.

In this study, the frequency of DIC was similar to that of VTE. VTE and DIC are the most important thrombotic complications in patients with malignant diseases, although their frequency depends on the stage and type of malignancy. DIC was frequently detected in the patients with hepatic cell carcinoma, hematopoietic malignancy, stomach cancer, breast cancer and urinary tract cancer, indicating similar findings to those of a questionnaire survey conducted by the Japanese Ministry Health and Welfare (7). These types of malignancies may be associated with either a high TF level or organ failure, such as that due to liver dysfunction or infection (22). VTE was frequently observed in the patients with colon cancer, breast cancer, urinary tract cancer, uterine cancer, pancreatic cancer and ovarian cancer. Colon cancer, uterine cancer, pancreatic cancer and ovarian cancer are not frequently associated with DIC, suggesting that the onset of most cases of VTE in patients with these types of malignant diseases depends on the condition of the host, including factors associated with surgery, anticancer agents, infections, etc. However, it is difficult to draw any valid conclusions due to the small number of subjects in the different subgroups, such as those with pancreatic cancer, breast cancer and ovarian cancer.

The levels of FRMs were significantly higher in the patients with malignant diseases than in those without malignant diseases, thus suggesting that both hypercoagulable and hyperfibrinolytic states exist in patients with malignant diseases (6, 7). Elevated D-dimer levels have been reported in elderly individuals (23), and many patients with malignant diseases are elderly, suggesting that the plasma D-dimer levels are slightly high in patients with malignant diseases. In addition, the plasma levels of D-dimer have been reported to be increased in patients with ascites (24). Furthermore, in the present study, among the patients with malignant diseases, the levels of FRMs were significantly higher in the patients with thrombosis than in those without thrombosis, although only the D-dimer levels were significantly higher in the patients with DIC than in those with VTE. These findings indicate that secondary fibrinolysis is more significant in patients with DIC than in those with VTE and that the D-dimer level is a sensitive marker of secondary fibrinolysis. There have been many reports regarding the SF and D-dimer levels in patients with DIC and VTE (9, 10). In this study, the levels of SF were significantly high only in

the patients with Hepatocellular carcinoma (HCC), suggesting that an elevated SF level is caused by thrombosis rather than malignancy. Meanwhile, the levels of FDP and D-dimer were significantly high in the patients with various types of malignant diseases, suggesting that elevated FDP and D-dimer levels are caused by both thrombotic states and malignant diseases. Elevated levels of FRMs, such as D-dimer and SF, are also observed in patients under a hypercoagulable state (25), such as those with DVT (26, 27), DIC (28) or hyperlipidemia (29). Therefore, it is important to determine the adequate cut-off values of FRMs for diagnosing thrombosis in patients with malignant diseases.

In the ROC analysis, the AUCs of the FRMs for the diagnosis of thrombosis were significantly high, suggesting that FRMs are useful for diagnosing thrombosis in patients with malignant or other diseases. The adequate cut-off values of SF, FDP and D-dimer for thrombosis were 6.3 μg/mL, 10.6 μg/mL and 7.4 μg/mL, respectively, although they were slightly different among the patients with thrombosis, DIC and VTE, indicating that FRMs are not useful for making the differential diagnosis between VTE and DIC. The cut-off value for SF is similar to that reported in previous studies (10); however, that for D-dimer was higher in the patients with malignant diseases than in those with other underlying diseases (approximately 4.0 μg/mL) (10). The odds ratios of SF and D-dimer were highest in the patients with DIC, while the odds ratio for VTE was higher in the patients with an elevated D-dimer level than in those with an increased SF value; however, these odds ratios were not sufficiently high.

Regarding the limitations of this study, the number of patients was small, and there were various underlying and thrombotic diseases. Complications of thrombosis, particularly DIC, increase mortality. These findings are similar for sepsis (2, 7) and highlight the importance of diagnosing thrombosis in patients with malignant diseases. In this study, the SF and FDP levels were useful for predicting poor outcomes, whereas the D-dimer level was not, suggesting that evaluating the FDP level is useful for diagnosing DIC (2, 7), while evaluating the D-dimer level is useful for diagnosing VTE (10).

In addition, this study was retrospective. The present study confirmed that measuring the FRMs levels is useful for making the diagnosis of thrombosis in patients with malignant diseases; however, the cut-off value for D-dimer used to diagnose thrombosis is high in comparison to that observed in patients with other underlying diseases.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

This study was supported in part by research grants from the Japanese Ministry of Health, Labour and Welfare and the Japanese Ministry of Education, Science, Sports and Culture.

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Int J Hematol (2014) 100:450–456
DOI 10.1007/s12185-014-1676-x

ORIGINAL ARTICLE

Elevated soluble platelet glycoprotein VI is a useful marker for DVT in postoperative patients treated with edoxaban

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Received: 4 July 2014 / Revised: 9 September 2014 / Accepted: 9 September 2014 / Published online: 25 September 2014

Abstract Prevention of deep vein thrombosis (DVT) is important in patients undergoing major orthopedic surgery. Although the detection of an elevated D-dimer level is useful for predicting DVT, it is not efficacious in postoperative patients being treated with anti-Xa agents. The soluble platelet glycoprotein VI (sGPVI) level is a marker of activated platelets, but not bleeding. Therefore, sGPVI levels are usually examined as a predictor of DVT in such patients. In the present study, 83 orthopedic patients were treated with 30 mg of edoxaban for prophylaxis of DVT. Fourteen patients developed DVT and 17 patients discontinued the prophylaxis due to decreased hemoglobin levels. Plasma levels of sGPVI in the patients were significantly higher after surgery than before surgery. On day 1, the sGPVI levels increased, while the platelet counts decreased. There were no significant differences in D-dimer, soluble fibrin, or FDP levels in orthopedic patients with and without DVT before surgery and on days 1, 4, and 8. Plasma sGPVI levels were significantly higher in the patients with DVT than in those without DVT on days 1 and 4. Plasma levels of D-dimer were significantly higher in patients with withdrawal than in those without. However, there were no significant differences in sGPVI levels between those with and without withdrawal. As D-dimer levels are known to increase in patients with withdrawal, this parameter is not useful for evaluating the risk of DVT in these patients. In contrast, the sGPVI level is not increased in those with withdrawal and may therefore be useful for evaluating the risk of DVT in postoperative patients treated with an anticoagulant.

