Table 1. Patient Characteristics[a]

	Steroid group	Nonsteroid group	*P* value
n	18	7	
Men/women (no)	14/4	5/2	NS
Age (years)	58 ± 16	58 ± 20	NS
BMI (kg/m^2)	22.8 ± 3.0	20.9 ± 1.5	NS
Duration (year)	2.7 ± 2.0	5.0 ± 3.5	NS
Steroid dosage			
Total dose (mg)	5233 ± 3541		
Therapy duration (day)	714 ± 717		
Daily dose (mg)	9.4 ± 5.2		
Platelet count (×10^4/mm)	11.8 ± 8.8	6.8 ± 3.3	NS
Total number of patients			
For osteoporosis by VD3	8	0	

NOTE: BMI = body mass index.
[a] Data are shown as mean ± SD. *P* value, steroid group versus nonsteroid group.

Table 2. Distribution of Bone Mineral Density[a]

		Steroid group	Nonsteroid group
Normal	>80%* of YAM	6 (33.3)	5 (71.4)
Osteopenia	70>, <80%* of YAM	5 (27.8)	1 (14.3)
Osteoporosis	<70%* of YAM	7 (38.9)	1 (14.3)

NOTE: YAM = Young Adult Mean (mean value of women aged 20-44 years)
( ): %
[a] Primary osteoporosis is classified according to the diagnostic criteria for this disease: the presence of a prior fracture and the percentage value of bone density compared with YAM. Mann-Whitney *U* test: NS. *T score (%) = (Observed value /YAM) × 100.

latter (0.75 ± 1.02). In the examination of the BMD as a function of the average daily steroid dose, the BMD was 0.59 ± 1.30 in the group receiving less than 5 mg daily, −0.43 ± 0.86 in the group receiving 5 to 10 mg daily, −0.65 ± 0.88 in the group receiving 10 to 15 mg daily, and −1.44 ± 1.77 in the group receiving more than 15 mg daily (Figure 1).

In regard to the relationship between the total steroid dose and the bone mass, the bone mass decreased as the total steroid dose increased. A negative correlation was observed between the total steroid dose and the bone mass (Figure 2).

In the relationship between the daily steroid dose and the bone mass, the bone mass decreased as the daily steroid dose increased. In addition, a negative correlation was observed between the daily steroid dose and the bone mass (Figure 3).

Alendronate was administered as the drug of first choice to patients recommended treatment for osteoporosis based on the guidelines of the Japanese Society for Bone and Mineral Metabolism, and its effects were examined. The serum level of the bone metabolism marker BAP was significantly lower in the group treated with alendronate than in the 5 patients in the steroid treatment group not treated with alendronate (28.1 ± 10.7 vs 44.7 ± 29.4, $P < .05$).

No differences were observed in the platelet count at the start of the drug administration and at 6 months and 12 months

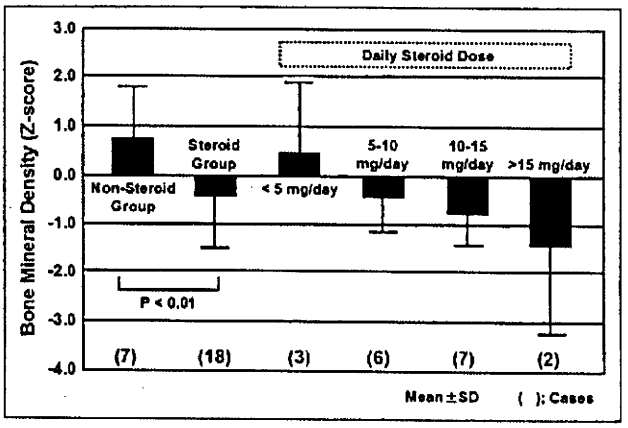


Figure 1. Impact of steroid treatment on bone mineral density (BMD). Mean level of BMD was decreased in steroid-treated patients compared with steroid nontreated patients.

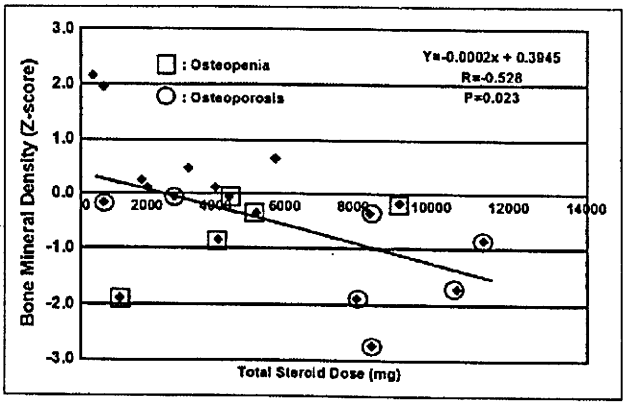


Figure 2. Correlation between total steroid dose and bone mineral density (BMD). Negative correlation between BMD and total steroid dose.

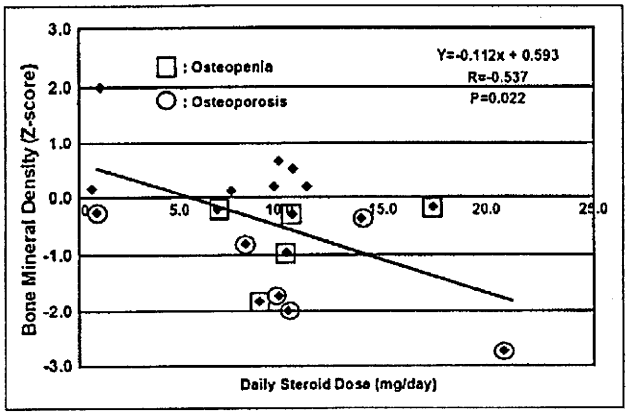


Figure 3. Correlation between daily steroid dose and bone mineral density (BMD). Relationship between BMD and daily steroid dose revealed negative correlation.

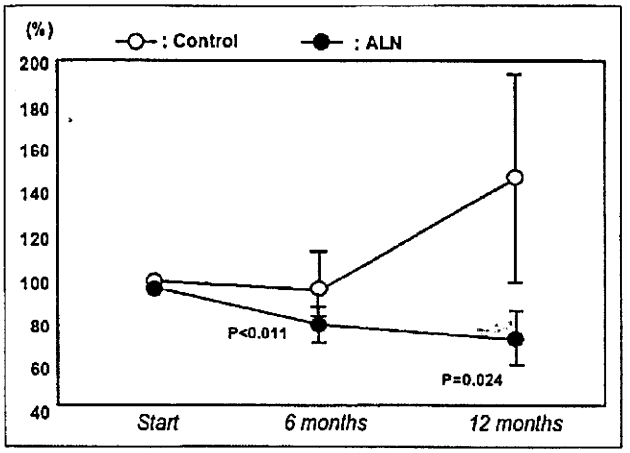


Figure 4. u-NTX%. u-NTX did not increase in alendronate group.

after the start of the drug administration between the alendronate group and the control group (data not shown).

In the alendronate group, the bone mass was $-2.4 \pm 1.3\%$ relative to the young adult mean (YAM) at the start of alendronate therapy but increased significantly to $2.8\% \pm 1.4\%$ at 6 months and $3.0\% \pm 5.4\%$ at 12 months after the start of treatment. The urinary level of the NTX, a bone resorption marker demonstrated an upward trend in the control group, while it decreased significantly in the alendronate group to $75.3\% \pm 37.8\%$ at 6 months and 71.7 ± 44.9 at 12 months after the start of alendronate therapy, relative to the level at the start of the drug administration (Figure 4).

The serum level of BAP decreased at 6 months and at 12 months after the start in both the control and the alendronate group and no differences were observed within both groups (data not shown).

Discussion

The secondary causes of osteoporosis have been classified into endocrine, nutritional drug-induced, immobility-related, congenital, and other causes. Glucocorticoid-induced osteoporosis is the most frequency-encountered form of secondary osteoporosis and poses a clinical problem in many cases. Rheumatoid arthritis is the most common underlying diseases causing glucocorticoid-induced osteoporosis; other common underlying diseases include collagen diseases, such as systemic lupus erythematosus, autoimmune diseases, bronchial asthma, and nephritic syndrome. According to the survey conducted by American College of Rheumatology in 1996, there are an estimated 20 million patients with osteoporosis in the United States, about 20% of which have glucocorticoid-induced osteoporosis. In regard to the underlying pathogenetic mechanism in glucocorticoid-induced osteoporosis, the direct actions of steroids on the bone are considered the most important.[13] When the administered steroid dose is relatively high, hyperparathyroidism may also be involved. Steroids act on osteoblastic cells to inhibit the formation of bone matrix, causing disorder of bone mineralization and inhibiting bone formation. In addition, inhibition of small intestinal Ca absorption and of renal tubular Ca reabsorption by steroids may lead to hyperparathyroidism, which is considered to promote bone absorption.

Van Staa et al[7] showed that the risks of bone fracture in patients with glucocorticoid-induced osteoporosis increase with escalating steroid dose and that the fracture risk is particularly high in the long bones. It has also been shown that after long-term steroid use in the elderly population, the BMD continues to decline even after discontinuation of the steroid.[8] Diagnosis and treatment guidelines were proposed in foreign countries based on these results.[14-17] According to these reports, treatment is indicated if a new bone fracture is found in a patient during oral steroid treatment for longer than 3 months or in patients with an existing fracture scheduled to receive steroid treatment. Even without fractures, if the BMD of a patient is less than 80% of the YAM value, that is, less than the bone mass loss, treatment is indicated. Treatment is also recommended for persons with normal bone mass scheduled to receive a prednisolone-equivalent dose of more than 5 mg daily. Bisphosphonates are considered the drugs of first choice for the treatment of glucocorticoid-induced osteoporosis.[18,19]

While there are numerous reports of the effects of steroid treatment on the bones in various diseases, all these are systemic diseases, and there is the possibility that the disease itself might also affect the BMD. Unlike chronic rheumatism or SLE, the clinical manifestation of ITP is limited to the decrease of the platelet count, with no systemic symptoms. Patients with ITP are thus considered the most suitable for the examination of glucocorticoid-induced osteoporosis. In this study, we conducted a retrospective examination of the effects of steroids on the bone mass in patients with ITP. The results revealed a high rate of osteopenia or osteoporosis (66.7%), diagnosed based on the diagnostic criteria for primary osteoporosis, in the group receiving steroid treatment. There were no differences in the patient background characteristics between the group

receiving steroid treatment and the group not treated with steroids; thus, it was considered that the risk of BMD loss was higher in the group receiving steroid treatment. In addition, after the elimination of confounding factors by expressing the BMD as *z* scores, which represent deviation scores from the mean values in sex- and age-matched participants, significant bone mass as compared with that in the group not treated with steroids was confirmed in the group receiving steroid treatment. The bone mass was also higher in patients receiving higher daily doses.

