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ORIGINAL ARTICLE

National questionnaire survey of TMA

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Abstract A questionnaire survey of Japanese patients with thrombotic microangiopathy (TMA) was carried out to investigate the frequency, laboratory abnormalities, and outcome in 2004. Out of 185 patients, there were 13 with familial TMA and 172 with acquired TMA. In acquired TMA, there were 66 with *Escherichia coli* O-157 infection (O-157)-related TMA, 35 with ADAMTS13-related TMA, and 22 with other types of TMA. The frequency of TMA in O-157-related TMA was high in patients from 0- to 15-year-old, and acquired TMA without O-157 was frequently observed in patients ranging from 31 to 65 years of age. In the treatment of acquired TMA, including plasma exchange (PE), steroid, antiplatelet agent, and anticoagulant, PE was carried out in 94.3% of ADAMTS13-related TMA, 77.3% of other TMA, and 7.6% of O-157-related TMA. The efficacy of PE and steroid therapy tended to be higher in ADAMTS13 TMA than in other types of TMA. The complete remission rate is the highest in O-157 TMA. The mortality rate was the lowest for O-157 TMA, and this rate also tended to be lower in ADAMTS13-related TMA than in other types of TMA. However, the determination of ADAMTS13 was not universal in Japan at the time of this questionnaire.

Keywords TMA · ADAMTS13 · Acquired TMA · Familial TMA · O-157

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1 Introduction

Thrombotic thrombocytopenic purpura (TTP) [1–3], which is characterized by thrombocytopenia and microangiopathic hemolytic anemia, is often associated with neurological dysfunction, renal failure, and fever. Unusually large Von Willebrand factor (VWF) multimers, produced in and then quickly released from vascular endothelial cells, are found in patients plasma in both familial and non-familial thrombotic thrombocytopenic purpura (TTP) [4, 5]. These unusually large VWF multimers are thought to interact with circulating platelets, thus resulting in platelet clumping due to an elevated shear stress [5]. ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13), which was identified in 2001 [6–8], is a zinc

metalloprotease that specifically cleaves unusual VWF multimer at the Tyr (1605)-Met(1606) bound located in the A2 region of VWF [9, 10].

TTP was previously a life-threatening syndrome, and the survival rate has increased from 20 to 80% since the development of plasma exchange (PE) [11], and recently it has reached about 90% [12]. The mainstay of treatment is therapeutic PE, both to remove the causative antibody to ADAMTS13 and to replace ADAMTS13 [13]. The current guidelines [14] for thrombotic microangiopathy (TMA) recommended that PE should be initiated within 24 h of presentation [15]; however, considerable discussion is ongoing with regard to the optional schedule for therapy and the type of replacement fluids to be used [11].

Several questionnaires survey for Japanese patients with TMA were carried out in 2004 and 2005 [16]. This questionnaire survey was carried out to investigate the frequency, laboratory abnormalities, and outcome in 2004.

2 Materials and methods

One hundred eighty-five patients diagnosed with TMA from January 1, 1999 to December 31, 2003 were examined by a national questionnaire survey. The questionnaire was mainly a selective type, and the contents of the questions were about age, sex, underlying disease, acute symptoms, laboratory data, including ADAMTS13, treatment, outcome, and so on. The questionnaire was sent to 994 departments of hematology in Japanese hospitals or institutes: 429 hospitals replied and 73 had 185 cases of TMA. The list of replying hospitals was as follows: Anjo Kosei Hospital, Akashi City General Hospital, Chiba Children's Hospital, Cyukyo Hospital, Fukui Prefectural Hospital, Gunma University Hospital, Higashiosaka City General Hospital, Higashi Sapporo Hospital, Himeji Medical Center, Hiroshima-Nishi Medical Center, Hiroshima University Hospital, Hyogo College of Medicine Hospital, Hyogo Prefectural Cancer Center, Hyogo Prefectural Nishinomiya Hospital, Iizuka Hospital, Ikeda Municipal Hospital, Iseikai Hospital, Juntendo University Shizuoka Hospital, Kagoshima City Hospital, Kagoshima Rousai Hospital, Kawasaki Medical School Hospital, Kitano Hospital, Kokura Medical Center, Kokura Memorial Hospital, Komaki City Hospital, Kure Medical Center, Kyoto City Hospital, Mie University Hospital, Nagaoka Red Cross Hospital, Nagasaki University Hospital of Medicine and Dentistry, Nara Medical University Hospital, National Defense Medical College Hospital, Nihon University Itabashi Hospital, Niigata City General Hospital, Nippon Medical School Hospital, Osaka City General Medical Center, Osaka City University Hospital, Osaka General Medical Center, Osaka University Hospital, Research Hospital, The Institute of Medical Science, The University of Tokyo, Rinku General Medical Center, Saga University Hospital, Saiseikai Maebashi Hospital, Saitama Medical University Hospital, Sapporo Hokuyu Hospital, Sapporo-Kosei general Hospital, Sasebo City General Hospital, SHIN-KOKURA Hospital, Shinsyu University Hospital, Shizuoka General Hospital, Showa University Fujigaoka Hospital, Takasaki National Hospital, Tokai University Hospital, Tokyo Medical And Dental University Hospital Faculty of Medicine, Tokyo Metropolitan Bokutoh Hospital, Tokyo Metropolitan Geriatric Hospital, Tokyo Women's Medical University Hospital, Tottori Prefectural Chuou Hospital, Toyama University Hospital, Tsukuba University Hospital, University of Fukui Hospital, University of Miyazaki Hospital, University of Occupational and Environmental Health Hospital, Usui Hospital, Uwajima City Hospital, Yaizu City Hospital, Yamada Red Cross Hospital, Yamagata Prefectural Central Hospital, Yamaguchi University Hospital, Yodogawa Christian Hospital, Yokkaichi Municipal Hospital, Yokohama City University Hospital, and Yokohama Minami Kyousai Hospital.

Derangement, lethargy, behavior disorder, convulsion, stupor, coma, and other neurological abnormalities were considered as neurological symptoms. Creatinine levels >1.3 mg/dl indicated renal injury. A body temperature >37.5°C was considered as fever.

TMA was classified to 4 groups; ADAMTS13-related TMA (ADAMTS13 TMA), ADAMTS13 levels was less than 20% or positive for inhibitor for ADAMTS13; *Escherichia coli* O-157 infection (O-157)-related TMA (O-157 TMA), the cause of TMA was due to an O-157 infection; other TMA, the cause of TMA was not known; not measured TMA (NM TMA), the ADAMTS13 level was not measured and the cause was not due to an O-157 infection.

The study protocol was approved by the Human Ethics Review Committees of Keio University School of Medicine and Mie University School of Medicine.

2.1 Statistical analysis

The data are expressed as the median (25–75% tile). Differences between the groups were examined for significance using the Chi-square for independence test. A P value of less than 0.05 was considered to indicate a significant difference. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

3 Results

The patients include 13 with familial TMA and 172 with acquired TMA. In familial TMA, the ADAMTS 13 level

was markedly reduced in 8 (ADAMTS13 TMA), but not in 1 (other TMA), and it was not measured in 4 (NM TMA). In acquired TMA, the 66 cases of TMA were caused by O-157 infection (O-157 TMA), and 35 were ADAMTS13 TMA, 22 were other TMA, and 49 were NM TMA (Table 1). There tended to be more females than males among those with ADAMTS13 TMA and O-157 TMA of acquired TMA. The frequency of TMA in O-157 TMA was high in patients from 0- to 15-year-old and that in acquired TMA without O-157 tended to be high in patients from 31- to 65-year-old (Fig. 1). O-157 infection was the most frequent underlying disease in patients with TMA, collagen disease was the second, malignant tumor was the third, and transplantation- and drug-induced TMA were the forth, etc. (Table 2). In collagen diseases, the rate of acquired ADAMTS13 TMA (42.9%) tended to be higher than that of other TMA ($P = 0.09$). In Malignant tumor, drug-induced TMA and post-operation, the rate of other TMA tended to be higher than that of ADAMTS13 TMA. In underlying disease, the rate of ADAMTS13-related TMA was significantly higher in collagen diseases than in malignant tumor, transplantation, and drug induced TMA ($P < 0.05$, respectively). Icterus neonatorum was observed in most patients with familial TMA (70%). The acute symptoms are shown in Table 3. Neurological symptoms tended to be more frequent in acquired TMA than in familial TMA ($P = 0.12$), and it was significantly lower in O-157 TMA than in all other types of acquired TMA ($P < 0.001$). The frequency of renal dysfunction was significantly higher in other TMA than in ADAMTS13 TMA ($P < 0.001$), and it tended to high in O-157 TMA. Fever was observed in more than 50% of acquired TMA. Respiratory symptoms were not regularly associated with TMA.