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Keywords Deep vein thrombosis · Orthopedic surgery · Major bleeding · sGPVI

Introduction

Platelet glycoprotein VI (GPVI), a type I transmembrane glycoprotein of the immunoreceptor family, is constitutively associated and expressed with the Fc receptor γ-chain (FcRγ), an immunoreceptor tyrosine-based activation motif-bearing (ITAM-bearing) receptor [1, 2]. Upon platelet activation, the platelet surface GPVI is cleaved off by proteases, such as ADAM10, releasing the soluble form GPVI (sGPVI) [3–5]. sGPVI has recently received much attention as a platelet activation marker, as described below. Several groups have reported that sGPVI is a useful biomarker of diseases caused by platelet activation, such as acute coronary syndrome (ACS) and stroke [6–10]. CLEC-2 is a similar transmembrane glycoprotein to GPVI that has been reported to be a potential thrombotic marker [11, 12].

Orthopedic surgery is associated with a high rate of postoperative venous thromboembolism (VTE) [13, 14]. The incidence of VTE ranges from 42 to 57 % after total hip arthroplasty (THA) and 41 to 85 % after total knee arthroplasty (TKA) [15] in the absence of thromboprophylaxis. Multiple studies [16–18] have established the efficacy of LMWH for VTE prophylaxis in orthopedic surgery patients. New oral anticoagulants, such as edoxaban, have recently become available for prophylaxis after surgery [19]. Although measurements of the D-dimer and soluble fibrin (SF) levels can be used to predict the incidence of deep vein thrombosis (DVT) after THA or TKA [20], this ability is canceled following the administration of fondaparinux [21, 22].

In this study, we measured the activation of platelets by measuring the sGPVI level in 83 patients undergoing major orthopedic surgery and examined the relationship between platelet activation and DVT.

Materials and methods

Eighty-three patients undergoing major orthopedic surgery treated with 30 mg of edoxaban (Daiichisankyo, Tokyo, Japan) and intermittent pneumatic compression for prophylaxis of DVT between January 1, 2013, and October 31, 2013, at Mie University Hospital were enrolled in this study. Blood samples were obtained 1 h after the administration of edoxaban. Patients treated with anticoagulant therapy and/or those with renal failure or a high risk of bleeding were excluded. These patients were registered with the Department of Molecular and Laboratory Medicine 3 days prior to surgery. These patients received 30 mg of edoxaban via oral administration once a day for 14 days beginning 24 h after lumbar anesthesia extubation.

Table 1 Subjects

	With DVT	Without DVT
Age (years old)	75.0 (68.0–78.0)*	66.0 (56.8–72.0)*
Sex (F:M)	11:3	55:14
THA:TKA	9:5	56:13
Weight (kg)	50.8 (45.5–73.1)	59.0 (50.1–68.1)
Height (cm)	152 (143–156)	153 (146–159)
Body mass index	24.6 (19.5–30.3)	24.6 (22.1–27.4)
Body surface area	1.45 (1.36–1.70)	1.55 (1.43–1.72)
Creatinine (mg/dl)	0.65 (0.54–0.91)	0.65 (0.55–0.74)
eGFR	74.5 (56.0–86.2)	73.5 (63.0–87.6)

* $p < 0.05$

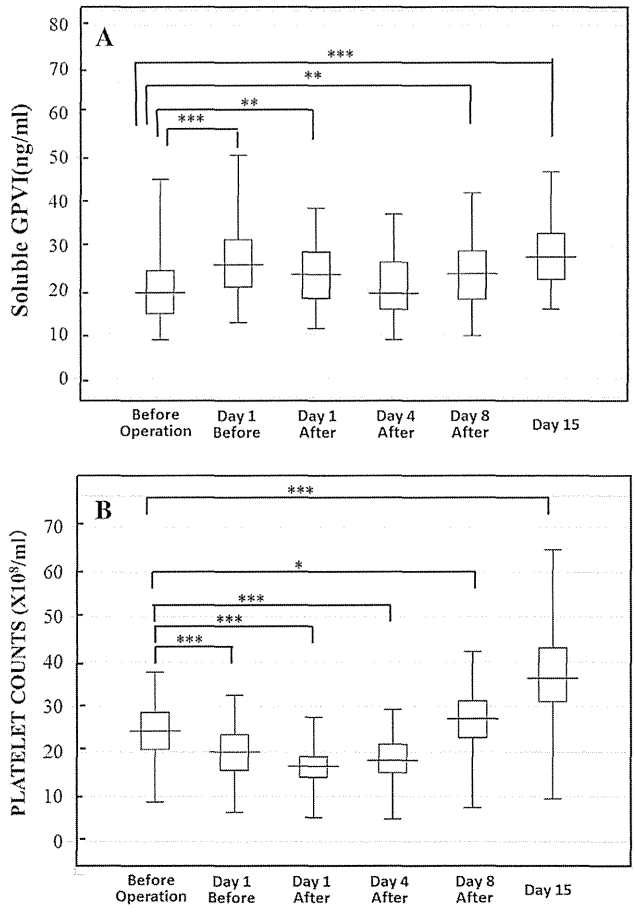

Fig. 1 Plasma levels of sGPVI and platelet counts during orthopedic surgery. **a** sGPVI, **b** platelet count. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. *Day 1 before*: day 1 before edoxaban administration, *day 1 after*: day 1 after edoxaban administration, *day 4 after*: day 4 after edoxaban administration, *day 8 after*: day 8 after edoxaban administration. The *upper* and *lower boxes* show the 75th and 25th percentiles. The *upper*, *middle*, and *lower lines* indicate the 97.5 %, median, and 2.5 % values

Table 2 Correlation between the sGPVI levels and other parameters

	r (significance)					
	Before operation	Day 1 before edoxaban	Day 1 after edoxaban	Day 4 after edoxaban	Day 8 after edoxaban	Day 15
Platelets counts	0.2160 (NS)	0.0101 (NS)	0.1974 (NS)	0.2726 (NS)	−0.0256 (NS)	0.4446 (NS)
FDP	−0.0397 (NS)	−0.1429 (NS)	−0.1053 (NS)	−0.0201 (NS)	0.1750 (NS)	−0.1103 (NS)
D-Dimer	−0.2850 (NS)	0.0018 (NS)	−0.0341 (NS)	−0.2222 (NS)	−0.0175 (NS)	−0.1086 (NS)
SF	0.2858 (NS)	−0.0878 (NS)	0.0524 (NS)	−0.0921 (NS)	−0.0849 (NS)	−0.3130 (NS)
AT	0.1861 (NS)	−0.0051 (NS)	0.0285 (NS)	−0.3342 (NS)	0.0975 (NS)	0.1944 (NS)