Based on the above, as in other diseases treated with steroids, it was concluded that the risk of bone mass loss is increased with the administration of steroids in patients with ITP. Particular attention to bone mass loss and the elevated risk of bone fractures should be paid in patients receiving large doses of steroids. Based on this background, we treated ITP patients with bone mass loss to osteopenia or osteoporosis levels with alendronate and followed their progress. Our results revealed a significant increase of the bone mass in these patients at 6 months and 12 months after the start of alendronate treatment as compared with that at the start of alendronate treatment.

Although the urinary levels of NTX, a bone resorption marker, demonstrated an upward trend in the control group, the levels decreased significantly in patients treated with alendronate, at 6 months and 12 months after the start of the treatment, relative to the levels at the start of alendronate administration. In other words, it was considered that bone resorption was inhibited by alendronate, preventing any increase in urinary NTX levels in patients treated with alendronate. However, the serum levels of the bone formation marker, BAP, also increased in the patients treated with alendronate at 6 months and 12 months after the start of the treatment. This result suggests that alendronate may have no effect on bone formation. Thus, based on our results, bisphosphonate appear to be useful for the prevention and treatment of glucocorticoid-induced osteoporosis in patients with ITP, because improvement of the serum and urinary levels of bone metabolism markers was observed following the administration of a bisphosphonate to patients with ITP on steroid treatment. Because the effects of steroids on bone are mainly exerted via effects on bone resorption, it may be reasonable to treat patients with steroid-induced osteoporosis with a bisphosphonate, which inhibits bone resorption. For patients with ITP under long-term steroid treatment or those who are scheduled for long-term treatment, aggressive treatment with a bisphosphonate may be expected to contribute to an improvement in the QOL of these patients. However, once bisphosphonate are incorporated into bone, they can remain there for up to 12 years without deteriorating.[20,21] Therefore, we should pay attention to the risk of osteonecrosis of the jaws (ONJ) due to bisphosphonate treatment.[22] Our study has some limitations: it was small sample size, and some clinical parameters were not routinely recorded. Furthermore, we could not investigate the effects of patient's sex or age in the current study. Further confirmation of these observations in larger studies would be useful.

Conclusion

Bone mass loss was confirmed in patients with ITP under long-term steroid treatment, as in patients with other diseases being treated with steroids, and thus the baseline BMDs should be measured prior to initiation of long-term steroids. In addition, alendronate may be an effective agent for the prevention and treatment of glucocorticoid-induced osteoporosis in patients with ITP scheduled to receive long-term steroid treatment.

Declaration of Conflicting Interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

References

1. Cines DB, Blanchette VS. Immune thrombocytopenic purpura. *N Engl J Med.* 2002;346(13):995-1008.
2. George JN. Management of patients with refractory immune thrombocytopenic purpura. *J Thromb Haemost.* 2006;4(8): 1664-1672.
3. Bussel JB. Treatment of immune thrombocytopenic purpura in adults. *Semin Hematol.* 2006;43(3 suppl 5):S3-S10.
4. Psaila B, Bussel JB. Refractory immune thrombocytopenic purpura: current strategies for investigation and management. *Br J Haematol.* 2008;143(1):16-26.
5. Stasi R, Evangelista ML, Stipa E, Buccisano F, Venditti A, Amadori S. Idiopathic thrombocytopenic purpura: current concepts in pathophysiology and management. *Thromb Haemost.* 2008;99(1):4-13.
6. Frederiksen H, Schmidt K. The incidence of idiopathic thrombocytopenic purpura in adults increase with age. *Blood.* 1999;94(3): 909-913.
7. Van Staa TP, Leufkens HG, Abenhaim L, Zhang B, Cooper C. Use of oral corticpsteroids and risk of fracture. *J Bone Miner Res.* 2000;15(6):993-1000.
8. Clowes JA, Peel N, Eastell R. Glucocorticoid-induced osteoporosis. *Curr Opin Rheumatol.* 2001;13(4):326-332.
9. Cram P, Schlechte J, Christensen A. A randomized trial to assess the impact of direct reporting of DXA scan results to patients on quality of osteoporosis care. *J Clin Densitom.* 2006;9(4):393-398.
10. Sosa M, Hernandez D, Segarra MC, Gómez A, de la Peña E, Betancor P. Effect of two forms of alendronate administration upon bone mass after two years of treatment. *J Clin Densitom.* 2002;5(1):27-34.
11. Sawka AM, Thabane L, Papaioannou A, Gafni A, Hanley DA, Adachi JD. A systemic review of the effect of alendronate on bone mineral density in men. *J Clin Densitom.* 2005;8(1):7-13.
12. Bruder JM, Ma JZ, Wing N, Basler J, Katselnik D. Effects of alendronate on bone mineral density in men with prostate cancer treated with androgen deprivation therapy. *J Clin Densitom.* 2006;9(4):431-437.
13. Patschan D, Loddenkemper K, Buttgereit F. Molecular mechanisms of glucocorticoid-induced osteoporosis. *Bone.* 2001;29(6): 498-505.

14. American College of Rheumatology Ad Hoc Committee on Glucocorticoid-Induced Osteoporosis. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis; 2001 update. *Arthritis Rheum.* 2001;44(7): 1496-1503.
15. Eastell R, Reid DM, Compston J, et al. A UK consensus group on management of glucocorticoid-induced osteoporosis: an update. *J Intern Med.* 1998;244(4):271-292.
16. Adachi JD, Olszynski WP, Hanley DA, et al. Management of corticosteroid-induced osteoporosis. *Semin Arthritis Rheum.* 2000;29(4):228-251.
17. Brown JP, Josse RG. 2002 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada. *CMAJ.* 2001;167(10 suppl):S1-S34.
18. Reid IR. Glucocorticoid-induced osteoporosis: assessment and treatment. *J Clin Densitom.* 1998;1(1):65-73.
19. Hanley DA, Ioannidis G, Adachi JD. Etironate therapy in the treatment and prevention of osteoporosis. *J Clin Densitom.* 2000;3(1):79-95.
20. Cheng A, Mavrokokki A, Carter G, et al. The dental implications of bisphosphonates and bone disease. *Aust Dent J.* 2005; 50(4 suppl 2):S4-S13.
21. Woo SB, Hellstein JW, Kalmar JR. Narrative [corrected] review: bisphosphonates and osteonecrosis of the jaws. *Ann Intern Med.* 2006;144(10):753-761.
22. Font RG, Garcia MLM, Martinez JMO. Osteochemonecrosis of the jaws due to bisphosphonate treatments: update. *Med Oral Patol Oral Cir Bucal.* 2008;13(5):E318-E324.

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Registry of 919 Patients with Thrombotic Microangiopathies across Japan: Database of Nara Medical University during 1998-2008

Yoshihiro Fujimura and Masanori Matsumoto

Abstract

Background Thrombotic microangiopathies (TMAs) are pathological conditions characterized by generalized microvascular occlusion by platelet thrombi, thrombocytopenia, and microangiopathic hemolytic anemia. Two typical phenotypes of TMAs are hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Severe deficiency of plasma ADAMTS13 activity (ADAMTS13: AC) is more specific for TTP, but not for HUS. Since 1998, our laboratory has functioned as a nationwide referral center for TMAs by analyzing ADAMTS13.

Methods Of 1,564 patients tested from 426 hospitals, 919 were positive for TMA. Levels of ADAMTS13: AC and the ADAMTS13 neutralizing autoantibody (ADAMTS13: INH) were determined by chromogenic act-ELISA and/or by classic von Willebrand factor multimer assay.

Results TMA patients consisted of two groups: severe (less than 3% of normal control) and non-severe deficiency of ADAMTS13: AC. Both groups were divided into congenital (n=65) and acquired (n=854) TMA. Of the former, 41 had congenital deficiency of ADAMTS13: AC, while the remaining 24 had disease of unknown etiology. The 854 patients with acquired TMA could be largely grouped into three categories: idiopathic TTP (n=284), idiopathic HUS (n=106), and secondary TMAs (n=464). The secondary TMAs were observed in heterogeneous patient groups and were associated with drugs, connective tissue diseases, malignancies, transplantation, pregnancy, *E. coli* O157: H7 infection, and other factors. All of the patients with acquired severe ADAMTS13: AC deficiency were positive for ADAMTS13: INH.

Conclusion Although TMAs are highly heterogeneous pathological conditions, one-third of TMA patients have severe deficiency of ADAMTS13: AC. Platelet transfusions to such patients are contraindicated. Rapid ADAMTS13: AC assays are therefore prerequisite to appropriately treat TMA patients.

Key words: TMA, TTP, HUS, USS, ADAMTS13, VWF

(Inter Med 49: 7-15, 2010)
(DOI: 10.2169/internalmedicine.49.2706)

Introduction

Thrombotic microangiopathies (TMAs) are pathological conditions that are characterized by microangiopathic hemolytic anemia, vast microvascular occlusions caused by platelet thrombi (common renal involvement), and thrombocytopenia (1). Two typical phenotypes of TMAs are hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), both of which are life-threatening diseases. HUS is characterized by the aforementioned three clinical signs (classic 'triad'), while TTP is characterized by a classic 'pentad,' which includes the 'triad' as well as fever and neurological signs; however, the two diseases are often indistinguishable. Further, these TMAs must be differentiated from disseminated intravascular coagulation (DIC) or consumptive thrombohemorrhagic disorders (2).

In 1996, a metalloprotease that specifically cleaves von Willebrand factor (VWF) was identified in normal plasma (3, 4), and 5 years later this enzyme was purified,

Department of Blood Transfusion Medicine, Nara Medical University, Kashihara
Received for publication July 22, 2009; Accepted for publication August 26, 2009
Correspondence to Dr. Yoshihiro Fujimura, malon@naramed-u.ac.jp

cloned, and termed ADAMTS13 (a disintegrin-like metalloproteinase with thrombospondin type 1 motifs 13) (5-8). ADAMTS13-producing cells were initially identified within the liver, and then more specifically as hepatic stellate cells (9), but now it is known that ADAMTS13 is also present in platelets (10), vascular endothelial cells (11), and kidney podocytes (12). Since the discovery of ADAMTS13, severe deficiency of ADAMTS13 activity (ADAMTS13: AC) has been thought to be a unique feature of TTP, and can be caused by genetic mutations or by acquired autoantibody (ADAMTS13: INH) to this enzyme; however, these alterations are not observed in HUS patients (13, 14). It is notable that a minor population of TTP patients with the 'pentad' of symptoms has almost normal or only slightly reduced ADAMTS13: AC (15). In this regard, Tandon et al (16) reported in 1994 that approximately 80% of the patients with acquired TTP had anautoantibody against CD36. In those days, however, this finding could not be directly linked to the pathogenesis of TTP. Recently, Davis et al (17) have shown that recombinant (r)-human ADAMTS13 specifically binds to r-human CD36 in vitro. CD36 is expressed in endothelial cells, platelets, and monocytes, and has been reported to bind thrombospondin-1 (18). ADAMTS13, after secretion into the circulation, is assumed to efficiently cleave unusually large VWF multimers (UL-VWFMs) released from vascular endothelial cells as a solid-phase enzyme by binding to the cell surface. It is currently unclear whether anti-CD36 autoantibodies block ADAMTS13 binding to vascular endothelial cells, but if so, this may interfere with the efficient cleavage of UL-VWFMs by ADAMTS13 and result in TTP.

