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High Prevalence of Anti-Prothrombin Antibody in Patients With Deep Vein Thrombosis

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The present study was designed to determine the prevalence of lupus anticoagulant (LA) antibody and several antibodies for antiphospholipid syndrome (APS) in patients with deep vein thrombosis (DVT)/pulmonary embolism (PE) ($n = 48$), cerebral thrombosis (CT, $n = 30$), systemic lupus erythematosus (SLE, $n = 22$), and idiopathic thrombocytopenic purpura (ITP, $n = 30$). The presence of antibodies was examined by using the respective ELISA kits. LA was positive in 38.6% of patients with DVT/PE, suggesting that LA is one of the most important risk factors in DVT/PE. The highest prevalence of anti-β_2 glycoprotein I (β_2GPI) IgG was in CT and SLE, followed by DVT, and none in ITP and healthy volunteers (control, $n = 40$), suggesting that it is related to thrombosis, particularly arterial thrombosis. The highest prevalence of anti-prothrombin (aPT) IgG antibody was in DVT, followed by CT and SLE, and none in ITP and the control, suggesting that it is related to thrombosis, especially venous thrombosis. The highest prevalence of antiphospholipid (aPL) IgG was in DVT, CT, and SLE, but 0% in ITP and control. On the other hand, aPL IgM, anti-annexin V IgG, and anti-annexin V IgM were positive in patients both with and without thrombosis, suggesting that they are not related to thrombosis. Our results indicated that among the anti-phospholipid antibodies, LA is the most sensitive marker for APS while anti-β_2GPI IgG, aPT IgG, and aPL IgG are risk factors for thrombosis. In particular, aPT IgG is a significant marker for DVT/PE. Am. J. Hematol. 76:338–342, 2004. © 2004 Wiley-Liss, Inc.

Key words: PE; DVT; DRVVT; anti-prothrombin antibody; anti-β2GPI antibody

INTRODUCTION

Patients with antiphospholipid syndrome (APS) develop episodes of thrombosis of the arteries and/or veins, miscarriage, and thrombocytopenia, associated with positivity for antiphospholipid antibody [1,2]. In patients with venous thromboembolism, the prevalences of anti-cardiolipin antibody (aCL) and lupus anticoagulant (LA) antibodies vary from 3% to 17% and from 3% to 14%, respectively [3–5]. A high prevalence of antiphospholipid antibodies is present in patients with systemic lupus erythematosus (SLE), with estimates varying between 30% and 60% [6,7]. In stroke patients, 18% of young patients (mean age, 38 years) were positive for antiphospholipid antibodies (LA and aCL) and 9.7% of patients who developed stroke for the first time were positive for aCL according to the Antiphospholipid Antibodies in Stroke Study Group (APASS) [8,9]. In patients with myocardial infarction, the prevalence of aCL is reported to be between 5% and 15% [10]. In 543

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blood donors under 65 years of age, 2% were positive for aCL [11]. Among 179 patients who were confirmed to be free of deep vein thrombosis (DVT), 18% were positive for aCL and 2% were positive for LA [12].

Approximately 50% of patients with APS do not have underlying systemic disease and are labeled as having primary APS [13]. The age of first thrombosis in primary APS ranges between 32 and 45 years [14], suggesting that APS tends to occur at a young age. However, many APS patients are more than 45 years of age. There are many tests for APS, such as aCL [15], LA [16], anti-β_2 glycoprotein I (β_2GPI) [17], and anti-prothrombin (aPT) antibody [18]. aCL antibody was first established as an anti-phospholipid antibody [15]. The phospholipid-dependent coagulation inhibitor, which did not appear to be associated with a bleeding tendency, was named LA. At present, however, LA is also known as anti-β_2GPI antibody or aPT antibody. The present study was designed to identify the most sensitive antibody marker(s) for thrombosis in APS and other conditions. For this purpose, we determined the prevalence of several antiphospholipid antibodies in 48 patients with DVT and examined the cause of antiphospholipid antibodies for LA.

MATERIALS AND METHODS

From 1 January 2000 to 31 June 2002, we examined several anti-phospholipid antibodies in 48 patients with DVT (ratio of females/males = 14:34, mean age, 55.5 ± 17.8 years), 30 with idiopathic thrombocytopenic purpura (ITP) (21:9, 44.4 ± 14.8 years), 22 with SLE (15:7, 43.9 ± 13.4 years), 30 cerebral thrombosis (CT) (10:20, 45.8 ± 11.3 years), and 40 healthy volunteers (control) (13:27, 38.0 ± 10.7 years). In SLE, six patients were associated with DVT and 5 patients were associated with CT. A total of 38 patients were diagnosed as having APS (22:16, 50.5 ± 15.6 years), including 21 patients with DVT and 17 with CT, and 18 patients were positive for LA without thrombosis (2:16, 49.8 ± 10.9 years). APS was diagnosed on the basis of Sapporo criteria [6]. None of the patients had congenital abnormalities of protein C, protein S, antithrombin, or Factor V Leiden.

The Diluted Russell Viper Venom test (DRVVT) was measured using the DVV-test (American Diagnostica Inc. [ADI], Greenwich, CT) and DVV-confirm (ADI). Patients with a DVV-test/DVV-confirm ratio of more than 1.2 were considered LA-positive.

Anti-phospholipid (aPL), anti-annexin V, aPT, and anti-β_2GPI antibodies were measured by enzyme-linked immunosorbent assay (ELISA) using the Imuclone aPL IgG ELISA kit (ADI), Imuclone aPL IgM ELISA kit (ADI), Imuclone Anti-annexin V IgG ELISA kit (ADI), Imuclone anti-Annexin V IgM ELISA kit (ADI), Imuclone anti-Prothrombin IgG ELISA kit (ADI), anti-prothrombin IgM ELISA kit ADI), Imuclone anti-β_2GPI IgG, ELISA kit (ADI), and anti-β_2GPI IgM ELISA kit (ADI), respectively. The aPT antibody ELISA uses human prothrombin for coating the solid ELISA plate. There are no anionic phospholipids on the aPT ELISA plate saturated with goat serum. The detecting antibody used is a goat anti-human Ig (G or M) antibody coupled with peroxidase. The buffer and diluent used in the kits are both calcium-free. In the Imuclone aPL IgG and IgM ELISA kits, the antigen is a mixture of negatively charged phospholipids instead of cardiolipin, and it has β_2GPI. In the Imuclone anti-β_2GPI IgG and IgM ELISA kits, anti-β_2GPI was prepared in human sera. The cutoff value is defined by testing a large group (>60) of normal individuals and some patients with APS. The cutoff value corresponds to the mean value of normal subjects ± 2SD in the LA assay and to the mean value of normal subjects ± 5SD in ELISA assays. All data were expressed as mean ± SD. Statistical analysis was performed by Fishers' test. A P value less than 0.05 denoted statistical significance. The likelihood ratio of a positive result (LR^+) was calculated by the formula LR^+ = sensitivity/(1 − specificity).

RESULTS

The prevalence of LA in patients with thrombosis (DVT and CT) was higher than in those free of thrombosis ($P < 0.01$) (Table I) and was 23.3% in patients with ITP and 54.5% in patients with SLE. The prevalence of anti-β_2GPI IgG in patients with thrombosis was higher than in those without thrombosis ($P < 0.01$) and was 45.5% in patients with SLE and 0% in patients with ITP. There was no significant difference in the prevalence of anti-β_2GPI IgM between patients with thrombosis and those without thrombosis, and the prevalence of the same antibody was 4.5% in SLE and 0% in ITP (Table I). The prevalence of aPT IgG antibody in patients with thrombosis was significantly higher than in those without thrombosis ($P < 0.01$) and was 18.2% in SLE and 0% in ITP. On the other hand, there was no significant difference in the prevalence of aPT IgM antibody between patients with thrombosis and those without thrombosis, and was 0% in SLE and 0% in ITP (Table I). The prevalence of aPL IgG was higher in patients with thrombosis than in those without thrombosis ($P < 0.01$), and was 40.9% in SLE and 6.7% in ITP. On the other hand, there was no

TABLE I. Prevalence of Lupus Anticoagulant (LA), Anti-β_2 Glycoprotein I (Anti-β_2GPI), Anti-prothrombin, Anti-phospholipid, and Anti-annexin V Antibodies in Patients With Deep Vein Thrombosis (DVT)/Pulmonary Embolism (PE), Cerebral Thrombosis (CT), Thrombosis, and Without Thrombosis and in Healthy Volunteers (Control)