NS not significant

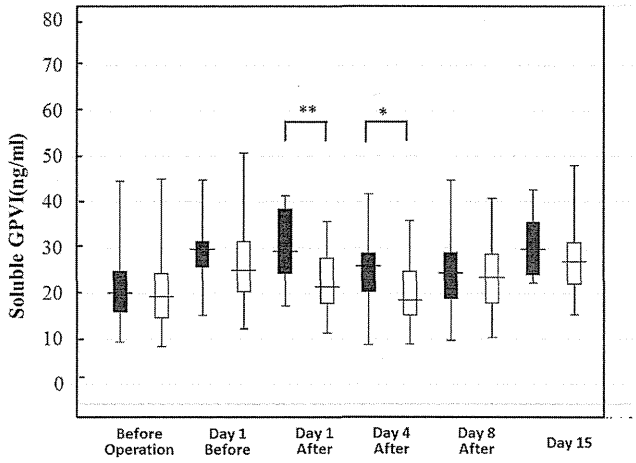

Fig. 2 Plasma levels of sGPVI in the patients with and without DVT during orthopedic surgery. $*p < 0.05$, $**p < 0.01$. *Closed bar* with DVT, *open bar* without DVT. *Day 1 before*: day 1 before edoxaban administration, *day 1 after*: day 1 after edoxaban administration, *day 4 after*: day 4 after edoxaban administration, *day 8 after*: day 8 after edoxaban administration. The *upper* and *lower boxes* show the 75th and 25th percentiles. The *upper*, *middle*, and *lower lines* indicate the 97.5 %, median, and 2.5 % values

Fourteen patients developed DVT (11 females and three males, median age 25–75 % tile; 75.0 years old: 68.0–78.0 years old), whereas 69 (55 females and 14 males, 66.0 years old; 56.8–72.0 years old) did not. Of these 83 patients, 17 discontinued the prophylaxis (withdrawal group), due to a reduction in the hemoglobin level of >2 g/dl compared with that observed on day 1 or to a hemoglobin level of <7 g/dl. The median and 25–75th percentiles of the number of days of edoxaban administration in withdrawal group were four (3–7 days).

The sGPVI, fibrin and fibrinogen degradation products (FDP), D-dimer, SF, and antithrombin (AT) activity levels were measured prospectively in the 83 patients who underwent THA or TKA and on days 1, 4, 8, and 15 of the administration of edoxaban. The diagnosis of DVT was assessed according to a whole-leg compression ultrasound examination using standardized ultrasound criteria for venous non-compressibility before surgery, as well as on days 4 and 14 [23]. No patients had DVT prior to surgery based on the ultrasound examinations. The study protocol was approved by the Human Ethics Review Committee of the Mie University School of Medicine, and a signed informed consent was obtained from each subject. This study was faithfully carried out in accordance with the principles of the Declaration of Helsinki.

The level of sGPVI in the plasma was quantified using sandwich ELISA, which consisted of two mouse anti-GPVI monoclonal antibodies, F1232-7-1 and F1232-10-2 able to recognize the extracellular domain I (D1) N-terminal loop and extracellular domain D2 loop of GPVI, respectively [24–26].

The plasma levels of FDP, D-dimer, and SF were measured according to the latex agglutination method using Nanopia FDP, Nanopia D-dimer, and Nanopia SF reagents (Sekisui Medical) [27]. The plasma levels of AT activity were measured using a Testzym S ATIII kit (Sekisui Medical).

Statistical analysis

The data are expressed as the median (25th–75th percentiles). Differences between groups were examined for significance using the Mann–Whitney *U* test. A *p* value of less than 0.05 was considered to indicate a significant difference.

All statistical analyses were performed using the Stat flex, version 6, software package (Artec Co., Ltd, Osaka, Japan).

Results

Fourteen patients developed DVT on day 4, although no patients experienced PE. These 14 patients presented with distal DVT with no symptoms and were continued on