Table 1 Subjects

	Number	Sex (F:M)		Number	Sex (F:M)
Familial	13	9:4	ADAMTS13 TMA	8	7:1
			Other TMA	1	0:1
			NM TMA	4	2:2
Acquired	172	92:79[a]	ADAMTS13 TMA	35	20:15
			O-157 TMA	66	40:25[a]
			Other TMA	22	11:11
			NM TMA	49	21:28

ADAMTS13 TMA ADAMTS13 activity markedly decreased; *Other TMA* ADAMTS13 activity did not markedly decrease; *NM TMA* ADAMTS13 activity was not measured; *O-157 TMA* O-157-related TMA

[a] One patient did not describe any symptom

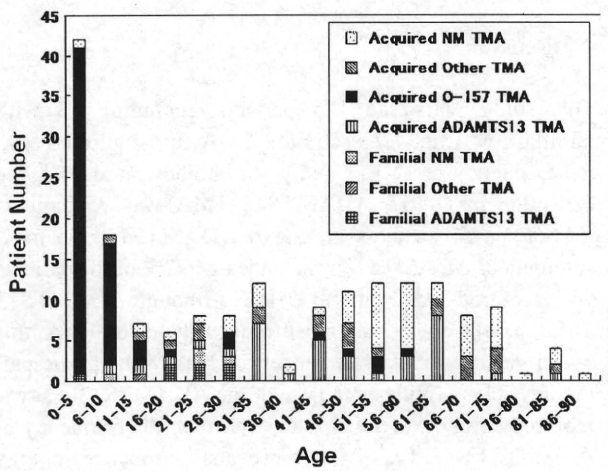

Fig. 1 Patient's age at the onset of TMA

The laboratory abnormalities are shown in Table 4. A decreased platelet count, red cell count, and hemoglobin and an increase of total bilirubin (T-bil) and lactate dehydrogenase (LDH) were frequently observed in each type of TMA. The platelet count was less than 110,000/μl in 98.9%, less than 50,000/μl in 85.4%, and 30,000/μl in 66.9% (Fig. 2). Hemoglobin was usually less than 13.0 g/dl and usually between 5.0 and 10.0 g/dl (Fig. 3). In acquired TMA, the frequency positive for antinuclear antibody was higher in ADAMTS13 TMA than in other TMA ($P < 0.05$). The frequency positive for PAIgG was higher in acquired TMA than in familiar TMA ($P < 0.01$). The Coombs test was negative in more than 90% of those with TMA. The haptoglobin level was reduced in most patients with TMA. Anticardiolipin antibodies (ACA) were not observed in most patients with TMA. Fibrin and fibrinogen degradation products (FDP) and D-dimer levels increased in most TMA patients, but fibrinogen was reduced in a few TMA patients.

Treatment of acquired TMA is summarized in Table 5. Plasma exchange (PE) was carried out in 94.3% of ADAMTS13 TMA, 77.3% of other TMA, and 7.6% of O-157 TMA. The efficacy of PE tended to be higher in ADAMTS13 TMA than in other TMA ($P = 0.052$). Transfusion of fresh frozen plasma (FFP) was frequently performed in familial TMA and ADAMTS13 TMA. The efficacy of FFP tended to be high in familial ADAMTS13 TMA (80.0%) but not high in acquired TMA. In acquired TMA, steroid treatment was carried out in 85.7% of ADAMTS13 TMA, in 72.7% of other TMA, and in 4.5% of O-157 TMA, and the efficacy of steroids tended to be higher in ADAMTS13 TMA than in other TMA ($P = 0.38$). Pulse therapy of methylprednisolone was frequently done in 65.7% of ADAMTS13 TMA and 68.2% of other TMA; but the efficacy was low. Antiplatelet therapy was carried out in 57.1% of ADAMTS13 TMA, 59.1% of other TMA, and 3.0% of O-157 TMA; but the efficacy was low. Hemodialysis was carried out in 37.9% of O-157 TMA and 31.8%

Table 2 Underlying disease

	Number	O-157 infection	Collagen diseases	Malignant tumor	Transplantation	Drug induced TMA	Pregnancy	Post-operation
		66	14	11	7	9	4	2
Familial								
ADAMTS13 TMA	8	0/66	0/14	0/11	0/7	0/9	2/4 (50.0%)	0/2
Other TMA	1	0/66	0/14	0/11	0/7	0/9	0/4	0/2
NM TMA	4	0/66	0/14	0/11	0/7	1/9 (11.1%)	0/4	0/2
Acquired								
ADAMTS13 TMA	35	0/66	6/14*[1],+[1] (42.9%)	1/11 (9.1%)	0/7	0/9	0/4	0/2
O-157 TMA	66	66/66	0/14	0/11	0/7	0/9	0/4	0/2
Other TMA	22	0/66	2/14 (14.3%)	5/11+[2] (45.5%)	2/7 (28.6%)	3/9+[2] (33.3%)	0/4	2/2+[2]
NM TMA	49	0/66	6/14 (42.9%)	5/11 (45.5%)	5/7 (71.4%)	5/9 (55.6%)	2/4 (50.0%)	0/2

*[1] $P < 0.05$ in comparison to acquired ADAMTS13 TMA in malignant tumor, transplantation, and drug-induced TMA

*[2] $P < 0.05$ in comparison to acquired ADAMTS13 TMA

+[1] $P = 0.09$ in comparison to acquired other TMA in collagen diseases

+[2] $P = 0.06$ in comparison to acquired ADAMTS13 TMA

Table 3 Acute symptoms

	Number	Neurological symptoms	Renal dysfunction	Fever (above 37.5°C)	Respiratory symptom
		82	82	113	14
Familial					
ADAMTS13 TMA	8	2/6 (33.3%)	3/8 (37.5%)	3/8 (37.5%)	1/8 (12.5%)
Other TMA	1	0/1	0/1	1/1	0/1
NM TMA	4	0/2	0/2	2/2	0/2
Total	13	2/9 (22.2%)	3/11 (27.3%)	6/11 (54.6%)	1/11 (9.1%)
Acquired					
ADAMTS13 TMA	35	26/34 (76.5%)	9/34 (26.5%)	25/32 (78.1%)	1/20 (5.0%)
O-157 TMA	66	11/64 (17.2%)**[1]	33/64 (51.6%)	36/64 (56.3%)	3/56 (5.4%)
Other TMA	22	14/22 (63.6%)	19/22 (86.4%)**[2]	14/20 (70.0%)	0/7
NM TMA	49	29/46 (63.0%)	18/49 (36.7%)	32/49 (65.3%)	9/49 (18.4%)
Total	172	80/166 (48.2%)	79/169 (46.7%)	107/165 (64.8%)	13/132 (9.8%)

**[1] $P < 0.001$ in comparison to all other type of Acquired TMA

**[2] $P < 0.001$ in comparison to Acquired ADAMTS13 TMA

of other TMA; the efficacy was relatively high in O-157 TMA. Anticoagulant therapy such as heparin and synthetic protease inhibitor was carried out in about 30% of acquired TMA, and the efficacy was higher in O-157 TMA than in other types of TMA ($P < 0.01$). Platelet concentrate (PC) transfusion was carried out in 49.0% of NM TMA, 34.8% of O-157 TMA, 22.7% of other TMA, and 20.0% of ADAMTS13 TMA; however, the efficacy was markedly low in ADAMTS13 TMA.

The outcome of acquired TMA is summarized in Table 6. The complete remission (CR) rate was the highest in O-157 TMA ($P < 0.001$), and the mortality rate was the lowest in O-157 TMA ($P < 0.001$). The mortality rate tended to be lower in ADAMTS13 TMA than in other TMA ($P = 0.53$).

4 Discussion

This study registered 185 patients, including 13 with familial TMA and 172 acquired TMA. In acquired TMA, the frequency of O-157 TMA was highest, and with the exception of O-157, ADAMTS13 TMA was 35 patients (61.4%) in 57 patients measured ADAMTS13. No measurement of ADAMTS13 was made in 46.2% of the patients with acquired TMA without O-157. Although ADAMTS13 TMA may be among the most frequent types of TMA, this questionnaire survey may reflect the bias of the participating physicians. Widespread use of the ADAMTS13 assay is required before it is possible to determine the frequency of ADAMTS13 TMA. A fluorescence resonance energy transfer (FRET) assay [17] for ADAMTS13 activity and an

Table 4 Frequency of abnormal laboratory data

	Number	Plt 176	RBC 137	Hb 155	T-bil 130	LDH 173	ANA 34	PAIgG 31	C-test 4	Haptoglobin 103	ACA 1	Fib 19	FDP 72	D-dimer 67
Familial														
ADAMTS13 TMA	8	7/7	5/7 (71.4%)	7/8 (87.5%)	6/8 (75.0%)	7/8 (87.5%)	0/5	2/4 (50.0%)	0/7	7/7	0/3	2/7 (28.6%)	2/5 (40.0%)	0/4
Other TMA	1	1/1	0/1	0/1	1/1	1/1	0/0	0/0	0/1	1/1	0/0	0/1	1/1	0/1
NM TMA	4	2/2	2/2	2/2	2/2	2/2	1/2	0/0	0/2	1/1	0/0	0/2	1/1	0/0
Total	13	10/10	7/10 (70.0%)	9/11 (81.8%)	9/11 (81.8%)	10/11 (90.9%)	1/7 (14.3%)	2/4 (50.0%)	0/10	9/9	0/3	2/10 (20.0%)	4/7 (57.1%)	0/5
Acquired														
ADAMTS13 TMA	35	34/34	30/32 (93.8%)	23/25 (92.0%)	29/33 (87.9%)	33/34 (97.1%)	17/30 (56.7%)*[1]	12/14 (85.7%)	1/21 (4.8%)	16/20 (80.0%)	0/12	4/29 (13.8%)	13/24 (54.2%)	12/15 (80.0%)
O-157 TMA	66	64/65 (98.5%)	37/39 (94.9%)	64/65 (98.5%)	41/62 (66.1%)	64/64	0/10	4/4	0/16	34/35 (97.1%)	0/1	4/35 (11.4%)	20/27 (74.1%)	18/21 (85.7%)
Other TMA	22	22/22	16/21 (76.2%)	14/15 (93.3%)	16/22 (72.7%)	22/22	3/14 (21.4%)	3/3	0/5	6/8 (75.0%)	0/1	5/20 (25.0%)	13/15 (86.7%)*[2]	11/11
NM TMA	49	46/47 (97.9%)	47/47	45/48 (93.8%)	35/47 (74.5%)	44/47 (93.6%)	13/37 (35.1%)	10/10	3/37 (8.1%)	38/40 (95.0%)	1/17 (5.9%)	4/43 (9.3%)	22/37 (59.5%)	26/26
Total	172	166/168 (98.8%)	130/139 (93.5%)	146/153 (95.4%)	121/164 (73.8%)	163/167 (97.6%)	33/81 (40.7%)	29/31**[3] (93.5%)	4/79 (5.1%)	94/103 (91.3%)	1/31 (3.2%)	17/127 (13.4%)	68/103 (66.0%)	67/73 (91.8%)

Plt platelet count; *RBC* red blood cell count; *Hb* hemoglobin; *T-bil* total bilirubin; *LDH* lactate dehydrogenase; *ANA* antinuclear antibody; *C-test* Coombs test; *ACA* anticardiolipin antibodies; *Fib* fibrinogen; *FDP* fibrin and fibrinogen degradation products

