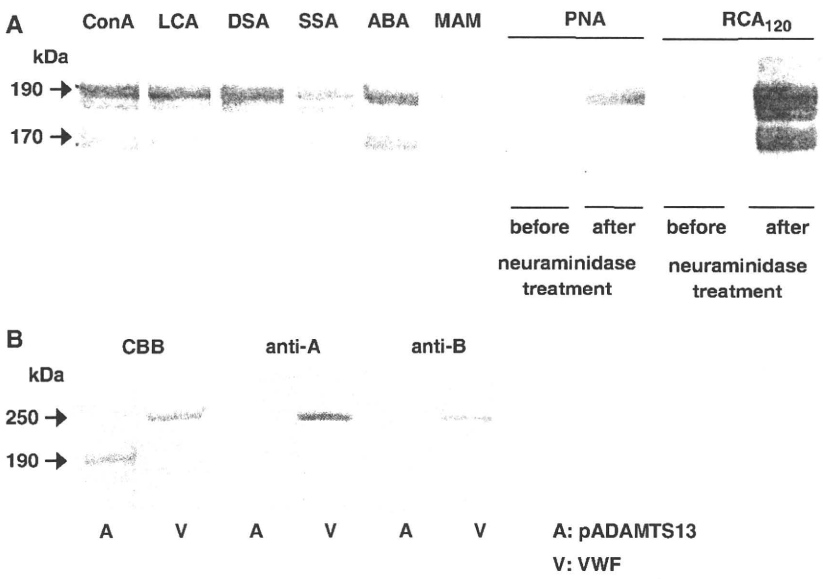

Fig. 5 Lectin blotting analysis of purified pADAMTS13 and detection of ABO blood group antigens on ADAMTS13. (A) Purified pADAMTS13 (0.4 μg) was subjected to SDS–5%PAGE under reducing conditions and transferred to a PVDF membrane. In some experiments, the blot proteins were treated with neuraminidase and then reacted with various lectins as described in 'Materials and Methods' section. Two protein bands, a major band of 190 kDa and a minor band of 170 kDa, are observed. Both bands positively reacted to Con A, LCA and DSA. PNA did not bind to purified pADAMTS13 before neuraminidase treatment, but did following neuraminidase treatment. (B) VWF (0.3 μg/lane) or pADAMTS13 (0.3 μg/lane) purified from pooled plasmas was subjected to SDS–5%PAGE under reducing conditions and transferred to a PVDF membrane as describe above. Protein bands on the membrane were stained with CBB. Reactivity to blood groups A or B antigen was clearly shown in VWF, but not in pADAMTS13.

enhanced by ~3-fold in the presence of 10 mM Ca^{2+}, but strongly inhibited in the presence of 10 mM Zn^{2+} and Ni^{2+}. This result appears to be in good agreement with that of Anderson *et al.* (*26*), who showed that Zn^{2+} at final concentrations of 1–3 mM enhanced the enzymatic activity of rADAMTS13 by the classic VWF multimer assay, but Zn^{2+} at higher concentrations (4–5 mM) inhibited activity. Gardner *et al.* (*27*) recently reported that rADAMTS13, extensively dialyzed against 0.15 M NaCl, 20 mM Tris–HCl (pH 7.8), requires pre-incubation with Ca^{2+} for 40–50 min to restore full enzyme activity. Furthermore, the activity of EDTA-treated enzyme, extensively dialyzed against 0.15 M NaCl, 20 mM Tris–HCl (pH 7.8) containing 5 mM Ca^{2+}, could be fully restored in a Zn^{2+}-dependent manner. These results indicated that dialysis can remove all functional Ca^{2+}, but does not remove the active site-bound Zn^{2+}. In addition, we have demonstrated here that 10 mM Ca^{2+} provides thermal resistance to pADAMTS13 activity, but it remains unaddressed how this happens in relation to the recently identified putative Ca^{2+} binding sites (Glu184 and Asp 187 of ADAMTS13).

Crawley *et al.* (*17*) showed that the three serine proteases, thrombin, plasmin and coagulation factor Xa, down-regulate ADAMTS13 activity by proteolysis. Lam *et al.* (*18*) identified the peptidyl bonds between Arg257–Ala258 and Arg1176–Arg1177 within ADAMTS13 as targets of thrombin. They predicted additional possible thrombin cleavable sites at Arg287, Arg393, Arg415, Arg910 and Arg968 of ADAMTS13 based on primary sequence analysis. In addition to the two thrombin cleavage sites reported by Lam *et al.* (*18*), we report two novel thrombin targets using the purified pADAMTS13: the peptidyl bonds between Arg459–Ser460 and Arg888–Thr889, neither of which was predicted. Of particular interest was the observation that plasmin cleaved the purified pADAMTS13 at the Arg257–Ala258, Arg888–Thr889 and Arg1174–Arg1177 sites, the same as thrombin.

Ono *et al.* (*15*) reported that ADAMTS13 antigen bands with lower molecular weights were found in plasmas of patients with sepsis-induced DIC that might have derived from digestion by bacterial proteases or leucocyte elastase. In this study, we therefore subjected the purified pADAMTS13 to leucocyte elastase digestion, and observed that the two peptidyl bonds between Ileu380–Ala381 and Tyr874–Ser875 were cleaved by this enzyme. These cleavage sites were different from those of thrombin or plasmin, and therefore also exclude the possibility that the three common cleavage sites of thrombin and plasmin were artificially generated during the purification process. According to the partial crystal structure of ADAMTS13 (*28*), Ileu380–Ala381 and Arg459–Ser460 were located on the surface exposure part between the Dis and thrombospondin1-1 domains and of the loop of the cystein-rich domain, respectively (Fig. 6).

Lam *et al.* (*18*) further reported that thrombin-cleaved rADAMTS13 remained partially active against intact VWF, and fully active against a recombinant VWF-A2 fragment (termed VWF115). However, the binding affinity of thrombin-treated rADAMTS13 for intact VWFM was significantly reduced compared

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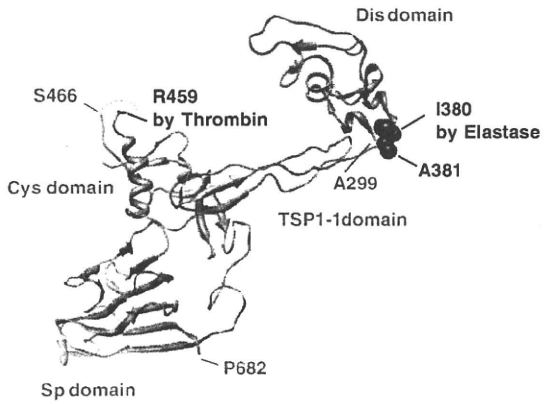

Fig. 6 Partial crystal structure of ADAMTS13 and location of the Ileu380–Ala381 and Arg459–Ser460 peptidyl bonds on ADAMTS13. Ribbon structure of ADAMTS13 (residues 299–682) was drawn with the UCSF Chimera package (*34*) using Protein Data Bank (PDB) entry as 3GHM. I380 and A381 are shown by space-filling model. Cleavage sites of I380–A381 by elastase and R459–S460 by thrombin (existing within the unsolved region) were located on the surface exposure part between the disintegrin-like and thrombospondin1-1 domains and of the loop of the cystein-rich domain, respectively. Dotted line indicates the unsolved region. Dis, disintegrin-like; TSP, thrombospondin1; Cys, cystein-rich; Sp, spacer domains.

to VWF115. As part of this study, we therefore also determined that >30% of the original pADAMTS13 activity against the GST-VWF73-His substrate remained during the incubation period (0.5–5.0 h) in each enzyme digestion.

ADAMTS13 has 10 putative *N*-glycosylation sites and *O*-fucosylated sugar chains in at least six thrombospondin type 1 repeats. Both the *N*- and *O*-glycans were reported to be prerequisite for enzyme secretion using rADAMTS13 (*29*, *30*). We here demonstrated by lectin blotting analysis that the purified pADAMTS13 possesses α2–6 (and partially α2–3-linked) sialic acid residues at the non-reducing terminus, and the presence of β1–4- and β1–3-linked galactose residues penultimate to sialic acid, through the observation that the reactivities of RCA_{120} and PNA were strongly enhanced after neuraminidase digestion. Since almost all sugar chains on pADAMTS13 are capped by sialic acid with no exposure of galactose residues, a clearance mechanism via hepatic asialogrycoprotein receptor might be involved, as is the case for other plasma glycoproteins (*31*, *32*). These sialo-sugar chains may also protect ADAMTS13 from proteolytic cleavage. In addition, neither blood group A nor B antigens were detected on the purified pADAMTS13, as previously reported by other investigators (*33*).

This study may in part contribute to understanding how ADAMTS13 activity is modulated by proteases generated in various clinical settings, and also to preparing the heat-treated pADAMTS13 concentrates.

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Conflict of interest
None declared.

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ORIGINAL ARTICLE

A second national questionnaire survey of TMA

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Abstract A second questionnaire survey of Japanese patients with thrombotic microangiopathy (TMA) was carried out to investigate the frequency, laboratory abnormalities and outcome in 2004 and 2005. The first and second surveys evaluated 397 patients including 19 with familial TMA and 378 with acquired TMA. The patients with acquired TMA included 165 with *Escherichia coli* O-157 infection-related TMA (O-157 TMA), 70 with ADAMTS13-related TMA (ADAMTS13 TMA) and 38 with other types of TMA (other TMA). The rate of ADAMTS13 TMA was significantly higher in patients with collagen diseases than in patients with all other underlying diseases ($p < 0.001$). The treatment of acquired TMA included plasma exchange (PE), steroids, antiplatelet agents, and anticoagulants, PE was carried out in 91.4% of patients with ADAMTS13 TMA, 68.4% of patients with other TMA and 12.7% of patients with O-157 TMA. The efficacy of PE and steroid therapy tended to be higher in patients with ADAMTS13 TMA than in those with other TMA. The complete remission rate was the highest and the mortality rate was the lowest in the patients with O-157 TMA. The mortality rate tended to be lower in patients with ADAMTS13 TMA than in those with other TMA. However, not all of the patients in our study were examined for ADAMTS13 at the time that this questionnaire survey was conducted.