	DVT/PE	CT	With thrombosis	Without thrombosis	Control
LA-positive[a]	21/48 (38.6%)	15/30 (50.0%)	35/77 (45.5%)*	28 (28.0%)	0/40 (0%)
Anti-β_2GPI antibodies					
IgG	8/48 (16.7%)	11/30 (36.7%)	18/77 (23.4%)*	4/100 (4.0%)	0/40 (0%)
IgM	0/48 (0.0%)	3/30 (10.0%)	3/77 (3.9%)	3/100 (3.0%)	0/40 (0%)
Anti-prothrombin antibodies					
IgG	14/48 (29.1%)	5/30 (16.7%)	18/77 (23.4%)*	1/100 (1.0%)	0/40 (0%)
IgM	4/48 (8.3%)	2/30 (6.7%)	6/77 (7.8%)	4/100 (4.0%)	1/40 (2.5%)
Anti-phospholipid antibodies					
IgG	10/48 (20.8%)	3/30 (10.0%)	20/77 (26.0%)*	6/100 (6.0%)	0/40 (0%)
IgM	9/48 (18.8%)	7/30 (23.3%)	15/77 (19.5%)	10/100 (10.0%)	2/40 (5%)
Anti-annexin V antibodies					
IgG	10/48 (20.8%)	8/30 (26.7%)	18/77 (23.4%)**	10/100 (10.0%)	2/40 (5%)
IgM	1/48 (2.1%)	3/30 (10%)	4 (5.2%)	6/100 (6.0%)	3/40 (7.5%)

[a]Based on prolonged DVV test of > 40 sec and a DVV-test/DVV-confirm ratio of > 1.2.
*$P < 0.01$, compared with patients without thrombosis.
**$P < 0.05$, compared with patients without thrombosis.

significant difference in prevalence of aPL IgM in patients with and without thrombosis, and was 27.3% in SLE and 6.7% in ITP (Table I). The prevalence of anti-annexin V IgG was higher in patients with thrombosis than in those without thrombosis ($P < 0.05$), and was 18.2% in SLE and 7.1% in ITP. There was no significant difference in the prevalence of anti-annexin V IgM among patients with thrombosis and those without thrombosis and was 4.5% in SLE and 6.7% in ITP (Table I). The likelihood ratio of positive results (LR^+) of anti-β_2GP1 IgG was markedly high in patients with CT and with thrombosis. Furthermore, LR^+ of PT IgG was significantly high in patients with DVT and thrombosis (Table II). The prevalences of anti-β_2GP1 IgG, aPT IgG, and aPL IgG were significantly higher in patients with APS than in those with LA without thrombosis ($P < 0.01$ each). However, the frequencies of LA and anti-annexin V IgG were not different in the two groups (Table III).

TABLE II. Likelihood Ratio of Positive Results (LR^+) and Likelihood Ratio of Negative Results (LR^-) for Lupus Anticoagulant (LA), Anti-β_2 Glycoprotein I (Anti-β_2GPI), Anti-prothrombin, Anti-phospholipid, and Anti-annexin V Antibodies in Patients With deep Vein Thrombosis (DVT)/Pulmonary Embolism (PE), Cerebral Thrombosis (CT), Thrombosis, and Without Thrombosis and in Healthy Volunteers (Control)

	DVT/PE		CT		With thrombosis	
	LR^+	LR^-	LR^+	LR^-	LR^+	LR^-
LA-positive[a]	1.344	0.834	1.531	0.742	1.623	0.758
Anti-β_2GPI antibodies						
IgG	1.536	0.935	4.90	0.685	5.884	0.798
IgM	0	1.049	4.90	0.919	1.299	0.991
Anti-prothrombin antibodies						
IgG	13.40	0.738	1.76	0.92	23.4	0.774
IgM	1.792	0.961	1.225	0.987	1.948	0.960
Anti-phospholipid antibodies						
IgG	1.680	0.904	3.593	0.705	4.329	0.788
IgM	1.512	0.928	1.906	0.874	1.948	0.895
Anti-annexin V antibodies						
IgG	1.493	0.920	1.960	0.849	2.338	0.851
IgM	0.299	1.053	2.100	0.945	0.866	1.009

[a]Based on prolonged DVV-test of > 40 sec and a DVV-test/DVV-confirm ratio of > 1.2.

TABLE III. Prevalence of Anti-phospholipid Antibodies in Patients With Anti-phospholipid Syndrome (APS) and Lupus Anticoagulant (LA)-Positive Patients Without Thrombosis

	LA	Anti-β_2GPI IgG	Anti-PT IgG	Anti-PL IgG	Anti-annexin V IgG
APS	36/38 (94.7%)	19/38* (50.0%)	11/39* (28.2%)	16/39* (29.2%)	10/39 (25.6%)
LA without thrombosis	18/18 (100%)	0/18 (0.0%)	0/18 (0.0%)	0/18 (0.0%)	3/18 (16.7%)

*$P < 0.01$, compared between patients with APS and LA patients without thrombosis.

DISCUSSION

Our results showed that LA was positive in 38.6% of patients with DVT/PE, suggesting that LA is the most important risk factor in DVT/PE. However, previous reports indicated that the prevalence of anti-phospholipid antibodies was less in patients with DVT/PE than in those with arterial thrombosis, although abnormalities of AT [19], protein C [20], and protein S [21] were reported to be more frequent in patients with DVT/PE. With respect to other conditions, our results showed that the prevalence of LA was the highest in collagen diseases. Previous studies reported that APS is frequently detected in patients with collagen diseases [6,7]. Because LA was observed in patients with ITP and those with SLE free of thrombosis as well as in healthy volunteers, this antibody does not seem to be a risk factor for thrombosis directly. In agreement with this conclusion, LA was also reported in patients without thrombosis [22].

Our results also showed a high prevalence of anti-β_2GP1 IgG in CT and SLE, followed by DVT, but was not detected in any of the patients with ITP and the control subjects, suggesting that this antibody is related to thrombosis, especially arterial thrombosis. Cerebral ischemia associated with anti-phospholipid antibody is the most common arterial thrombotic manifestation [23,24]; however, the importance of this antibody as a cardiovascular risk factor is controversial. Since only a few patients with thrombosis were positive for anti-β_2GP1 IgM, this antibody may be low risk factor for thrombosis. In this regard, previous studies [25,26] reported the detection of IgG, and indicated that the presence of IgM anti-β_2GP1 correlated significantly with a history of thrombosis. In particular, Guerin et al. [25] used both anti-β_2GP1 and conventional aCL assays and found that anti-β_2GP1 IgG assays improved the diagnostic specificity relative to the conventional aCL assays.

The present study also showed a moderately high prevalence of aPT IgG antibody in patients with DVT, followed by those with CT and SLE, but in none of the patients with ITP and healthy volunteers, suggesting that it is related to thrombosis, especially venous thrombosis. In 1959, Loeliger [27] was the first to propose that prothrombin, another phospholipid binding protein, is a possible cofactor for LA. A positive correlation between the presence of aPT antibody and DVT was also reported in patients with SLE [28]. In a study of 265 cases with DVT/PE, the risk of thrombotic events was significantly high in carriers of aPT antibody [29]. Because the plasma levels of activated protein C are significantly elevated in patients with DVT/PE [30,31] and LA [32], activation of the coagulation system plays an important role in the onset of DVT/PE, suggesting that aPT is important in these states. On the other hand, activation of platelet plays an important role in the onset of arterial thrombosis. Anti-β_2GP1 antibody may play a more important role in the onset of arterial thrombosis than aPT antibody.

Finally, our results showed moderately high prevalences of aPTIgG, anti-β_2GP1, and aPL IgG antibodies in patients with DVT, CT, and SLE, but these were not present in patients with ITP or in healthy volunteers. Based on the experiences of our hospital, which is a referral center for patients with autoimmune diseases, the prevalence of anti-phospholipid antibodies in our patients with DVT seems to be high. Similarly to the antibodies named above, this antibody may be related to thrombosis. On the other hand, our results demonstrated the presence of aPT IgM, aPL IgM, anti-annexin V IgG, and anti-annexin V IgM antibodies in patients with and without thrombosis, suggesting that they are not strongly related to thrombosis.