*[1] $P < 0.05$ in comparison to Acquired Other TMA

*[2] $P < 0.05$ in comparison to Acquired ADAMTS13 TMA

**[3] $P < 0.01$ in comparison to Familial TMA

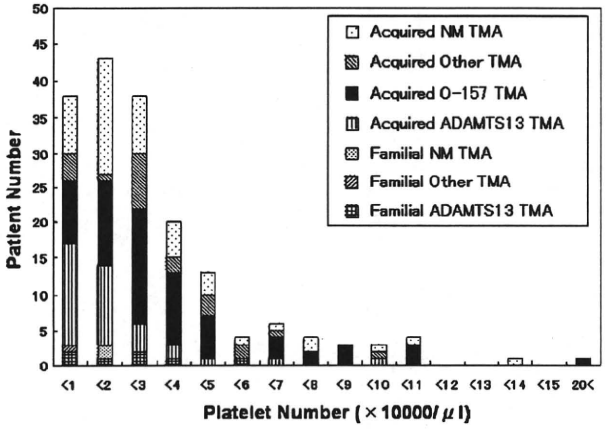

Fig. 2 Platelet number in patients with TMA

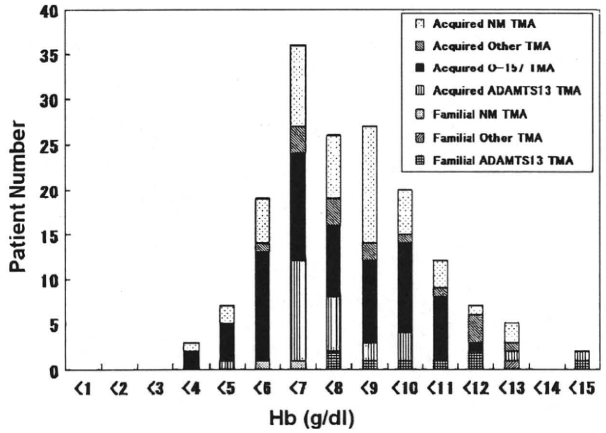

Fig. 3 Hemoglobin levels in patients with TMA

enzyme immunoassay (EIA) [18] of the ADAMTS13 activity have recently been developed, and is very easy to perform and not time-consuming. There was a high frequency of collagen disease, administration of ticlopidine, and the idiopathic type associated with ADAMTS13 TMA. Auto-antibodies for ADAMTS13 may be produced in collagen disease, administration of ticlopidine [19] or idiopathic state. Indeed, the frequency of positive antinuclear antibody was higher in ADAMTS13 TMA than in other TMA. While the detection of auto-antibodies for ADAMTS13 is rare in malignant diseases, post-operation, post-transplantation, infections etc., these conditions may cause TMA via vascular endothelial injuries and inflammation [20].

The frequency of several symptoms of TMA such as neurological symptoms, renal dysfunction, and fever were different in each type of TMA, but may depend on the sensitivity of diagnosis of TMA. In O-157 TMA, O-157 infection is initially diagnosed and then TMA is secondarily detected. The diagnosis of O-157 TMA is easily following the diagnosis of O-157 infection, and it is possible to diagnose the early state without other symptoms. With the exception of O-157 TMA, microangiopathic hemolytic anemia (MHA) with markedly decreased ADAMTS13 might be diagnosed as TMA, but MHA without markedly decreased ADAMTS13 requires a neurological symptom, renal dysfunction or fever for the diagnosis of TMA. Therefore, only about 25% of ADAMTS13 TMA has the symptoms of renal dysfunction.

The platelet count was decreased in 98.9% of TMA, but the frequency of a markedly decreased platelet count (less than 30,000/μl) was 66.9%. Abnormal red cell counts, hemoglobin, T-Bil, and LDH were frequently observed, but these abnormalities were not significantly dominant. Therefore, it is difficult to diagnose as TMA using only these laboratory abnormalities. FDP and D-dimer levels increased in most TMA patients, but fibrinogen was reduced in a few, suggesting that TMA includes thrombotic events but not marked secondary fibrinolysis. However, a few cases of TMA were either associated with disseminated intravascular coagulation (DIC) or were considered to have been caused by DIC.

PE is administered to treat most TMA patients without O-157 TMA, and the efficacy of PE tended to be higher in ADAMTS13 TMA than in other TMA, indicating that PE is usually applied as the standard therapy in Japan. It is clear that PE is effective by both removing the antibody to ADAMTS13 and to replacing ADAMTS13 in ADAMTS13 TMA [13], but it is not clear how PE affects other TMA. PE may remove platelet aggregation factors [21] and inflammatory cytokines [22]. Transfusion of FFP was frequently performed in familial TMA and ADAMTS13 TMA. The efficacy of FFP transfusion tends to be high in familial ADAMTS13 TMA but not high in acquired TMA. A Canadian Apheresis Study suggested that PE was more useful than FFP transfusion [23]. Steroid treatment was administered to most patients with acquired TMA without O-157 TMA, and the efficacy of steroids tends to be higher in ADAMTS13 TMA than in other TMA. Immunosuppressive therapy, including steroid therapy [24], is performed to inhibit of the synthesis of autoantibody against ADAMTS13. Anti-platelet and anti-coagulant therapies were administered to from 30 to 60% of the patients with acquired TMA without O-157 TMA, but these were not effective in this study. Although PC transfusion was not recommended in TMA, this therapy was still carried out in acquired TMA, and the efficacy was markedly low in ADAMTS13 TMA.

The mortality rate of TMA was the lowest in O-157 TMA and tended to be lower in patients with ADAMTS13 TMA in comparison to those with other TMA. The mortality of TMA in Japan was 26.8% in 1988 [25],

Table 5 Treatment of TMA

	Number		PE 90	FFP 78	Steroid 87	Pulse 57	Antiplatelet 58	Hemodialysis 43	Anticoagulant 53	PC 63
Familial										
ADAMTS13 TMA	8	Enforcement	2 (25.0%)	5 (62.5%)	2 (25.0%)	0 (0.0%)	2 (25.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)
		Efficacy	50.0%	80.0%	50.0%	0.0%	100.0%	0.0%	0.0%	0.0%
Other TMA	1	Enforcement	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
		Efficacy	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	100.00%
NM TMA	4	Enforcement	1 (25.0%)	2 (50.0%)	1 (25.0%)	1 (25.0%)	0 (0.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)
		Efficacy	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Total	13	Enforcement	3 (23.1%)	7 (53.8%)	3 (23.1%)	1 (7.7%)	3 (23.1%)	1 (7.7%)	1 (7.7%)	4 (30.8%)
		Efficacy	66.7%	57.1%	33.3%	0.0%	100.0%	0.0%	0.0%	25.0%
Acquired										
ADAMTS13 TMA	35	Enforcement	33 (94.3%)	23 (65.7%)	30 (85.7%)	23 (65.7%)	20 (57.1%)	1 (2.9%)	7 (20.0%)	7 (20.0%)
		Efficacy	45.5%$^{+}$	21.7%	23.3%	13.0%	20.00%	100.0%	0.0%	0.00%
O-157 TMA	66	Enforcement	5 (7.6%)	12 (18.2%)	3 (4.5%)	2 (3.0%)	2 (3.0%)	25 (37.9%)	23 (34.8%)	23 (34.8%)
		Efficacy	60.0%	41.7%	33.3%	50.0%	50.0%	48.0%	47.8%**	43.4%
Other TMA	22	Enforcement	17 (77.3%)	7 (31.8%)	16 (72.7%)	15 (68.2%)	13 (59.1%)	7 (31.8%)	7 (31.8%)	5 (22.7%)
		Efficacy	17.7%	0.0%	12.5%	13.3%	7.7%	0.0%	0.0%	20.0%
NM TMA	49	Enforcement	32 (65.3%)	29 (59.2%)	35 (71.4%)	16 (32.7%)	20 (40.8%)	9 (18.4%)	15 (30.6%)	24 (49.0%)
		Efficacy	46.9%	34.5%	34.6%	37.5%	20.0%	66.7%	20.0%	16.7%
Total	172	Enforcement	87 (50.6%)	71 (41.3%)	84 (48.8%)	56 (32.6%)	55 (32.0%)	42 (24.4%)	52 (30.2%)	59 (34.3%)
		Efficacy	41.4%	28.2%	26.2%	21.4%	18.2%	45.2%	26.9%	25.4%

PE plasma exchange; *FFP* fresh frozen plasma; *PC* platelet concentrate

$^{+}$ $P = 0.052$ in comparison to acquired other TMA

** $P < 0.01$ in comparison to all other types of Acquired TMA

Table 6 Outcome

	Number[a]	CR 98	Without remission 8	Mortality 28
Familial				
ADAMTS13 TMA	4	0	4	0
Other TMA	1	1	0	0
NM TMA	2	1 (50.0%)	1 (50.0%)	0
Acquired				
ADAMTS13 TMA	19	13 (68.4%)	2 (10.5%)	4 (21.1%)
O-157 TMA	55	53 (96.4%)**	0	2 (3.6%)**
Other TMA	6	4 (66.7%)	0	2 (33.3%)
NM TMA	47	26 (55.3%)	1 (2.1%)	20 (42.6%)

** $P < 0.001$ in comparison to all other types of Acquired TMA

[a] Number of patients where the outcome was reported

26.0% in 1999 [26], and 22.0% in 2005, thus suggesting that the mortality of TMA is improving. An early diagnosis of TMA due to the development of an ADAMTS13 assay and the spread of PE treatment have all contributed to the improvement in the mortality of TMA patients.

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Regular Article

Decreased ADAMTS13 Levels in Patients after Living Donor Liver Transplantation

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ABSTRACT

Introduction: Thrombotic microangiopathy (TMA) is a complication occurring after liver transplantation (LT), and an unusually large multimer (ULM) of Von Willebrand factor (VWF) and ADAMTS13 may play an important role in the onset of TMA during LT.