In contrast to TTP, HUS is rarely induced by genetic mutations in complement regulatory factors (factors B, H, and I, and membrane cofactor protein or CD46). HUS can also be acquired, typically following acute enterocolitis due to shigatoxin-producing *Echerichia coli* O157: H7 infection, but also rarely due to autoantibody against factor H (19).

Since 1998, our laboratory at Nara Medical University has functioned as a nation-wide referral center for TMAs via assaying ADAMTS13: AC in a large Japan-wide patient population with thrombocytopenia suspected of being TMA. As of the end of 2008, we have established a registry of 919 patients with TMAs, and have analyzed their clinical and laboratory information. Here, we describe the results of this study, and discuss the divergence of TMAs among patient groups with masked or unmasked thrombocytopenia.

Materials and Methods

Patients

Between July 1998 and December 2008, plasma samples from 1,564 patients with thrombocytopenia suspected of TMAs were referred to our laboratory with clinical and laboratory information from 426 medical institutions across Japan. All subjects provided informed consent to participate in this study. The study protocol was approved by the Ethics Committee of Nara Medical University Hospital.

Blood sampling

Before therapeutic approaches including plasma infusion, plasma exchange, and the use of immunosuppressants, whole blood samples (~5 mL) were taken from each patient into plastic tubes containing 1/10 volume of 3.8% sodium citrate. The plasma was separated by centrifugation at 3,000 × g for 15 min at 4℃, kept in aliquots at -80℃ until testing, and sent to our laboratory.

Assays of plasma ADAMTS13: AC and ADAMTS13: INH

Until March 2005, ADAMTS13: AC was determined by classic VWFM assay (3) with a detection limit of 3% of the normal control (20). Thereafter, a chromogenic ADAMTS 13-act-ELISA with a detection limit of 0.5% of the normal control was developed (21), and replaced the VWFM assay. Measurement of plasma levels of ADAMTS13: AC by these assays were highly correlated (R^2=0.72, $p<0.01$) and provided similar results for mean ± SD in healthy individuals (102.4±23.0% vs. 99.1±21.5%), as shown previously (21, 22). Thus, we re-examined the plasma of 724 of the 774 TMA patients determined by the VWFM assay by act-ELISA, and the latter data were used in this study. For 50 TMA patients we were unable to re-examine by act-ELISA, the VWFM assay data were used. We have therefore tentatively categorized plasma levels of ADAMTS13: AC of <3%, 3%-<25%, and 25%-<50% of the normal as severe, moderate, and mild deficiency, respectively.

Plasma ADAMTS13: INH titers were also evaluated either by classic VWFM assay or chromogenic ADAMTS13-act-ELISA using heat-inactivated plasma at 56℃ for 30 minutes (13, 14). One Bethesda unit (U) is defined as the amount necessary to reduce ADAMTS13: AC to 50% of control levels (23). Titers greater than 0.5 Bethesda U/mL were classified as inhibitor positive.

Diagnostic criteria for TMAs

According to previous reports (2, 24, 25), TMAs were defined as having all of the following: (i) microangiopathic hemolytic anemia (hemoglobin ≤12 g/dL), Coombs test negative, undetectable serum haptoglobin (<10 mg/dL), more than 2 fragmented red cells (schistocytes) in a microscopic field with a magnification of 100, and concurrent increased serum lactate dehydrogenase (LDH) above institutional baseline; (ii) thrombocytopenia (platelet count ≤100× 10^9/L); and (iii) a variable severity of organ dysfunction (renal or neurological involvement) devoid of the stigmata of DIC (26).

A differential diagnosis of HUS or TTP based on routine laboratory data is usually difficult. As a rule, plasma levels of ADAMTS13: AC were first determined on all patients suspected of TMAs, and patients with severe deficiency of ADAMTS13: AC were classified as TTP regardless of clini-

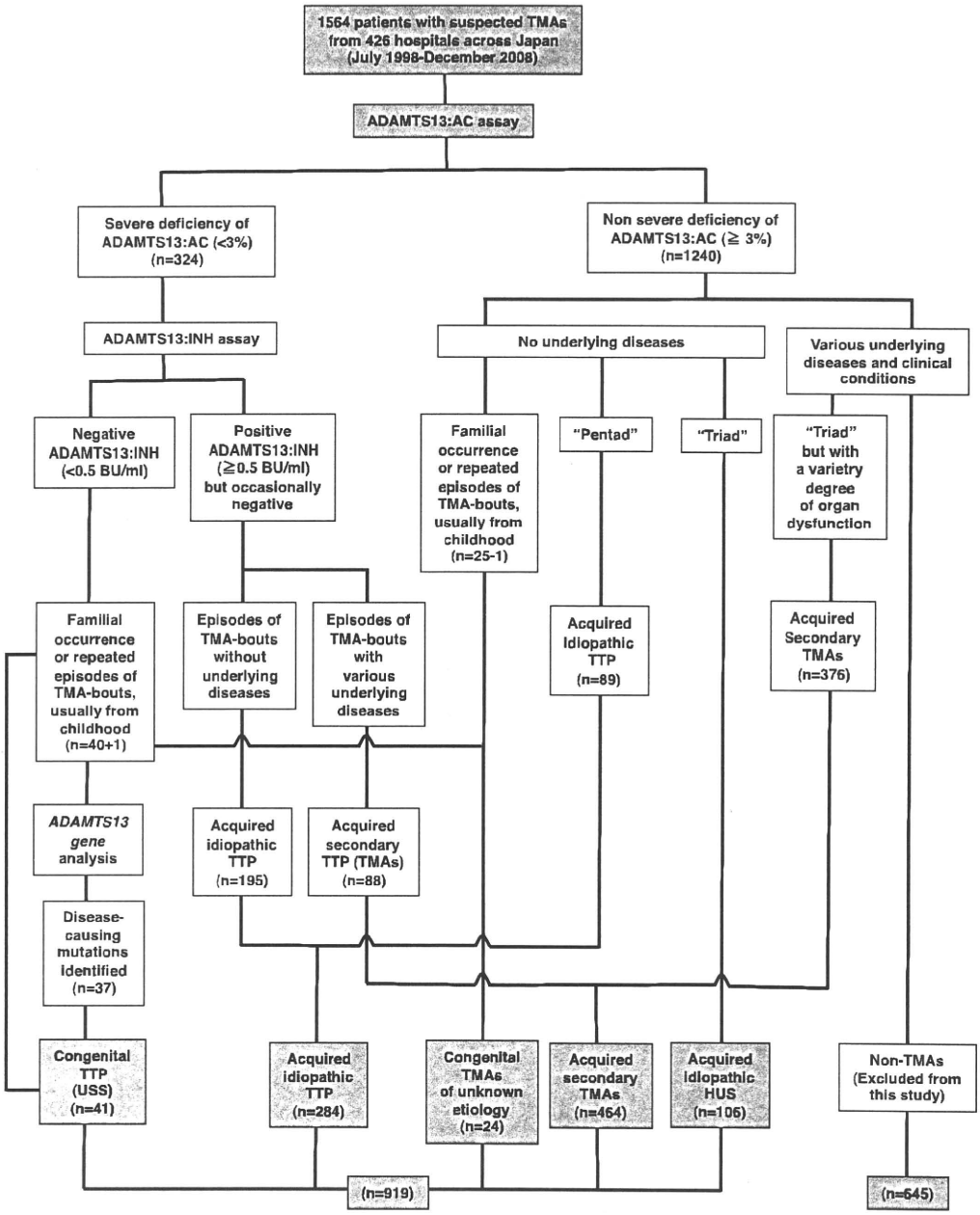


Figure 1. Flow chart of categorization of patients with suspected thrombotic microangiopathies (TMAs) based on ADAMTS13 analysis. Of 1,564 patients with suspected TMAs, 324 had severe deficiency of ADAMTS13 activity and 1,240 did not. In the former category, 40 patients were categorized as USS and 284 as acquired TTP. In the latter category, 24 patients were categorized as congenital TMAs of the unknown etiology, 570 as acquired TMAs, and one patient as USS with moderately reduced plasma ADAMTS13:AC (3.4%), to whom frequent plasma infusions had been made to prevent further aggravation of cerebral infarction. The remaining 645 patients did not have TMAs and were therefore excluded from this study.

cal signs. Second, the patients were grouped as HUS or TTP based on the 'triad' or 'pentad' of clinical signs. This protocol appeared to be important, because our registry includes patients with hereditary deficiency of ADAMTS13: AC or congenital TTP (Upshaw-Schulman syndrome, USS), which generally have less severe clinical signs (often isolated thrombocytopenia) than acquired TTP.

Results and Discussion

A flow chart of patient categorization based on ADAMTS 13 analysis is shown in Fig. 1. Of the 1,564 patients referred to our laboratory, 324 (minor population) had severe deficiency of ADAMTS13: AC and 1,240 (major population) did not. In the population with severe ADAMTS13: AC de-

Table 1. Plasma Levels of ADAMTS13: AC and ADAMTS13: INH in 919 Patients with Thrombotic Microangiopathies (TMAs) Registered at Nara Medical University during July 1988- December 2008

	Congenital TMAs		Acquired TMAs											Total
			Idiopathic		Secondary									
					Drug-Induced									
	Upshaw-Schulman syndrome (USS) (n=41)	Unknown etiology (n=24)	Thrombotic thrombocytopenic purpura (TTP) (n=284)	Hemolytic-uraemic syndrome (HUS) (n=106)	Ticlopidine (n=22)/ Clopidogrel (n=1)	Mitomycin C (n=10)	Pegylated-interferon (n=1) / Sildenafil (n=1)	Connective tissue diseases and their allied diseases (CTDs/ADs) (n=221)	Malignancies (n=61)	Hematopoietic stem cell transplantation (HSCT) (n=54)	Pregnancy (n=15)	E. coli O157: H7 infection (n=32)	Others (Liver cirrhosis, etc) (n=46)	(n=919)
ADAMTS13:AC (%)	(n=41)	(n=24)	(n=284)	(n=106)	(n=22/n=1)	(n=10)	(n=1/n=1)	(n=221)	(n=61)	(n=54)	(n=15)	(n=32)	(n=46)	(n=919)
<3	40	0	185	0	18	0	2	46	5	0	4	0	13	324
3 ~ <25	1	4	72	20	2	2	0	66	23	18	4	5	16	233
25 ~ <50	0	9	14	48	1	5	0	66	22	24	4	17	6	216
≥50	0	11	3	38	1	3	0	43	11	12	3	10	11	146
ADAMTS13:INH (U/ml)	(n=41)	(n=23)	(n=282)	(n=43)	(n=22/n=1)	(n=7)	(n=1/n=1)	(n=187)	(n=26)	(n=15)	(n=8)	(n=17)	(n=23)	(n=697)
≥2	0	0	120	0	15	0	0	28	5	0	3	0	9	180
0.5 ~ <2	0	0	128	2	6	0	2	80	8	4	2	1	8	242
<0.5	41	23	33	41	2	7	0	79	13	11	3	16	6	275

( ) Sample number determined

ficiency, 40 patients were categorized as USS and 284 as acquired TTP, and no patients with DIC or septic DIC were included. In the population without severe ADAMTS13: AC deficiency, 24 patients were categorized as congenital TMAs of unknown etiology, 570 as acquired TMAs, and only one patient (GG in Table 2) as USS with moderately reduced plasma ADAMTS13: AC (3.4%), to whom frequent plasma infusions had been made to prevent further aggravation of cerebral infarction. Thus, a diagnosis of USS in this patient GG was made after identifying the disease-causing mutations (C1024R/C1024R) in exon 24 by *ADAMTS13* gene analysis. These data will be published elsewhere in detail. The remaining 645 patients did not have TMAs, and were therefore excluded from this study; this group included 64 patients with DIC or septic DIC.