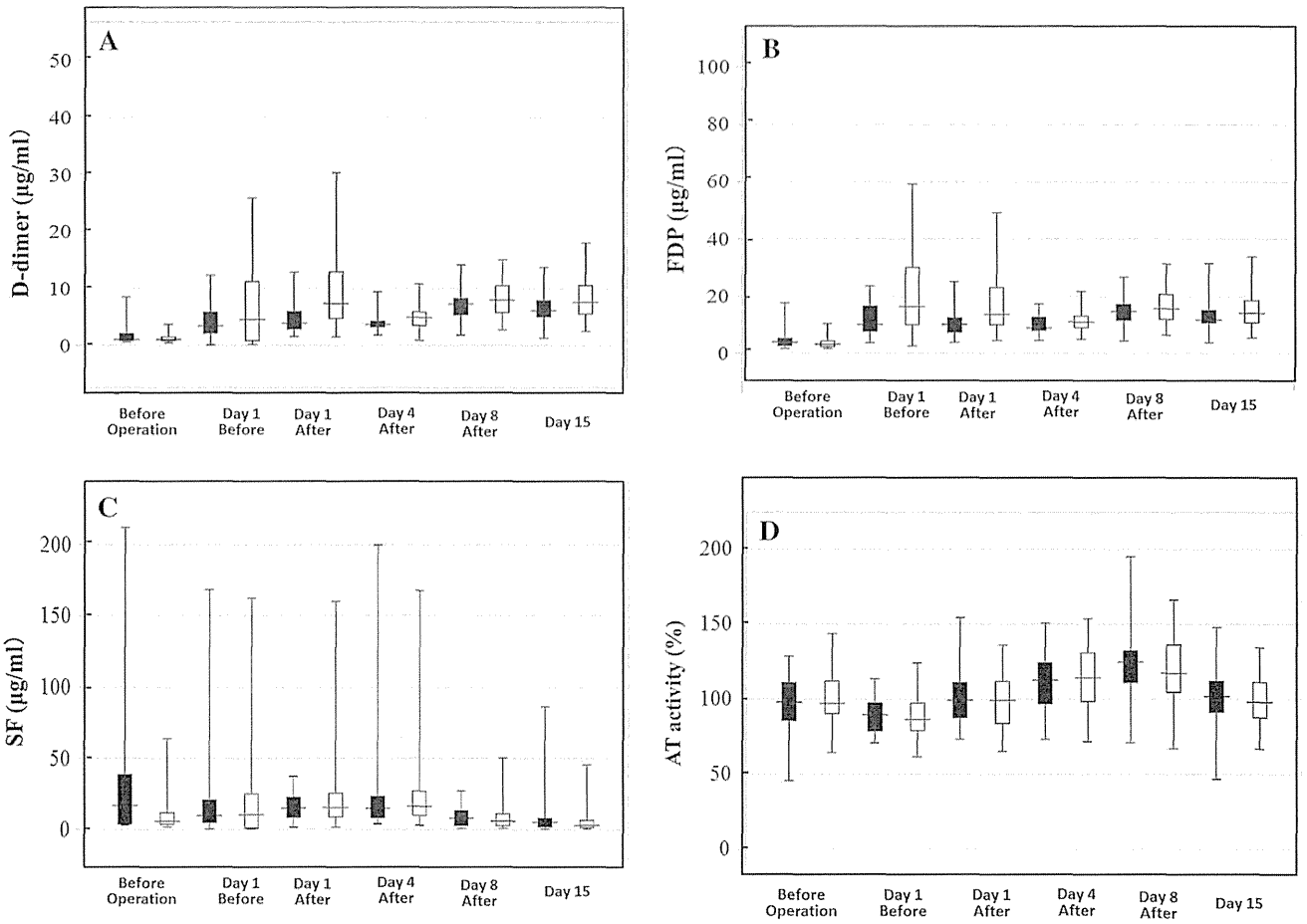

Fig. 3 Plasma levels of D-dimer (**a**), FDP (**b**), SF (**c**), and AT activity (**d**) in the patients with and without DVT during orthopedic surgery. *Closed bar* with DVT, *open bar* without DVT. *Day 1 before*: day 1 before edoxaban administration, *day 1 after*: day 1 after edoxaban administration, *day 4 after*: day 4 after edoxaban administration, *day 8 after*: day 8 after edoxaban administration. The *upper* and *lower boxes* show the 75th and 25th percentiles. The *upper*, *middle*, and *lower lines* indicate the 97.5 %, median, and 2.5 % values

prophylaxis with edoxaban. Eleven of these patients were again found to have DVT on day 14 and followed by 3 months of warfarin therapy. The patient age was significantly higher among those with DVT than those without DVT ($p < 0.05$, Table 1). There were no significant differences in the TKA/THA, sex, body weight, height, body mass index (BMI), body surface area (BSA), creatinine or estimated glomerular filtration rate (eGFR) values between the patients with and without DVT. The plasma levels of FDP (3.5 μg/ml; 2.60–5.50 vs 15.10 μg/ml; 10.20–25.30 μg/ml, $p < 0.001$), D-dimer (1.09 μg/ml; 0.81–1.84 vs 3.99 μg/ml; 1.36–10.0 μg/ml, $p < 0.001$), and SF (6.60 μg/ml; 3.30–9.15 vs 13.5 μg/ml; 8.3–25.1 μg/ml, $p < 0.001$) were found to have significantly increased on day 1. The plasma levels of sGPVI in the patients who underwent major orthopedic surgery were significantly higher on day 1 (25.8 ng/ml; 20.7–31.4 ng/ml, $p < 0.001$), day 8 (23.7 ng/ml; 17.9–28.8 ng/ml, $p < 0.01$), and day 15 (27.5 ng/ml; 22.5–32.8 ng/ml, $p < 0.001$) than those observed before surgery (19.4 ng/ml; 14.7–24.4 ng/ml) (Fig. 1a). The platelet counts in these patients were significantly lower on day 1 (16.8×10^8/ml; $14.3–18.9 \times 10^8$/ml, $p < 0.001$) and day 4 (18.1×10^8/ml; $15.4–21.7 \times 10^8$/ml, $p < 0.001$) and significantly higher on day 8 (27.4×10^8/ml; $23.1–31.3 \times 10^8$/ml, $p < 0.05$) and day 15 (36.4×10^8/ml; $31.2–43.1 \times 10^8$/ml, $p < 0.001$) than those observed before surgery (24.6×10^8/ml; $20.5–28.8 \times 10^8$/ml) (Fig. 1b). On day 1, the sGPVI levels increased and the platelet counts decreased. There were no significant correlations between the sGPVI level and the platelet count, D-dimer, FDP, SF, or AT activity (Table 2). The plasma levels of sGPVI in these patients with DVT were significantly higher on day 1 (29.3 ng/ml; 24.5–38.5 ng/ml, $p < 0.01$) and day 4 (26.0 ng/ml; 20.4–28.8 ng/ml, $p < 0.05$) than in those without DVT (21.5 ng/ml; 17.8–27.7 ng/ml, $p < 0.01$ and 18.6 ng/ml; 15.2–24.8 ng/ml, $p < 0.05$, respectively) (Fig. 2). There were no significant differences in the plasma levels of D-dimer, FDP, SF, AT, and platelet counts between the patients with and without DVT (Fig. 3a–d).

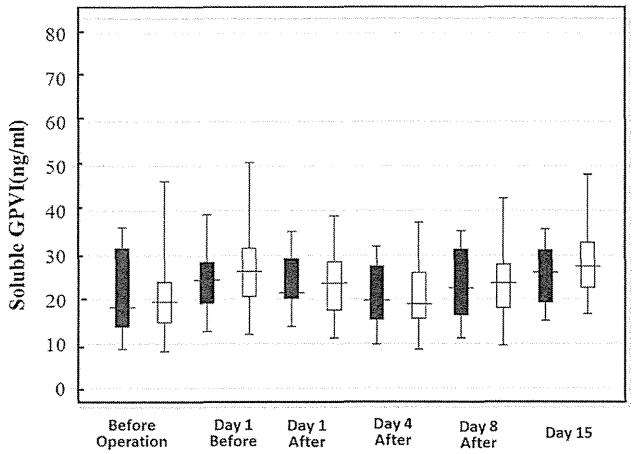

Fig. 4 Plasma levels of sGPVI in the patients with and without withdrawal due to increased bleeding. *Closed bar* with withdrawal due to bleeding, *open bar* without withdrawal. *Day 1 before*: day 1 before edoxaban administration, *day 1 after*: day 1 after edoxaban administration, *day 4 after*: day 4 after edoxaban administration, *day 8 after*: day 8 after edoxaban administration. The *upper* and *lower boxes* show the 75th and 25th percentiles. The *upper*, *middle*, and *lower lines* indicate the 97.5 %, median, and 2.5 % values