In conclusion, we have demonstrated in the present study that LA is the most sensitive antibody for APS among those tested in the study but that it is not specific to APS and LA without thrombosis. Our results also showed that the prevalence of antiphospholipid antibody was high in specific states: aPT IgG in DVT/PE, anti-β_2GP1 IgG in CT and SLE, and aPL in SLE.

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The Japanese Experience With Thrombotic Thrombocytopenic Purpura–Hemolytic Uremic Syndrome

Masanori Matsumoto, Hideo Yagi, Hiromichi Ishizashi, Hideo Wada, and Yoshihiro Fujimura

A total of 290 Japanese patients with thrombotic thrombocytopenic purpura–hemolytic uremic syndrome (TTP-HUS) were analyzed with respect to ADAMTS-13 activity and its inhibitor. Twenty-one patients (17 families) had Upshaw-Schulman syndrome, and 12 patients (six families) had familial HUS of undetermined etiology. The number of patients with acquired HUS and TTP was 44 and 213, respectively. In acquired TTP, patients with severe deficiency of ADAMTS-13 activity secondary to the presence of an inhibitor were high responders to plasma exchange, but others were low responders to plasma exchange. The former patients were associated with "idiopathic" TTP, drugs, and pregnancy, and the latter patients with malignancy and stem cell transplantation. Patients with autoimmune disease-associated TTP fit into both groups.

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THROMBOTIC thrombocytopenic purpura (TTP) is a life-threatening disease, characterized by Moschcowitz's pentad[1]: thrombocytopenia, microangiopathic hemolytic anemia (MAHA), fluctuating neurological signs, renal failure, and fever. The majority of patients with TTP are adults who suddenly develop clinical signs without any underlying disease; this is referred to as "idiopathic" TTP. However, in a significant number of patients TTP is associated with a variety of clinical conditions, including autoimmune disease, stem cell transplantation, malignancy, drugs, pregnancy, and infections. These patients are classified as having "secondary" TTP.[2-4]

By contrast, hemolytic-uremic syndrome (HUS) is characterized by Gasser's triad[5]: MAHA, thrombocytopenia, and renal insufficiency. Most childhood HUS patients have Shiga-like toxin-producing *Escherichia coli* 0157:H7 infection, usually accompanied by bloody diarrhea. Except for these distinctive cases, however, differential diagnosis between TTP and HUS is often difficult in clinical practice, and therefore the comprehensive term TTP-HUS or thrombotic microangiopathy (TMA) has been used.[2-4]

Recent advances in elucidating the proteolytic processing of plasma von Willebrand factor (VWF) multimers have established assays for VWF-cleaving protease (VWF-CP) activity and its inhibitor (autoantibody).[6,7] These assays made it possible to distinguish TTP from HUS, because the former shows defective enzymatic activity with or without presence of its inhibitor, and the latter has exclusively normal activity. VWF-CP is now identified as a metalloproteinase belonging to the ADAMTS (A Disintegrin And Metalloproteinase domain, with ThromboSpondin type I motif) family, termed ADAMTS-13.[8-11] ADAMTS-13 is produced in the liver, exclusively in the perisinusoidal cells.[12]

In early 1998, we began to quantify VWF-CP/ADAMTS-13 activity and its inhibitor, based on previous methods.[13] Over the past 5 years, our laboratory has been the only facility in Japan able to assay these activities. In this regard, we were fortunate to have the opportunity to collect blood specimens and analyze the activities in a variety of diseases, at the request of hospitals across Japan. This system still works, and analysis of the accumulated data is still underway. Here we report on data from 290 patients with TTP-HUS, clinically diagnosed by physicians in referring hospitals, with special reference to the activity of ADAMTS-13 and its inhibitor.

Methods and Patients

Plasma ADAMTS-13 activity was assayed based on VWF multimer analysis,[13] with a slight modification as previously described.[14] The normal range of ADAMTS-13 activity (n = 60) in our laboratory was

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Table 1. Classification of 290 Japanese Patients With TTP-HUS

Congenital or Familial TTP-HUS	33
Upshaw-Schulman syndrome	21 (17 families)
Etiology undetermined	12 (6 families)
Acquired	257
HUS	44
Idiopathic	34
Secondary	10
E coli O157:H7	7
Mitomycin	3
TTP	213
Idiopathic	108
Secondary	105
Autoimmune disease	43
Malignancy	21
Stem cell transplantation	19
Pregnancy	9
Drugs	8
Others	5

102% ± 23% (mean ± SD).[15] The inhibitor against ADAMTS-13 activity was also measured according to previously described methods[6,7] using heat-inactivated (56°C for 60 minutes) patient plasma samples. The inhibitor titer was expressed as Bethesda units,[16] in which one unit was defined as the amount that reduces the ADAMTS-13 activity to 50% of normal control. In this assay, a titer of more than 0.5 U/mL was considered significant.

A total of 290 patients with TTP-HUS, from 121 different hospitals in Japan, were studied. After informed consent, patient blood was collected, and citrated platelet-poor plasma was prepared by centrifugation, kept frozen in dry ice, and then sent to our laboratory together with clinical information.

Congenital or familial TTP-HUS was defined as the following: (1) severe deficiency of ADAMTS-13 activity (<3% of the normal control), but without its inhibitor. Each assay was performed on at least two occasions, with an interval of more than 6 months. Most patients in this category develop their clinical symptoms soon after birth, requiring exchange blood transfusion; this condition is also called Upshaw-Schulman syndrome (USS).[17-19] (2) Regardless of the ADAMTS-13 activity, patients have one or more family members with repeated clinical episodes of TTP-HUS. The number of patients in these two categories in our study was 33, from 23 different families (Table 1).

The diagnosis of acquired HUS or TTP was made by physicians in the referring hospitals, on the basis of clinical symptoms and routine laboratory findings. As a result, 44 patients with acquired HUS and 213 patients with acquired TTP were enrolled (Table 1).

Congenital or Familial TTP-HUS

Upshaw-Schulman Syndrome

The most striking clinical feature of USS is severe hyperbilirubinemia with negative Coombs test soon after birth, requiring exchange blood transfusions.[19] The ADAMTS-13 activity of USS patients is consistently below 3%, but without its inhibitor, and their parents have roughly half the activity of normal controls (~50%). Unusually large VWF multimers (UL-VWFM) are often noted in plasma,[20,21] which soon disappear after infusion of fresh frozen plasma.[21] The UL-VWFM induce platelet aggregation under high shear stress, and therefore play a key role in the pathogenesis of thrombocytopenia.[21] Twenty-one patients from 17 different families with USS were identified. After obtaining approval from the ethics committee, the responsible mutations were identified in the *ADAMTS13* gene in seven families (designated A through G) (Table 2).[22,23] As a consequence, we found that five patients (A, D, E, F, and G) with USS were compound heterozygotes of *ADAMTS13* gene mutations, and two patients (B and C) were homozy-

Table 2. Clinical and Genetic Profiles in Seven Japanese Patients With Upshaw-Schulman Syndrome

Patients	A	B	C	D	E	F	G
Year of birth	1986	1986	1972	1978	1985	1993	1987
Sex	M	F	M	F	M	F	F
Neonatal jaundice	Severe	Severe	Moderate	Severe	Severe	Severe	Severe
Exchange blood transfusion	Yes	Yes	No	Yes	Yes	Yes	Yes
Genetic transmission	Compound heterozygote	Homozygote	Homozygote	Compound heterozygote	Compound heterozygote	Compound heterozygote	Compound heterozygote
Relation of the parents	Unrelated	Unrelated*	Cousin	Unrelated	Unrelated	Unrelated	Unrelated

* Two great-grandparents of patient B moved to Hokkaido Island from the same village in the northeastern region of the Japanese mainland at the end of 19th century.

Table 3. ADAMTS-13 Activity of the Patients With Familial HUS of Etiology Undetermined and Acquired HUS

ADAMTS-13 Activity (%)	Familial HUS of Etiology Undetermined (n = 12.6 families)	Acquired HUS (n = 44)		
		Idiopathic (n = 34)	O157:H7 (n = 7)	Mitomycin (n = 3)
<3	0	0	0	0
3-25	4	4	0	1
26-50	7	18	5	1
>50	1	12	2	1

gotes. The parents of patients in the former group were all unrelated, but in the latter group those of patient C were cousins. The parents of patient B were unrelated, but subsequent careful genealogical analysis revealed that two great-grandparents of patient B moved to Hokkaido island as farmers from the same village in the Northeastern region of the Japanese mainland at the end of 19th century.