Material and Methods: Eight-one patients underwent living donor LT (LDLT). Seventeen of those patients had both severe thrombocytopenia and hemolytic anemia with fragmented red cells and were diagnosed as TMA-like syndrome (TMALS).

Results and Conclusions: A significant reduction of ADAMTS13 and an increase of VWF were observed in the patients with TMALS. The ADAMTS13 activity in patients after LDLT was significantly reduced from day 1 to day 21, and it was significantly low in those with TMALS at day 14 and 28. The VWF levels in patients with LDLT were significantly high, and the VWF/ADAMTS13 ratio was significantly increased in patients at 7, 14 and 28 days after LDLT, especially in patients with TMALS at day 14 and 28 after LDLT. High molecular weight multimers of VWF were observed to have increased in patients with LDLT, and the high molecular weight multimers of VWF were further increased in those with mild TMALS but they decreased in those with severe TMA. These findings suggest that ULM- VWF and ADAMTS13 might be associated with the onset of TMA after LT.

Introduction

Thrombotic microangiopathy (TMA) is a microvascular occlusive disorder induced by endothelial damage, primary platelet aggregation and microangiopathic hemolytic anemia (MHA) [1–3]. This complication is a well-recognized disorder that may occur in up to 6% of patients following bone marrow transplantation [4] and shows symptoms similar to thrombotic thrombocytopenic purpura (TTP) [5]. TMA stands out as an infrequent but severe life-threatening complication, often requiring intense therapy [2]. According to previous reports, TMA develops after solid-organ transplantation with an incidence of 0.5 to 3% [6–8]. This complication is most prevalent in kidney transplant recipients, but it has also been reported in liver transplant (LT) recipients [9,10].

The possible causative factors of TMA following liver transplantation include calcineurin inhibitors [11,12] and infections [13], including hepatitis C virus [14]. However, the specific pathophysiological mechanism of TMA is still not fully understood. Recently, the kinetics of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13) and unusually large Von Willebrand factor (VWF) multimer (UL-VWFM) have been reported to be good indicators for the occurrence of adverse events after LT [15]. ADAMTS13 is a metalloprotease that specifically cleaves the multimeric VWF [16–20]. A severely deficient ADAMTS13 activity (less than 5% of that in normal plasma) is caused by either a mutation of the ADAMTS13 gene [17,21] or by inhibitory antibodies against ADAMTS13 (14–16). UL-VWFM produced in and then quickly released from vascular endothelial cells, has often been found in patient's plasma in familial and nonfamilial TTP [22,23].

Abbreviations: TMA, thrombotic microangiopathy; LT, liver transplantation; VWF, von Willebrand factor; LDLT, living donor liver transplantation; TMALS, thrombotic microangiopathy - like syndrome; MHA, microangiopathic hemolytic anemia; TTP, thrombotic thrombocytopenic purpura; UL-VWFM, unusually large Von Willebrand factor multimer; ULM, unusually large multimer; FRC, fragmented red blood cell; ELISA, enzyme immunosorbent assay; Hb, hemoglobin.

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Table 1
TMALS patients.

Age	Sex	Underlying disease	Day	PLT ($x10^3/\mu l$)	Hb (g/dl)	LDH (g/L)	ADAMTS13 (%)	VWF (%)
66	F	Hepatocellular Carcinoma	14	77	9.4	2002	27.5	578
53	M	Hepatocellular Carcinoma	14	27	7.6	198	1.0	377
54	F	Liver Cirrhosis	6	46	9.1	758	22.5	617
49	M	Liver Cirrhosis	22	44	10.4	1432	16.3	309
54	M	Hepatocellular Carcinoma	15	19	9.7	342	7.5	430
45	F	Primary Biliary Cholangitis	14	33	6.4	6112	1.0	528
51	M	Liver Cirrhosis	7	40	12.4	330	2.5	560
70	F	Hepatocellular Carcinoma	14	81	8.8	409	37.5	526
45	F	Alcoholic Cirrhosis	16	44	9.0	679	18.8	164
60	M	Liver Cirrhosis	14	51	6.9	546	8.8	303
47	F	Primary Biliary Cholangitis	15	64	8.7	596	1.0	561
6	M	Cholestasis	18	62	9.0	641	13.8	321
62	F	Liver Cirrhosis	15	68	4.7	1909	33.8	601
64	F	Primary Biliary Cholangitis	10	24	9.4	458	12.5	550
45	F	Fulminant Hepatitis	18	50	8.3	801	11.3	502
51	F	Liver Cirrhosis	17	43	7.9	589	12.5	241
59	F	Liver Cirrhosis	21	46	9.3	904	18.8	439

TMALS; thrombotic microangiopathy - like syndrome.

VWF is a large glycoprotein which is essential for high-shear stress associated platelet adhesion and aggregation [24]. These UL-VWFMs have been thought to interact with circulating platelets, thus resulting in platelet clumping due to an elevated shear stress [22].

This study measured the ADAMTS13 activity and VWF antigen in the plasma of 81 patients during living donor LT (LDLT) in order to examine the usefulness of a diagnosis of TMA after LT.

Materials and Methods

The ADAMTS13 activity, VWF antigen and fragmented red blood cell (FRC) were measured in 50 healthy volunteers (31 females and 19 males; median age, 31 years; range, 19-51 years) and 81 patients after LDLT (35 females and 46 males; median age, 47 years; range, 0-70 years) from January 1, 2002 to December 31, 2005. The underlying diseases of the LDLT patients were 24 with hepatic cell carcinoma, 20 with liver cirrhosis due to viral infection, 11 with primary biliary cirrhosis, 10 with hepatitis due to other causes, 6 with cholestatic disease due to other cause, 7 with biliary atresia, and 3 with other diseases.

The diagnosis of TMA-like syndrome (TMALS) is mainly based on thrombocytopenia due to consumption and hemolytic anemia due to the microangiopathy and, in addition, it also includes the laboratory data and clinical symptoms such as liver dysfunction, neurological dysfunction, renal failure, or fever.

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.

Human plasma was obtained by centrifugation at 3000×g at 4 for 15 min from whole blood that was treated with a 1/10 volume of 3.8% sodium citrate as an anti-coagulant. All plasma specimens were stored at -80 before the assay.

ADAMTS13 was measured using a FRETS-VWF73, which was chemically synthesized by the Peptide Institute, Inc. (Osaka, Japan) according to the method of Kokame *et al* [25]. VWF was measured using an IMUBIND vWF ELISA (ADI, CT, USA) using an enzyme immunosorbent assay (ELISA). Plasma UL-VWFM was evaluated by SDS- 0.9% agarose gel electrophoresis followed by Western blotting with luminographic detection [26,27]. The hemoglobin (Hb) levels and platelet counts were measured by automated the fully automated hematology analyzer XE-2100 (Sysmex, Kobe, Japan).

Statistical analysis

The data are expressed as the median (25%tile - 75%tile). The differences between the groups were examined for statistical significance using Mann- Whitney's U test. A P value of less than 0.05 was considered to indicate the presence of a statistically significant difference.

Results

Seventeen patients were diagnosed to have TMALS after LDLT; 4 hepatic carcinoma (16.7%), 7 liver cirrhosis due to viral infection (35%), 3 primary biliary cirrhosis (27.3%), 1 fulminant hepatitis and 1 alcoholic cirrhosis, and 1 cholestasis (16.6%; Table 1 and 2). The mortality at 30 days and 90 days was significantly higher in TMALS (17.5% and 41.2%) than in the patients without TMALS (0% and 5.2%). In the laboratory data of patients with TMA, the platelet count and hemoglobin (Hb) levels were reduced and the LDH (lactate dehydrogenase) and VWF antigen levels were increased. In the plasma levels of ADAMTS13 activity in healthy volunteers, the median value was 108.0% (25% tile - 75% tile; 68.0% - 148.0%). A significant reduction of ADAMTS13 (median; 25% tile – 75% tile) was observed in the patients with TMALS (12.5%; 6.3 -19.7%) in comparison to the healthy volunteers ($p<0.01$).

The laboratory data during LT is shown in Table 3. The platelet count was significantly ($p<0.01$) increased in patients without TMA in day 7 and 14 in comparison to day 0, but was not increased in those with TMALS from day 0 to 28 days. Therefore, the platelet count was significantly lower in patients with TMALS than in those without TMALS (day 0, 1 and 7; $p<0.05$, day 14 and 28; $p<0.01$; Fig. 1). The level of Hb significantly increased in the patients without TMALS on day 1 ($p<0.05$) and on day 7, 14 and 28 ($p<0.01$, respectively) in comparison to day 0, but it was not increased in those with TMALS from 0 day to 28 days. The level of Hb was significantly lower in the patients with TMALS than in those without TMALS on day 14 ($p<0.01$) and on day 28 ($p<0.05$; Fig. 2). The level of T-Bil significantly increased in the patients with TMALS on days 14 and 28 ($p<0.01$) in comparison to day 0 and the level of T-Bil was significantly higher in the patients with TMALS than in those without TMALS ($p<0.01$) on days 7, 14 and 28.

The level of LDH was significantly increased in patients without TMALS at day1 and 7 ($p<0.01$) and day 14 ($p<0.05$), and in patients with TMALS at day 7 and 14 ($p<0.05$) and day 28 ($p<0.01$) in comparison to day 0. The LDH level was significantly higher in patients with TMALS than in those without TMALS on days 14 ($p<0.05$) and 28 ($p<0.01$). There was no significant difference in the creatinine levels on days 0, 1, 7, 14 and 28 in the patients with and without TMALS. No significant difference was observed in the ADAMTS13 levels before the operation among the patients with various underlying diseases (Table 2). The ADAMTS13 activity in patients with TMALS and without TMALS significantly decreased from day 1 to day 28 ($p<0.01$, except on day 28 in those without TMALS; $p<0.05$) in comparison to day 0, and

Table 2
Frequency of TMA and ADAMTS13 activity in various underlying diseases.