Congenital TMAs

Patients with repeated TMA episodes usually starting in early childhood with or without familial occurrence are usually considered as congenital TMAs; these patients are largely separated into the following two categories, on the basis of plasma levels of ADAMTS13: AC and ADAMTS 13: INH.

1. Upshaw-Schulman syndrome (USS)

USS is alternatively termed congenital TTP and is characterized by severe deficiency of ADAMTS13: AC due to genetic mutations (27). Forty-one patients (25 females and 16 males) belonging to 36 different families, were placed in this category (Table 2). All of these patients were negative for ADAMTS13: INH. USS is inherited in an autosomal recessive fashion, and therefore, the parents of patients are asymptomatic carriers with significantly reduced plasma levels of ADAMTS13: AC. The female-to-male ratio in the USS patient population is theoretically one-to-one, but our results indicate an apparent female predominance (25 to 16). Of the 41 patients, 17 (41%) had a history of exchange blood transfusions during the newborn period, and 32 (78%) had a history of thrombocytopenia during childhood. For the remaining 9 (22%), it was unclear whether their platelet counts had been checked during that period.

ADAMTS13 gene analysis was performed for 38 USS patients, and the disease-causing mutations were identified in 37 of the 38. Of the 37 genotyped patients, 8 were homozygotes and 29 were compound heterozygotes [one *de novo* mutation (28)] for *ADAMTS13* gene mutations. Of the 8 homozygous patients, the parents of 6 had consanguineous marriages.

2. Congenital TMAs of unknown etiology

Patients in this category were characterized by repeated TMA episodes with predominant renal involvement from early childhood, and often with familial occurrence. Twenty-four patients belonging to 12 families were identified, but the etiology of TMAs in these patients remained completely unclear.

In this regard, it is well known that gene mutations in complement regulatory cofactors (factor H, factor I, factor B, and CD46 or membrane cofactor protein) cause excessive complement activation by impairing C3b inactivation, resulting in severe hemolysis, which triggers TMA episodes. Therefore, these patients are commonly termed 'congenital atypical HUS' (19). It is possible that among the patients of this category in this study, some disease might be related to gene mutations of complement regulatory cofactors, but at the time such analysis had not been done in Japan. As a first step toward such analysis, we determined the plasma levels of factor H antigen by immunoassay in our patients, and did not observe reduced levels in any patients (data not shown).

Table 2. Registration of 41 Japanese Patient with Upshaw-Schulman Syndrome (USS)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	Plasma ADAMTS13:AC (%)	*ADAMTS13* gene mutations
1	A	1999	M	+	+	< 0.5	C-Hetero
2	B	1986	F	+	+	< 0.5	Homo
3	C	1972	M	-	+	< 0.5	Homo
4	D	1978	F	+	+	< 0.5	C-Hetero
5	E	1985	M	+	+	< 0.5	C-Hetero
6	F	1983	M	+	+	0.6	C-Hetero
7	G	1987	F	+	+	< 0.5	C-Hetero
8	H	1951	M	-	-	0.6	C-Hetero
9	I	1972	M	-	+	< 0.5	C-Hetero
10	J-3	1977	F	-	+	< 0.5	C-Hetero
11	J-4	1979	M	-	+	< 0.5	C-Hetero
12	K-3	1976	F	-	+	< 0.5	C-Hetero
13	K-4	1978	F	+	+	< 0.5	C-Hetero
14	L-2	1967	F	-	-	< 0.5	C-Hetero
15	L-3	1972	F	-	+	< 0.5	C-Hetero
16	M-3	1969	F	-	-	< 0.5	C-Hetero
17	M-4	1971	F	-	-	< 0.5	C-Hetero
18	N	1986	F	+	+	< 0.5	C-Hetero
19	O-4	1958	F	-	-	< 0.5	C-Hetero
20	P	1971	M	-	+	< 0.5	C-Hetero
21	Q (1)	1983	M	+	+	< 0.5	C-Hetero
22	Q (2)	1988	M	+	+	< 0.5	C-Hetero
23	R-5	1982	F	-	+	< 0.5	C-Hetero
24	S	1982	F	-	+	0.9	※
25	T	1981	F	-	+	< 0.5	C-Hetero
26	U	1980	F	+	+	< 0.5	Homo
27	V	1983	F	+	+	< 0.5	C-Hetero
28	W-4	1990	F	-	+	< 0.5	C-Hetero
29	X-5	1963	F	-	-	< 0.5	※
30	Y	1960	F	-	+	< 0.5	C-Hetero
31	Z-3	1971	F	-	+	< 0.5	Homo
32	AA	1987	F	-	-	< 0.5	※
33	BB	1947	M	-	-	< 0.5	Homo
34	CC-5	2004	M	+	+	< 0.5	C-Hetero
35	DD	2007	F	-	+	< 0.5	C-Hetero
36	EE	2003	M	+	+	< 0.5	Homo
37	FF	1981	F	+	+	< 0.5	Homo
38	GG	1931	M	-	-	3.4	Homo
39	HH	2004	F	+	+	< 0.5	C-Hetero
40	II	1977	F	+	+	< 0.5	※
41	JJ	1977	M	-	+	< 0.5	C-Hetero

C-Hetero: Compound heterozygotes, Homo: Homozygotes, ※: Not determined.

Acquired TMAs

Patients with acquired TMAs are characterized by the following: 1) usually no familial occurrence, 2) presence or absence of underlying diseases or medications associated with TMAs, and 3) common sudden onset of TMA episodes during adulthood. Patients with acquired TMAs are grouped as primary (idiopathic) or secondary, and then further separated into categories as follows, based on the results of ADAMTS 13: AC and ADAMTS13: INH assays.

1. Idiopathic TMAs

The patients in this group lack apparent underlying diseases or medications related to TMA episodes. Idiopathic TMAs can be further categorized into TTP and HUS subgroups. Idiopathic TTP (n=284) included two patient populations: 1) patients (n=195) with severe deficiency of ADAMTS13: AC, commonly positive for ADAMTS13: INH, and 2) patients (n=89) with clinical 'pentad' signs, regardless of plasma ADAMTS13: AC levels. Distribution of plasma ADAMTS13: AC is shown in Table 1. Detailed analysis of the clinical and laboratory features of these patients will be published elsewhere.

In contrast, idiopathic HUS (n=106) consisted of one patient population with clinical 'triad' signs, without severe deficiency of ADAMTS13: AC. Two patients of this category exhibited low levels of ADAMTS13: INH (0.5-<2 BU/mL).

2. Secondary TMAs

Secondary TMAs develop in the setting of various clinical conditions, such as infection, medication, and various underlying diseases. For instance, acquired TMAs are often associated with connective tissue diseases, and also treatment using several specific drugs. In these patients, clinical signs are often highly variable, so diagnostic differentiation of TTP or HUS appears to be insignificant.

(1) Drug-induced TMAs

A significant number of drugs have been associated with TMAs, including anti-platelet thienopyridine derivative drugs, antineoplastic drugs such as mitomycin C, and quinine (29). We have no experience with quinine-associated TMAs, but observed two suspected drug-associated TMAs:

one with sildenafil (Viagra) and the other with pegylated-interferon. Thus, drug-induced TMAs will be discussed in the following 3 subgroups.

a) Thienopyridine derivative-induced TMAs

Ticlopidine (TC) and clopidogrel (CL) are two typical thienopyridine derivatives (30). We identified 22 patients with TC-induced TMAs and one with CL-induced TMA. Nineteen of the 22 patients with TC-TMAs (86%) had severe ADAMTS13: AC deficiency and were positive for ADAMTS13: INH. The mechanism by which TC induces TMAs is still unclear, but it is speculated that TC becomes active in circulation and binds to ADAMTS13, forming a hapten-carrier complex. Antibodies formed against such a complex may be specific for the hapten, the combination hapten-carrier site, or the carrier alone, in a similar fashion to alpha-methyldopa, which may cause the development of anti-red cell antibodies. In approximately 90% of patients with TC-induced TMAs, the onset of TMA episodes occurred within 40 days of treatment (30). The frequency of TC-induced TMAs is estimated to be one per 1,600 to 5,000 patients. In contrast, only one female patient with CL-induced TMA, who developed TMA episodes 4 days after treatment, has been reported in Japan (31). This patient had slightly reduced plasma ADAMTS13: AC (34%), and was negative for ADAMTS13: INH. The pathogenesis of CL-induced TMAs is unclear, but recent studies suggest that ADAMTS13 is released from the liver into circulation, binds to endothelial cell surfaces, and efficiently cleaves UL-VWFMs. Thus, if endothelial cell injuries are present, ADAMTS13 cannot effectively cleave UL-VWFMs; this may lead to TMA episodes. In this regard, Zakarija et al (32) recently addressed two mechanistic pathways in TMAs related to thienopyridine derivatives.

b) Mitomycin C-induced TMAs

Ten patients with mitomycin C (MMC)-induced TMAs were identified. None had severe deficiency of ADAMTS13: AC, and all were negative for ADAMTS13: INH. Previous reports (33) suggest that MMC-induced TMAs develop with a frequency of 4-15% of the patients treated with this drug. The pathophysiology of MMC-TMAs is not well understood, but it is assumed that MMC may cause vascular endothelial cell injuries.

c) TMAs associated with other drugs

We observed two other TMA patients with severe deficiency of ADAMTS13: AC and positive ADAMTS13: INH. Both of these patients were assumed to have drug-associated TMA. One patient was a 62-year-old male with chronic hepatitis C. This patient developed TMA a month after long-term treatment with pegylated-interferon; the detailed clinical course of this patient was previously reported (34). The other patient with possible drug-induced TMA was a 65-year-old male who had taken sildenafil. The patient had taken sildenafil once several months prior to development of TMA, and then he had taken the drug twice within the 2 weeks prior to TMA. Two days after his third intake of sildenafil, the patient developed a low-grade fever, hemolytic anemia (hemoglobin 10.3 g/dL and reticulocyte 3.9%), thrombocytopenia (11,000/μL), and hematuria. ADAMTS13 analysis identified severe deficiency of ADAMTS13: AC (<3%) and ADAMTS13: INH positivity (1.5 Bethesda U/mL). The patient was treated by oral administration of the anti-platelet drug dipyridamole without plasma exchange. Since then, he has recovered, and his ADAMTS13: AC returned to normal range 3 months later.