The number of subjects tested is still too small, but our findings suggest that among USS patients consanguinity gives rise to the minor group with homozygous mutation of the *ADAMTS13* gene, whereas the major group consists of individuals with compound heterozygous mutations not arising from consanguinity.[23]

Of asymptomatic carriers among USS patients, the father of patient A was particularly interesting, because he had an extremely low level of plasma ADAMTS-13 activity, 4.5% to 7% of the normal controls on three different occasions, but so far, at the age of 36, he has shown no apparent clinical signs.[14,22,23] This unusually low level of ADAMTS-13 activity was confirmed by proving that both the father and the sister of patient A had the P475S mutation, and by performing an expression experiment in vitro. These findings indicate that plasma ADAMTS-13 activity of more than 5% is enough to prevent clinical manifestations of the disease, unless other risk factors for thrombosis are present.

Further, we examined two interesting but separate families (Y and Z) with congenital deficiency of ADAMTS-13 activity (<3% of the normal). Each family had two female patients (siblings) aged 23 to 27 years old, who had been referred to our laboratory because of repeated episodes of TTP-HUS, resulting in unsustainable pregnancy. History during the neonatal period of patients in family Y is presently unknown, but that for family Z has shown that a younger sister (Z2) received exchange blood transfusion soon after birth, and had an episode of thrombocytopenia at age 3, indicating that Z2 had USS. The elder sister (Z1), however, lacked such episodes during the newborn period, but at age 7 she was diagnosed with idiopathic thrombocytopenic purpura. We cannot address why differences in clinical onset exist in siblings, or even assume that they have the same degree of deficiency of ADAMTS-13 activity. However, this experience indicates that pregnancy aggravates the clinical or subclinical condition of TTP-HUS, presumably triggered by an increased amount of VWF together with a higher ratio of UL-VWFM than in the nonpregnant state. From this point of view, we are interested to know whether the fact that ADAMTS-13 activity decreases to roughly half that of normal controls in the third trimester of gestation is a cause or result of this mechanism.[24]

Etiology Undetermined

We investigated 12 patients from six unrelated families with familial HUS (Table 3). All the patients, except one, had mild-to-moderately decreased ADAMTS-13 activity (<50% of the normal controls) in their plasma. In one family (data not shown), the proposita had 22% of normal plasma ADAMTS-13 activity and a cousin of her mother had merely 5%. Determination of both the *ADAMTS13* gene mutations and the plasma factor H levels[25-27] is currently being carried out in these patients.

Acquired TTP-HUS

Hemolytic-Uremic Syndrome

Of 44 cases of acquired HUS, 34 cases were idiopathic and 10 were secondary, seven of which were *E coli* 0-157:H7–associated and three mitomycin-associated (Tables 1 and 3). None of the patients with acquired HUS had a severe deficiency of ADAMTS-13 activity, five patients showed moderately decreased activity (3% to 25%), 24 patients had mildly decreased activity (26% to 50%), and 15 patients demonstrated normal activity (>50%). No inhibitor of ADAMTS-13 activity was detected in any of these patients (data not shown).

Thrombotic Thrombocytopenic Purpura

A total of 213 patients were diagnosed with TTP (Table 4). Of these, 108 were idiopathic and 105 were secondary. Secondary TTP was categorized according to underlying disease as follows: (1) autoimmune disease (n = 43), (2) malignancy (n = 21), (3) stem

Table 4. ADAMTS-13 Activity and Its Inhibitor of the Patients With Acquired TTP

	TTP (n = 213)						
		Secondary (n = 105)					
ADAMTS-13 Activity (%)	Idiopathic (n = 108)	Autoimmune Disease (n = 43)	Malignancy (n = 21)	Stem Cell Transplantation (n = 19)	Pregnancy (n = 9)	Drugs (n = 8)	Others (n = 5)
<3	56	10	1	1	5	8	1
3-25	50	16	9	8	3	0	3
26-50	2	8	8	4	1	0	1
>50	0	9	3	6	0	0	0
Inhibitor (Bethesda U/mL)	(n = 68)*	(n = 27)*	(n = 7)*	(n = 9)*	(n = 6)*	(n = 8)*	(n = 2)*
<0.5	14	14	6	8	2	0	0
0.5-2	33	10	1	1	3	5	2
>2	21	3	0	0	1	3	0

* The inhibitor was looked for in 127 of 213 patients.

cell transplantation (n = 19), (4) pregnancy (n = 9), (5) drugs (n = 8), and (6) other causes (n = 5).

Idiopathic TTP

Of 108 patients in this category, 56 had severely decreased ADAMTS-13 activity (<3% of normal), 50 had moderately decreased activity, and two mildly decreased activity (Table 4). A plasma inhibitor level of more than 0.5 Bethesda U/mL was detected in 54 of 68 patients tested, but the remaining 40 patients have not been tested yet. From these 68 patients, 64 samples of purified IgG from their plasma were found to have inhibitory activity for ADAMTS-13, confirming that most patients with idiopathic TTP have the IgG-type autoantibody (data not shown).

To evaluate the relationship between plasma ADAMTS-13 activity before treatment and clinical outcome, we chose one hospital where 18 patients were treated with the same strategy[15] (Table 5). All but one were treated with plasma exchange and steroid therapy. Only one patient (no. 9) had a relapsing clinical course afterward, and therefore received splenectomy. Ten of 12 patients with severe deficiency of ADAMTS-13 activity due to the presence of an inhib-

Table 5. Relationship Between ADAMTS-13 Activity/Its Inhibitor Before Treatment and Clinical Outcome in the Patients With Acquired "Idiopathic" TTP

Patient No.	Age (yr)	Sex	ADAMTS-13 Activity (%)	Inhibitor (Bethesda U/mL)	Therapy	Outcome
1	43	F	<3	1.1	PE/ST/AT	CR
2	45	F	<3	1	PE/ST/AT	CR
3	34	F	<3	5	PE/ST	CR
4	61	M	<3	1.6	PE/ST/AT	CR
5	64	F	<3	1	PE/ST/AT	CR
6	34	F	<3	1.6	PE/ST/AT	CR
7	14	F	<3	3.2	PE/ST/VCR	CR
8	79	M	<3	0.9	PE/ST/AT	CR
9	16	F	<3	55	PE/ST/AT/SP	CR
10	49	M	<3	4.5	PE/ST/AT	CR
11	41	M	<3	4	PE/ST/AT	Death
12	72	M	<3	8	PE/ST	Death
13	55	F	6	<0.5	PE/ST	CR
14	59	M	12	1	PE/ST/AT	CR
15	51	F	24	<0.5	PE/ST/AT	Death
16	72	F	25	<0.5	PE/ST	Death
17	67	M	26	<0.5	PE	Death
18	48	M	28	<0.5	PE/ST/AT	Death

Abbreviations: PE, plasma exchange; ST, steroid therapy; AT, antiplatelet medicine; VCR, vincristine; SP, splenectomy; CR, complete remission.

itor showed a complete remission on this regimen. However, only two of six patients with mild-to-moderately decreased ADAMTS-13 activity showed a complete remission. It is notable that four patients who died had no inhibitor against ADAMTS-13.

Further, we have previously reported a rare case of acquired TTP in a 9-month-old girl with high titers of inhibitor against ADAMTS-13 (>100 Bethesda U/mL).[28] She had diarrhea associated with Rota virus infection on admission, but its relationship with TTP was unclear; thus she was categorized as "idiopathic." Soon after admission, she was in a critical condition characterized by generalized convulsion followed by right-hemiplegia associated with left-brain infarction. Extensive treatment with plasma exchange and steroid pulse therapy saved her life, and now almost 4 years later she is in good health without significant sequelae.

Secondary TTP

Autoimmune disease. There are 43 patients in this category with the following disease distribution: systemic lupus erythematosus (n = 17), systemic sclerosis (n = 7), mixed connective tissue disease (n = 5), antiphospholipid syndrome (n = 5), polymyositis (n = 3), polyarteritis nodosa (n = 2), rheumatoid arthritis (n = 1), Sjögren's syndrome (n = 1), Goodpasture's syndrome (n = 1), and vasculitis syndrome (n = 1). Ten of these patients had a severe deficiency of ADAMTS-13 activity (<3% of the normal), and 24 patients had mild-to-moderately decreased activity. The remaining nine patients had normal activity. The inhibitor to ADAMTS-13 was detected in 13 of 27 patients tested, whereas the remaining 14 patients had no inhibitor. Interestingly, of the 10 patients with severe deficiency of ADAMTS-13 activity who were treated with plasma exchange, only one died. In contrast, of the remaining 33 patients with mild-to-moderately decreased or normal activity of ADAMTS-13, 11 patients died despite receiving plasma exchange (data not shown). This finding appears to coincide with idiopathic TTP.