	Frequency of TMA	ADAMTS13 activity
hepatic carcinoma	4(16.7%)	96.3% (55.3% - 120.3%)
liver cirrhosis due to viral infection	7(35.0%)	71.3% (53.8% - 98.8%)
Primary biliary cirrhosis	3(27.3%)	52.5% (37.5% - 72.5%)
cholestasis	1(16.6%)	53.8% (31.6% - 100.0%)
biliary atresia	0(0%)	57.5% (50.0% - 68.8%)
Others	2(20.0%)	41.3% (23.4% - 58.8%)

ADAMTS13 activity was shown as median (25%tile -75%tile).

Table 3
Laboratory data during liver transplantation.

		Day 0	Day1	Day7	Day 14	Day 28
PLT ($x10^3/\mu l$)	TMALS	44 (32 - 60)	50 (44 - 67)	58 (41 - 85)	52 (42 - 86)	71 (55 - 122)
	p	p<0.05	p<0.05	p<0.05	p<0.01	p<0.01
	Without TMALS	56 (46 - 77)	69 (52 - 86)	91 (61 - 120)**	180 (121 - 270)**	147 (108 - 226)**
Hb (g/dl)	TMALS	8.6 (7.9 - 9.5)	9.5 (8.6 - 10.7)	9.1 (8.8 - 10.5)	8.3 (7.0 - 9.1)	8.6 (7.0 - 9.8)
	P	NS	NS	NS	p<0.01	p<0.05
	Without TMALS	8.7 (7.8 - 10.2)	9.5 (9.0 - 10.7) *	10.2 (9.4 - 10.8)**	9.9 (8.7 - 10.8)**	9.5 (9.1 - 10.5)**
T-Bil (mg/dl)	TMALS	4.2 (1.8 - 8.2)	4.6 (2.6 - 6.9)	7.6 (4.0 - 9.8)	7.7 (4.6 - 18.0)**	17.8 (6.8 - 22.9)**
	P	NS	NS	p<0.01	p<0.01	p<0.01
	Without TMALS	3.9 (1.7 - 7.0)	4.4 (2.7 - 7.3)	2.8 (1.7 – 6.2)	1.7 (0.9 - 3.9)	1.0 (0.6 - 2.0)
LDH (IU/L)	TMALS	243 (195 – 415)	377 (232 - 505)	368 (264 - 522)*	409 (269 - 1094)*	396 (329 - 544)**
	P	NS	NS	NS	p<0.05	p<0.01
	Without TMALS	219 (191 – 300)	324 (276 - 437)**	326 (260 - 387)**	283 (221 - 347)*	225 (199 - 331)
Creatinine (mg/dl)	TMALS	1.0 (0.7 - 2.2)	1.0 (0.8 - 1.8)	1.1 (0.8 - 1.6)	1.0 (0.9 - 1.6)	1.7 (0.8 - 1.7)
	P	NS	p<0.05	NS	NS	NS
	Without TMALS	0.8 (0.7 - 1.0)	0.9 (0.6 - 1.0)*	0.8 (0.6 - 1.2)	0.9 (0.6 - 1.2)	1.0 (0.7 - 1.5)
ADAMTS13 (%)	TMALS	74.5 (40.0-96.0)	29.0 (14.0-38.0)**	29.0 (18.8-45.0)**	12.5 (4.5-27.0)**	15.5 (5.0-25.0)**
	P	NS	NS	NS	p<0.01	p<0.01
	Without TMALS	61.0(40.8 – 102)	26.5(20.5 - 38.0)**	36.0 (24.5 - 45.0)**	41.0 (23.0 - 51.0)**	49.5 (30.0 – 65.0)*
VWF (%)	TMALS	360 (222 – 432)	225 (178 – 374)	528 (386 - 623)*	431 (306 - 560)	433 (330 - 583)
	P	NS	NS	NS	P<0.01	NS
	Without TMALS	414 (193 – 568)	274 (133 - 434)**	522 (398 - 643)**	532 (388 – 632)**	459 (354 - 594)
ADAMTS13/ VWF	TMALS	4.6 (2.7 - 12.1)	8.0 (5.0 - 15.0)	19.9 (10.1 - 41.9)**	22.2 (14.6 - 51.0)**	40.1 (14.6 - 78.9)**
		NS	NS	NS	P<0.05	P<0.01
	Without TMALS	5.1(2.7 - 11.3)	8.0(5.3 - 13.0)*	14.8(10.2 - 22.4)**	14.0(9.6 - 20.9)**	8.7(5.4 – 16.9)**

NS; not significant, P<0.05 and p<0.01 show difference between patients with TMALS and those without, *; p<0.05 in comparison to day 0, **; p<0.01 in comparison to day 0. Data shows median (25%tile - 75%tile).

it was significantly lower in patients with TMALS than in those without TMALS on days 14 and 28 ($p<0.01$; Fig. 3).

The VWF antigen levels in patients with LT were significantly higher before the operation in comparison to healthy volunteers and they significantly decreased at 1 day in those without TMALS (p<0.01), but they thereafter significantly increased again at day 7 and 14 in those without TMALS (p<0.01) and at day 7 in those with TMALS (p<0.05) in comparison to day 0. The VWF antigen levels were significantly higher in those without TMALS than those with TMALS at day 14 (p<0.01; Fig. 4). The ratio of VWF/ADAMTS13 significantly increased in both groups at 7, 14, and 28 days (p<0.01) after the operation, and those ratios were also significantly higher in patients with TMALS than in those without TMALS at day 14 ($p<0.05$) and 28 ($p<0.01$; Fig. 5).

In the VWF multimer analysis, the high molecular weight multimers of VWF increased in the patients without TMALS at 7 and 14 days after the LDLT, and the high molecular weight multimers of VWF, furthermore, increased in the patients with mild TMALS, while the multimers decreased in the patients with severe TMALS (Fig. 6).

Discussion

In this study, 17 patients (21.0%) were diagnosed to have TMALS after LDLT. The most common underlying disease associated with TMALS was liver cirrhosis due to viral infection, but there was no significant difference in the frequency of TMA among various underlying diseases. The ratio of complications with TMALS was markedly high in comparison to that of TMA in the previous reports [6–8], because, almost all of the current patients had severe hepatic dysfunction before the LDLT and the transplanted liver was not full-sized in LDLT. It follows that the improvement in the liver function requires more time and that complications frequently occur. FRC and reticulated platelets [28] are routinely examined by an automated hematology analyzer XE-2100, thus suggesting that a highly sensitive diagnosis for TMALS could be carried out in this study. A significant reduction of ADAMTS13 activity and increase of VWF antigen were observed in these patients with TMALS, suggesting that this pathophysiological state is similar to TTP. TTP, a life-threatening syndrome characterized by thrombocytopenia and microangiopathic hemolytic anemia, is often associated with neurological dysfunction,

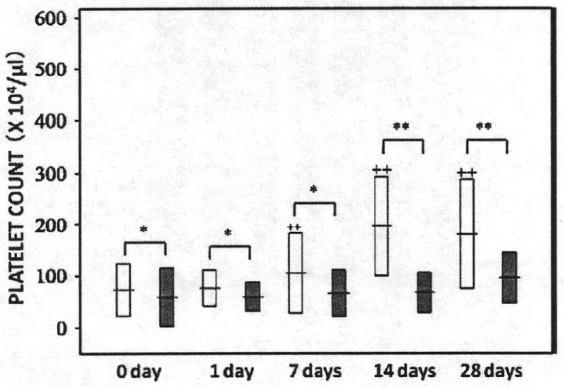

Fig. 1. Platelet counts during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. *; p<0.05, **; p<0.01. ++; p<0.05 in comparison to day 0.

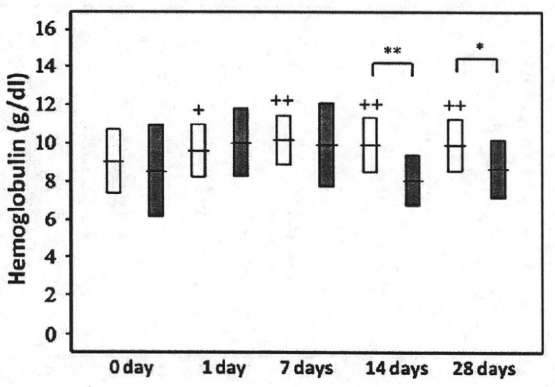

Fig. 2. Hemoglobin levels during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. *; p<0.05, **; p<0.01. +; p<0.05 in comparison to day 0. ++; p<0.05 in comparison to day 0.

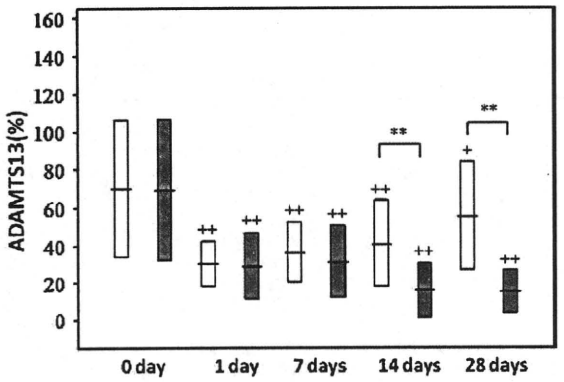

Fig. 3. ADAMTS13 activity during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. **; $p<0.01$. +; $p<0.05$ in comparison to day 0. ++; $p<0.05$ in comparison to day 0.