Keywords TMA · TTP · HUS · ADAMTS13

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1 Introduction

Thrombotic microangiopathies (TMAs) are defined by the association of acute mechanical hemolytic anemia, thrombocytopenia, and visceral ischemic manifestations related to the formation of platelet thrombi in the microcirculation [1]. Clinically, TMA includes mainly thrombotic thrombocytopenic purpura (TTP) [2–4] and the hemolytic uremic syndrome (HUS) [5] characterized by multivisceral ischemia, fever and renal ischemia. In 1982, Moake et al. [6] demonstrated the presence of "unusually large multimers of Von Willebrand factor" (UL-VWF) in the plasma of patients suffering from chronic relapsing TTP. UL-VWF produced in and then quickly released from vascular endothelial cells is found in the plasma of patients

with both familial and non-familial TTP [6, 7]. These UL-VWF are thought to interact with circulating platelets, thus resulting in platelet clumping due to elevated shear stress [7]. Platelet-rich microthrombi in the small vessels are the pathologic hallmark of this disease [8].

ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13), which was identified in 2001 [9–11], is a zinc metalloprotease that specifically cleaves UL-VWF at the Tyr (1605)-Met(1606) boundary located in the A2 region of VWF [12, 13]. TTP was previously a life-threatening syndrome, although the survival rate has increased from 20 to 80% since the development of plasma exchange (PE) [14] and it recently has reached about 90% [15]. The mainstay of treatment is therapeutic PE, both to remove the causative antibodies to ADAMTS13, UL-VWF and cytokines, and to replace ADAMTS13 and normal size VWF [16]. The current guidelines [17] for thrombotic microangiopathy (TMA) recommend that PE should be initiated within 24 h of diagnosis.

Two questionnaire surveys for Japanese patients with TMA were carried out in 2004 [18, 19] and 2005. In the present study, the frequency, underlying diseases, symptoms, laboratory abnormalities, treatments, efficacy of treatment and outcome were analyzed from all of the data collected in the two questionnaire surveys conducted in 2004 and 2005.

2 Materials and methods

One hundred and eighty-five patients and two hundred and twelve patients diagnosed with TMA between 1 January 1999 and 31 December 2003 and between 1 January 2004 and 31 December 2004 were examined by a national questionnaire survey. The questionnaire was mainly of a selective type, and the contents of the questionnaire were about age, sex, underlying disease(s), acute symptoms, laboratory data including ADAMTS13, treatment, outcomes, etc. The first questionnaire in 2004 was sent to 994 departments of hematology in Japanese hospitals or institutes. A total of 429 hospitals responded and 73 had 185 cases of TMA.

In 2005, the second questionnaire concerning the number of patients was sent to 3,301 departments of rheumatism-collagen disease, internal medicine, pediatrics, urology and emergency and dialysis-renal centers in Japanese hospitals. A total of 2,275 departments responded and 457 cases of TMA (familial TMA: 20 cases, acquired TMA: 437 cases) were reported. The next questionnaire was sent to the hospitals where the 457 patients had received their diagnosis and treatment. In this case, 146 hospitals responded and 212 cases of TMA were included in the analysis. The hospital name was made anonymous. Finally, 397 cases in total (185 cases in 2004 and 212 cases in 2005) were analyzed.

Derangement, lethargy, behavioral disorders, convulsions, stupor, coma and other neurological abnormalities were considered to be neurological symptoms. Creatinine levels >1.3 mg/dl indicated renal injury. A body temperature >37.5°C was considered as a fever. Cough, sputum, and other symptoms of bronchitis and pneumonia were considered to be respiratory symptoms.

TMA patients were classified into 4 groups: those with ADAMTS13-related TMA (ADAMTS13 TMA), where the ADAMTS13 level was less than 20% or when patients were positive for an inhibitor of ADAMTS13; *Escherichia coli* O-157 infection-related TMA (O-157 TMA), in which TMA was caused by an O-157 infection; other TMA, the cause of which, was not known; or those in which TMA was not measured (NM TMA). In these patients, the ADAMTS13 level was not measured, and the disease was not induced by an O-157 infection.

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

2.1 Statistical analysis

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

3 Results

The patients included 19 with familial TMA and 378 with acquired TMA. In the patients with familial TMA, the ADAMTS 13 level was markedly reduced in 13 of the patients (ADAMTS13 TMA), but not in 2 of the patients (other TMA). The level of ADAMTS13 was not measured in the other 4 patients (NM TMA). In the patients with acquired TMA, 165 cases of TMA were caused by O-157 infection (O-157 TMA), and 70 were due to ADAMTS13 (ADAMTS13 TMA), 38 were due to other causes (other TMA), and 105 were NM TMA (Table 1). There tended to be more females than males among those with ADAMTS13 TMA and O-157 TMA within those with acquired TMA. There were more patients from 0 to 15 years in the O-157 TMA group, while those with acquired TMA not due to an O-157 infected were generally between the ages of 31 and 65 (Fig. 1). O-157 infection was the most frequent underlying disease in patients with TMA, while collagen disease was the second, the presence of a malignant tumor and transplantation were the third, and drug-induced TMA was

Table 1 Subjects

	Number			Sex (F:M)		
	First	Second	Total	First	Second	Total
Familial	13	6	19	9:4	2:4	11:8
ADAMTS13 TMA	8	5	13	7:1	2:3	9:4
Other TMA	1	1	2	0:1	0:1	0:2
NM TMA	4	0	4	2:2	0:0	2:2
Acquired	172	206	378	92:79[a]	133:73	225:152[a]
ADAMTS13 TMA	35	35	70	20:15	21:14	41:29
O-157 TMA	66	99	165	40:25[a]	69:30	109:55[a]
Other TMA	22	16	38	11:11	5:11	16:22
NM TMA	49	56	105	21:28	38:18	59:46

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

[a] 1 patient is not described

the fourth, etc. (Table 2). In patients with collagen diseases, the rate of acquired ADAMTS13 TMA (47.6%) was significantly higher than that of other TMA ($p < 0.01$). With regard to the underlying disease, the rate of ADAMTS13 TMA was significantly higher in patients with collagen diseases than in those with all other types of underlying disease ($p < 0.001$). In patients with an underlying O-157 infection, the rate of ADAMTS13 TMA was 0%. In patients with familial TMA, icterus neonatorum was observed in most patients (68.8%). The acute symptoms reported are shown in Table 3. The incidence of neurological symptoms was significantly lower in patients with O-157 TMA than in those with all other types of acquired TMA ($p < 0.001$) and their incidence tended to be higher in patients with ADAMTS13 TMA than with other TMA ($p = 0.089$). In patients with acquired TMA, the frequency of renal dysfunction was significantly higher in patients with other TMA than in those with ADAMTS13 TMA ($p < 0.001$). A fever was observed in 70.7% of patients with acquired TMA. Respiratory symptoms were not regularly associated with TMA although they occurred with significantly lower frequency in patients with O-157 TMA than in those with all other types of acquired TMA ($p < 0.001$).

The laboratory data are shown in Table 4. A decreased platelet count and a decreased hemoglobin level, and an increase in total bilirubin (T-bil) and lactate dehydrogenase (LDH) were frequently observed in each type of TMA. The platelet count (median value) was significantly lower in patients with familial TMA than in those with acquired TMA ($p < 0.05$), and tended to be lower in patients with ADAMTS13 TMA than in those with other TMA ($p < 0.061$). However, the platelet count was significantly higher in patients with O-157 TMA than in those with all other types of acquired TMA ($p < 0.001$).

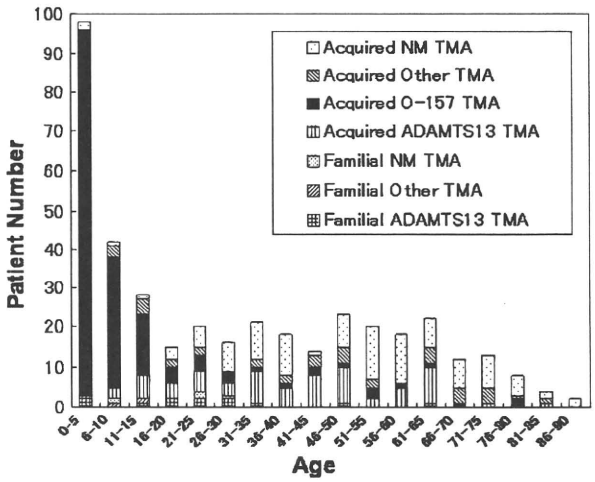

Fig. 1 The age of patients at the onset of TMA

The hemoglobin level was usually less than 13.0 g/dl and typically was between 5.0 and 10.0 g/dl. It was significantly lower in patients with acquired TMA than those with familial TMA ($p < 0.05$), and significantly lower in patients with O-157 TMA than in all patients with other types of acquired TMA ($p < 0.01$). The T-bil level was significantly lower in patients with O-157 TMA than in those with all other types of acquired TMA ($p < 0.01$). The LDH level was significantly higher in patients with other TMA than those with ADAMTS13 TMA ($p < 0.05$) and in patients with O-157 TMA compared to those with all other types of acquired TMA ($p < 0.001$). The levels of fibrin and fibrinogen degradation products (FDP) were slightly increased in most TMA patients, although the fibrinogen level was reduced in a few TMA patients. In those with acquired TMA, the frequency of positivity for antinuclear antibodies was higher in those with ADAMTS13 TMA

Table 2 Underlying diseases and conditions

		O-157 infection	Collagen diseases	Malignant tumor	Trans-plantation	Drug-induced TMA	Pregnancy	Post-surgery	Other	None
TMA	F:M	94:70[a]	33:9	9:10	7:12	5:9	10:0	2:0	9:19	59:56
Familial										
ADAMTS13	9:4	0	0	0	0	0	3:0 (30.0%)	0	0	6:4 (10.2%)
Other	0:2	0	0	0	0	0	0	0	0	0:2 (2.0%)
NM	2:2	0	0	0	0	1:0 (7.1%)	0	0	0	1:2 (3.1%)
Acquired										
ADAMTS13	43:30[b]	0	17:3 (47.6%)*,+	3:1 (21.1%)	1:2 (15.8%)	1:0 (7.1%)	1:0 (10.0%)	0	1:7 (28.6%)	19:17 (33.7%)
O-157	94:70[a]	94:70[a]	0	0	0	0	0	0	0	0
Other	16:28[c]	0	4:3 (16.7%)	1:5 (31.6%)	0:4 (21.1%)	1:3 (28.6%)	0	2:0 (5.3%)	1:3 (14.3%)	7:10 (11.2%)
NM	64:49[d]	0	12:3 (35.7%)	5:4 (47.4%)	6:6 (63.2%)	2:6 (57.1%)	6:0 (60.0%)	0	7:9 (57.1%)	26:21 (39.8%)