(2) Connective tissue diseases and their allied diseases (CTD/AD)-associated TMAs

A close relationship between systemic lupus erythematosus (SLE) and TTP was first described in 1939 (35). It is now known that TMAs are frequently associated with CTDs with a frequency of 1-6% of the patient population (36). We have recently reported that severe deficiency of ADAMTS 13: AC and positive ADAMTS13: INH was predominantly detected in patients with rheumatoid arthritis (RA)- and SLE-associated TMAs, via the analysis of 127 patients with CTD-associated TMAs, whose samples were collected between 1998-2006 (37).

In this study, we included other miscellaneous autoimmune diseases, such as antiphospholipid syndrome (APS), as listed in Table 3, in the analysis. Thus, we examined 221 patients with CTD/AD-associated TMAs (Tables 1, 3), of whom 46 (21%) had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH, while the remaining 175 (79%) had mild-to-moderate deficiency. We presume that the high prevalence of TMA episodes in patients with CTD/AD is closely related to high plasma levels of VWF over the low levels of ADAMTS13: AC (37). Anatomical changes of the microvasculature, namely narrowed vessel cavities due to the proliferation of vascular endothelial cells, result in altered circulation hemodynamics and contribute to the formation of platelet thrombi at sites of vascular injury.

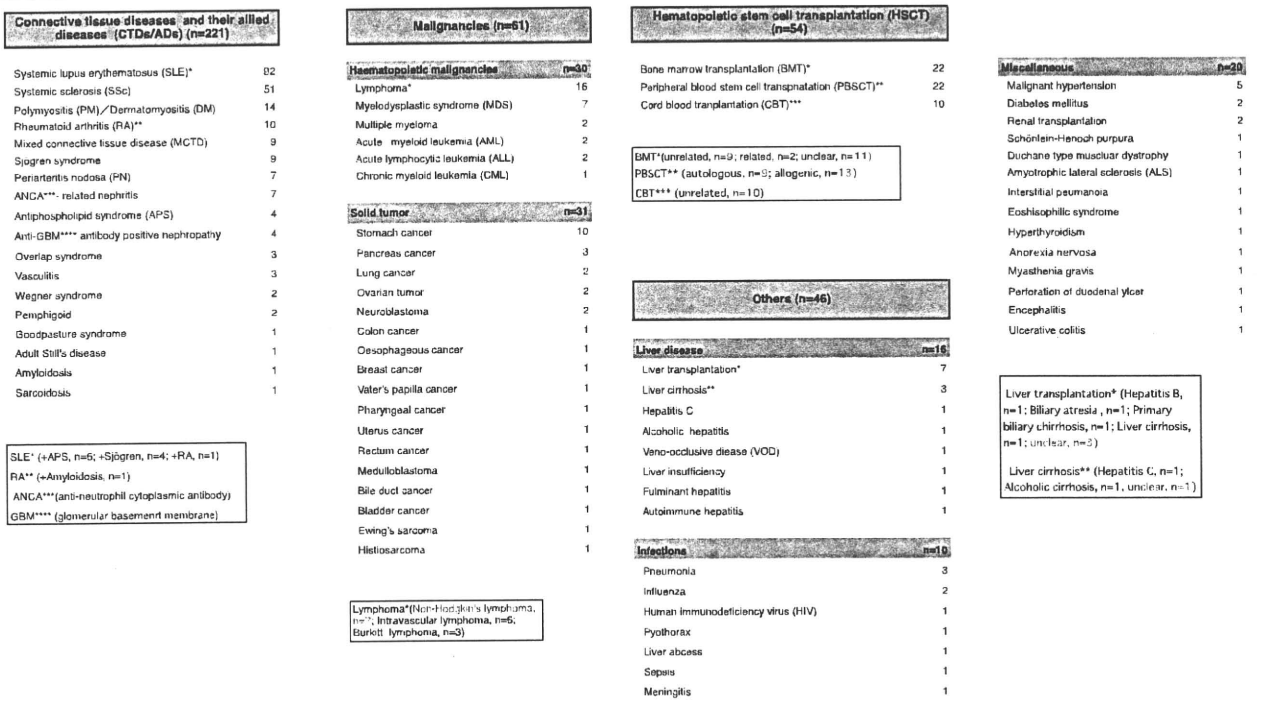
(3) Malignancy-associated TMAs

Sixty-one patients were classified into this category, which largely consisted of 2 groups: one group of patients with hematological malignancies (n=30) and the other group with malignant solid tumors (n=31) (Table 2).

Of the hematological malignancies, lymphoma was the most frequently seen (n=16), and four of the 16 patients had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH. The clinical course of one patient with intravascular lymphoma (IVL)-associated TMA was previously reported (38). In this case, the aggravation of TMA was dependent on the treatment efficacy of chemotherapy during the early stage of disease progression, but in the later stage was dependent on rituximab after several relapses during a 4-year observation period (39).

Of 31 patients with malignant solid tumor-associated TMAs, stomach cancer (n=10) was most commonly seen,

Table 3. Details of Underlying Diseases or Clinical Conditions of Patients with Acquired Secondary Thrombotic Microangiopathies (TMAs)



Connective tissue diseases and their allied diseases (CTDs/ADs) (n=221)

Systemic lupus erythematosus (SLE)*	92
Systemic sclerosis (SSc)	51
Polymyositis (PM)／Dermatomyositis (DM)	14
Rheumatoid arthritis (RA)**	10
Mixed connective tissue disease (MCTD)	9
Sjogren syndrome	9
Periarteritis nodosa (PN)	7
ANCA***- related nephritis	7
Antiphospholipid syndrome (APS)	4
Anti-GBM**** antibody positive nephropathy	4
Overlap syndrome	3
Vasculitis	3
Wegner syndrome	2
Pemphigoid	2
Goodpasture syndrome	1
Adult Still's disease	1
Amyloidosis	1
Sarcoidosis	1

SLE* (+APS, n=6; +Sjögren, n=4; +RA, n=1)
RA** (+Amyloidosis, n=1)
ANCA***(anti-neutrophil cytoplasmic antibody)
GBM**** (glomerular basement membrane)

Malignancies (n=61)

Hematopoietic malignancies	n=30
Lymphoma*	16
Myelodysplastic syndrome (MDS)	7
Multiple myeloma	2
Acute myeloid leukemia (AML)	2
Acute lymphocytic leukemia (ALL)	2
Chronic myeloid leukemia (CML)	1

Solid tumor	n=31
Stomach cancer	10
Pancreas cancer	3
Lung cancer	2
Ovarian tumor	2
Neuroblastoma	2
Colon cancer	1
Oesophageous cancer	1
Breast cancer	1
Vater's papilla cancer	1
Pharyngeal cancer	1
Uterus cancer	1
Rectum cancer	1
Medulloblastoma	1
Bile duct cancer	1
Bladder cancer	1
Ewing's sarcoma	1
Histiosarcoma	1

Lymphoma*(Non-Hodgkin's lymphoma, n=7; Intravascular lymphoma, n=6; Burkitt lymphoma, n=3)

Hematopoietic stem cell transplantation (HSCT) (n=54)

Bone marrow transplantation (BMT)*	22
Peripheral blood stem cell transpnatation (PBSCT)**	22
Cord blood tranplantation (CBT)***	10

BMT*(unrelated, n=9; related, n=2; unclear, n=11)
PBSCT** (autologous, n=9; allogenic, n=13)
CBT*** (unrelated, n=10)

Others (n=46)

Liver disease	n=16
Liver transplantation*	7
Liver cirrhosis**	3
Hepatitis C	1
Alcoholic hepatitis	1
Veno-occlusive disease (VOD)	1
Liver insufficiency	1
Fulminant hepatitis	1
Autoimmune hepatitis	1

Infections	n=10
Pneumonia	3
Influenza	2
Human immunodeficiency virus (HIV)	1
Pyothorax	1
Liver abcess	1
Sepsis	1
Meningitis	1

Miscellaneous	n=20
Malignant hypertension	5
Diabetes mellitus	2
Renal transplantation	2
Schönlein-Henoch purpura	1
Duchane type muscluar dystrophy	1
Amyotrophic lateral sclerosis (ALS)	1
Interstitial peumanoia	1
Eoshisophilic syndrome	1
Hyperthyroidism	1
Anorexia nervosa	1
Myasthenia gravis	1
Perforation of duodenal ylcer	1
Encephalitis	1
Ulcerative colitis	1

Liver transplantation* (Hepatitis B, n=1; Biliary atresia , n=1; Primary biliary chirrhosis, n=1; Liver cirrhosis, n=1; unclear, n=3)
Liver cirrhosis** (Hepatitis C, n=1; Alcoholic cirrhosis, n=1, unclear, n=1)

but a variety of organs were involved as listed in Table 3. One patient with Vater's papilla cancer showed severe deficiency of ADAMTS13: AC with the presence of ADAMTS 13: INH.

(4) Hematopoietic stem cell transplantation (HSCT)-associated TMAs

Fifty-four patients with TMAs were classified in this category. Of these, 22 were associated with bone marrow transplantation, 22 with peripheral blood SCT, and 10 with cord blood SCT (Table 3). The pathogenesis of TMAs in this category is highly complicated by pre-conditioning regimens of chemotherapies and body irradiation, as well as post-transplantation complications, such as bacterial or viral infections and graft-versus-host disease (GVHD). It was remarkable that none of the patients in this category had severe deficiency of ADAMTS13: AC, and all were negative for ADAMTS13: INH, as previously reported by others (40).

(5) Pregnancy-associated TMAs

TMA episodes are sometimes precipitated by pregnancy and postpartum, and require a rapid differential diagnosis from other thrombocytopenic status, such as ITP, pregnancy toxemia, eclampsia, and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome. The category of pregnancy-associated TMAs was present in two clinical settings: USS (congenital TTP) and acquired TTP. We have recently reported that pregnancy induced isolated thrombocytopenia or TTP in all female patients with USS examined, and their babies were stillborn or premature when the appropriate plasma therapy was not performed (41).