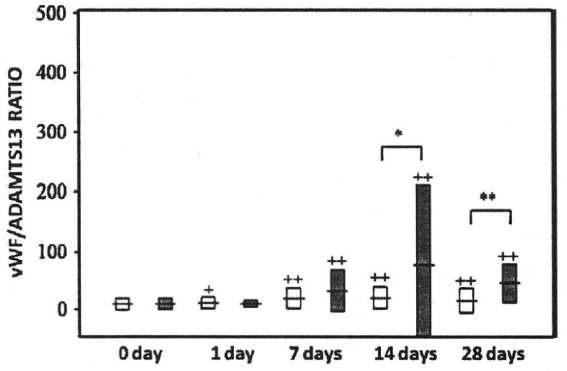

Fig. 5. VWF/ADAMTS13 Ratio during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. *; $p<0.05$, **; $p<0.01$. +; $p<0.05$ in comparison to day 0. ++; $p<0.05$ in comparison to day 0.

renal failure, and fever [5,29]. UL-VWFM, and significant reduce of ADAMTS13 have often been found in patients' plasma in familial and non-familial TTP [22,23]. The ADAMTS13 levels were affected by the production from the liver and consumption or reduction of ADAMTS13 might occur during LT. The VWF levels decrease in persons with blood type "O" [30]. The activity of ADAMTS13 was also low in the patients undergoing hematopoietic stem cell transplantation. A decreased activity has been reported in patients with hepatic veno-occlusive disease (VOD) after stem cell transplantation [31]. These findings suggest that a reduced amount ADAMTS13 may therefore be a risk factor for the onset of VOD or TMA after LDLT.

The platelet counts and Hb levels in the patients with TMALS did not significantly increase on days 7 and 14 in comparison to those without TMALS, and they were also significantly lower in the patients with TMA than in those without TMALS. The levels of LDH and T-Bil significantly increased in the patients with TMALS on day 14 and those were significantly higher in patients with TMALS than in those without TMALS. Indeed, the onset of TMALS frequently occurred on about day 14. The ratio of VWF/ADAMTS13 significantly increased at 7, 14 and 28 days after the LDLT, especially in the patients with TMALS. The plasma ADAMTS13 levels before the operation were not related to the onset of TMA, thus suggesting that the reduction of ADAMTS13 depends on the LDLT. The ratio of VWF/ ADAMTS13 was closely related to the laboratory data, such as the platelet count, Hb, LDH and T-Bil, thus suggesting that decreased ADAMTS13 and UL-VWFM might be related to the onset of TMA after LDLT.

TMA occurs more commonly in cadaveric transplants, thus suggesting that prolonged cold ischemia time and reperfusion injury may cause endothelial injury [32,33]. However, TMA also can occur in LDLT and, to date, there have been sporadic reports of TMA in LDLT recipients [9,10,34,35]. In a VWF multimer analysis, the high molecular weight multimers of VWF were observed to increase in patients without TMALS on days 7 and 14 after the LDLT, and the high molecular weight multimers of VWF, which might be called UL-VWFM, also increased in patients with TMALS while the multimers decreased in patients with severe TMALS. These findings correlate with those from a previous report [15] and suggest that UL-VWFM may cause the consumption of platelets and vascular endothelial cell injuries. These pathophysiological states are similar to those in TTP, thus suggesting that UL-VWFM and decreased ADAMTS13 might thus be associated with the onset of TMA during LT.

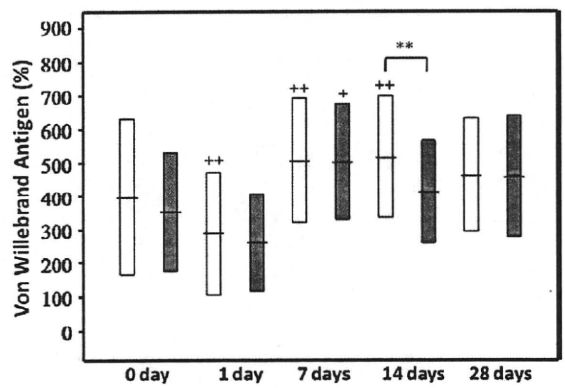

Fig. 4. VWF antigens during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. **; $p<0.01$. +; $p<0.05$ in comparison to day 0. ++; $p<0.05$ in comparison to day 0.

Conflict of interest statement

All authors disclose no financial and personal relationship with other people or organizations that could inappropriately influence their work.

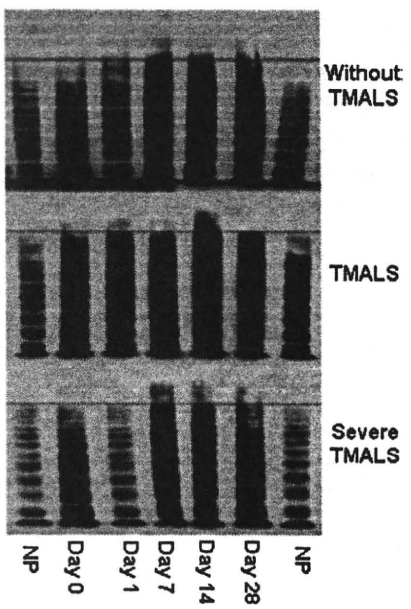

Fig. 6. VWF multimer analysis in mild or severe patients with TMALS, and patients without TMA after LDLT. Plasma VWFM was evaluated by SDS- 0.9% agarose gel electrophoresis followed by Western blotting with luminographic detection. NP; normal plasma. The multimers over the upper line are suggested to be UL-VWFM.

Acknowledgments

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ORIGINAL ARTICLE

Cut-off values of D-dimer and soluble fibrin for prediction of deep vein thrombosis after orthopaedic surgery

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Abstract Soluble fibrin (SF) and D-dimer are considered to be useful for the diagnosis of thrombosis; however, the efficacy of the diagnosis of deep vein thrombosis (DVT) after orthopaedic surgery by SF and D-dimer is still not well established. The present study was designed to evaluate the efficacy of SF and D-dimer in the diagnosis of DVT after orthopaedic surgery. The plasma concentrations of SF and D-dimer were measured in 99 patients following orthopaedic surgery. The plasma concentrations of D-dimer and SF in patients undergoing orthopaedic surgery were markedly high in comparison to healthy volunteers, and these markers were increased after surgery. The plasma concentrations of D-dimer were significantly higher in patients with DVT than in those without DVT at days 4, 7, 10 and 14, and those of SF were significantly higher in patients with DVT than in those without DVT at days 1, 4 and 14. A receiver operating characteristic (ROC) analysis of SF and D-dimer for diagnosis of DVT after surgery generated an ROC curve that showed SF to be better than D-dimer at day 1, while D-dimer was better than SF at day 4. In addition, less than 7.2 μg/ml of D-dimer or 3.6 μg/ml of SF at day 1 after surgery, or less than 7.0 μg/ml of D-dimer at day 4 excluded DVT. These findings suggest that the D-dimer and SF are useful for the diagnosis and exclusion of DVT after orthopaedic surgery.

Keywords SF · D-dimer · Orthopaedic surgery · Total hip arthroplasty · Total knee arthroplasty

1 Introduction

Soluble fibrin (SF) and D-dimer are sensitive markers for thrombotic diseases [1, 2]. These markers are reported to be elevated in deep vein thrombosis (DVT)/pulmonary embolism (PE) [3–5], disseminated intravascular coagulation (DIC) [6–8], acute myocardial infarction (AMI) [9, 10] and thrombotic thrombocytopenic purpura (TTP) [11]. The International Society of Thrombosis and Haemostasis (ISTH) established the diagnostic criteria for overt-DIC using SF and D-dimer [12]. Since PE is a common, frequently undiagnosed and potentially fatal event, and the symptoms of PE are common, including dyspnoea and chest pain, the early recognition of DVT and PE by D-dimer and SF is clinically important [13, 14]. D-dimer is widely used to diagnose thrombosis such as DVT, but many of the commercially available D-dimer assay kits contain different monoclonal antibodies and standard substances, and are based on different assay systems. Since the issue of standardization of D-dimer assays remains to be

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resolved, several studies [15, 16] reported the basic data for the standardization of D-dimer. The *elevated* soluble fibrin (SF) [17] in plasma is an indicator of thrombin activation in the blood, as are the thrombin–antithrombin complex and prothrombin fragment F1+2.

Orthopaedic surgeries, such as total hip arthroplasty (THA) and total knee arthroplasty (TKA), are frequently associated with DVT/PE [18, 19]. Several clinical trials have been recently reported for the prevention of DVT [20, 21]. The diagnosis of DVT depends on venography or echography, but these methods are time consuming and expensive. The exclusion of DVT by D-dimer or SF might be helpful. But the usual cut-off value of D-dimer is not appopriate for patients after surgery, since all patients after surgery have high plasma concentrations of D-dimer and SF.

The present study was designed to evaluate the efficacy of the SF and D-dimer in the diagnosis of DVT after surgery. For this purpose, the changes of the plasma concentration of D-dimer and SF were examined in the clinical course after surgery of 99 patients who underwent orthopaedic surgery.

2 Materials and methods

2.1 Subjects

From January 1, 2006 to May 31, 2007, 99 patients (median age 66 years of age, 25–75% percentile, range 58–73 years of age; sex, 86 females and 13 males) had orthopaedic surgery (71 THA and 28 TKA) in the Mie University Graduate School of Medicine. The plasma concentrations of SF and D-dimer were examined in these patients before the operation, and 1, 4, 7, 10, 14, 21, 24 and 32 days after surgery. At 4 and 10 days after orthopaedic surgery, echography was carried out for DVT. The study protocol was approved by the Human Ethics Review Committees of the Mie University Graduate School of Medicine and a signed consent form was obtained from each subject. Among these patients, 84 patients (66 years of age, 57–73 years of age, 73 females and 11 males) had no thrombosis, and 15 patients had a DVT (68 years of age, 60–73 years of age, 13 females and 2 males). Confirmation of DVT was diagnosed with echography or venography. Several mechanical prophylaxis were carried out in all patients until 4 days after surgery and 10 patients with high risk (evaluated by a physician) or superficial vein thrombosis were treated with unfractionated heparin (UFH) or low-dose warfarin 4 days after orthopaedic surgery. Fifteen patients, who were diagnosed to have DVT, were treated with UFH.

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 analysed within 4 h. The same parameters were also measured in 99 healthy subjects (mean age 22 years, range 21–30 years; 41 females and 58 males), who were free of any diseases including thrombotic disease or hyperlipidaemia, as confirmed by an annual medical checkup.