Malignancy. Of 21 patients in this category, hematological malignancy occurred in 12 as follows: lymphoma with leukemic phase (intravascular lymphoma) (n = 4), myelodysplastic syndrome (n = 3), acute myeloblastic leukemia (n = 1), acute lymphoblastic leukemia (n = 1), chronic myeloblastic leukemia (n = 1), non-Hodgkin's lymphoma (n = 1), plasmacytoma (n = 1). The remaining nine were gastric cancer (n = 3), lung cancer (n = 2), breast cancer (n = 1), cholangiocarcinoma (n = 1), ovarian cancer (n = 1), and Ewing's sarcoma (n = 1). Except for one, the patients in this category showed neither severe deficiency of ADAMTS-13 activity nor its inhibitor. One noteworthy case was a patient with intravascular lymphoma who showed severe thrombocytopenia and neurological signs on admission, accompanied by a deficiency of ADAMTS-13 activity (<3% of the normal) due to the presence of its inhibitor (1.4 Bethesda U/mL). These symptoms were successfully treated by a combination drug therapy including anti-CD20 chimeric monoclonal antibody (rituximab), but without plasma exchange (unpublished data).

Stem cell transplantation. Nineteen patients with stem cell transplantation–associated TTP were included: bone marrow transplantation (n = 9), peripheral blood stem cell transplantation (n = 7), and cord blood stem cell transplantation (n = 3). Of these, only one patient showed a severe deficiency of the ADAMTS-13 activity due to its inhibitor, eight had mild-to-moderately decreased activity without the inhibitor, and the remaining six showed normal activity. Nine of 19 patients died, with or without plasma exchange. Our findings appear to correlate well with a 1999 report.[29]

Pregnancy. There were nine patients in this category, five of whom developed TTP at 10 to 30 weeks of gestation; the remaining four had clinical signs within 3 weeks after delivery. Five of the nine patients had severe deficiency of ADAMTS-13 activity, and four had mild-to-moderately decreased activity. The inhibitor to ADAMTS-13 was positive in four patients who showed severe deficiency of enzyme activity. All patients except one were successfully treated with plasma exchange, with normalization of defective ADAMTS-13 activity, totally excluding the possibility of USS in these patients. One patient who died had 22% ADAMTS-13 activity without its inhibitor before treatment, but did not recover after extensive plasma exchange (22 times).

Drugs. Of eight patients in this category, seven were associated with ticlopidine,[30,31] and one was assumed to be related to sildenafil. All patients had severe deficiency of ADAMTS-13 activity due to the presence of its inhibitor. All patients with ticlopidine-associated TTP recovered in response to plasma exchange, and the one patient with sildenafil-associated TTP without neurological signs recovered after antiplatelet therapy (dipyridamole) without plasma exchange.

Other causes. Five patients were excluded from the previous categories. Three of the cases were associated with liver cirrhosis. They had less than 3%, 18%, and 32% of ADAMTS-13 activity, respectively; the patient with severely deficient activity had the inhibitor. The fourth patient was a child with an ADAMTS-13 activity of 14%, who received living-related liver transplantation; the fifth patient had Duchenne muscular dystrophy with an activity of 21%.

Conclusion

Based on our assays for measuring ADAMTS-13 activity and its inhibitor, we can tentatively summarize our results as follows: (1) Patients with a severe deficiency of ADAMTS-13 activity (<3% of the normal) due to the presence of its inhibitor are high responders to plasma exchange. A majority of patients diagnosed as having "idiopathic," pregnancy- or drug-associated TTP, belong to this group. (2) Patients with mild-to-moderately decreased (3% to 50%) or roughly normal (>50%) levels of ADAMTS-13 activity without its inhibitor appear to be low responders to plasma exchange. Most patients with malignancy- and stem cell transplantation–associated TTP belong to this group. (3) Patients with autoimmune disease–associated TTP fit into both groups.

Our experience suggests that plasma exchange for TTP patients with ADAMTS-13 inhibitors appears to have the following five effects[32]: (1) removal of ADAMTS-13 inhibitor, (2) removal of UL-VWFM, (3) supply of ADAMTS-13, (4) supply of VWF required for normal hemostasis, and presumably (5) reduction of various cytokines, which induce endothelial cell damage and platelet activation.[33,34]

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Diffuse neurodeficits in intravascular lymphomatosis with ADAMTS13 inhibitor

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Abstract—Inhibitory antibody to von Willebrand factor (vWF)-cleaving protease (ADAMTS13) was detected in a patient with intravascular lymphomatosis. The increased serum level of the antibody paralleled an increase in the expression of uncleaved vWF, which might cause microvascular thrombosis and platelet consumption. Malignant cell proliferations with superimposed thrombosis within the lumina throughout the entire vasculature account for diffuse neurodeficits observed in the patient.

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Intravascular lymphomatosis (IVL) is a rare, high mortality disease characterized by vascular occlusions with malignant cells and commonly presenting with diffuse CNS manifestations.[1] Most patients are diagnosed postmortem because of rapidly progressive and misleading clinical features that mimic degenerative dementia, vasculitis, stroke, infections, or other neoplasms.[2] Several authors have reported dramatic responses of the clinical symptoms to plasmapheresis, suggesting the presence of antibodies or other factors in the patient's serum.[3] However, a humoral mechanism in this disease is unclear. The present article concerns a patient with IVL with clinical and laboratory findings of thrombotic thrombocytopenic purpura (TTP). We demonstrated the presence of an inhibitory autoantibody to von Willebrand factor (vWF)-cleaving protease (ADAMTS13), and our data suggest that malignant cell proliferations with superimposed thrombosis may cause multiple neurodeficits in a patient with IVL.

Case report. A 54-year-old man was hospitalized because of 2 months of decreased motivation, disorientation, and increasing generalized convulsive seizures. On admission, the patient was febrile with somnolence but had no adenopathy or skin lesions. A neurologic examination revealed left hemiparesis, vertical gaze palsy, dysphagia, and dysarthria. Brain MRI showed scattered high-intensity lesions in the white matter on T2-weighted images without enhanced gadolinium–diethylenetriamine penta-acetic acid imaging (figure 1A). These neurodeficits and radiologic findings were consistent with demyelinating diseases. The patient received a 5-day course of methylprednisolone (1000 mg/d), which temporally shrunk the subcortical lesions without marked improvements of clinical symptoms. Perfusion MRI revealed widespread reduction in cerebral blood flow in the right parietal and left frontal lobes and brainstem (figure 1, D through F). EEG showed a moderate slowing of background activity and focal delta activity over the right parietal area. Laboratory studies during the first week in the hospital showed anemia (hemoglobin, 11.1 g/dL), decreased platelet count (3.6×10^{10}/L), and elevated soluble interleukin-2 receptor (sIL-2R, 1,510 U/mL), but normal findings for the leukocyte count, erythrocyte sedimentation rate, serum lactate dehydrogenase (LDH), albumin, and electrolytes. CSF was normal in pressure, glucose concentration, cell counts, and cytologic findings except for an elevated protein concentration (73 mg/dL). Bone marrow aspiration was normocellular without hemophagocytosis and atypical cells. On day 7 after admission, the platelet count decreased to 2.3×10^{10}/L, with normal level of serum fibrinogen degradation products, presumably eliminating disseminated intravascular coagulation (DIC). On day 12, prophylactic platelet transfusions increased the convulsions and respiration difficulty, which prevented execution of a brain biopsy, but, at the same time, reminded us of the aggravation of underlying TTP. The plasma ADAMTS13 activity severely decreased (<3% of the normal control), and heated plasma (1.4 Bethesda units/mL) and purified immunoglobulin (Ig) G were positive for ADAMTS13 autoantibody (figure 2). These clinical and laboratory findings strongly suggested acquired TTP but not as a causative disease. On day 17, brain MRI demonstrated massive lesions with hematoma and surrounding edema (figure 1, B and C) with elevated serum levels of LDH (1,098 IU/L) and sIL-2R (12,400 U/mL). The patient developed tachypnea, unresponsiveness to painful stimulation, and bilateral adrenal gland tumors (right, 6.6 × 4.1 cm; left, 3.5 × 2.4 cm) on abdominal CT scans. We tentatively diagnosed the patient with IVL, obtained informed consent from the family members, and began the combination chemotherapy (CHOP), cyclophosphamide, doxorubicin, vincristine, and prednisolone, followed by 2 days of infusion with 5 units of fresh-frozen plasma. The serum LDH and sIL-2R decreased, and the platelet count normalized at 7 days after the start of treatment. The six courses of biweekly CHOP therapy remarkably improved the symptoms, with only inferior quadrantanopsia left. The increased ADAMTS13 activity paralleled the antibody reduction and clinical improvement. Skin, lung, and liver biopsies during the clinical course were unsuccessful in determining IVL, and only transbronchial lung biopsy performed on day 343 after admission revealed atypical cells within the lumina of the venules. The cells were positive for CD20 (figure 3, A and B), confirming B-cell lineage IVL underlying acquired TTP. Aggressive combination chemotherapy and immunotherapy, CHOP, ESHAP (i.e., etoposide, methylprednisolone, cytarabine, and cisplatin), autologous peripheral blood stem cell transplant, and rituximab (anti-CD20 monoclonal antibody) have prolonged a symptom-free survival time that currently exceeds 20 months.