In the present study, we were able to identify 15 patients with acquired TMAs associated with pregnancy or postpartum. Of these, eight patients developed TMA episodes at 10-40 weeks of gestation, six patients developed TMA episodes soon after delivery, and one patient developed TMA episodes 3 months postpartum. Four of the 15 patients (27%) had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH (Table 1). These results suggest that the pathogenesis of acquired pregnancy-associated TMAs may be multifactorial, a different setting from USS.

(6) Escherichia coli O157 H7-associated TMAs:

Shigatoxin (1 and 2), composed of one A-subunit of 33 kDa and five B-subunits of 7 kDa each, is produced by the *E. coli* O157: H7 strain. Shigatoxin binds to a receptor, termed globotriaosyl ceramide, which is richly expressed in glomerular endothelial cells. After binding, shigatoxin is internalized and it induces endothelial cell apoptosis; this releases significant levels of UL-VWFMs into the circulation, resulting in platelet thrombi within microvasculatures. Hence, *E. coli* O157: H7-associated TMAs appear to be induced independent of plasma levels of ADAMTS13: AC. Thirty-two patients with TMAs were in this category, and in fact none of them had severe deficiency of ADAMTS13: AC. However, 22 patients had slightly reduced ADAMTS13: AC (Table 1). The reason underlying this is unclear, but we postulate either that ADAMTS13 is partially consumed to cleave the increased plasma VWF or that shigatoxin directly targets ADAMTS13-producing cells.

(7) TMAs associated with other causes

Forty-six TMA patients, who did not fit the aforementioned categories, were classified in this category (Table 3). Because of high heterogeneity in this category, it was subcategorized into patients with liver diseases (n=16), those with infections (n=10), and miscellaneous causes (n=20).

We have reported that numerous liver diseases are associated with reduced plasma ADAMTS13: AC. Notably, plasma levels of ADAMTS13: AC decline in parallel to the progression of liver cirrhosis (42). More interestingly, several patients with advanced liver cirrhosis had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH. These patients were assumed to have cryptic clinical signs of TMA; therefore, the term 'subclinical TTP' was introduced. In addition, we have reported on recipients of liver transplants with early allograft dysfunction who showed severe thrombocytopenia accompanied by a marked reduction of ADAMTS13: AC one or two days after transplantation, but without any apparent clinical features of TMAs (43). This observation has been confirmed by two recent reports (44, 45), but the mechanism has not yet been addressed.

Viral or bacterial infections can trigger TMA episodes, but the mechanism has not yet been addressed. Most recently, influenza has been revisited by researchers, due to a close relationship between influenza and TMA originally reported in 1980 (46). It is now known that influenza vaccine may induce TTP or disease relapse (47). We have two patients with influenza A-associated TMAs, and one of them had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH. Influenza virus or vaccination often worsens underlying diseases or conditions, including diabetes mellitus, pregnancy, and ongoing hemodialysis, resulting in multiorgan failure (MOF). Is it possible that such MOF is caused by microcirculatory disturbances, resembling the pathogenesis of TTP.

Human immunodeficiency virus (HIV) infection is also a known trigger of TMAs (48). In our registry, only one HIV-positive patient with severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH was identified.

Finally, the TMAs that fell into the miscellaneous subcategory are too variable to address in this report. The details of some of these patients will be reported in detail elsewhere by referral physicians.

Acknowledgement

The authors thank Dr. Masahito Uemura for his critical reading and valuable comments. We also thank Ms Ayami Isonishi, and Drs. Hideo Yagi and Hiromichi Ishizashi for their excellent technical assistance performing ADAMTS13: AC assays.

Grant Support: This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan and from the Ministry of Health, Labor, and Welfare of Japan for Blood Coagulation Abnormalities (Successive Directors; Drs. Masao Nakagawa, Yasuo Ikeda, and Mitsuru Murata), and by the reward of Erwin von Bälz prize in 2008.

References

1. Moake JL. Thrombotic microangiopathies. N Engl J Med **347**: 589-600, 2002.
2. George JN. Clinical practice. Thrombotic thrombocytopenic purpura. N Engl J Med **354**: 1927-1935, 2006.
3. Furlan M, Robles R, Lammle B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood **87**: 4223-4234, 1996.
4. Tsai HM. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. Blood **87**: 4235-4244, 1996.
5. Gerritsen HE, Robles R, Lammle B, Furlan M. Partial amino acid sequence of purified von Willebrand factor-cleaving protease. Blood **98**: 1654-1661, 2001.
6. Fujikawa K, Suzuki H, McMullen B, Chung D. Purification of human von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family. Blood **98**: 1662-1666, 2001.
7. Soejima K, Mimura N, Hirashima M, et al. A novel human metalloprotease synthesized in the liver and secreted into the blood: possibly, the von Willebrand factor-cleaving protease? J Biochem (Tokyo) **130**: 475-480, 2001.
8. Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem **276**: 41059-41063, 2001.
9. Uemura M, Tatsumi K, Matsumoto M, et al. Localization of ADAMTS13 to the stellate cells of human liver. Blood **106**: 922-924, 2005.
10. Suzuki M, Murata M, Matsubara Y, et al. Detection of von Willebrand factor-cleaving protease (ADAMTS-13) in human platelets. Biochem Biophys Res Commun **313**: 212-216, 2004.
11. Turner N, Nolasco L, Tao Z, Dong JF, Moake J. Human endothelial cells synthesize and release ADAMTS-13. J Thromb Haemost **4**: 1396-1404, 2006.
12. Manea M, Kristoffersson A, Schneppenheim R, et al. Podocytes express ADAMTS13 in normal renal cortex and in patients with thrombotic thrombocytopenic purpura. Br J Haematol **138**: 651-662, 2007.
13. Furlan M, Robles R, Galbusera M, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med **339**: 1578-1584, 1998.
14. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med **339**: 1585-1594, 1998.
15. Sadler JE. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. Blood **112**: 11-18, 2008.
16. Tandon NN, Rock G, Jamieson GA. Anti-CD36 antibodies in thrombotic thrombocytopenic purpura. Br J Haematol **88**: 816-825, 1994.
17. Davis AK, Makar RS, Stowell CP, Kuter DJ, Dzik WH. ADAMTS 13 binds to CD36: a potential mechanism for platelet and endothelial localization of ADAMTS13. Transfusion **49**: 206-213, 2009.

18. Febbraio M, Hajjar DP, Silverstein RL. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. J Clin Invest **108**: 785-791, 2001.
19. Coppo P, Veyradier A. Thrombotic microangiopathies: towards a pathophysiology-based classification. Cardiovasc Hematol Disord Drug Targets **9**: 36-50, 2009.
20. Kinoshita S, Yoshioka A, Park YD, et al. Upshaw-Schulman syndrome revisited: a concept of congenital thrombotic thrombocytopenic purpura. Int J Hematol **74**: 101-108, 2001.
21. Kato S, Matsumoto M, Matsuyama T, Isonishi A, Hiura H, Fujimura Y. Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity. Transfusion **46**: 1444-1452, 2006.
22. Mori Y, Wada H, Gabazza EC, et al. Predicting response to plasma exchange in patients with thrombotic thrombocytopenic purpura with measurement of vWF-cleaving protease activity. Transfusion **42**: 572-580, 2002.
23. Kasper CK, Aledort L, Aronson D, et al. Proceedings: A more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh **34**: 612, 1975.
24. Ho VT, Cutler C, Carter S, et al. Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant **11**: 571-575, 2005.
25. Ruutu T, Barosi G, Benjamin RJ, et al. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. Haematologica **92**: 95-100, 2007.
26. Wada H, Wakita Y, Nakase T, et al. Increased plasma-soluble fibrin monomer levels in patients with disseminated intravascular coagulation. Am J Hematol **51**: 255-260, 1996.
27. Fujimura Y, Matsumoto M, Yagi H, Yoshioka A, Matsui T, Titani K. Von Willebrand factor-cleaving protease and Upshaw-Schulman syndrome. Int J Hematol **75**: 25-34, 2002.
28. Kokame K, Aoyama Y, Matsumoto M, Fujimura Y, Miyata T. Inherited and de novo mutations of ADAMTS13 in a patient with Upshaw-Schulman syndrome. J Thromb Haemost **6**: 213-215, 2008.
29. Medina PJ, Sipols JM, George JN. Drug-associated thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Curr Opin Hematol **8**: 286-293, 2001.
30. Bennett CL, Kim B, Zakarija A, et al. Two mechanistic pathways for thienopyridine-associated thrombotic thrombocytopenic purpura: a report from the SERF-TTP Research Group and the RADAR Project. J Am Coll Cardiol **50**: 1138-1143, 2007.
31. Fukusako T, Yamashita H, Omoto M, Matsuda K, Shinohara K, Fujimura Y. A case of thrombotic thrombocytopenic purpura associated with clopidogrel. Clin Neurol **47**: 635-638, 2007.
32. Zakarija A, Kwaan HC, Moake JL, et al. Ticlopidine- and clopidogrel-associated thrombotic thrombocytopenic purpura (TTP): review of clinical, laboratory, epidemiological, and pharmacovigilance findings (1989-2008). Kidney Int **75** (Suppl 112): S20-S24, 2009.
33. Zakarija A, Bennett C. Drug-induced thrombotic microangiopathy. Semin Thromb Hemost **31**: 681-690, 2005.
34. Kitano K, Gibo Y, Kamijo A, et al. Thrombotic thrombocytopenic purpura associated with pegylated-interferon alpha-2a by an ADAMTS13 inhibitor in a patient with chronic hepatitis C. Haematologica **91**: ECR34, 2006.
35. Gitlow S, Goldmark C. Generalized capillary and arteriolar thrombosis. Report of two cases with a discussion of the literature. Ann Intern Med **13**: 1046-1067, 1939.
36. Sato T, Hanaoka R, Ohshima M, et al. Analyses of ADAMTS13 activity and its inhibitor in patients with thrombotic thrombocytopenic purpura secondary to connective tissue diseases: Observations in a single hospital. Clin Exp Rheumatol **24**: 454-455, 2006.
37. Matsuyama T, Kuwana M, Matsumoto M, Isonishi A, Inokuma S, Fujimura Y. Heterogeneous pathogenic processes of thrombotic microangiopathies in patients with connective tissue diseases. Thrombosis and Haemostasis **102**: 371-378, 2009.
38. Kawahara M, Kanno M, Matsumoto M, Nakamura S, Fujimura Y, Ueno S. Diffuse neurodeficits in intravascular lymphomatosis with ADAMTS13 inhibitor. Neurology **63**: 1731-1733, 2004.
39. Kanno M, Nakamura S, Kawahara M, et al. Chemotherapy-resistant intravascular lymphoma accompanied by ADAMTS13 inhibitor successfully treated with rituximab. Int J Hematol **88**: 345-347, 2008.
40. van der Plas RM, Schiphorst ME, Huizinga EG, et al. von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. Blood **93**: 3798-3802, 1999.
41. Fujimura Y, Matsumoto M, Kokame K, et al. Pregnancy-induced thrombocytopenia and TTP, and the risk of fetal death, in Upshaw-Schulman syndrome: a series of 15 pregnancies in 9 genotyped patients. Br J Haematol **144**: 742-754, 2009.
42. Uemura M, Fujimura Y, Matsumoto M, et al. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. Thromb Haemost **99**: 1019-1029, 2008.
43. Ko S, Okano E, Kanehiro H, et al. Plasma ADAMTS13 activity may predict early adverse events in living donor liver transplantation: observations in 3 cases. Liver Transpl **12**: 859-869, 2006.
44. Kobayashi T, Wada H, Usui M, et al. Decreased ADAMTS13 levels in patients after living donor liver transplantation. Thromb Res **124**: 541-545, 2009.
45. Pereboom IT, Adelmeijer J, van Leeuwen Y, Hendriks HG, Porte RJ, Lisman T. Development of a severe von Willebrand factor/ADAMTS13 dysbalance during orthotopic liver transplantation. Am J Transplant **9**: 1189-1196, 2009.
46. Wasserstein A, Hill G, Goldfarb S, Goldberg M. Recurrent thrombotic thrombocytopenic purpura after viral infection. Clinical and histologic simulation of chronic glomerulonephritis. Arch Intern Med **141**: 685-687, 1981.
47. Dias PJ, Gopal S. Refractory thrombotic thrombocytopenic purpura following influenza vaccination. Anaesthesia **64**: 444-446, 2009.
48. Bell WR, Chulay JD, Feinberg JE. Manifestations resembling thrombotic microangiopathy in patients with advanced human immunodeficiency virus (HIV) disease in a cytomegalovirus prophylaxis trial (ACTG 204). Medicine (Baltimore) **76**: 369-380, 1997.