2.2 Measurement of plasma concentrations of D-dimer and soluble fibrin

Plasma D-dimer levels were measured with LPIA-ACE D-dimer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) using JIF23 monoclonal antibody. The JIF23 monoclonal antibody, which recognizes plasmin-digested N-terminus of the γ chain on the D region, was used for latex agglutination [22]. SF was also determined by the latex agglutination method using IATRO SF (Mitsubishi Kagaku Iatron Inc.) containing the monoclonal antibody IF-43, which recognizes a segment of the fibrin $A\alpha$ chain [($A\alpha$-17–78) residue segment] exposed in the E region of fibrin monomer (FM) when the FM molecule binds the D region of another FM or fibrinogen. The antibody is coated for the SF assay [23].

2.3 Statistical analysis

The data are expressed as the median (25–75% percentile). The differences between the groups were examined for statistical significance using the Mann–Whitney U test. A P value of less than 0.05 was considered to be significant. The usefulness of D-dimer and SF levels in the diagnosis of thrombosis and VTE was examined by a receiver operating characteristic (ROC) analysis [24]. The cut-off values were determined by the ROC analysis. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

3 Results

The plasma concentrations of D-dimer in patients without DVT were significantly increased on day 1 (median 13.4 μg/ml, 25–75 percentile, 8.4–24.1 μg/ml, $P < 0.01$) and day 7 (9.4 μg/ml, 7.6–12.6 μg/ml, $P < 0.05$) after surgery in comparison to day 0 (7.5 μg/ml, 5.9–9.3 μg/ml). While in patients with DVT, the plasma concentrations of D-dimer were significantly increased on day 1 (16.1 μg/ml, 9.9–26.3 μg/ml, $P < 0.01$), day 4 (19.0 μg/ml, 10.6–28.7 μg/ml, $P < 0.01$), day 7 (12.3 μg/ml, 8.9–25.4 μg/ml, $P < 0.05$) and day 14 (16.0 μg/ml, 9.6–23.0 μg/ml, $P < 0.01$) after surgery in

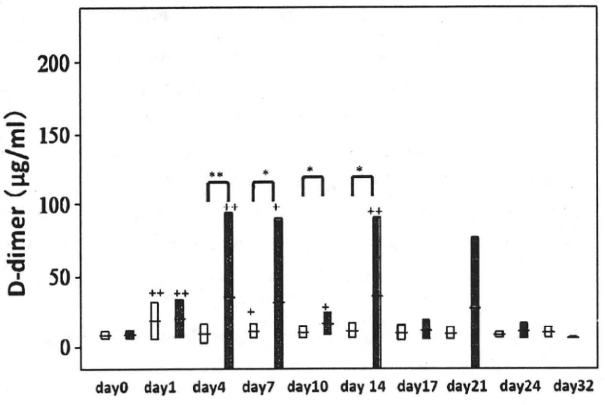

Fig. 1 Plasma concentrations of D-dimer in patients after orthopaedic surgery. *Open bar* patients without DVT, *closed bar* patients with DVT. $*P < 0.05$, $**P < 0.01$ difference between patients without DVT and those with DVT. $^{+}P < 0.05$, $^{++}P < 0.01$ in comparison to day 0

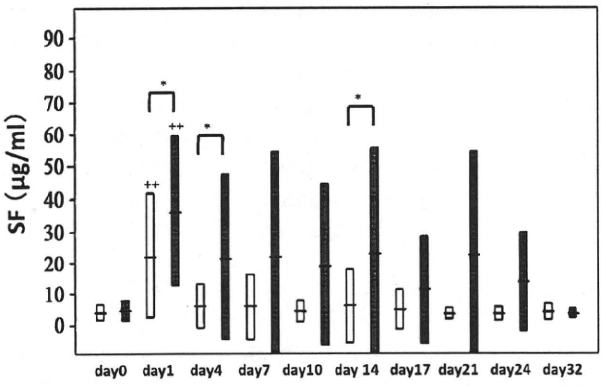

Fig. 2 Plasma concentrations of SF in patients after orthopaedic surgery. *Open bar* patients without DVT, *filled bar* patients with DVT. $*P < 0.05$, $**P < 0.01$ difference between patients without DVT and those with DVT. $^{+}P < 0.05$, $^{++}P < 0.01$ in comparison to day 0

comparison to day 0 (7.6 μg/ml, 6.1–9.5 μg/ml). The plasma concentrations of D-dimer were significantly higher in patients with DVT than in those without DVT on day 4 ($P < 0.01$), 7 ($P < 0.05$), 10 ($P < 0.05$) and 14 ($P < 0.05$; Fig. 1). The plasma concentration of D-dimer was markedly high in each of the days after surgery in comparison to healthy volunteers (0.4 μg/ml, 0.2–0.5 μg/ml).

The plasma concentrations of SF in patients without DVT were significantly increased on day 1 (18.2 μg/ml, 6.0–33.1 μg/ml, $P < 0.01$) after surgery in comparison to day 0 (3.7 μg/ml, 2.5–8.3 μg/ml). While in patients with DVT, the plasma concentrations of SF were significantly increased on day 1 (32.0 μg/ml, 16.7–48.0 μg/ml, $P < 0.01$) after surgery in comparison to day 0 (3.5 μg/ml, 2.8–10.1 μg/ml). The plasma concentrations of SF were significantly higher in patients with DVT than in those without DVT on days 1, 4 and

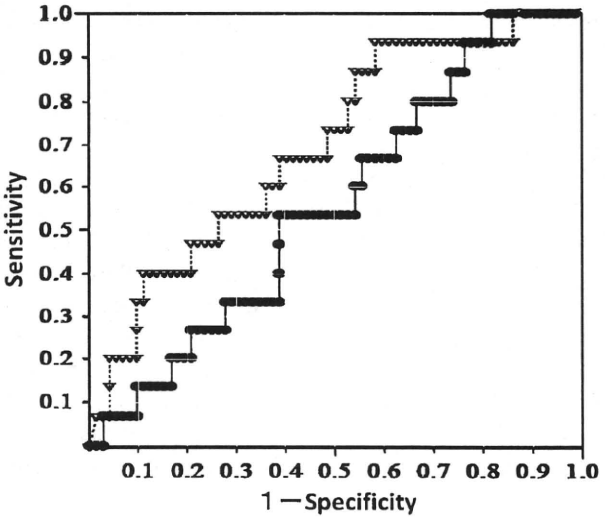

Fig. 3 ROC analysis for diagnosis of DVT 1 day after orthopaedic surgery. *Filled circle* D-dimer, *open triangle* SF. AUC: D-dimer 0.557, SF 0.692

14, respectively ($P < 0.05$) (Fig. 2). The plasma concentration of SF was markedly high in each of the days after surgery in comparison to healthy volunteers (0 μg/ml, 0–0.6 μg/ml).

In an ROC analysis of SF and D-dimer for diagnosis of DVT at day 1 after surgery (Fig. 3), the ROC curve showed that SF was better than D-dimer on day 1. The area under the curve (AUC) was 0.692 in SF and 0.557 in D-dimer. The 100% negative predictive value (NPV) of SF and D-dimer was 3.6 and 7.2 μg/ml, respectively. In the highest odds ratio, D-dimer was 8.0 μg/ml and SF was 11.9 μg/ml (Table 1). The ROC analysis of SF and D-dimer for diagnosis of DVT at day 4 after surgery (Fig. 4) showed that D-dimer was better than SF on day 4. The AUC was 0.737 in SF and 0.862 in D-dimer. The 100% of NPV for SF was not detected, but that of D-dimer was 7.0 μg/ml. In the highest odds ratio, D-dimer was 17.7 μg/ml and SF was 17.8 μg/ml (Table 2).

4 Discussion

In the present study, the plasma concentrations of D-dimer and SF in patients after orthopaedic surgery were markedly high in comparison to those in healthy volunteer. These findings are consistent with previous reports [1, 2, 14]. Under these conditions, the cut-off value in previous reports might not be useful for the diagnosis of thrombosis. Therefore, a new cut-off value of SF or D-dimer for thrombosis should be determined in the patients after orthopaedic surgery. Patients with DVT had high levels of D-dimer on day 0 in comparison to healthy volunteers. This reason for this may be due to the presence of some occult thrombosis in these patients.

Table 1 Cut-off values of SF and D-dimer on day 1 after surgery

Cut-off value (μg/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Odds ratio
SF					
3.6	100	12.5	19.5	100	–
11.9	93.3	41.7	25.0	96.8	10.0
D-dimer					
7.2	100	18.1	20.3	100	–
8.0	93.3	23.7	20.3	94.4	4.33

PPV positive predictive value, *NPV* negative predictive value

Table 2 Cut-off values of SF and D-dimer on day 4 after surgery

Cut-off value (μg/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Odds ratio
SF					
–	100			100	–
17.8	36.4	95.6	66.7	86.0	12.3
D-dimer					
7.0	100	55.6	35.5	100	–
17.7	54.5	97.8	85.7	90.0	52.8

PPV positive predictive value, *NPV* negative predictive value

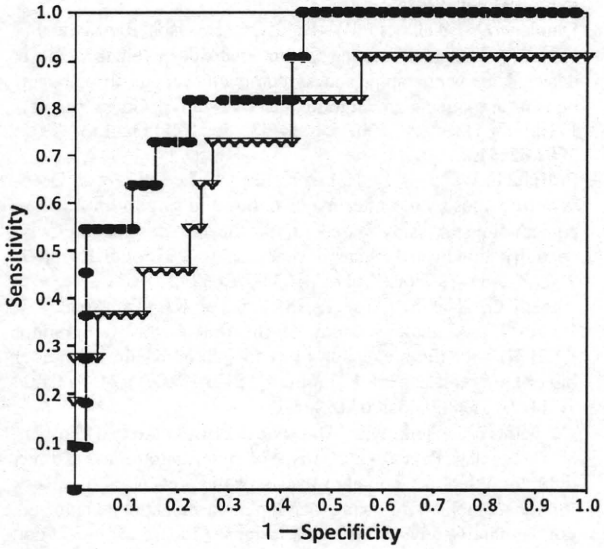

Fig. 4 ROC analysis for diagnosis of DVT 4 days after orthopaedic surgery. *Filled circle* D-dimer, *open triangle* SF. AUC: D-dimer 0.862 SF 0.737

From the significant difference in the plasma concentrations of D-dimer between patients with DVT and those without DVT, D-dimer may be useful for the diagnosis of DVT in patients on day 4, 7, 10 and 14 after orthopaedic surgery. However, no significant difference of plasma D-dimer concentration was observed between the two groups on day 1 after surgery, suggesting that D-dimer was not useful for the diagnosis of DVT at that time. The ROC analysis also showed that D-dimer was not useful for the diagnosis of DVT on day 1 after surgery. As the half life of the plasma SF levels is short, the elevation of SF due to the operation decreased on day 1. The onset of DVT is thus considered to begin on day 1. Therefore, an elevation of SF on day 1 after operation is a useful marker for the prediction of DVT. In contrast, the half life of the plasma D-dimer levels is long, and therefore the elevation of D-dimer due to the operation continues for several days, thus making it difficult to detect the onset of DVT on day 1.