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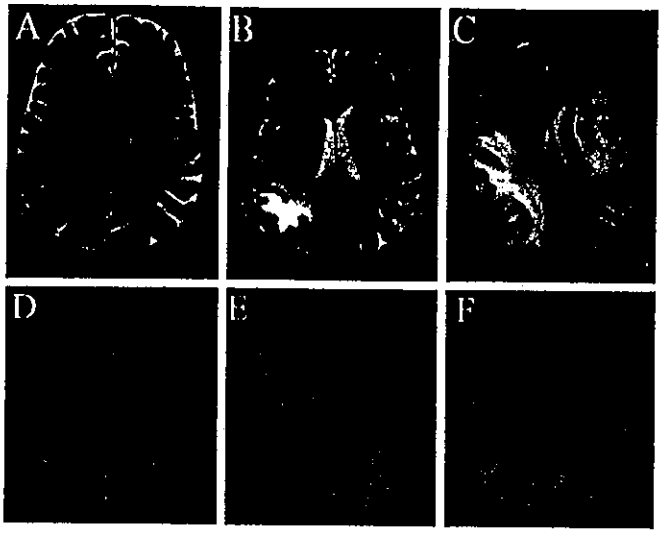

Figure 1. T2-weighted MRI shows scattered high-intensity lesions in the subcortical white matter on day 3 (A) and enlarged lesions with surrounding edema and hematoma on days 10 (B) and 17 (C). Perfusion MRI shows a widespread reduction in brain blood flow on day 3 (D–F).

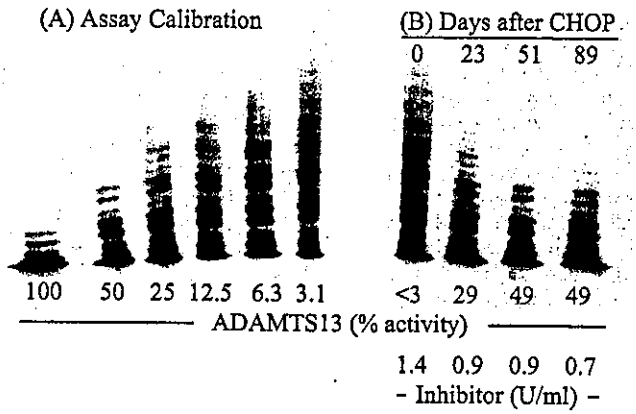

Figure 2. Activity of ADAMTS13 and the level of its inhibitor in the patient with intravascular lymphomatosis. An assay calibration of plasma ADAMTS13 activity was performed from pooled normal human plasma. Serially diluted plasma was incubated with purified von Willebrand factor (vWF), and the reaction mixture was electrophoresed on a 1.4% sodium dodecyl sulfate gel and subjected to Western blot analysis. The extent of vWF degradation was visualized by luminography and scanned by a densitometer. A value of 100% activity was defined as the amount of ADAMTS13 contained in 1 mL of the normal plasma (A). The extent of vWF degradation paralleled the increase of ADAMTS13 activity and the decrease of its inhibitor in the patient on days 0, 23, 51, and 89 after the start of CHOP therapy. The inhibitor activity was determined by measuring ADAMTS13 activity in a mixture of heat-inactivated plasma from the patient and normal subjects. One unit of the inhibitor was defined as the amount inhibiting 50% of ADAMTS13 activity of the control based on the Bethesda method (B). The methods were detailed in our previous report.[10]

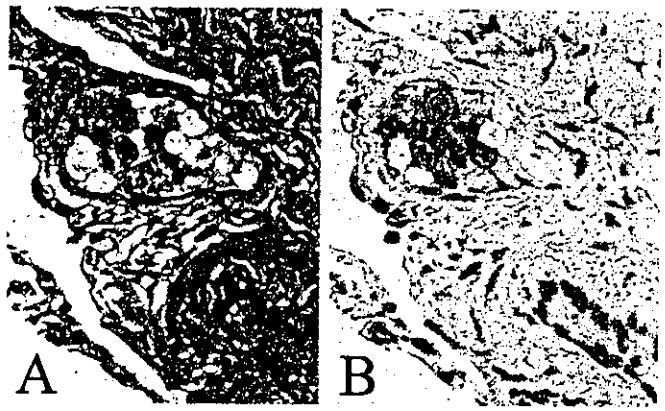

Figure 3. Transbronchial lung biopsy demonstrates infiltration of atypical cells in the lumina of the venules. (H-E, ×400; A). The atypical cells are positive for CD20 (immunohistochemical staining for CD20, ×400; B).

Discussion. A definitive diagnosis of IVL was largely delayed in our patient because of rapidly progressive and confusing clinical findings with severe thrombocytopenia. The temporal reduction of MRI signals mimicking multiple demyelination (e.g., multiple sclerosis [MS]) with steroid treatment, and clinical exacerbation by the platelet transfusion resulted in complications in diagnosis. Several authors reported difficulties in differentiating CNS lymphomas from MS,[4] and IVL was not weighted in our patient. More focal EEG and circumscribed MRI changes in the right hemisphere reflecting a local maximum thrombosis with infarctions or hemorrhages indicated the complicated pathologic process occurring within the CNS of this patient. These symptoms are indistinguishable from those of TTP, of which triad, anemia, thrombocytopenia, and bizarre neurologic abnormalities have been observed in 74% of the patients with TTP.[5]

In most patients with IVL, the vascular occlusions responsible for multiple neurodeficits have been explained by the malignant cell proliferations within the lumen of the small vessels. Several authors have proposed that antibody or other serum factors cause capillary sludging based on the dramatic relief of symptoms with plasmapheresis.[3] However, such a humoral mechanism remains unclear. The present patient is the first IVL case with detectable ADAMTS13 autoantibody, which may explain the pathophysiology of thrombocytopenia and neurodeficits. In a normal individual, vWF is secreted from endothelial cells in an unusual multimeric form (UM-vWF) that is rapidly cleaved into smaller vWF by ADAMTS13.[6] In our patient, uncleaved vWF multimers are released into plasma as UM-vWF, which spontaneously reacts with platelets and leads to microvascular thrombosis and platelet consumption. This pathomechanism is supported by adverse effect of the platelet transfusion and the serial relationship of the reduction in the ADAMTS13 inhibitor and the improvement in the clinical and laboratory findings. It is noteworthy that TTP showed the occlusive mi-

croangiopathy preferentially localized to terminal arterioles and capillaries, whereas IVL showed occlusive microangiopathy in venules.[7] Thus, malignant cell proliferation with superimposed thrombosis within the lumina throughout the whole vascular system may account for the diffuse and severe vascular occlusions in our patient with IVL and TTP symptoms. This explanation is supported by the widespread reduction in cerebral blood flow on the perfusion MRI. The association of thrombocytopenia and IVL has been reported, but the pathomechanism of thrombocytopenia remains unclear except for a minor population of patients with bone marrow infiltration of malignant cells, hemophagocytic syndrome, or DIC. In this study, we have demonstrated that IVL is another cause of TTP symptoms. Accumulated data have shown that the mortality of patients with TTP exceeds 90% without plasmapheresis, currently the most effective treatment for patients with TTP.[8] Our patient received no plasmapheresis but recovered with the combination of chemotherapy and immunotherapy. The reduction in ADAMTS13 antibody titers also paralleled the clinical improvement. These findings suggest that the malignant B-lymphoma cells, which may produce the IgG-type autoantibody against ADAMTS13, were deeply related to thrombosis in our patient.