* $p < 0.01$ in comparison to acquired other TMA to collagen-related diseases

+ $p < 0.001$ in comparison to other underlying diseases without any collagen-related disease in acquired ADAMTS13 TMA

[a] 1 patient is not described

[b] 3 patients overlapped in collagen diseases–malignant tumor, collagen diseases–drug-induced TMA and malignant tumor–transplantation

[c] 6 patients overlapped in malignant tumor–transplantation (2 patients), malignant tumor–drug-induced TMA (3 patients) and drug-induced TMA–other

[d] 7 patients overlapped in collagen diseases–other, collagen disease–pregnancy, malignant tumor–transplantation (2 patients), malignant tumor–other, malignant tumor–pregnancy and drug-induced TMA–other

Table 3 Acute symptoms

	Number	Neurological symptoms 168	Renal dysfunction 199	Fever (above 37.5°C) 267	Respiratory symptoms 33
Familial					
ADAMTS13 TMA	13	4/11 (36.4%)	4/11 (36.4%)	5/12 (41.7%)	1/12 (8.3%)
Other TMA	2	0/2	1/2 (50.0%)	2/2	0/2
NM TMA	4	0/2	0/2	2/2	0/2
Total	19	4/15 (26.7%)	5/15 (33.3%)	9/16 (56.3%)	1/16 (6.3%)
Acquired					
ADAMTS13 TMA	70	51/69*[1] (73.9%)	27/66 (40.9%)	47/65 (72.3%)	3/52 (5.8%)
O-157 TMA	165	32/163#[1] (19.6%)	91/160 (56.9%)	113/162 (69.8%)	5/155#[1] (3.2%)
Other TMA	38	22/38 (57.9%)	28/37*[2] (75.7%)	26/36 (72.2%)	4/23 (17.4%)
NM TMA	105	59/98 (60.2%)	48/97 (49.5%)	72/102 (70.6%)	20/102 (19.6%)
Total	378	164/368 (44.6%)	194/360 (53.9%)	258/365 (70.7%)	32/332 (9.6%)

*[1] $p = 0.089$ in comparison to acquired Other TMA

*[2] $p < 0.001$ in comparison to acquired ADAMTS13 TMA

#[1] $p < 0.001$ in comparison to all other types of acquired TMA

than in those with other TMA ($p < 0.01$). The Coombs test was negative in more than 85% of all patients with TMA. The haptoglobin level was reduced in most patients with TMA. Anticardiolipin antibodies (ACA) were not observed in most of the patients with TMA.

The treatment of patients with acquired TMA is summarized in Table 5. PE was carried out in 91.4% of those with ADAMTS13 TMA, 68.4% of those with other TMA and 12.7% of those with O-157 TMA. The efficacy of PE tended to be higher in patients with ADAMTS13 TMA than in those with other TMA. Transfusion of fresh frozen plasma (FFP) was frequently performed in patients with familial TMA and ADAMTS13 TMA. The efficacy of FFP tended to be high in patients with familial ADAMTS13 TMA (75.0%), and was not high in patients with acquired TMA. In the patients with acquired TMA, steroid treatment was carried out in 85.7% of those with ADAMTS13 TMA, in 71.1% of those with other TMA, and in 6.1% of those with O-157 TMA. The efficacy of steroids tended to be higher in patients with ADAMTS13 TMA than in those with other TMA ($p = 0.067$). Pulse therapy with methylprednisolone was administered to 58.6% of patients with

Table 4 Laboratory data

		Median (25–75 percentile)				
	Number	Plt ($\times 10^4/\mu l$) 382	Hb (g/dl) 369	T-bil (mg/dl) 369	LDH (IU/l) 381	FDP (μg/ml) 251
Familial						
ADAMTS13 TMA	13	2.00 (0.90–2.95)	9.65 (7.80–12.05)	3.20 (1.35–5.20)	828 (426–1,229)	7.50 (2.47–33.9)
Total	19	1.70 (0.93–2.38)$^{+1}$	9.20 (6.95–12.05)$^{+1}$	2.65 (1.45–3.50)	1,173 (505–2,675)	10.7 (3.53–40.0)
Acquired						
ADAMTS13 TMA	70	1.60 (0.80–4.00)^{*1}	7.25 (6.50–8.45)	2.15 (1.25–3.95)	1,078 (718–1,843)	12.2 (8.40–20.3)
O-157 TMA	165	2.80 (1.90–4.68)$^{\#1}$	6.90 (5.90–8.40)$^{\#2}$	1.85 (1.20–2.70)$^{\#2}$	2,141 (1,373–3,461)$^{\#1}$	13.7 (8.05–37.3)
Other TMA	38	2.35 (1.60–4.80)	7.90 (6.65–9.85)	2.45 (1.00–4.60)	1,779 (844–3,243)^{*2}	20.0 (8.35–37.0)
NM TMA	105	2.30 (1.20–4.70)	7.60 (6.50–8.90)	2.07 (1.38–3.83)	1,264 (615–1,919)	14.9 (6.10–32.0)
Total	378	2.50 (1.30–4.60)	7.20 (6.20–8.70)	2.00 (1.20–3.10)	1,710 (869–2,848)	14.0 (7.60–32.1)

Plt platelet count, *Hb* hemoglobin, *T-bil* total bilirubin, *LDH* lactate dehydrogenase, *FDP* fibrin and fibrinogen degradation products

^{*1} $p = 0.061$ in comparison to acquired other TMA

^{*2} $p < 0.05$ in comparison to acquired ADAMTS13 TMA

$^{\#1}$ $p < 0.001$ in comparison to all other types of acquired TMA

$^{\#2}$ $p < 0.01$ in comparison to all other types of acquired TMA

$^{+1}$ $p < 0.05$ in comparison to acquired TMA

ADAMTS13 TMA and 60.5% of patients with other TMA, but the efficacy in all patients was low. The efficacy of pulse therapy tended to be higher in those with ADAMTS13 TMA than in those with other TMA ($p = 0.084$). Antiplatelet therapy was carried out in 51.4% of patients with ADAMTS13 TMA, 50.0% of those with other TMA, and 8.5% of those with O-157 TMA; however, the efficacy of this treatment was also low. Hemodialysis was carried out in 34.5% of the patients with O-157 TMA and 31.6% of the patients with other TMA. The efficacy of the treatment was significantly higher in patients with O-157 TMA than in patients with all other types of acquired TMA ($p < 0.05$). Anticoagulant therapy, such as heparin and synthetic protease inhibitors was carried out in approximately 35% of acquired TMA patients and the efficacy was significantly higher in those with O-157 TMA than in those with other types of TMA ($p < 0.001$). Platelet concentrate (PC) transfusion was carried out in 47.6% of the patients with NM TMA, 39.5% of patients with other TMA, 30.9% of patients with O-157 TMA, and 30.0% of patients with ADAMTS13 TMA. The efficacy was significantly lower in patients with ADAMTS13 TMA than in patients with other TMA ($p < 0.01$).

The outcomes of patients with acquired TMA are summarized in Table 6. The complete remission (CR) rate was the highest, and the mortality rate was the lowest in patients with O-157 TMA ($p < 0.001$). The mortality rate of acquired TMA was 22.0% in the first survey, 18.0% in the second survey, and 19.6% in the combined patients from both surveys. The mortality rate tended to be lower in patients with ADAMTS13 TMA than in those with other TMA.

4 Discussion

The first questionnaire survey [19] was sent to specialists in hematology, and the second questionnaire survey was sent to general hospital departments, including hematology, rheumatology and hemodialysis departments. There were no significant differences in the data collected between the patients recruited for the two surveys. It is expected that the accuracy of analysis improved, because the patient number increased.

There were 19 patients with familial TMA, and the overall frequency of familial TMA was about 4.8% in our study. About 86.7% (13/15) of the patients examined for ADAMTS13 were found to have abnormalities in ADAMTS13, but bias by the participating physician might have affected the results. The highest percentage of acquired TMA was due to O-157 TMA (43.7%). In the case of acquired TMA not induced by O-157 infection, 64.8% of the patients who were examined for ADAMTS13 were found to have ADAMTS13 TMA. However, 49.3% of the patients with acquired TMA not induced by O-157 infection were not examined for their ADAMTS13 status. As the decrease of ADAMTS13 may be the most frequent cause of TMA in patients without O-157, widespread use of the ADAMTS13 assay should be employed. Further studies in a large number of patients will be necessary to determine the true frequency of ADAMTS13 TMA in the population. A fluorescence resonance energy transfer (FRET) assay [20] and an enzyme immunoassay (EIA) [21] for ADAMTS13 activity have recently been developed.

Table 5 Treatment of TMA

	Number		PE 189	FFP 167	Steroid 169	Pulse 106	Antiplatelet 105	Hemodialysis 113	Anticoagulant 133	PC 143
Familial										
ADAMTS13 TMA	13	Enforcement	5 (38.5%)	8 (61.5%)	4 (30.8%)	0 (0.0%)	2 (15.4%)	0 (0.0%)	0 (0.0%)	3 (23.1%)
		Efficacy	40.0%	75.0%	25.0%	0.0%	100.0%	0.0%	0.0%	0.0%
Other TMA	2	Enforcement	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	2 (100.0%)
		Efficacy	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%	100.0%	100.00%
NM TMA	4	Enforcement	1 (25.0%)	2 (50.0%)	1 (25.0%)	1 (25.0%)	0 (0.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)
		Efficacy	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Total	19	Enforcement	7 (36.8%)	11 (57.9%)	5 (26.3%)	1 (5.3%)	3 (15.8%)	2 (10.5%)	2 (10.5%)	6 (31.6%)
		Efficacy	42.9%	54.5%	20.0%	0.0%	100.0%	50.0%	50.0%	33.3%
Acquired										
ADAMTS13 TMA	70	Enforcement	64 (91.4%)	44 (62.9%)	60 (85.7%)	41 (58.6%)	36 (51.4%)	11 (15.7%)	17 (24.3%)	21 (30.0%)
		Efficacy	50.0%	22.7%	38.3%*[1]	26.8%*[2]	25.0%	45.5%	17.6%	0.00%*[3]
O-157 TMA	165	Enforcement	21 (12.7%)	32 (19.4%)	10 (6.1%)	6 (3.6%)	14 (8.5%)	57 (34.5%)	57 (34.5%)	51 (30.9%)
		Efficacy	66.7%	46.9%	30.0%	33.3%	42.9%	68.4%#[1]	50.9%#[2]	45.1%
Other TMA	38	Enforcement	26 (68.4%)	16 (42.1%)	27 (71.1%)	23 (60.5%)	19 (50.0%)	12 (31.6%)	18 (47.4%)	15 (39.5%)
		Efficacy	34.6%	25.0%	18.5%	8.7%	10.5%	16.7%	5.6%	33.3%
NM TMA	105	Enforcement	71 (67.6%)	64 (61.0%)	67 (63.8%)	35 (33.3%)	33 (31.4%)	31 (29.5%)	39 (37.1%)	50 (47.6%)
		Efficacy	56.3%	37.5%	34.3%	34.3%	33.3%	58.1%	33.3%	22.0%
Total	378	Enforcement	182 (48.1%)	156 (41.3%)	164 (43.4%)	105 (27.8%)	102 (27.0%)	111 (29.4%)	131 (34.7%)	137 (36.2%)
		Efficacy	52.2%	34.0%	32.9%	25.7%	27.5%	57.7%	35.1%	28.5%