From the significant difference of plasma concentrations of SF between patients with DVT and those without DVT, SF may be useful for the diagnosis of DVT on day 1, 4 and 14 after orthopaedic surgery. The ROC analysis also showed that SF was useful for the diagnosis of DVT on day 1 after surgery. As the patients with high risk and with superficial vein thrombosis were treated with UFH or low-dose warfarin from day 4 after surgery, the value of D-dimer and SF at only 1 and 4 days after orthopaedic surgery were not affected by the use of anticoagulant drugs in this study.

In previous reports [2, 14, 25], the high concentrations of SF and D-dimer could be considered to be markers of thrombosis including venous thromboembolism (VTE). An appropriate cut-off value for the diagnosis of VTE in patients without operation was reported to be 5.9 μg/ml in SF and 4.8 μg/ml in D-dimer with different assays (data from SF and D-dimer by different assays were similar; unpublished data) [25]. In this study, more than 11.9 μg/ml of SF on day 1 or more than 17.7 μg/ml of D-dimer on day 4 was suggested to be associated with DVT in the patients after surgery.

In Europe and North America, D-dimer concentrations of less than 0.5 μg/ml are considered to exclude DVT/PE in patients without surgery [26], but some D-dimer kits that are frequently used in Japan have a different cut-off value (1.2 μg/ml) for the exclusion of DVT/PE [14]. In this study, a new cut-off value for exclusion of DVT in patients after orthopaedic surgery could be determined: for D-dimer 7.2 μg/ml on day 1 and 7.0 μg/ml on day 4, and for SF 3.6 μg/ml on day 1. This discrepancy in the cut-off values between outpatients and postoperative patients may be due to thrombin formation as a result of the operation.

Fondaparinux and enoxaparin were recently approved as prophylaxis drugs for orthopaedic surgery by the Japanese Ministry Health, Labour and Welfare, but these sometimes cause severe bleeding [27]. The current results could allow recommending treatment with fondaparinux or enoxaparin in patients with more than 11.9 μg/ml of SF on day 1 or more than 17.7 μg/ml of D-dimer on day 4, but not in those with less than 7.2 μg/ml of D-dimer or less than 3.6 μg/ml of SF on day 1. For the prevention of DVT, it is therefore

considered important to administer anticoagulant treatment within 4 days after orthopaedic surgery.

These findings suggest that the appropriate cut-off values of D-dimer or SF is useful for the diagnosis of DVT after orthopaedic surgery.

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LETTER TO THE EDITOR

Definite diagnosis in Japanese patients with protein C deficiency by identification of causative *PROC* mutations

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Protein C is a vitamin K-dependent plasma glycoprotein that functions as an important regulator of blood coagulation, and its deficiency is known to be a risk factor for thrombosis [1]. Congenital protein C deficiency is an autosomally inherited disorder, and has been classified into 2 types: a quantitative deficiency (type I), and qualitative deficiency (type II) [2]. Heterozygous patients with inherited protein C deficiency are mildly affected, and are either symptomatic (1 in 16,000 of the general population [3]) or asymptomatic (1 in 500 of the healthy population [4]). Thus, protein C deficiency in itself is thought to be a relatively mild risk factor for thromboembolism, and it is suggested that thrombosis-prone protein C deficient families might carry additional genetic factors that increase the risk, such as FV Leiden mutation and prothrombin 20210G > A mutation in Caucasian populations [5]. Severe congenital protein C deficiency is a much rarer disease, most often caused by a homozygous (or compound heterozygous) protein C gene (*PROC*) mutation(s). Some homozygous subjects develop purpura fulminans or skin necrosis and intravascular disseminated coagulation at birth [6–8], while heterozygous deficiency predisposes to venous thrombosis in adulthood. This clinical heterogeneity could reflect a variety of molecular mechanisms.

The prevalence of protein C deficiency in the general Japanese population was also estimated similarly to be approximately 1 in 500 [9]. In this study, we investigated the molecular defects of protein C deficiency in 6 Japanese patients, and defined respective causative mutations in the *PROC*. All patients were diagnosed as having protein C deficiency (Table 1) [10]. After informed consents were obtained, genomic DNAs were isolated from peripheral blood leukocytes of the patients and studied under approval of the study from the Ethics Committee of the Nagoya University School of Medicine. We amplified each of the 8 exons including the exon/intron boundaries of the *PROC* by polymerase chain reaction (PCR), and directly sequenced them as described previously [7]. We identified 6 distinct heterozygous *PROC* mutations in the 6 Japanese patients with protein C deficiency: 5 missense mutations (p.Arg42Ser [c.124C > A], p.Met406Ile [c.1218G > A], p.Cys147Tyr [c.439G > A], p.Arg211Trp [c.631C > T],

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Table 1 Gene abnormalities and clinical features of patients with PC deficiency

Case	Age	Sex	PC Ac (%)	PC Ag (%)	Mutation	Location	Predicted AA change	Thrombosis
1	0	F	<10	ND	c.124C > A	Exon 3	p.Arg42Ser	PF
					c.1218G > A	Exon 9	p.Met406Ile	
2	38	M	48	35	c.1268delG	Exon 9	p.Gly423ValfsX82	PE
3	30	F	52	42	c.439G > A	Exon 7	p.Cys147Tyr	DVT
4	30	F	38	44	c.631C > T	Exon 6	p.Arg211Trp	DVT
5	35	F	39	42	c.1015G > A	Exon 9	p.Val339Met	PVT
6	0	M	10	5	c.631C > T	Exon 6	p.Arg211Trp	PF
					c.1268delG	Exn 9	p.Gly423ValfsX82	

Nucleotide and amino acid numbers are described according to den Dunnen et al. [10]. (GenBank accession number: NT_022135)

M male, *F* female, *ND* not done, *PF* purpura fulminans, *PE* pulmonary embolism, *DVT* deep vein thrombosis, *PVT* portal vein thrombosis

and p.Val339Met [c.1015G > A]) and a G deletion (p.Gly423ValfsX82 [c.1268delG]). All mutations were previously reported in Japanese patients with protein C deficiency, except for c.439G > A which had been already reported in 2 Dutch families [11–13].

Two patients (Cases 1 and 6) with severe protein C deficiency, who both developed purpura fulminans shortly after birth, were found to be compound heterozygous for c.124C > A and c.1218G > A mutations, and c.631C > T and c.1268delG mutations, respectively. In Case 1, we could not obtained samples from the other family members including the parents, but we performed PCR including exons 3–9 of the *PROC* gene of the patient to define compound heterozygosity for the identified mutations (i.e., c.631C > T in exon 3 and c.1268delG in exon 9), as described previously [14]. Direct sequencing data from the subclones of the PCR fragments showed c.631C > T or c.1268delG mutation independently, and no subclone with both mutations was obtained, indicating that the respective *PROC* mutations might have been inherited from his parents separately (data not shown). In Case 6, we performed PCR-RFLP analyses as described in previously [15], to examine inheritance of the mutations (c.631C > T and c.1268delG) identified in the patient. PCR-*Sac* II RFLP analysis to detect the c.631C > T mutation in the *PROC* showed that the patient and his mother had a mutant allele, but his father did not (Fig. 1a). We also employed mismatch PCR-*Eco*N I RFLP analysis to detect the c.1268delG mutation in the *PROC*, showing that the patient and his father had a mutant allele, but his mother did not (Fig. 1b). These data indicated that the patient inherited the c.631C > T mutation from his mother and the c.1268delG mutation from his father, suggesting that the patient could be compound heterozygous. These observations were consistence with previous reports that severe protein C deficiency caused by a homozygous (or compound heterozygous) *PROC* mutation(s) frequently led to infantile purpura fulminans [2]. Diagnosing homozygote infants with protein C deficiency depends upon the appropriate clinical picture, a protein C level that is essentially unmeasurable, and the confirmation of heterozygous parents. To confirm the diagnosis of this rare disorder, it may be necessary to ascertain the presence of *PROC* mutations in the family members including the parents.

Numerous cases of hereditary protein C deficiency have been reported, showing that individuals with protein C deficiency tend to have an increased risk of thromboembolism [2], and over two hundred different *PROC* mutations have been identified so far. It was reported that five recurrent defects (c.541T > G, c.631C > T, c.1015G > A, c.1218G > A, and c.1268delG, which were founder or hot spot mutations) may account for 49% of Japanese families with protein C deficiency [11]. Consistently, we found in this study that 5 out of the 6 patients had either one or two of these recurrent mutations. Especially, one (Case 6) of the compound heterozygous patients with severe protein C deficiency had two of the Japanese recurrent *PROC* mutations, and the other (Case 1) had one of the recurrent mutations and the previously reported *PROC* mutation in Japanese. These data again suggest that it might be necessary to survey *PROC* mutations including Japanese recurrent *PROC* mutations in Japanese patients with such severe protein C deficiency in order to make an accurate diagnosis and start appropriate treatment immediately.

In summary, we investigated molecular defects of protein C deficiency in 6 Japanese patients, and defined respective causative mutations in the *PROC*, most of which were recurrent *PROC* mutations in the Japanese population.