Neurologists should consider IVL in any patient with diffuse neurodeficits because, if diagnosed at the beginning of the disease, aggressive chemotherapy potentially prolongs the lifespan. In a review of 77 patients with IVL and nervous system involvement, only 29% were diagnosed antemortem.[9] Brain and adrenal gland biopsy is reportedly diagnostic but rarely made because the symptoms give few hints as to the nature of the underlying disease. Under these circumstances, the rarity of IVL makes anecdotal case reports useful to advance knowledge concerning diagnosis and treatment, especially with the pathogenesis of accompanied thrombocytopenia.

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Regular Article

Elevated plasma levels of fibrin degradation products by granulocyte-derived elastase in patients with deep vein thrombosis

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Abstract Plasma levels of granulocyte-derived elastase (GE-XDP), D-dimer and soluble fibrin (SF) were examined in 53 patients with deep vein thrombosis (DVT) and in 100 healthy volunteers. The mean plasma level of D-dimer was 0.92±0.81 μg/ml (±S.D.) in healthy volunteers and the mean+2 S.D. value (cutoff value for DVT) was 2.53 μg/ml, which was higher than that used in Europe and North America. Plasma levels of GE-XDP, D-dimer and SF were significantly higher in patients with DVT than in healthy volunteers, and diminished after 1 week of treatment with heparin, urokinase or tissue type plasminogen activator, though were still higher than those of the control subjects. The sensitivity of GE-XDP, D-dimer and SF for DVT was 81.1%, 75.5% and 79.2%, respectively. GE-XDP levels correlated with those of D-dimer and SF. Our results indicate that GE-XDP is a potentially useful marker for the diagnosis of DVT, suggesting that granulocytes are activated in patients with DVT. In our system, the cutoff value of D-dimer for the diagnosis of DVT is higher than in western countries, probably due to the use of different analytical assays.

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Introduction

Granulocyte-derived elastase (GE) is released extracellularly in response to various stimuli such as endotoxins and cytokines [1]. Released GE may degrade components of the extracellular matrix, such as elastin and various proteoglycans, as well as plasma proteins including fibrin(ogen) [2,3] and is thus implicated in the pathogenesis of a wide variety of diseases. Although GE is regulated predominantly by α1-proteinase inhibitor (α1-PI) in vivo, the granulocyte-associated elastase has been shown to be more resistant to α1-PI than free elastase [4-6]. GE is released into the blood circulation where it can digest fibrinogen and fibrin clots in certain diseases such as disseminated intravascular coagulation (DIC) [7,8], before it is neutralized by α1-PI. Thus, GE plays a role in fibrin(ogen) degradation in vitro [9] as well as in vivo [10].

Cross-linked fibrin degradation products (XDP) resulting from cleavage by plasmin are not uniform in their molecular structure. They include D-dimer, which contains D-D domains. D-dimer is a marker of endogenous fibrinolysis and reported to be increased in patients with DIC [11], deep vein thrombosis (DVT)/pulmonary embolism (PE) [12] and acute myocardial infarction [13]. D-dimer is adopted in the diagnostic criteria of overt-DIC by the International Society of Thrombosis and Haemostasis [14]. D-dimer is also reported to be a negative predictor for DVT [15]. Plasma levels of soluble fibrin (SF) were reported to be significantly high in patients with DIC [16]. Since PE is a fatal disease caused by DVT, evaluation of DVT [15] and PE [17] is important.

The present study was designed to evaluate the usefulness of GE-XDP, D-dimer and SF in the diagnosis of DVT and PE. For this purpose, we determined plasma levels of these molecules in 53 patients with DVT or PE and 100 healthy volunteers.

Materials and methods

Subjects

From January 1, 2001 to December 31, 2003, 53 patients were admitted to Mie University School of Medicine and diagnosed with deep vein thrombosis or pulmonary embolism (mean age: 53.2 years, range: 19-80 years; 35 females and 18 males). There were 11 patients with malignant disease, 6 patients after operation, 3 with autoimmune disease, 2 with trauma, 1 with encephalitis, 1 with pregnancy and 1 with protein S deficiency, and 19 patients without underlying disease.

Patients were treated with 20,000-40,000 units of heparin and urokinase or tissue type plasminogen activator (t-PA). Plasma levels of SF, D-dimer and GE-XDP were measured in these patients at the onset of DVT or PE (baseline) and at 1 week after onset (posttreatment). The same parameters were also measured in 100 healthy volunteers (HV: mean age: 41.5 years, range: 20-58 years; 47 males and 53 females) as control. The study protocol was approved by the Human Ethics Review Committees of participating institutions and a signed consent form was obtained from each subject.

Measurement of plasma concentrations of GE-XDP, D-dimer and soluble fibrin

GE-fibrin degradation products (GE-XDP) were measured by latex-agglutination assay using monoclonal antibody IF-123. IF-123 specifically recognizes elastase-digests of human fibrinogen and fibrin, but not their plasmin-digests. The epitope for this antibody is located at Aα Leu-196 to Ile-204 peptide segment [18]. For D-dimer determination, JIF23 monoclonal antibody, which recognizes plasmin digested N-terminus of γ chain on the D region, was used for latex agglutination [19]. SF was also determined by latex agglutination method using monoclonal antibody IF-43. Monoclonal antibody IF-43, which recognizes a segment of the fibrin Aα chain [(Aα-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, is coated for SF assay [20].

Statistical analysis

Data are expressed as mean±S.D. Differences between groups were examined for statistical significance using the Mann-Whitney's *U*-test, while correlation between two variables was tested by Pearson's correlation analysis. A p-value less than 0.05 denoted the presence of a statistically significant difference. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

Results

Plasma levels of GE-XDP of patients with DVT at baseline (16.8±24.5 U/ml) were significantly

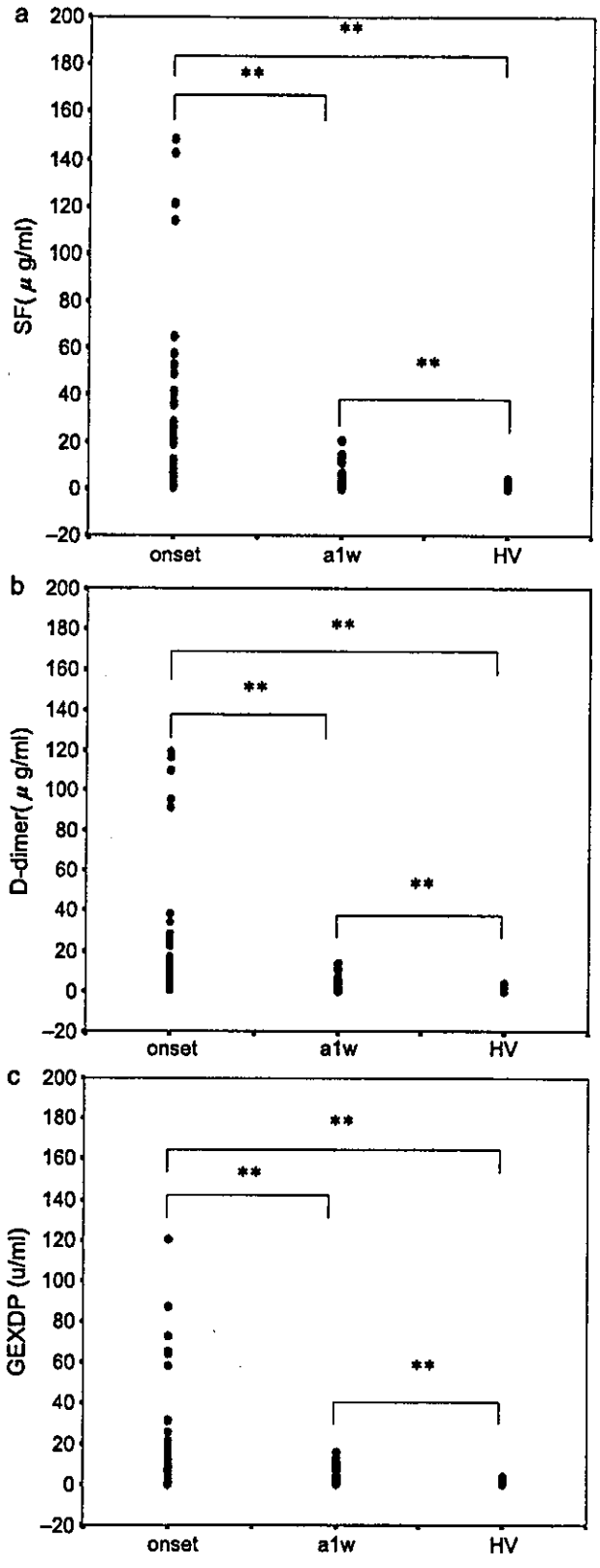

Figure 1 Plasma levels of SF, D-dimer and GE-XDP in patients with DVT. a1w: 1 week after onset of DVT; HV: healthy volunteers; **: $p<0.01$.