*[1] $p = 0.067$ in comparison to acquired other TMA

*[2] $p = 0.084$ in comparison to acquired other TMA

*[3] $p < 0.01$ in comparison to acquired other TMA

#[1] $p < 0.05$ in comparison to all other type of acquired TMA

#[2] $p < 0.001$ in comparison to all other type of acquired TMA

Table 6 Patient outcome

	CR			Mortality		
	First	Second	Total	First	Second	Total
Familial						
ADAMTS13 TMA	0/4	1/5 (20.0%)	1/9 (11.1%)	0/4	0/5	0/9
Other TMA	1/1	1/1	2/2	0/1	0/1	0/2
NM TMA	1/2 (50.0%)	0/0	1/2 (50.0%)	0/2	0/0	0/2
Total	2/7	2/6	4/13	0/7	0/6	0/13
Acquired						
ADAMTS13 TMA	13/19 (68.4%)	24/35 (68.6%)	37/54 (68.5%)	4/19 (21.1%)	8/35 (22.9%)	12/54 (22.2%)
O-157 TMA	53/55 (96.4%)#	82/94 (87.2%)#	135/149 (90.6%)#	2/55 (3.6%)#	5/94 (5.3%)#	7/149 (4.7%)#
Other TMA	4/6 (66.7%)	9/16 (56.3%)	13/22 (59.1%)	2/6 (33.3%)	5/16 (31.3%)	7/22 (31.8%)
NM TMA	26/47 (55.3%)	31/55 (56.4%)	57/102 (55.9%)	20/47 (42.6%)	18/55 (32.7%)	38/102 (37.3%)
Total	96/107 (89.7%)	146/200 (70.6%)	242/327 (74.0%)	28/127 (22.0%)	36/200 (18.0%)	64/327 (19.6%)

# $p < 0.001$ in comparison to all other types of acquired TMA

In our study, a female-to-male ratio of approximately 1.48 was observed, suggesting that TTP, especially ADAMTS13 TMA and O-157 TMA, may occur more frequently in women than men. In a similar report [22], the female-to-male ratio was found to be 3:2. Our results may have demonstrated a higher proportion of female patients because the collagen-related diseases were the most frequent non-infectious diseases underlying acquired TMA in this survey, and collagen disease is more common in women. The rate of acquired ADAMTS13 TMA, which was more frequent among female patients, was markedly higher in patients with collagen diseases. This may be

because auto-antibodies against ADAMTS13 may be frequently produced in collagen diseases. This is further supported by our finding that the frequency of positivity for antinuclear antibodies was high in patients with ADAMTS13 TMA. In contrast, auto-antibodies against ADAMTS13 were rarely detected in patients with malignant diseases or infections, and those that were post-surgery or post-transplantation, all of which may cause TMA via vascular endothelial injuries and inflammation [23]. Neurological symptoms tended to be high in patients with ADAMTS13 TMA, and the frequency of renal dysfunction was high in those with other TMA, suggesting that ADAMTS13 TMA might be suitable for a typical TTP, while the other TMA might be suitable for typical HUS.

Although decreased platelet count (98.4%) and decreased hemoglobin (95.1%) were frequently observed in patients with all types of TMA, decreased platelet count was not observed in all patients. In this survey, a few patients with platelet counts greater than 120,000/μl were diagnosed to have TMA based on clinical symptoms and other laboratory data such as stool culture for O-157 or ADAMTS13. It was previously reported that thrombocytopenia was found in 98.4% of patients with TMA [24]. In acquired TMA, the platelet count tended to be low in the patients with ADAMTS13 TMA. It was also previously reported that patients with severe ADAMTS13 deficiency had a lower platelet count than patients with detectable ADAMTS13 activity (49.5×10^9/l; range $6–103 \times 10^9$/l; $p = 0.0004$) [25].

The fact that the hemoglobin level was lower in patients with acquired TMA than those with familial TMA suggests that microangiopathic hemolytic anemia might be predominantly observed in acquired TMA.

Moreover, PE is performed in most TMA patients without O-157 TMA, and the efficacy of this treatment tended to be high in patients with ADAMTS13 TMA, supporting the use of PE, which is usually applied in typical TTP as the standard therapy in Japan. It is clear that PE can exert its effects by both removing the antibody to ADAMTS13 and by replacing ADAMTS13 in the ADAMTS13 TMA [16]. However, it is not clear how PE affects other TMA. The transfusion of FFP was frequently performed in patients with familial TMA and ADAMTS13 TMA, and the efficacy tended to be high in those with familial ADAMTS13 TMA, but was not high in patients with acquired TMA. PE was previously reported to be more useful than FFP transfusion [26]. Both findings suggest that removing the antibody to ADAMTS13 is necessary to treat acquired ADAMTS13 TMA.

Steroid treatment, including pulse therapy with methylprednisolone, was administered to most patients with acquired TMA without underlying O-157 infection, and the efficacy of pulse therapy tended to be high in patients with ADAMTS13 TMA. Immunosuppressive therapy, including steroid therapy [27] is used to inhibit the production of autoantibodies against ADAMTS13. Recently, the efficacy of rituximab was reported in refractory or relapsing TTP as the strongest immunosuppressive therapy [28], further studies examining its efficacy are needed. Hemodialysis and anticoagulant therapy were carried out in patients with acquired TMA, and the efficacy was high in patients with O-157 TMA, leading to a high complete remission (CR) rate for these patients. PC transfusion was not recommended in TTP, but the therapy was still carried out in patients with ADAMTS13 TMA. As expected, it had relatively low efficacy. The CR rate was the highest and the mortality rate was the lowest in patients with O-157 TMA. The mortality rate tended to be low in patients with ADAMTS13 TMA. This is likely because, PE and steroids are more effective against ADAMTS13 TMA than against other TMA. The mortality rate of TMA in Japan was 26.8% in 1988 [27], 26.0% in 1999 [29], 22.0% in 2005 [19] and 18.0% in 2006, suggesting that the mortality rate of TMA is improving.

The evaluation of TMA by measurement of ADAMTS13 might promote better diagnosis and early treatment using PE and steroid therapy in those with ADAMTS13 TMA. This could lead to further improvement in the mortality rate.

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ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

Potential Role of Enhanced Cytokinemia and Plasma Inhibitor on the Decreased Activity of Plasma ADAMTS13 in Patients With Alcoholic Hepatitis: Relationship to Endotoxemia

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Background: Deficiency of ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) results in an increase in unusually large von Willebrand factor multimer (UL-VWFM) of the plasma and finally causes microcirculatory disturbance. Our previous study demonstrated that the imbalance of increased UL-VWFM over decreased ADAMTS13 activity may contribute to the development of multiorgan failure in patients with alcoholic hepatitis (AH). The aim of this study was to explore the potential mechanism to reduce the activity of plasma ADAMTS13.

Methods: Plasma cytokine levels including interleukin (IL)-6, IL-8, and tumor necrosis factor-α (TNF-α), plasma endotoxin concentration, and the plasma inhibitor against ADAMTS13 were determined together with ADAMTS13 activity, VWF antigen (VWF:Ag), and UL-VWFM in 24 patients with AH and 5 patients with severe alcoholic hepatitis (SAH).

Results: The concentrations of IL-6, IL-8, and TNF-α on admission were significantly higher in patients with SAH than in those with AH and controls. The ADAMTS13 activity concomitantly decreased, and the VWF:Ag progressively elevated with increasing concentrations of these cytokines from normal range to over 100 pg/ml. Plasma endotoxin concentration was markedly higher in patients with SAH (mean 52.3 pg/ml) and AH (21.7 pg/ml) than in controls (7.9 pg/ml). The endotoxin concentration inversely correlated with ADAMTS13 activity and was higher in patients with UL-VWFM than those without. The inhibitor was detected in 4 patients with SAH (0.9 to 2.1 BU/ml) and 6 patients with AH (0.5 to 1.6 BU/ml). Patients with the inhibitor showed lower functional liver capacity, higher endotoxin concentration, and marked inflammatory signs than those without. At the recovery stage, the ADAMTS13 activity increased to normal range, the VWF:Ag decreased, and the UL-VWFM disappeared with the decrease in the concentrations of cytokines and endotoxin, and the disappearance of the inhibitor.

Conclusion: Decreased ADAMTS13 activity and increased VWF:Ag could be induced not only by pro-inflammatory cytokinemia, but also by its inhibitor, both of which may be closely related to enhanced endotoxemia in patients with AH and SAH.

Key Words: ADAMTS13, Cytokines, Inhibitor, Endotoxin, Alcoholic Hepatitis.

ALCOHOLIC HEPATITIS (AH) is a potentially life-threatening complication of alcoholic abuse, and its severe form, severe AH (SAH) frequently develops multi-organ failure with manifestations of acute hepatic failure, which is associated with high morbidity and mortality (Ishii et al., 1993; Maddrey et al., 1978; Mookerjee et al., 2003). The pathogenesis of AH is uncertain, but relevant factors include metabolism of alcohol to toxic products, oxidant stress, acetaldehyde adducts, the action of endotoxin on Kupffer cells, and impaired hepatic regeneration (Haber et al., 2003).