Pediatric Hematology and Oncology, 27:56–61, 2010

ISSN: 0888-0018 print / 1521-0669 online
DOI: 10.3109/08880010903401752

informa
healthcare

A 9-MONTH-OLD INFANT WITH ACQUIRED IDIOPATHIC THROMBOTIC THROMBOCYTOPENIC PURPURA CAUSED BY INHIBITORY IgG-AUTOANTIBODY TO ADAMTS13

Atsushi Sato, MD, PhD, Yoshiyuki Hoshi, MD, PhD, and Masaei Onuma, MD □ *Department of Hematology and Oncology, Miyagi Children's Hospital, Sendai, Japan*

Ryusuke Sato, MD, PhD □ *Department of Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan*

Yukiko Tsunematsu, MD, PhD □ *Department of Strategic Medicine, Division of Pediatric Oncology, National Center for Child Health and Development, Tokyo, Japan*

Ayami Isonishi, BS, Masanori Matsumoto, MD, PhD, and Yoshihiro Fujimura, MD, PhD □ *Department of Blood Transfusion Medicine, Nara Medical University, Nara, Japan*

Masue Imaizumi, MD, PhD □ *Department of Hematology and Oncology, Miyagi Children's Hospital, Sendai, Japan*

□ *Although acquired idiopathic thrombotic thrombocytopenic purpura (ai-TTP) is rare in children, the authors present the case of a 9-month-old boy with ai-TTP showing severe deficiency of ADAMTS13 activity by its inhibitory IgG-autoantibody (4.8 Bethesda units/mL). Plasma exchange therapy was clinically effective but transient. Deficient activity of ADAMTS13 with the presence of its inhibitor persisted for 7 months after the initial diagnosis. However, other laboratory findings improved gradually with steroid (pulse) therapy. The hitherto insufficiently characterized clinical settings of ai-TTP during early childhood underscore the importance of measuring ADAMTS13 activity and its inhibitors for differential diagnosis in patients with thrombocytopenia of unknown etiology.*

Keywords acquired TTP, ADAMTS13, infancy, von Willebrand factor-cleaving protease

Thrombotic thrombocytopenic purpura (TTP) is prominent in disorders with thrombotic microangiopathy characterized by hemolytic anemia, thrombocytopenia, and organ dysfunctions such as neurological

Received 1 December 2008; Accepted 9 October 2009.
Address correspondence to Atsushi Sato, MD, Department of Hematology and Oncology, Miyagi Children's Hospital, Aoba-ku, Sendai 989-3126, Japan. E-mail: asatoh@miyagi-children.or.jp

abnormalities or renal insufficiency. Recent reports have described that von Willebrand factor (vWF) cleaving protease, designated as ADAMTS13, plays important roles in TTP pathophysiology. The lack of ADAMTS13 activity causes the accumulation of unusually large vWF multimers in the plasma, resulting in the disseminated platelet thrombi characteristic of TTP [1, 2]. Previous reports described that 18–72% of clinically diagnosed TTP patients had severe deficiency of ADAMTS13 activity [3].

Decreased activity of ADAMTS13 in patients with TTP might be associated with either inherited or acquired mechanisms. Hereditary TTP, known as Upshaw-Schulman syndrome (USS), results from ADAMTS13 gene mutations [4, 5]. In contrast, acquired TTP—either idiopathic or secondary to drugs, pregnancy, or diseases such as infections, cancers, and autoimmune diseases—is caused mostly by autoantibody against ADAMTS13 [6, 7].

While approximately one-third of USS cases with an inherent deficiency of ADAMTS13 are diagnosed in adolescence or adulthood after passing early childhood [8], acquired TTP in children, including infant patients, has been reported in the relevant literature. This report describes a young infant with acquired idiopathic TTP caused by IgG autoantibody against ADAMTS13. Clinical findings for this patient suggest that assays of ADAMTS13 activity and its inhibitor are indispensable for differential diagnosis with USS and other thrombocytopenic diseases during childhood.

CASE REPORT

In January 2005, a 9-month-old boy with petechial hemorrhage was referred to the Tokyo Medical and Dental University Hospital for suspected idiopathic thrombocytopenic purpura (ITP). He showed nonimmune hemolytic anemia as well as thrombocytopenia. Subsequent examinations revealed that his plasma ADMATS13 activity by vWF multimer assay [6] was markedly decreased (<3%). Moreover, inhibitor activity was detected in the titer of 4.8 BU/mL. This inhibitor activity resided on the purified IgG (data not shown). Based on these findings, he was diagnosed as having acquired idiopathic TTP. Treatment with plasma exchange (PE) performed at the National Center for Child Health and Development was effective to decrease the inhibitor activity (0.2 BU/mL) and increase the serum ADAMTS13 activity to 62.8%, engendering the improvement of his anemia and thrombocytopenia. However, this effect of 6 courses of PE was transient: after about 1 month, the inhibitor activity rebounded to the higher titer at 10 BU/mL with recurrence of low ADAMTS13 activity (<3%) and hematological abnormalities. No effect of administration of fresh frozen plasma (FFP) was observed, nor any obvious sign of renal insufficiency. After PE treatment, as his platelet count decreased rapidly to the critical level lower than 10×10^9/L, presenting the increased risk of hemorrhage, attending

physicians chose to administer low doses of continuous platelet infusion under close observation and started treatment with oral prednisolone (PSL) (2 mg/kg/day). His platelet counts were constantly higher than 1.0×10^9/L after beginning these treatments.

At admission to our hospital in March 2005, he had no petechiae, hepatomegaly, or neurological abnormality. His mental status was normal. His peripheral blood examination showed severe thrombocytopenia (1.6×10^9/L), mild anemia (Hb 7.9 g/dL), and elevation of reticulocyte counts (16.1%). In fact, RBC fragmentation was found in his peripheral blood smear (0.16%). Biochemical examination revealed mild elevation of lactate dehydrogenase (672 IU/L) and indirect bilirubin (0.88 mg/dL), in addition to decreased haptoglobin: lower than 10 mg/dL. Both blood urea nitrogen (14.2 mg/dL) and serum creatinine (0.19 mg/dL) values were within normal ranges. Results of both direct and indirect Coombs tests were negative. Hemostatic examination showed that PT, APTT, and FDP were within normal ranges, but a slightly elevated value of D-dimer was observed. *Escherichia coli* O157 was not detected in his stool culture. His ADAMTS13 activity remained at an undetectable level (<3%). Furthermore, the inhibitor of ADAMTS13 was detected in serum with the titer of 4.3 BU/mL. Moreover, ADAMTS13 gene analysis in this patient revealed no disease-causing mutations for USS (data not shown).

During his stay at our hospital, he received pulse therapy using methylprednisolone (mPSL) (30 mg/kg/day for 3 days). Although the effect of this therapy was not apparent initially, platelet counts of his peripheral blood increased gradually over the 7 weeks subsequent to pulse therapy. Thereafter, the recovery of ADAMTS13 activity with a concomitant disappearance of its inhibitory activity was observed after approximately 5 and 7 months, respectively, following pulse therapy and initial treatment (Figure 1). Oral PSL was stopped when the recovery of ADAMTS13 activity was detected.

DISCUSSION

We report here an infant case with acquired TTP caused by anti-ADAMTS13 autoantibody. Based on findings of low ADAMTS13 activity with serum inhibitors, our patient was considered to have an acquired form of TTP. Only 2 cases diagnosed as acquired TTP during the first year of life have been reported in the relevant literature [9, 10] (Table 1). Ashida et al. reported a 9-month-old girl with high titer of ADMAMTS13 inhibitor (200 Bethesda units/mL), who was treated successfully with mPSL pulse therapy following PE. Schneppenheim et al. also reported an 11-month-old boy with recurrent thrombocytopenia who responded to corticosteroids. For our patient, however, we were unable to conclude simply that steroid therapy was effective because low ADAMTS13 activity with inhibitors was sustained for a certain time after hematological improvement was achieved.