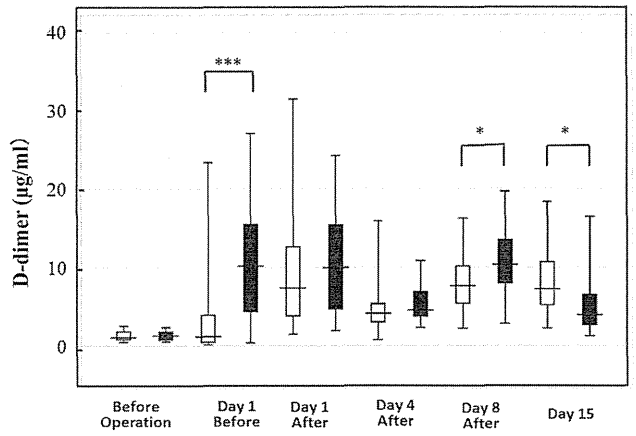

Fig. 5 Plasma levels of D-dimer in the patients with and without withdrawal due to increased bleeding. $*p < 0.05$, $***p < 0.001$. *Closed bars* with withdrawal group, *open bars* without withdrawal group. *Day 1 before*: day 1 before edoxaban administration, *day 1 after*: day 1 after edoxaban administration, *day 4 after*: day 4 after edoxaban administration, *day 8 after*: day 8 after edoxaban administration. The *upper* and *lower boxes* show the 75th and 25th percentiles. The *upper*, *middle*, and *lower lines* indicate the 97.5 %, median, and 2.5 % values

There were also no significant differences in age, sex, THA/TKA, weight, hight, BMI, BSA, creatinine, or eGFR between the edoxaban withdrawal group (Table 2) and those who received complete administration (CA; 14 days of treatment). Furthermore, there were no significant differences in the plasma levels of sGPVI (Fig. 4), SF, or AT activity between the two groups. The plasma levels of D-dimer were significantly higher in the withdrawal group than in the CA group on days 1 (10.2 μg/ml; 4.4–15.5 vs 1.2 μg/ml; 0.5–4.0 μg/ml, $p < 0.001$) and 8 (10.4 μg/ml; 8.0–13.6 vs 7.7 μg/ml; 5.4–10.2 μg/ml, $p < 0.05$) after surgery (Fig. 5). Meanwhile, the plasma levels of FDP were significantly higher in the withdrawal group than in the CA group on days 15 ($p < 0.05$) after surgery.

Table 3 Patients with and without increased bleeding

	With IB	Without IB
Age (years old)	71.0 (65.8–74.5)	65.5 (56.0 -73.0)
Sex (F:M)	11:6	55:11
THA:TKA	12:5	52:14
Weight (kg)	52.0 (47.6–64.7)	59.5 (49.8–70.5)
Height (cm)	152 (146–158)	153 (147–158)
BMI	23.2 (22.3–25.8)	25.1 (21.8–28.1)
BSA	1.47 (1.38–1.70)	1.56 (1.42–1.73)
Creatinine (mg/dl)	0.63 (0.57–0.93)	0.66 (0.55–0.75)
eGFR	74.4 (62.4–86.7)	74.0 (62.8–87.4)

IB increased bleeding

Discussion

The plasma sGPVI levels have been reported to be significantly increased in patients during postoperative period and those with disseminated intravascular coagulation (DIC) [28] or thrombotic microangiopathy (TMA), thus suggesting that the plasma sGPVI levels are increased in a thrombotic state, which activates platelets [26]. Several groups have previously reported the plasma levels of sGPVI [29, 30]. Although one group reported that the levels of sGPVI were elevated in patients with platelet activated diseases [7, 8, 31] such as DIC, stroke, and shear-dependent platelet activation (SAP), another group demonstrated that the levels of sGPVI were negatively associated with the development of ACS [30, 32].

Preventing VTE is important in patients undergoing major orthopedic surgery. Although the detection of elevated D-dimer and SF levels is useful for predicting DVT in outpatients and postoperative subjects, these parameters are not efficacious in postoperative patients treated with an anti-Xa agents. The sGPVI level is useful marker of activated platelets, but not bleeding or vascular endothelial cell injury such as soluble P-selectin [33]. In the present study, of the 83 orthopedic patients treated with 30 mg of edoxaban for prophylaxis of DVT, 14 (16.9 %) developed DVT and 17 (20.5 %) discontinued the prophylaxis due to a reduction in the level of hemoglobin. The frequency of DVT or withdrawal of anti-Xa agents is similar to that observed in previous reports of prophylaxis with fondaparinux at the same institute [22] (Table 3).

In the present study, the plasma levels of GPVI as well as FDP, D-dimer, and SF were significantly higher after surgery than before surgery, and the sGPVI levels increased, while the platelet counts decreased on day 1. These findings suggest that platelets were significantly activated after surgery and that the sGPVI level is a useful marker of thrombosis similar to the levels of FDP, D-dimer, and SF. In addition, there were no significant differences in the D-dimer, SF, or FDP levels between the orthopedic patients with and without DVT, although these parameters were significantly increased after surgery. This finding may be due to the fact that the FDP and D-dimer levels were also increased in the patients with major bleeding resulting from anti-Xa agents [21, 22]. Although, the plasma sGPVI levels were significantly higher in the patients with DVT than in those without DVT, there were no significant differences in the sGPVI levels between those with and without withdrawal, suggesting that the sGPVI levels were not increased due to increased bleeding in contrast to those of FDP or D-dimer. Although elevated FDP and D-dimer levels are known to reflect the degree of fibrinolysis in hematomas within outer blood vessels, there are no reports of elevated sGPVI levels due to hematomas in outer blood vessels. These findings indicate that sGPVI is the only useful marker for predicting VTE in postoperative patients treated with edoxaban. Furthermore, the plasma levels of sGPVI have been reported to be not well correlated with ADAMTS13, von Willebrand factor (VWF), VWF propeptide or thrombomodulin in patients with TMA, suggesting that this parameter may function as a new marker of the activation of platelets but not the activation or injury of vascular endothelial cells [26].