Recently, ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) has been focused on the occurrence of thrombotic thrombocytopenic purpura (TTP) (Fujimura et al., 2002; Furlan et al., 1997; Tsai and Lian, 1998), which is characterized by thrombocytopenia, renal dysfunction, fluctuating neurological symptoms, microangiopathic hemolytic anemia, and fever (Moschcowitz, 1924). ADAMTS13 is a metalloproteinase that specifically cleaves the multimeric von Willebrand factor (VWF) between

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Tyr1605 and Met1606 within the VWF A2 domain (Levy et al., 2001; Plaimauer et al., 2002; Soejima et al., 2001; Zheng et al., 2001). VWF is synthesized in the vascular endothelial cells, and released into the plasma as "unusually large" VWF multimers (UL-VWFM) (Moake, 2002; Ruggeri, 1997). Deficiency of ADAMTS13 caused either by mutations of the *ADAMTS13* gene (Kokame et al., 2002) or by inhibitory autoantibodies against ADAMTS13 (Furlan et al., 1998; Tsai and Lian, 1998) increases the plasma levels of UL-VWFM, which leads to platelet clumping and/or thrombi under high shear stress, resulting in microcirculatory disturbance (Furlan et al., 1998; Moake, 2002; Ruggeri, 1997; Tsai and Lian, 1998). We recently demonstrated that the ADAMTS13 is produced exclusively in the hepatic stellate cells adjacent to the endothelial cells (Uemura et al., 2005a), where VWF is produced.

A little information has been available on the ADAMTS13 activity associated with liver diseases. The activity was low in the patients with liver cirrhosis (Mannucci et al., 2001; Uemura et al., 2008) and acute hepatitis (Kavakli, 2002). We showed the significant reduction in the ADAMTS13 activity in patients with hepatic veno-occlusive disease after stem cell transplantation (Park et al., 2002), and a prompt decrease in the protease activity associated with early adverse events including ischemia-reperfusion injury and/or acute graft rejection in living-donor related liver transplantation (Ko et al., 2006). In our previous reports, the ADAMTS13 activity was extremely low in the nonsurvivors with SAH and multiorgan failure, and the imbalance of increased production of UL-VWFM over decreased activity of ADAMTS13 may, in part, contribute to the progression of liver disturbance and the development of multiorgan failure through microcirculatory disturbance in SAH in addition to AH (Matsuyama et al., 2007; Uemura et al., 2005b). However, it remains unclear why the ADAMTS13 activity decrease in patients with AH.

Alternatively, endotoxemia due to hepatic reticuloendothelial dysfunction and increased intestinal permeability may be thought to trigger the enhancement of proinflammatory cytokines, which may cause systemic inflammatory response syndrome together with microcirculatory disturbance and finally lead to multiorgan failure in SAH (Fukui et al., 1991; Ishii et al., 1993; Mookerjee et al., 2003). It was, recently, demonstrated that inflammatory cytokines are associated with the decrease in the ADAMTS13 activity and the increase in UL-VWFM released from endothelial cells in vitro (Bernardo et al., 2004) and that inflammation-associated ADAMTS13 deficiency promotes formation of UL-VWFM in patients with sepsis (Bockmeyer et al., 2008), indicating the close linkage among cytokinemia, endotoxemia, and the ADAMTS13 activity in AH.

In the present study, we determined the plasma cytokine levels, plasma endotoxin concentration, and the inhibitor against the ADAMTS13, and tried to explore the potential mechanism to reduce the activity of plasma ADAMTS13 in patients with AH and SAH.

MATERIALS AND METHODS

Patients

The study was carried out in 28 patients with AH (26 men and 2 women; mean age: 55.1 years) and 5 patients with SAH (4 men and 1 woman; mean age: 41.2 years), who were principally same patients previously described (Matsuyama et al., 2007; Uemura et al., 2005b) (Table 1). All patients were originally admitted in our

Table 1. Clinical Data of Patients With Alcoholic Hepatitis

Variable	Alcoholic hepatitis	Severe alcoholic hepatitis	Normal range
Age (year)	55.1 (23–67)	41.2[b] (30–61)	
Sex (male/female)	26/2	4/1	
Serum total bilirubin (mg/dl)	4.4 (0.3–22.1)	13.5[c] (8.0–24.3)	0.3–1.1
Aspartate aminotransferase (IU/l)	180 (40–673)	320 (119–709)	12–32
Alanine aminotransferase (IU/l)	116 (25–407)	87 (63–165)	5–36
Lactate dehydrogenase (IU/l)	278 (132–450)	538[c] (283–836)	116–230
γ-Glutamyl transpeptidase (IU/l)	670 (37–2388)	472 (145–1000)	11–69
White blood cell count (/mm^3)	7,474 (3000–17100)	12,620[a] (3500–26600)	3,900–9,800
Polymorphonuclear neutrophil (/mm^3)	5,260 (1462–14877)	11,345[c] (3220–25004)	2,000–7,500
Hemoglobin (g/dl)	13.3 (9.1–17.0)	9.0[c] (7.3–11.1)	13.5–17.6
Platelet count (×10^4/mm^3)	16.8 (6.9–27.9)	8.8[a] (2.8–16.4)	13.1–36.2
C-reactive protein (mg/dl)	1.2 (0.1–13.8)	4.0 (0.5–12.2)	0–0.6
Serum albumin (g/dl)	4.0 (2.3–4.9)	3.0[c] (1.8–3.1)	3.8–5.0
Prothrombin time (%)	83 (58–100)	36[c] (27–39)	70–100
Blood urea nitrogen (mg/dl)	17 (4–60)	33[a] (11–89)	8–20
Serum creatinine (mg/dl)	1.0 (0.6–1.8)	2.8[c] (0.4–4.7)	0.3–0.9
Liver cirrhosis (+)	11	5	
Hepatic encephalopathy (Grade II-III)	0	3	
Renal failure/pneumonia/heart failure/DIC	0/0/0/0	4/4/3/1	
Treatment (FFP/prednisolone/HD)	–	5/2/1	
Outcome (alive/dead)	28/0	2/3	

DIC, disseminated intravascular coagulation; FFP, fresh frozen plasma; HD, hemodialysis.
[a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.005$ versus alcoholic hepatitis.

hospital between June 2001 and January 2006. Any patients with a known history of coagulopathies, sepsis, or platelet disorders were excluded from this study. The diagnosis of AH and SAH was based on the physical findings, laboratory tests, and confirmed by the liver histology in 2 patients with SAH and 11 patients with AH; the remaining 3 cases with SAH and 17 cases with AH were clinically diagnosed, according to the Diagnostic Criteria for Alcoholic Liver Injury, established by Takada, and a Japanese study group for alcoholic liver disease (1993). In brief, the etiological diagnosis of alcoholics with liver disease was classified into 3 groups: alcohol alone, combination with alcohol and virus, and others. In the alcohol alone group, virus markers were negative, and serum transaminase decreased less than 80 units during 4 weeks after abstinence. Serum γ-glutamyl transpeptidase (γ-GTP) also decreased either 1.5 times of normal value or less than 40% of the initial levels, during 4 weeks after abstinence. In addition, in the absence of liver histology, AH was clinically diagnosed in patients who showed augmented liver dysfunction following the increase in alcohol consumption, the increase in aspartate aminotransferase higher than alanine aminotransferase, and the increase in serum total bilirubin more than 2.0 mg/dl, in addition to more than 3 clinical features among abdominal pain, fever up, leukocytosis, the increase in alkaline phosphatase more than 1.5 times of normal value, and the increase in γ-GTP more than 2.0 times of normal value. The severity of SAH was estimated according to Maddrey score (Carithers et al., 1989). Hepatic encephalopathy was graded according to the classification of Trey and colleagues (1966). The diagnosis of disseminated intravascular coagulation (DIC) was made by the scoring system (Taylor et al., 2001). Standard therapy for patients with AH was abstinence from alcohol and supportive care including nutritional supplementation of at least 25 kcal/d, 1 g protein/kg/d, vitamins, and minerals via oral or enteral routes, but if difficulties arised, a parenteral route was used. All subjects gave informed consent to participate in the study. The study protocol was approved by the Nara Medical University Hospital Ethics Committee.

Assays of ADAMTS13 Activity, VWF Antigen, UL-VWFM, and Inhibitor Against ADAMTS13

Blood was taken from the patients on and/or during admission in plastic tubes with 1/10th volume of 3.8% sodium citrate as an anticoagulant. In 8 patients with AH and 2 survivors with SAH, a second plasma sample was taken between 7 and 90 days at the recovery stage when serum total bilirubin has been normalized and/or transaminase decreased within 2 times of normal range; in a nonsurvivor with SAH, plasma was sequentially taken every 2 week for 2 months until the terminal stage. Platelet-poor plasma was prepared by centrifugation of the plasma at $3000 \times g$ at 4°C for 15 minutes, and was stored in aliquots at −80°C until analysis. Plasma ADAMTS13 activity was assayed according to the method of Furlan et al. (1998) with slight modification (Mori et al., 2002). The detection limit of the activity was approximately 3%, and its normal value was 102 ± 23% (mean ± SD) (n = 60; 30 women and 30 men, 20 to 39 years old) (Mori et al., 2002). We, therefore, considered the activity low when it was less than 50% of the healthy subjects (mean—2SD). The plasma UL-VWFM was analyzed by SDS-0.9% agarose gel electrophoresis using 1 μl of samples (Park et al., 2002). The plasma VWF:Ag was measured by ELISA (Dako, Kyoto, Japan), and its normal level was 100 ± 53% (n = 60, 20 to 39 years of age). The inhibitor activity against ADAMTS13 was measured using heat-inactivated plasmas at 56°C for 30 minutes (Furlan et al., 1998; Tsai and Lian, 1998). One Bethesda's unit (BU) of the inhibitor was defined as the amount that reduces the ADAMTS13 activity to 50% of the control (Kasper et al., 1975), and its titer was estimated to be significant in more than 0.5 BU/ml.

Measurements of Cytokines

Plasma concentrations of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-8 were determined by Immunoassay Kits (BioSource International, Camarillo, CA).