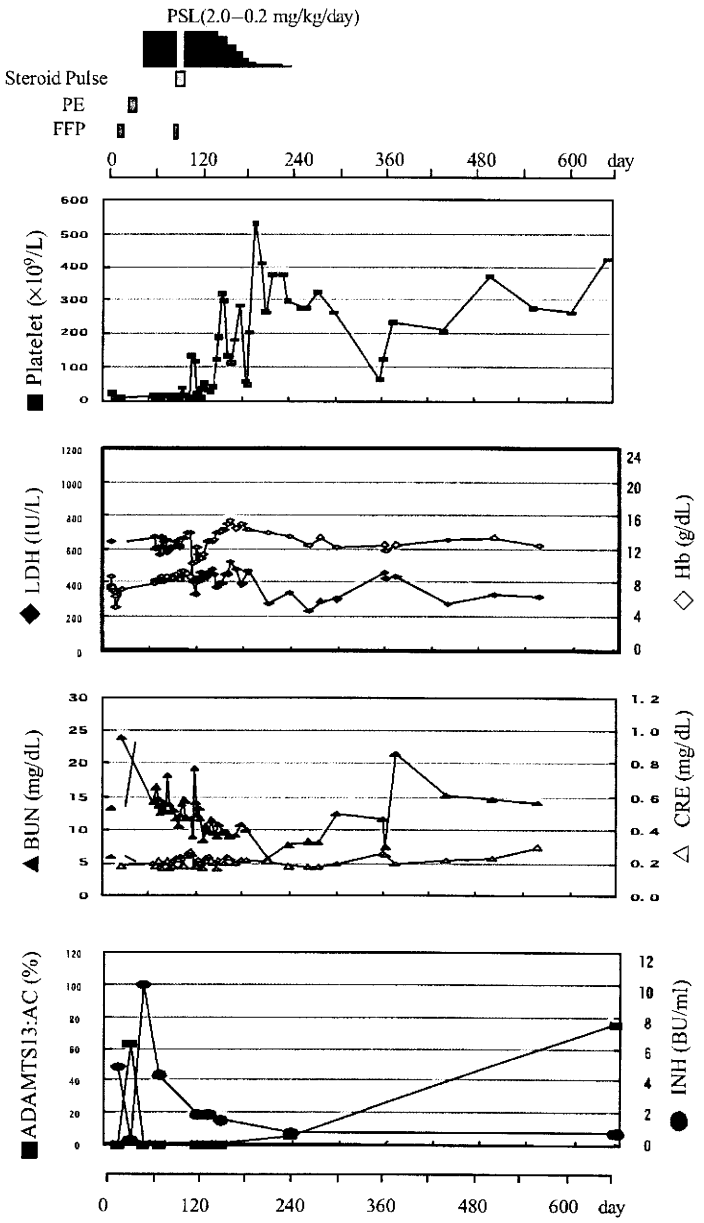


FIGURE 1 Clinical course of the present case of acquired TTP in infancy. ADAMTS13 activity and its autoantibodies were examined using frozen plasma stocked in −80°C with sensitive chromogenic ADAMTS13-act-ELISA method described in a prior study [16]. *Abbreviations*: PSL, prednisolone; FFP, fresh frozen plasma; INH, inhibitors; BU, Bethesda unit.

Neurological abnormalities and renal insufficiency are the hallmarks of acquired TTP in adults. These symptoms are worsened by platelet transfusions through expanding thrombus formation [11]. However, our patient, with a low titer of antibodies, showed no such symptoms at onset or

TABLE 1. Infants with acquired TTP showing anti-ADAMTS13 autoantibody

Patient no.	Sex	Age (months)	Renal impairment	CNS complication	Initial diagnosis	ADAMTS13 activity (%)	Inhibitor (BU/mL)	Ref.
1	F	9	Hematuria	Hemiconvulsion	TTP	<3	200	[9]
2	M	11	(−)	(−)	ITP	<2	(+)	[10]
Present case	M	9	(−)	(−)	ITP	<3	4.8	

Note. M, male; F, female; CNS, central nervous system; ITP, idiopathic thrombocytopenic purpura; TTP, thrombotic thrombocytopenic purpura; BU, Bethesda unit.

worsening in the hospital. In contrast, an infant TTP with high titer of antibodies showed both neurological and renal symptoms [9]. Results show that clinical symptoms become more severe in patients with high titer of inhibitor [12]. Therefore, results suggest that the titer of inhibitors might be a critical factor determining the severity of clinical manifestations of acquired TTP in infancy.

It is noteworthy that, during approximately 3 months, our patient showed hematological improvement despite the sustained low ADAMTS13 activity and the presence of inhibitor, thereby indicating that low ADAMTS13 activity does not necessarily worsen thrombotic microangiopathy. This finding in our patient might resemble the clinical picture of a subset of patients with USS who might be asymptomatic during infancy despite the inherent impairment of ADAMTS13 function [8, 13].

The classical 'pentad' of TTP is known to be fully present in only a minority of patients [3], indicating a limitation of diagnosis based solely on symptoms and routine examinations. Childhood TTP might be diagnosed initially as hemolytic anemia, Evans syndrome, or ITP [10, 14]; the assessment of ADAMTS13 would be of value for differential diagnosis of these diseases. Recently, rapid assays for measuring ADAMTS13 activity have been developed [15, 16]. Therefore, the assessment of ADAMTS13 activity and its inhibitor would be of value as routine laboratory tests for differential diagnosis of thrombocytopenia of unknown etiology during childhood.

ACKNOWLEDGMENT

This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology and from the Ministry of Health, Labor and Welfare of Japan.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Fujimura Y, Matsumoto M, Yagi H, et al. Von Willebrand factor-cleaving protease and Upshaw-Schulman syndrome. *Int J Hematol.* 2002;75:25–34.

[2] Loirat C, Girma JP, Desconclois C, et al. Thrombotic thrombocytopenic purpura related to severe ADAMTS13 deficiency in children. *Pediatr Nephrol.* 2008;24:Epub.

[3] Mannucci PM, Peyvandi F. TTP and ADAMTS13: When is testing appropriate? *Hematol Am Soc Hematol Educ Program.* 2007;121–126.

[4] Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature.* 2001;413:488–494.

[5] Shibagaki Y, Matsumoto M, Kokame K, et al. Novel compound heterozygote mutations (H234Q/R1206X) of the ADAMTS13 gene in an adult patient with Upshaw–Schulman syndrome showing predominant episodes of repeated acute renal failure. *Nephrol Dial Transplant.* 2006;21:1289–1292.

[6] Furlan M, Robles R, Galbusera M, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic–uremic syndrome. *N Engl J Med.* 1998;339:1578–1584.

[7] Sadler JE, Moake JL, Miyata T, et al. Recent advances in thrombotic thrombocytopenic purpura. *Hematol Am Soc Hematol Educ Program.* 2004;407–423.

[8] Loirat C, Veyradier A, Girma JP, et al. Thrombotic thrombocytopenic purpura associated with von Willebrand factor-cleaving protease (ADAMTS13) deficiency in children. *Semin Thromb Hemost.* 2006;32:90–97.

[9] Ashida A, Nakamura H, Yoden A, et al. Successful treatment of a young infant who developed high-titer inhibitors against VWF-cleaving protease (ADAMTS-13): important discrimination from Upshaw–Schulman syndrome. *Am J Hematol.* 2002;71:318–322.

[10] Schneppenheim R, Budde U, Hassenpflug W, et al. Severe ADAMTS-13 deficiency in childhood. *Semin Hematol.* 2004;41:83–89.

[11] Fontana S, Kremer Hovinga JA, Lammle B, et al. Treatment of thrombotic thrombocytopenic purpura. *Vox Sang.* 2006;90:245–254.

[12] Tsai HM, Li A, Rock G. Inhibitors of von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura. *Clin Lab.* 2001;47:387–392.

[13] Tsai H-M. Thrombotic thrombocytopenic purpura: a thrombotic disorder caused by ADAMTS13 deficiency. *Hematol Oncol Clin North Am.* 2007;21:609–632.

[14] Horton TM, Stone JD, Yee D, et al. Case series of thrombotic thrombocytopenic purpura in children and adolescents. *J Pediatr Hematol Oncol.* 2003;25:336–339.

[15] Kokame K, Kokubo Y, Okayama A, et al. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol.* 2005;129:93–100.

[16] Kato S, Matsumoto M, Matsuyama T, et al. Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity. *Transfusion.* 2006;46:1444–1452.

Int J Hematol (2010) 91:20–29
DOI 10.1007/s12185-009-0481-4

Pivotal role of ADAMTS13 function in liver diseases

Masahito Uemura · Yoshihiro Fujimura · Saiho Ko · Masanori Matsumoto · Yoshiyuki Nakajima · Hiroshi Fukui

Received: 1 July 2009 / Revised: 17 December 2009 / Accepted: 21 December 2009 / Published online: 7 January 2010

Abstract The liver is a major source of clotting and fibrinolytic proteins, and plays a central role in thromboregulation. Patients with advanced liver diseases tend to bleed because of reduced plasma levels of several clotting factors and thrombocytopenia, but they do also exhibit thrombotic complications. ADAMTS13 is a metalloproteinase, produced exclusively in hepatic stellate cells, and specifically cleaves highly multimeric von Willebrand factor (VWF). VWF plays a pivotal role in hemostasis and thrombosis, and its function is dependent on its multimeric state. Deficiency of ADAMTS13 results in accumulation of unusually large VWF multimers (UL-VWFM) in plasma, in turn induces platelet clumping or thrombi under high shear stress, followed by microcirculatory disturbances. Considering that UL-VWFM, the substrate of ADAMTS13, is produced in transformed vascular endothelial cells at sites of liver injury, decreased ADAMTS13 activity may be involved in not only sinusoidal microcirculatory disturbances, but also subsequent progression of liver injuries, eventually leading to multiorgan failure. This concept can be applied to the development or aggravation of liver diseases, including liver cirrhosis, alcoholic hepatitis, veno-occlusive disease, and adverse events after liver transplantation. These results promise to bring further understanding of the pathophysiology of liver diseases, and offer new insight for development of therapeutic strategies.

Keywords ADAMTS13 · Von Willebrand factor · Liver cirrhosis · Alcoholic hepatitis · Veno-occlusive disease · Liver transplantation · Microcirculatory disturbance · Multiorgan failure

1 Introduction

The liver plays a central role in hemostasis by synthesizing clotting factors, coagulation inhibitors, and fibrinolytic proteins [1]. The hemostatic system is normally in a delicate balance between pro-hemostatic and anti-hemostatic processes [1]. Severe liver diseases are accompanied by multiple changes in the hemostatic system, and the alterations in the system may lead to either a bleeding or thrombosis [1, 2]. Bleeding is clinically evident but hypercoagulability is also an important role in many aspects including poor hepatic blood flow, vasculopathy, and portal and hepatic vein thrombosis, which are closely related to microcirculatory disturbance [2]. Deficiency of anticoagulant proteins and high levels of several procoagulant factors may favor hypercoagulability [2], but the mechanisms underlying this disorder have not been fully elucidated.

ADAMTS13 (*a d*isintegrin-like *a*nd *m*etalloproteinase with *th*rombo*s*pondin type-1 motifs *13*) is a metalloproteinase that specifically cleaves multimeric von Willebrand factor (VWF) between Tyr1605 and Met1606 within its A2 domain [3, 4]. ADAMTS13 deficiency, caused either by mutations in the *ADAMTS13* gene [3–6] or by inhibitory

M. Uemura and Y. Fujimura contributed equally to this study.

M. Uemura (✉) · H. Fukui
Third Department of Internal Medicine, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan
e-mail: muemura@naramed-u.ac.jp

Y. Fujimura · M. Matsumoto
Department of Blood Transfusion Medicine, Nara Medical University, Kashihara, Nara 634-8522, Japan

S. Ko · Y. Nakajima
Department of Surgery, Nara Medical University, Kashihara, Nara 634-8522, Japan