In conclusion, measuring the level of sGPVI may be useful for assessing the activation of platelets in postoperative patients treated with anti-Xa agents and also for predicting the incidence of VTE in this population.

Acknowledgments This work was supported in part by a Grant-in-Aid from Mochida Pharmaceutical Co., Ltd, the Ministry of Health, Labour, and Welfare of Japan for Blood Coagulation Abnormalities and the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Conflict of interest The study was funded by Mochida Pharmaceutical Co., Ltd, and the sGPVI ELISA system was provided by the company. KN contributed to obtaining the measurements of sGPVI but was not involved in interpreting the results. KN is an employee of Mochida Pharmaceutical Co., Ltd. All authors have no other COI to report.

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A first bout of thrombotic thrombocytopenic purpura triggered by herpes simplex infection in a 45-year-old nulliparous female with Upshaw-Schulman syndrome

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Dear Sir,

Upshaw-Schulman syndrome (USS) is a congenital deficiency of ADMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motifs, 13) activity caused by gene mutations. ADAMTS13 specifically cleaves unusually large von Willebrand factor multimers produced in and released from vascular endothelial cells under high shear stress conditions in the microvasculature[1,2]. Thus, in the absence of ADAMTS13 activity, the uncleaved unusually large von Willebrand factor multimers are released into the circulation, causing a life-threatening systemic disease termed thrombotic thrombocytopenic purpura (TTP). Most cases of TTP are induced by acquired autoantibodies against this enzyme. USS is an extremely rare disease, and to date approximately 100 affected patients have been reported in the literature, of whom 43 are in Japan[3].

According to our experience in Japan, bouts of TTP in USS patients are triggered by various stimuli, including pregnancy, severe infection, administration of 1-deamino-8-D-arginine vasopressin (DDAVP) and drinking large amounts of alcohol. Pregnancy is the single most common trigger in female patients. In fact, in an analysis of the natural history of our 43 USS patients in Japan, we found that 26 (60%) were diagnosed during childhood (early-onset phenotype), and the remaining 17 (40%) were diagnosed after 15 years of age (late-onset phenotype). In the early-onset group, the female:male ratio was 13:13, while it was 14:3 in the late-onset group. These 14 female patients were aged between 15 and 45 years, and nine were diagnosed during pregnancy. In contrast, all three male patients had their first bouts after 45 years of age. With regards to ADAMTS13 activity, 35 patients had extremely low levels (<0.5% of normal), seven had trace amounts (0.5-0.8% of normal), and one male patient (USS-GG2) who had his first bout of TTP at 63 years of age had some activity (2.4-3.6%). Thus, one important determinant of the late-onset phenotype in USS patients is the level of ADAMTS13 activity. However, here we present the late-onset phenotype found in a middle-aged nulliparous USS female with severe deficiency of ADAMTS13 activity (<0.5% of the normal), whose first bout of TTP was triggered by an oral herpes simplex infection at the age of 45.

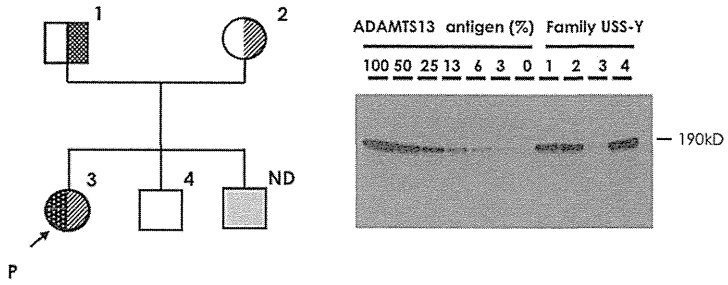
The proband (USS-Y3), born in Sapporo in 1960, was the first of three siblings born to non-consanguineous parents. Her parents and two brothers have had no episodes of thrombosis or excessive bleeding. Her perinatal medical history was unclear, but she did not have any exchange blood transfusions as a neonate. By the age of 3, she suffered from repeated episodes of thrombocytopenia and was diagnosed with idiopathic thrombocytopenic purpura, for which she received transfusions of fresh whole blood on a few occasions. Further details of her medical history during childhood were unavailable. Since the age of 38, her platelet count has been occasionally evaluated at a nearby hospital. The counts were almost normal (104-175×10^9/L). However, when she has a cold, her platelet count temporarily drops to less than 50×10^9/L (minimum 19×10^9/L), but normalises without any specific medical therapy. At the age of 45, she suffered from an oral herpes simplex infection complicated by thrombocytopenia (11×10^9/L), for which the antiviral aciclovir (1,000 mg/day) was prescribed. Subsequently, she has had repeated episodes of oral herpes simplex infection; therefore, she received a prescription of acyclovir for 5 months but has not had an appreciable clinical improvement. She was referred to our hospital for analysis of the cause of her thrombocytopenia. Laboratory findings on admission were as follows: thrombocytopenia (9×10^9/L), haemolytic anaemia (red cell count 1.84×10^9/L, haemoglobin 6.7 g/dL, reticulocyte 168‰, schistocytes on a peripheral smear [2+], total bilirubin 2.8 mg/dL, lactate dehydrogenase 872 IU/L, and haptoglobin <10 mg/dL), near-normal renal function (blood urea nitrogen 19 mg/dL, creatinine 1.07 mg/dL, and positive occult blood in urine), C-reactive protein 0.1 mg/dL, negative direct and indirect Coombs' tests, and normal haemostatic

Blood Transfus 2014; 12 Suppl 1: s153-5 DOI 10.2450/2013.0307-12

tests. She was initially treated with oral prednisolone (50 mg/day) for a diagnosis of Coombs-negative Evans syndrome, but soon thereafter her general condition worsened, and ADAMTS13 analyses were performed for diagnostic purposes.