Determination of Endotoxin

All blood specimens from 20 healthy controls (10 men and 10 women, 20 to 39 years old) and from patients with AH and SAH were obtained under aseptic conditions by peripheral venipuncture using pyrogen-free syringe and needles. The blood samples were mixed in pyrogen-free tubes with 1/10th volume of 3.8% sodium citrate as an anticoagulant, placed on ice, and transported immediately to the laboratory. Plasma was immediately separated in a refrigerated centrifuge at $3000 \times g$ at 4°C for 15 minutes, and stored at −20°C for subsequent analysis. Endotoxin activity was measured by a chromogenic substrate assay (Toxicolor LS–M Set, Seikagaku Kogyo Co., Tokyo, Japan) with kinetics analysis (Obayashi et al., 1985). In brief, 50 μl of plasma samples was mixed with 450 μl of 0.02% Triton X-100. The mixture was heated at 70°C for 10 minutes to inactivate the inhibitor reacted with endotoxin, and serial standard solution was made to final exogenous endotoxin concentration of 180, 90, 45, 22.5, 11.3, and 5.6 pg/ml. The absorbance was measured at 37°C every 15 second until 30 minutes by a microprocessor controlled reader (Wellreader, SK603; Seikagaku Co., Tokyo, Japan). Liner part of the kinetics curve was read and endogenous plasma endotoxin concentrations were calculated from the obtained standard curve. Determinations were done in duplicate, and the mean value was utilized.

Statistics

The differences between the paired and unpaired groups were analyzed using the Mann–Whitney U-test. Correlations were calculated with the Spearman rank test. Categorical data were analyzed using the chi-squared test (Fisher's exact test). The analysis was carried out using the statistical software Statview (version 5.0; SAS Institute, Cary, NC). The data are expressed as mean ± SD. A 2-tailed p-value less than 0.05 was considered significant.

RESULTS

Clinical Characteristics and Laboratory Values

The clinical data of patients with AH and SAH are shown in Table 1. The patients with SAH were younger than those with AH, and the gender was predominant in male both in patients with AH and SAH. Serum total bilirubin, lactate dehydogenase, white blood cell, and peripheral polymorphonuclear neutrophil (PMN) count were higher in patients with SAH than those with AH, whereas hemoglobin, platelet count, serum albumin, and prothrombin time were lower in patients with SAH than those in AH. Maddrey score of patients with SAH was 52 to 71 (mean: 60) on admission. Eleven of 24 patients with AH and all patients with SAH were complicated by liver cirrhosis (LC). All patients with AH survived, and 3 of 5 patients with SAH died of hepatic failure within 2 to 61 days. Three nonsurvivors with SAH showed hepatic encephalopathy of grade II to III, ascites, renal failure, pneumonia, and heart failure on admission, indicating the occurrence of multiorgan failure. One of them had DIC, but the others did not. Of the remaining 2 survivors with SAH, one was complicated by renal

failure and pneumonia, but not by hepatic encephalopathy, and the other had moderate ascites, but not multiorgan failure. All patients with SAH were treated with fresh frozen plasma (FFP) together with standard therapy. Of the 2 survivors, one completely recovered in 30 days and the other in 90 days. One of the 3 nonsurvivors was treated with hemodialysis because of acute renal failure, but finally died in 61 days. The other 2 was treated with prednisolone, but died within a week. In 3 nonsurvivors, plasma exchange was not performed because of systemic circulatory disturbance (Table 1).

Plasma ADAMTS13 Activity, VWF:Ag, and UL-VWFM

As previously reported (Matsuyama et al., 2007; Uemura et al., 2005b), the plasma ADAMTS13 activity on admission was significantly lower in patients with AH (61 ± 34%, $p < 0.001$) and SAH (24 ± 22%, $p < 0.001$) than in healthy subjects (102 ± 23%). The activity further decreased in patients with SAH as compared with those with AH ($p < 0.02$). The values of plasma VWF:Ag were higher in patients with AH (381 ± 207%, $p < 0.001$) and SAH (806 ± 326%, $p < 0.001$) than in healthy subjects (100 ± 53%), and it was higher in patients with SAH than those with AH ($p < 0.005$). The ratio of VWF:Ag to ADAMTS13 activity was higher in patients with AH (10.6 ± 11.6, $p < 0.001$) and SAH (102.2 ± 112.6, $p < 0.001$) than in healthy subjects (1.0 ± 0.4), and it was higher in patients with SAH than those with AH ($p < 0.005$). Plasma UL-VWFM was detected in 4 (80.0%) of 5 patients with SAH, and in 5 (17.9%) of 28 patients with AH, who had moderate deficiency of ADAMTS13 activity together with markedly high VWF values.

Plasma Cytokine Levels and Their Relationships to ADAMTS13 Activity, VWF:Ag, and UL-VWFM

Plasma IL-6 concentration on admission was significantly higher in patients with AH (25 ± 32 pg/ml, $p < 0.05$) and SAH (504 ± 681 pg/ml, $p < 0.01$) than in healthy subjects (< 7.8 pg/ml), and it was higher in patients with SAH ($p < 0.001$) compared with those with AH (Fig. 1*A*). Plasma concentration of IL-8 was significantly higher in patients with SAH (216 ± 304 pg/ml) than in healthy subjects (< 15.6 pg/ml, $p < 0.01$) and patients with AH (37 ± 77 pg/ml, $p < 0.05$), whereas it did not differ between patients with AH and healthy subjects (Fig. 1*B*). Plasma TNF-α concentration was higher in patients with SAH (29 ± 18 pg/ml) than those with AH (17 ± 6 pg/ml, $p < 0.005$) and healthy subjects (< 15.6 pg/ml, $p < 0.01$), although it did not differ between patients with AH and healthy subjects (Fig. 1*C*).

The ADAMTS13 activity on admission concomitantly decreased from the highest in patients with normal range of IL-6 (68 ± 31%) and IL-8 (70 ± 32%), to those with normal range to 100 pg/ml of IL-6 (37 ± 14%, $p < 0.02$) and IL-8 (37 ± 14%, $p < 0.02$), and to the lowest in those with more than 100 pg/ml of IL-6 (13 ± 10%, $p < 0.02$) and IL-8 (9 ± 7%, $p < 0.05$) (Fig. 2*A* and 2*B*). In addition, the

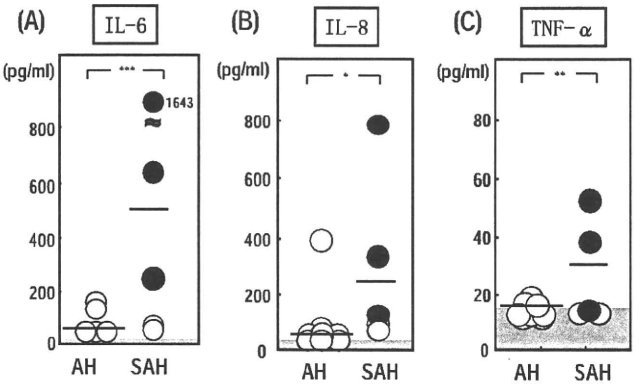

Fig. 1. Plasma levels of cytokines in the patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH) on admission. The shaded area shows the normal range. The open circles indicate survivors and the closed circles indicate nonsurvivors. The concentrations of IL-6 (**A**), IL-8 (**B**), and TNFα (**C**) were significantly higher in the patients with SAH than those in AH. IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor-α; AH, alcoholic hepatitis; SAH, severe alcoholic hepatitis. $*p < 0.05$, $**p < 0.005$, and $***p < 0.001$: significantly different from the 2 groups.

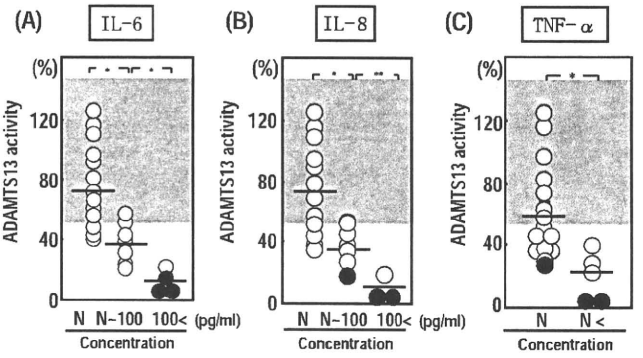

Fig. 2. Relationship between plasma cytokine levels and ADAMTS13 activity in the patients with alcoholic hepatitis and severe alcoholic hepatitis on admission. The shaded area shows the normal range. The open circles indicate survivors and the closed circles indicate nonsurvivors. The ADAMTS13 activity concomitantly decreased with increasing levels of plasma concentration of IL-6 (**A**) and IL-8 (**B**). In addition, the activity decreased in patients with higher TNF-α concentrations over normal range compared to those without (**C**). IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor-α; N, normal range. $*p < 0.02$ and $**p < 0.005$: significantly different from the 2 groups.

activity decreased in patients with higher TNF-α concentrations over normal range (22 ± 18%, $p < 0.02$) compared to those without (57 ± 31%) (Fig. 2*C*).

The VWF:Ag on admission progressively increased from the lowest in patients with normal range of IL-6 (298 ± 107%) and IL-8 (309 ± 107%), to those with normal range to 100 pg/ml of IL-6 (509 ± 232%, $p < 0.005$) and IL-8 (425 ± 190%, $p < 0.05$), and to the highest in those with more than 100 pg/ml of IL-6 (624 ± 394%, $p < 0.001$) and IL-8 (880 ± 354%, $p < 0.02$) (Fig 3*A* and 3*B*). In addition, the VWF:Ag increased in patients with higher TNF-α concentrations over normal range (609 ± 328%, $p < 0.02$) compared to those without (352 ± 178%) (Fig. 3*C*). The incidence of UL-VWFM was

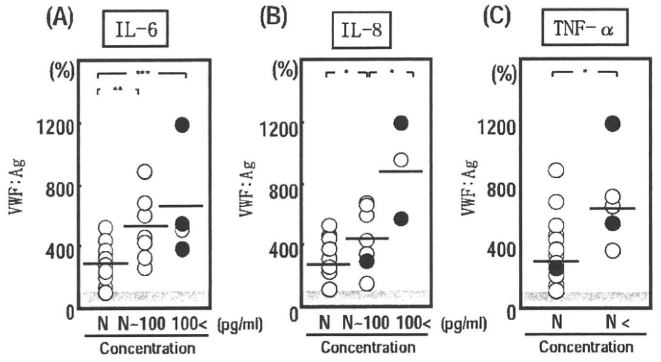

Fig. 3. Relationship between plasma levels of cytokines and VWF antigen (VWF:Ag) in the patients with alcoholic hepatitis and severe alcoholic hepatitis on admission. The shaded area shows the normal range. The open circles indicate survivors and the closed circles indicate nonsurvivors. The VWF:Ag concomitantly increased with increasing levels of plasma concentration of IL-6 (**A**) and IL-8 (**B**). In addition, the antigen increased in patients with higher TNF-α concentrations over normal range compared to those without (**C**). IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor-α; N, normal range. $*p < 0.05$, $**p < 0.005$, and $***p < 0.001$: significantly different from the 2 groups.