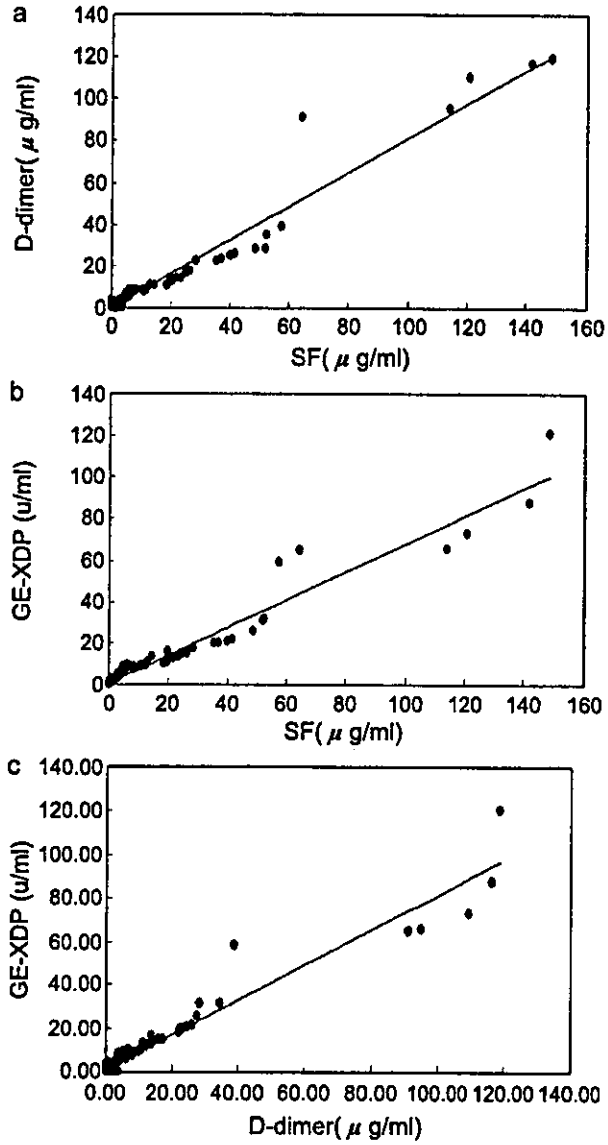

Figure 2 Relationships among SF, D-dimer and GE-XDP in patients with DVT.

higher than those of 1-week posttreatment (4.07±4.02 U/ml, $p<0.01$) and healthy volunteers (0.53±0.94 U/ml, $p<0.01$) (Fig. 1a). Plasma levels of D-dimer of patients with DVT at baseline (19.1±30.1 μg/ml) were significantly higher than those of 1-week posttreatment (2.79±3.32 μg/ml,

Table 1 Relationships among GE-XDP, D-dimer and SF

	GE-XDP		D-dimer		SF	
	r	p level	r	p level	r	p level
GE-XDP			0.972	<0.01	0.975	<0.01
D-dimer	0.972	<0.01			0.977	<0.01
SF	0.975	<0.01	0.977	<0.01		

r=correlation coefficient.

$p<0.01$) and healthy volunteers (0.92±0.81 μg/ml, $p<0.01$) (Fig. 1b). Plasma levels of SF of patients with DVT at baseline (24.4±35.4 μg/ml) were significantly higher than those of 1-week posttreatment (3.58±4.62 μg/ml, $p<0.01$) and of healthy volunteers (0.54±0.94 μg/ml, $p<0.01$) (Fig. 1c). Plasma levels of GE-XDP, D-dimer and SF were significantly higher in patients with DVT at 1-week posttreatment than healthy volunteers ($p<0.01$). Plasma levels of GE-XDP, D-dimer and SF were significantly correlated with each other ($p<0.01$, Fig. 2a-c), and the correlation coefficients were markedly high (Table 1). A cutoff value was selected for every parameter and represented the mean value+2SD. Accordingly, the cutoff values of GE-XDP, D-dimer and SF for DVT were 2.41 U/ml, 2.53 μg/ml and 2.42 μg/ml, respectively. The sensitivity of GE-XDP, D-dimer and SF for DVT was 81.1%, 75.5% and 79.2%, respectively.

Discussion

In our healthy subjects, the mean±S.D. level of plasma D-dimer was 0.92±0.81 μg/ml with a range of 0.10-2.42 μg/ml. In Europe and North America, less than 0.5 μg/ml of D-dimer is considered to exclude DVT/PE [15]. However, in Japan, the D-dimer value in many patients is more than 0.5 μg/ml and this cutoff value is not useful as a negative predictive value for DVT/PE in Japan. The underlying difference in the normal value of D-dimer between Japan and Europe/North America may be differences in assay kits, of standard substances or represent racial differences. Since D-dimer is currently widely used clinically as a parameter for detection of in vivo fibrin formation, the issue of standardization of D-dimer assays remains to be resolved. Several studies [21,22] were designed to generate basic data for standardization of D-dimer. In general, the smallest detection concentration is lower in enzyme-linked immunosorbent assay (ELISA) than in latex agglutination assay, which is frequently used in Japan. In this regard, latex agglutination assay is easier assay and the results become available more rapidly than ELISA (Table 2).

Baseline plasma levels of GE-XDP, D-dimer and SF of patients with DVT were significantly higher than the control. The high plasma levels of D-dimer and SF in patients with DVT were expected because previous studies considered both parameters as markers of hypercoagulable state and were reported to be elevated in DVT [12,23], DIC [24,25] and hyperlipidemia [26]. The high plasma levels of GE-XDP are probably due to activation of granulocytes and have been reported to be elevated in DIC [27]. Our findings suggest that granulocytes are activated in patients with DVT/PE and that activated granulocyte may play an important role in the onset/pathology of DVT and PE. Our results also showed that plasma levels of GE-XDP, D-dimer and SF were significantly correlated with each other. Although plasma concentrations of SF reflect thrombin generation, those of GE-XDP and D-dimer reflect generation of thrombin, plasmin and GE in the plasma. In patients with DVT, secondary fibrinolysis may immediately occur after fibrin clot formation.

Table 2 Evaluation of SF, D-dimer and GE-XDP for the diagnosis of DVT/PE

	SF	D-dimer	GE-XDP
Sensitivity (%)	79.2	75.5	81.1
Specificity (%)	93.0	95.0	95.0
Positive predictive value (%)	85.7	88.9	89.6
Negative predictive value (%)	89.4	90.7	90.5
Odds ratio	0.019	0.017	0.012

The sensitivity of GE-XDP, D-dimer and SF for DVT was 81.1%, 75.5% and 79.2%, respectively. In about 20% of patients with DVT, the levels of these markers were lower than the cutoff values, suggesting that the cutoff values are too high, or that the size of DVT is small or that blood-sampling time is different from the actual time of onset of DVT. Plasma levels of D-dimer and SF were still significantly higher in patients with DVT at 1-week posttreatment, relative to the normal control, suggesting that the hypercoagulable state persisted up to 1 week after treatment. It is reasonable that heparin treatment was continued up to 1 week after the onset of DVT/PE. D-dimer might be released from thrombi undergoing fibrinolysis and thus these molecules may not only reflect the onset of DVT but also secondary fibrinolysis of DVT.

Our results indicate that GE-XDP is a potentially useful marker for the diagnosis of DVT, suggesting that granulocytes are activated in patients with DVT. In our system, the cutoff value of D-dimer for the diagnosis of DVT might be higher than that used in other reports.

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