The family pedigree of this patient is shown in Figure 1A (left). The patient had very low ADAMTS13 activity (<0.5% of normal) and an absence of ADAMTS13 inhibitor (<0.5 BU/mL). Both of her parents had mild deficiencies of ADAMTS13 activity (both 34%) without its inhibitor. Plasma levels of ADAMTS13 activity in one of her younger brothers were normal (74%). Plasma levels of ADAMTS13 antigen as analysed by enzyme-linked immunosorbent assay (Figure 1B) were 2.1% of the normal control in the patient, and 25%, 22%, and 104% of normal in her father, mother, and

A



B

ADAMTS13 analysis in USS –Y family

	ADAMTS13 activity (%)	ADAMTS13 antigen (%)		*ADAMTS13 gene*		
	ELISA	ELISA	WB	Gly385	Gln448	Arg1206
Y-1	34	25	52	G/E	Q/E	R/R
Y-2	34	22	58	G/G	Q/Q	R/*
Y-3	<0.5	2.1	4	G/E	Q/Q	R/*
Y-4	74	104	102	G/G	Q/E	R/R

C

***ADAMTS13* mutations found in family Y**

Exon	Family member	Nucleotide	Amino acid	Remarks
5	Y1, Y4	c. 420 T>C	silent	common SNP
10	Y1, Y3	c. 1154 G>A	p.G385E	disintegrin-like domain
12	Y1, Y4	c. 1342 C>G	p.Q448E	common SNP
15	Y1, Y4	c. 1716 G>A	silent	common SNP
19	Y1, Y2, Y3, Y4	c. 2280 C>T	silent	common SNP
26	Y2, Y3	c. 3616 C>T	p.R1206*	CUB-1 domain
29	Y1, Y4	c. 4221 C>A	silent	common SNP

Figure 1 - The family pedigree of USS-Y is shown in Figure 1A (left). Squares and circles indicate males and females, respectively, and the arrow with P indicates the proposita. Filled symbols represent a patient of USS-Y3. The half-filled symbols represent asymptomatic carriers. Figure 1A (right) shows western blot (WB) analysis of ADAMTS13 antigen followed by anti-ADAMTS13 monoclonal antibody detection using plasma samples, according to the previous method. Note a trace amount of ADAMTS13 antigen in USS-Y3 (patient) in lane 3 of Figure 1A (right). ND indicates not determined. ADAMTS13 activity was measured by chromogenic act-enzyme linked immunosorbent assay (ELISA), and the ADAMTS13 antigen was determined by both WB and antigen-ELISA (Figure 1B). The ADAMTS13 gene mutations found in this family are shown as one-letter amino acid abbreviations (Figure 1B). The ADAMTS13 single nucleotide polymorphisms (SNP) are also shown in Figure 1C.

younger brother, respectively. Furthermore, as analysed by western blot, plasma levels of ADAMTS13 antigen (Figure 1A right and B) were 4% of the normal control in the patient, and 52%, 58%, and 102% of normal in the father, mother, and younger brother, respectively. *ADAMTS13* gene analysis revealed that the patient was a compound heterozygote for two mutations in *ADAMTS13*: p.G385E (c.1154G>A, exon 10) from her father and p.R1206* (c.3616 C>T, exon 26) from her mother. Her parents were heterozygous carriers of each of the two mutations (Figure 1B). These two mutations were not found in her younger brother. p.Q448E was reported as a single nucleotide polymorphism causing a missense mutation[4]. All mutations found in this family are shown in Figure 1C, including common single nucleotide polymorphism without amino acid substitutions. We previously reported the p.R1206X nonsense mutation in a USS-I4 patient[5]. The p.G385E missense mutation presented here is novel. Our experience indicates that the clinical phenotype of females with USS who have never been pregnant is almost indistinguishable from that of males.

Acknowledgements

This work was supported in part by grants-in-aid from the Ministry of Health, Labour and Welfare of Japan, the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Takeda Science Foundation.

Conflict of interest disclosure

Yoshihiro Fujimura is a member of clinical advisory boards for Baxter BioScience.

All other Authors declare no conflicts of interest.

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Arrived: 28 December 2012 - Revision accepted: 28 February 2013
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THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/jog.12125 J. Obstet. Gynaecol. Res. Vol. 40, No. 1: 247–249, January 2014

Case of maternal and fetal deaths due to severe congenital thrombotic thrombocytopenic purpura (Upshaw–Schulman syndrome) during pregnancy

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Abstract

Upshaw–Schulman syndrome (USS) involves a congenital deficiency of von Willebrand factor-cleaving metalloprotease (ADAMTS13) activity due to gene mutations. Female patients develop overt thrombotic thrombocytopenic purpura (TTP) caused by a decline of ADAMTS13 activity in pregnancy. A 23-year-old nulliparous Japanese woman died due to severe, rapid progression of TTP with intrauterine fetal death at 20 weeks of gestation after its onset, even though she underwent intensive treatment which included plasma exchange. She had a history of idiopathic thrombocytopenic purpura at the age of 3 years. The patient's ADAMTS13 activity was of very low level. It should be borne in mind that there is the possibility of rapidly progressive fulminant USS during pregnancy.

Key words: ADAMTS13, intrauterine fetal death, maternal death, pregnancy, thrombotic thrombocytopenic purpura, Upshaw–Schulman syndrome.

Introduction

Thrombotic thrombocytopenic purpura (TTP) is characterized by thrombocytopenia, hemolytic anemia, fever, renal dysfunction and neurological dysfunctions.[1] Congenital TTP (Upshaw–Schulman syndrome, USS) involves a congenital deficiency of von Willebrand factor (VWF) cleaving metalloprotease (ADAMTS13) activity caused by ADAMTS13 gene mutations.[2–4] 'When ADAMTS13 activity is deficient, unusually large VWF multimers accumulate in the circulation that can cause platelet thrombi under high shear stress of the microcirculation'.[5] We present herein a very rare case of congenital TTP with both maternal and fetal death in the second trimester of pregnancy.

Case Report

A 23-year-old pregnant Japanese woman, gravida 1, para 0, had epigastric pain and hematuria at 20 weeks and 3 days of gestation. She was treated for gastritis in a local private clinic, but the severe languid feeling and vomiting showed aggravation. She was referred to our university hospital at 20 weeks and 5 days. She had a history of severe thrombocytopenia at the age of 3 years, which was diagnosed as idiopathic thrombocytopenic purpura (ITP).

There was a decline in her consciousness. Moreover, she showed tonic and clonic convulsions. Sonographic examination revealed intrauterine fetal death (IUFD) on her visit. Cerebral hemorrhage and infarction were

Received: October 3 2012
Accepted: March 4 2013.
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Conflict of interest: The authors declare no conflict of interest.