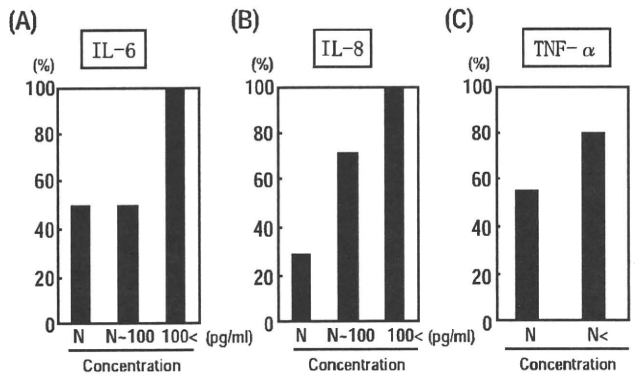

Fig. 4. Relationship between plasma levels of cytokines and the incidence of unusually large von Willebrand factor multimer (UL-VFWM) in the patients with alcoholic hepatitis and severe alcoholic hepatitis on admission. The incidence reached 100% in patients with higher concentration more than 100 pg/ml of IL-6 (**A**), and increased with increasing levels of plasma concentration of IL-8 (**B**). In addition, it increased in patients with higher TNF-α concentration over normal range than those without (**C**). IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor-α; N, normal range.

50% both in patients with normal range and normal range to 100 pg/ml of IL-6, and reached 100% in those with more than 100 pg/ml of IL-6 (Fig. 4*A*). The incidence concomitantly increased from the lowest in patients with normal range of IL-8 (30%), to those with normal range to 100 pg/ml of IL-8 (70%), and to the highest in those with more than 100 pg/ml of IL-8 (100%) (Fig. 4*B*). In addition, it tended to be higher in patients with higher TNF-α concentrations over normal range (80%) than those without (55%) (Fig. 4*C*).

Plasma Endotoxin Concentration and Their Relationships to ADAMTS13 Activity, VWF:Ag, and UL-VWFM

In normal healthy subjects, plasma endotoxin concentration was below 10 pg/ml, and averaged 7.9 ± 1.7 pg/ml.

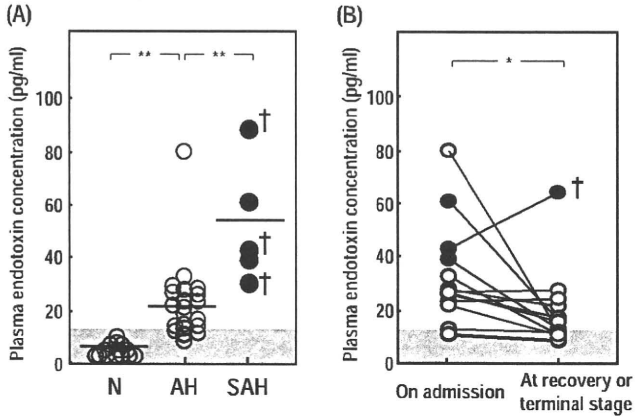

Fig. 5. Plasma endotoxin concentration in patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH). The shaded area shows the normal range. The open circles indicate AH and the closed circles indicate SAH. The crosses indicate nonsurvivors. Plasma endotoxin concentration on admission was significantly higher in patients with AH and SAH than in normal subjects, and it was higher in patients with SAH compared to those with AH (**A**). The concentration on admission significantly decreased at the recovery phase in 8 patients with AH and 2 survivors with SAH, whereas a nonsurvivor with SAH showed further increase at the terminal stage (**B**). N, normal subjects; AH, alcoholic hepatitis; SAH, severe alcoholic hepatitis. $*p < 0.02$ and $**p < 0.001$: significantly different from the 2 groups.

The concentration on admission was significantly higher in patients with AH (21.7 ± 14.0 pg/ml, $p < 0.001$) and SAH (52.3 ± 23.1 pg/ml, $p < 0.001$) than in healthy subjects, and it was higher in patients with SAH ($p < 0.001$) as compared with those with AH (Fig. 5*A*). The concentration on admission significantly decreased at the recovery phase in 8 patients with AH and 2 survivors with SAH (31.0 ± 19.8 to 15.0 ± 6.0 pg/ml, $p < 0.02$), whereas a nonsurvivor with SAH showed further increase at the terminal stage (42.8 to 64.5 pg/ml) (Fig. 5*B*). The endotoxin concentration on admission inversely correlated with plasma ADAMTS13 activity ($r = -0.474$, $p < 0.01$) (Fig. 6), and was higher in patients with UL-VWFM than those without UL-VWFM (46.6 ± 24.0 vs. 18.5 ± 7.9 pg/ml, $p < 0.001$). In addition, plasma endotoxin concentration correlated positively with white blood cell count ($r = 0.486$, $p < 0.005$), PMN count ($r = 0.814$, $p < 0.001$), serum total bilirubin ($r = 0.493$, $p < 0.005$), blood urea nitrogen ($r = 0.677$, $p < 0.001$), and serum creatinine ($r = 0.749$, $p < 0.001$), and correlated inversely with hemoglobin ($r = -0.512$, $p < 0.005$) and prothrombin time ($r = -0.665$, $p < 0.001$).

Plasma Inhibitor Against ADAMTS13 and Its Relationship to ADAMTS13 Activity, VWF:Ag, Plasma Endotoxin Concentration, and Clinical Features

The plasma inhibitor against ADAMTS13 on admission was detected in 4 patients with SAH (80%, $p < 0.05$) and 6 patients with AH (21.4%). The inhibitory activity averaged 1.5 BU/ml (range 0.9 to 2.1 BU/ml) in SAH and 1.0 BU (0.5 to 1.6 BU/ml) in AH, respectively. Patients with the inhibitor showed lower ADAMTS13 activity (Fig. 7*A*),

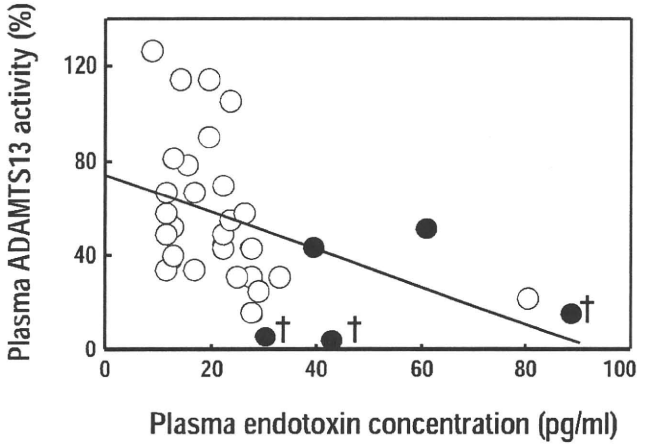

Fig. 6. Correlation between plasma endotoxin concentration and plasma ADAMTS13 activity in patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH) on admission. The open circles indicate AH and the closed circles indicate SAH. The crosses indicate nonsurvivors. The endotoxin concentration inversely correlated with plasma ADAMTS13 activity ($r = -0.474$, $p < 0.01$).

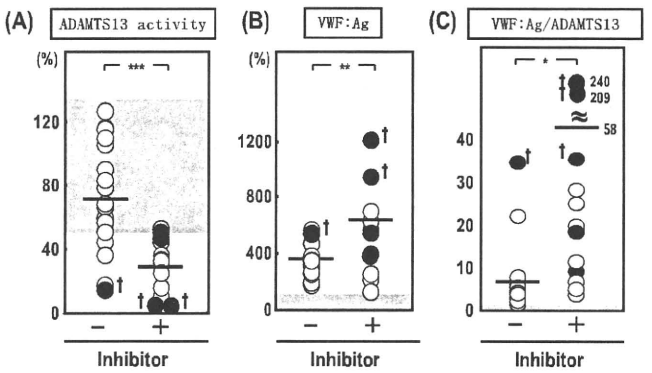

Fig. 7. Relationship of plasma inhibitor against ADAMTS13 to ADAMTS13 activity, VWF antigen (VWF:Ag), and the ratio of VWF:Ag to ADAMTS13 activity in patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH) on admission. The shaded area shows the normal range. The open circles indicate AH and the closed circles indicate SAH. Crosses indicate nonsurvivors. Patients with the inhibitor showed lower ADAMTS13 activity (**A**), higher VWF:Ag (**B**), and higher ratio of VWF:Ag to ADAMTS13 activity (**C**) than those without. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$: significantly different from the 2 groups.

higher VWF:Ag (Fig. 7*B*), and higher ratio of VWF:Ag to ADAMTS13 activity (Fig. 7*C*) than those without (ADAMTS13 activity: 26 ± 15% vs. 68 ± 32%, $p < 0.001$; VWF:Ag: 609 ± 316% vs. 374 ± 199%, $p < 0.01$; the ratio of VWF:Ag to ADAMTS13 activity: 58.4 ± 88.2 vs. 7.3 ± 7.9, $p < 0.02$; respectively). In addition, patients with AH and SAH who had inhibitor showed lower serum albumin level and higher levels of serum total bilirubin, PMN count, C-reactive protein, and plasma endotoxin concentration than those with AH who had no inhibitor (Table 2).

Changes in Plasma ADAMTS13 Activity and Its Related Parameters During Hospitalization

At the recovery stage in survivors with AH and SAH, the ADAMTS13 activity significantly increased to normal range, the VWF:Ag decreased, and the UL-VWFM disappeared with the decrease in the concentrations of IL-6, IL-8, and endotoxin, and with the disappearance of the inhibitor against ADAMTS13 (Table 3). On the other hand, in a nonsurvivor with SAH, the activity of ADAMTS13 during FFP infusion showed transient increase but finally decreased, the VWF:Ag remained high, and the UL-VWFM was still present with the increase in the concentrations of IL-6, IL-8, TNF-α, and endotoxin, and the presence of the ADAMTS13 inhibitor at the terminal stage (Table 3).

