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

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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

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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,

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 an autoantibody 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 *E. 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 $3,000 \times g$ for 15 min at 4°C , kept in aliquots at -80°C 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 ADAMTS13-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 $\pm$ SD in healthy individuals ($102.4 \pm 23.0\%$ vs. $99.1 \pm 21.5\%$), 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°C 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 $\leq 100 \times 10^9/\text{L}$); 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 clinical

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 ADAMTS13 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
	Upshaw-Schulman syndrome (USS) (n=41)	Unknown etiology (n=24)	Idiopathic		Secondary									(n=919)
			Thrombotic thrombocytopenic purpura (TTP) (n=284)	Hemolytic-uremic syndrome (HUS) (n=106)	Drug-induced			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)	
					Ticlopidine (n=22)/ Clopidogrel (n=1)	Mitomycin C (n=10)	Pegylated-interferon (n=1) / Sildenafil (n=1)							
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	195	0	19	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	129	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

iciency, 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 ADAMTS13: 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	1993	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	1990	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	1991	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 ADAMTS13: 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 pa-

tients 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-VWFs. Thus, if endothelial cell injuries are present, ADAMTS13 cannot effectively cleave UL-VWFs; 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/ μ L), 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 ADAMTS13: 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,

(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 sub-categorized 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 sub-category are too variable to address in this report. The details of some of these patients will be reported in detail elsewhere by referral physicians.

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