Table 1 Clinical data and laboratory findings

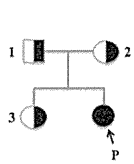
BP (mmHg)	110/80		BIL-T (mg/dL)	3.5	(0.1–1.2)
PR (b.p.m.)	90		Pro	4+	(–)
Fever (°C)	37.4		LDH (U/L)	3384	(110–220)
Purpura	No		BUN (mg/dL)	41	(7.0–20.0)
Jaundice	No		Cr (mg/dL)	1.37	(0.50–1.00)
Oliguria	+		FDP (μg/dL)	29.5	(0.0–5.0)
NS sign	Coma		D-dimmer (μg/dL)	8.7	(0.0–1.0)
Hb (g/L)	54	(110–150)	APTT (s)	28.0	(27.0–40.0)
Plt ($\times 10^9$/L)	16	(150–350)	PT-INR	1.77	(0.85–1.15)
ALT (U/L)	93	(5–40)	Distorted RBC	+	(–)
AST (U/L)	15	(10–35)	P-Hb (mg%)	13.4	

Data in parentheses indicate ranges. ALT, alanine transaminase; APTT, activated partial thromboplastin time; AST, aspartate transaminase; BIL-T, total bilirubin; BP, blood pressure; BUN, blood urea nitrogen; Cr, creatinine; FDP, fibrin/fibrinogen degradation products; Hb, hemoglobin; LDH, lactate dehydrogenase; NS, neulogis system; P-Hb, plasma-free hemoglobin; Plt, platelet; PR, pulse rate; Pro, proteinuria; PT-INR, prothrombin time – international normalized ratio; RBC, red blood cell.

not observed with computed tomography, magnetic resonance imaging and magnetic resonance angiography. Clinical data and laboratory findings on admission are shown in Table 1. Acute disseminated intravascular coagulation such as HELLP syndrome and dead fetus syndrome was ruled out based on the data and clinical symptoms. We diagnosed her with TTP because of severe hemolytic anemia, thrombocytopenia, fever, renal dysfunction and neurological deficits (Table 1).

She was treated with fresh frozen plasma (FFP) transfusion (2880 mL) 5 h after admission, continuous hemodiafiltration with FFP (2440 mL) 9 h after admission and plasma exchange with FFP (4800 mL) 25 h after admission. Induced abortion with dilatation of the cervical canal and artificial rupture of membrane was also initiated. However, she died 32 h after admission without delivery. Consent for autopsy could not be obtained from the family.

The plasma level of ADAMTS13 activity of the patient was 10% or less, and those of her family were moderately deficient (Fig. 1). The ADAMTS13 mutation was p.R193W (c.577C>T, exon 6) in her father and p.P1220Sfs*12 (c.3657_3658insT, exon 26) in her mother. Her sister had the ADAMTS13 mutation from the father (p.R193W, c.577C>T, exon 6) (Fig. 1). They were heterozygous carriers of the mutations. However, the ADAMTS13 gene sequence analysis ended in failure. Therefore, we inferred from the familial genetic analysis that the patient was a compound heterozygote of p.R193W (c.577C>T, exon 6) and p.P1220Sfs*12 (c.3657_3658insT, exon 26). The family of our patient was proven to be a new family line of USS by genetic analysis.



	ADAMTS13: activity (%)	ADAMTS13: gene mutation
1	18 (ELISA)	R193W (c. 577C > T, exon 6)
2	20 (ELISA)	P1220SfsX12 (c.3657_3658insT, exon 26)
3	28 (ELISA)	R193W (c. 577C > T, exon 6)
P	< 10 (FRET)	-

Normal lower limit: 50 %

Figure 1 The plasma level of ADAMTS13 activity and genetic analysis of ADAMTS13 gene. Her parents and sister were heterozygous carriers of ADAMTS13 mutation. 1, father; 2, mother; 3, older sister; ELISA, enzyme-linked immunosorbent assay; FRET, fluorescence resonance energy transfer method; P, patient.

Discussion

Certain clinical symptoms of USS may be absent during childhood in many cases. Therefore, many female patients are not diagnosed accurately before their first pregnancy. However, it is a significant feature of USS that many patients are treated for ITP in childhood. Twenty-nine of 37 USS patients had a history of thrombocytopenia during their childhood, and six of the nine with their first pregnancies had episodes misdiagnosed as ITP.[5] Our patient also received a diagnosis of ITP at 3 years of age, and underwent platelet transfusion.

Plasma ADAMTS13 activity decreases due to the progression of pregnancy,[6] and plasma levels of von Willebrand factor antigen increase markedly during normal pregnancy.[7] Fujimura *et al.*[5] pointed out that the rapidly increased plasma level of unusually large VWF

multimers due to a defect of the VWF cleaving enzyme plays a critical role in precipitating TTP in pregnant women with USS. They showed that pregnancy consistently induced thrombocytopenia during the second–third trimester, as occurred in the present case.

In the previous and present 12 patients with USS during pregnancy, thrombocytopenia was confirmed at the first pregnancy from 12–28 weeks (mean, 22 weeks).[5,8,9] It was diagnosed in one of the 12 patients based on an episode in the older sister, and was treated early. In the other 11 patients, the fetal or neonatal infants' prognoses were extremely poor, and a favorable outcome was reported in only one of the 11. Another three cases that were diagnosed with USS during previous pregnancies or due to an episode involving an older sister's pregnancy received FFP or Octaplas transfusion during pregnancy from an early stage. Their newborn baby prognoses were favorable. Regarding the mothers' prognoses, the symptoms of TTP were serious except for in three cases, in which USS was diagnosed before becoming pregnant. However, it improved immediately on treatment for TTP after delivery or miscarriage. Only one case died at 3 months after discharge from the hospital. In the present case, the patient died a few days after symptom appearance during pregnancy. We could not induce abortion because of the rapid, severe progression. The fetus remaining *in utero* after IUFD may be one of the causes of a poor prognosis. There may be a number of cases in which USS is not proved when there is maternal death due to rapidly severe TTP with pregnancy. It is necessary to diagnose precisely by assay of ADAMTS13 activity and gene analysis when the possibility of USS is suspected based on the clinical course and natural history of TTP with pregnancy. Moreover, we must be aware of the possibility of rapidly progressive fulminant USS with pregnancy.

In conclusion, we should examine ADAMTS13 activity based on the possibility of USS, when we confirm that pregnant women have a history of thrombocytopenia, a low platelet count in early pregnancy and unclear thrombocytopenia after the second trimester of pregnancy.

Acknowledgments

This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no. 23591425) and from the Ministry of Health, Labor, and Welfare of Japan (H23-Nanchi-Ippan-002).

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