DISCUSSION

In the present study, the ADAMTS13 activity gradually decreased, and the VWF:Ag progressively elevated with concomitant increase in concentrations of IL-6, IL-8, and TNF-α from normal range to over 100 g/ml, on admission (Figs. 2 and 3). The incidence of UL-VWFM detected in plasma became higher as concentrations of IL-6, IL-8, and TNF-α increased (Fig. 4).

At the recovery stage in survivors with AH and SAH, the ADAMTS13 activity significantly increased to normal range, the VWF:Ag decreased, and the UL-VWFM disappeared with the decrease in the concentration of IL-6 and IL-8, whereas in a nonsurvivor with SAH, the ADAMTS13 activity remained extremely in low levels, the VWF:Ag was still high, and the UL-VWFM was persistently present with the increase in concentrations of these cytokines (Table 3). These results indicate that the decrease in the ADAMTS13 activity and the increase in VWF:Ag in addition to UL-VWFM may be closely associated with increased proinflammatory cytokines including IL-6, IL-8, and TNF-α. It was, recently, demonstrated that IL-6 inhibited the action of ADAMTS13 under flow condition, and both IL-8 and TNF-α stimulated the release of UL-VWFM in a dose-dependent manner from human umbilical vein endothelial cells in vitro (Bernardo et al., 2004). Considering that high concentrations of proinflammatory cytokines such as IL-8 and TNFα are closely related to a poor outcome of AH (Fujimoto et al., 2000; Ishii et al., 1993; Mookerjee et al., 2003), enhanced cytokinemia may, in part, cause the decrease in the ADAMTS13 activity together with the increase in the VWF:Ag and UL-VWFM, finally resulting in the occurrence of multiorgan failure through microcirculatory disturbance in patients with SAH.

On the other hand, endotoxemia has been known to play an important role in the initiation and aggravation of AH through the enhancement of proinflammatory cytokines including IL-6, IL-8, and TNF-α (Fujimoto et al., 2000; Fukui et al., 1991; Ishii et al.,1993; Mookerjee et al., 2003). In our study, the concentrations of IL-6, IL-8, and TNF-α on

Table 2. Relationship of Presence or Absence of Plasma Inhibitor Against ADAMTS13 to Laboratory Findings and Plasma Endotoxin Concentration in Patients With Alcoholic Hepatitis

	Alcoholic hepatitis		Severe alcoholic hepatitis
Variable	Inhibitor (−) (n = 22)	Inhibitor (+) (n = 6)	Inhibitor (+) (n = 4)
Serum total bilirubin (mg/dl)	2.5 ± 4.0	11. 1 ± 10.0[b]	10.0 ± 2.7[b]
Polymorphonuclear neutrophil (/mm^3)	4063 ± 1750	8762 ± 3118[c]	7931 ± 4316[b]
C-reactive protein (mg/dl)	1.1 ± 2.1	4.6 ± 4.9[a]	4.3 ± 5.4[a]
Serum albumin (g/dl)	4.2 ± 1.1	3.3 ± 1.2[a]	3.1 ± 1.2[b]
Plasma endotoxin concentration (pg/ml)	17.3 ± 6.1	39.4 ± 23.0[b]	43. 3 ± 12.9[c]

[a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.001$ versus alcoholic hepatitis without inhibitor against ADAMTS13.

Table 3. Changes in Plasma ADAMTS13 Activity and Its Related Parameters in Survivors and a Nonsurvivor in Patients With Alcoholic Hepatitis

	Survivors (n = 10)		Nonsurvivors (n = I)		
Variables	On admission	Recovery state	On admission	During FFP infusion	Terminal stage
ADAMTS13 activity (%)	42 ± 14	72 ± 26[c]	4.5	12.0	4.5
VWF:Ag	533 ± 367	335 ± 241[a]	940	501	750
VWF:Ag/ADAMTS13	17.7 ± 19.5	5.6 ± 5.1[a]	209	42	167
UL-VWFM (positive/negative)	5/5	0/10[b]	1/0	1/0	1/0
Interleukin-6 (pg/ml)	21 ± 14	12 ± 7[a]	563	649	1756
Interleukin-8 (pg/ml)	28 ± 18	15 ± 13[b]	211	213	322
Tumor necrosis factor-α (pg/ml)	16.1 ± 1.8	<15.6	42	53	138
Plasma endotoxin concentration (pg/ml)	31.0 ± 19.8	15.0 ± 6.0[b]	42.8	55.2	64.5
Inhibitor against ADAMTS13 (positive/negative)	7/3	0/10[c]	1/0	1/0	1/0

VWF:Ag, von Willebrand factor; UL-VWFM, unusually large von Willebrand factor; FFP, fresh frozen plasma.
[a]$p < 0.05$, [b]$p < 0.02$, and [c]$p < 0.005$ versus on admission.

admission were significantly higher in patients with SAH than in those with AH and controls (Fig. 1). Plasma endotoxin concentration was higher in patients with SAH and AH than in healthy subjects, and was markedly higher in patients with SAH than in AH (Fig. 5*A*). The endotoxin concentration determined by the chromogenic substrate assay after pretreatment with detergent, Triton X-100, and heating at 70°C for 10 min was consistent with that described by the previous report (Fukui et al., 1991, Lumsden et al., 1988; Obayashi, 1984; Obayashi et al., 1985). The endotoxin concentration on admission inversely correlated with ADAMTS13 activity (Fig. 6), and was higher in patients with UL-VWFM than those without. At the recovery stage, the endotoxin concentration significantly decreased with increased ADAMTS13 activity and decreased VWF:Ag, and the disappearance of UL-VWFM together with the reduction of IL-6 and IL-8 concentrations (Table 3). These results indicate that enhanced endotoxemia may be closely related to the decrease in the ADAMTS13 activity and the appearance of UL-VWFM through the enhanced cytokinemia. This is the first report to demonstrate a potential linkage of endotoxemia to enhanced inflammatory cytokines and the imbalance of increased VWF:Ag over decreased activity of ADAMTS13 leading to systemic microcirculatory disturbance especially in patients with SAH. Recent study demonstrated that inflammation-associated ADAMTS13 deficiency promotes formation of UL-VWFM (Bockmeyer et al., 2008), and that severe secondary ADAMTS13 deficiency can be associated with sepsis-induced DIC and may contribute to the development of renal failure (Ono et al., 2006), which may support our data and hypothesis.

Alternatively, another mechanism to reduce the activity of ADAMTS13 is the presence of plasma inhibitor against ADAMTS13. In our study, the inhibitor on admission was detected in 80% in patients with SAH and 21.4% in patients with AH, and its inhibitory activity averaged 1.5 BU/ml in SAH and 1.0 BU/ml in AH. Patients with the inhibitor showed lower ADAMTS13 activity and higher VWF:Ag than those without (Fig. 7). At the recovery stage, the inhibitor detected in 5 patients disappeared with increased ADAMTS13 activity and decreased VWF:Ag, together with the decrease in concentrations of cytokines and endotoxin (Table 3). Interestingly, patients with AH in addition to SAH who had inhibitor showed higher levels of serum total bilirubin, PMN count, C-reactive protein, and plasma endotoxin concentration, and lower serum albumin level than those with AH who had no inhibitor (Table 2). These results indicate that the decrease in the ADAMTS13 activity may be caused by the presence of its inhibitor, which is closely related to lower functional liver capacity, marked inflammation, and enhanced endotoxemia in patients with AH and SAH. It was recently reported that the intravenous infusion of endotoxin to healthy volunteers brought the decrease in plasma ADAMTS13

activity together with the increase in VWF:Ag and the appearance of UL-VWFM during acute systemic inflammation (Reiter et al., 2005). From our results and the previous finding (Reiter et al., 2005), endotoxemia itself might be a candidate to reduce the plasma activity of ADAMTS13 together with inflammatory cytokines in patients with AH. It will be, then, necessary to clarify what kinds of the inhibitor would be involved in the association with inflammatory cytokines and endotoxin. We, recently, encountered 2 patient who developed TTP; one occurred in the course of hepatitis C virus (HCV)-related advanced liver cirrhosis (Yagita et al., 2005) and another did in a month after pegylated-interferon alpha-2a therapy in a HCV-related chronic hepatitis (Kitano et al., 2006). In both of them, plasma ADAMTS13 activity was extremely low, and the inhibitor against ADAMTS13 was detected in the patient's heated plasma (2.0 and 1.6 BU/ml, respectively) and purified IgG (0.19 and 0.4 BU/mg IgG, respectively). Furthermore, we could detect IgG-inhibitor by western blot in 4 patients with advanced liver cirrhosis, who showed extremely lower ADAMTS13 activity (< 3% of controls), but had no apparent clinical features of TTP (Uemura et al., 2008). Of 108 patients with idiopathic TTP whose plasma samples were sent to our department of Blood Transfusion Medicine across Japan, the inhibitor was detected in 54 (79.4%) of 68 patients analyzed, and its inhibitor activity was 0.5 to 2.0 BU/ml in 33 cases (61.1%), and more than 2.0 BU/ml in remaining 21 cases (38.9%) (Matsumoto et al., 2004). Taken these considerations together, the inhibitor activity detected in our patients with SAH and AH would be enough to reduce the activity of plasma ADAMTS13.

As for the relationship of the treatment to ADAMTS13 activity and outcome, all AH patients treated with supportive care including nutritional supplementation survived with the increase in the ADAMTS13 activity. All 5 patients with SAH were treated with FFP infusion together with supportive care, and 2 of them survived, but remaining 3 did not. One of the nonsurvivors showed transient increase in ADAMTS13 activity during FFP infusion, but finally decreased, and the other 2 died of hepatic failure in spite of the administration of prednisolone within a week. The administration of FFP might be, in part, useful as the supplementation of ADAMTS13, but the effect might depend on the severity of liver disturbance and the degree of multiorgan failure prior to the administration.

In conclusion, decreased ADAMTS13 activity and increased VWF:Ag could be induced not only by enhanced cytokinemia, but also by its inhibitor, both of which are closely related to enhanced endotoxemia in patients with AH and SAH. The cytokinemia and the presence of inhibitor may cause the imbalance of the enzyme to substrate, resulting in multiorgan failure especially in patients with SAH. These results will raise the possibility of novel supportive therapies for patients with AH, such as ADAMTS13 supplementation or anti-inflammatory cytokine agents.

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CONFLICTS OF INTEREST STATEMENT

The authors have declared no conflicts of interest. [Correction added after online publication 16 December 2008: Conflicts of Interest Statement added.]

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