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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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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ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

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

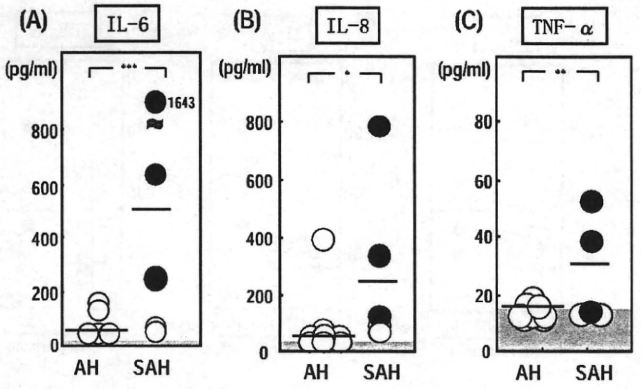

Fig. 1. Plasma levels of cytokines in the patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH) on admission. The shaded area shows the normal range. The open circles indicate survivors and the closed circles indicate nonsurvivors. The concentrations of IL-6 (**A**), IL-8 (**B**), 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.

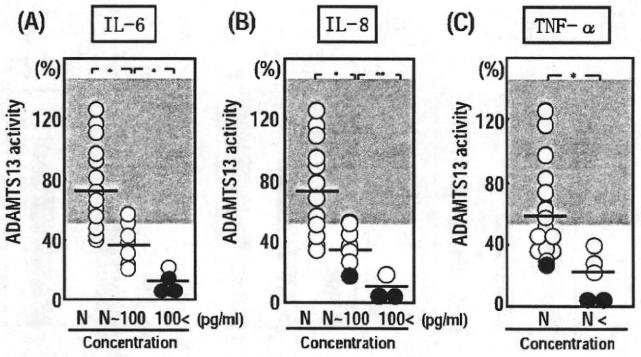

Fig. 2. Relationship between plasma cytokine levels and ADAMTS13 activity in the patients with alcoholic hepatitis and severe alcoholic hepatitis on admission. The shaded area shows the normal range. The open circles indicate survivors and the closed circles indicate nonsurvivors. The ADAMTS13 activity 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

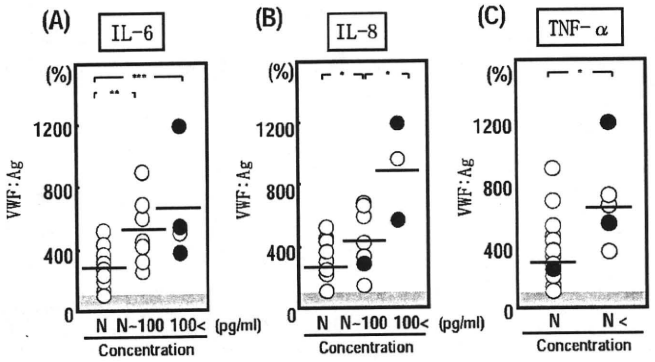

Fig. 3. Relationship between plasma levels of cytokines and VWF antigen (VWF:Ag) in the patients with alcoholic hepatitis and severe alcoholic hepatitis on admission. The shaded area shows the normal range. The open circles indicate survivors and the closed circles indicate nonsurvivors. The VWF:Ag concomitantly 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.

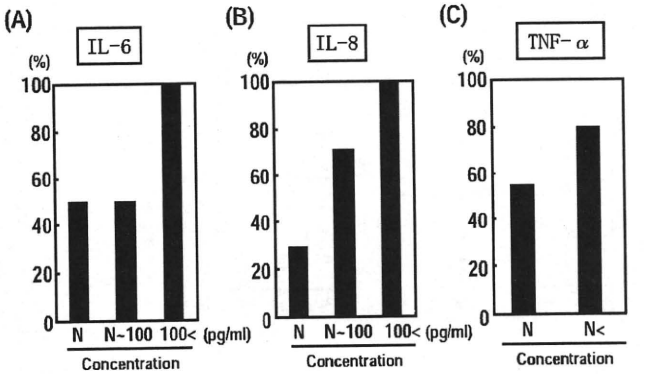

Fig. 4. Relationship between plasma levels of cytokines and the incidence of unusually large von Willebrand factor multimer (UL-VFWM) in the patients with alcoholic hepatitis and severe alcoholic hepatitis on admission. The incidence reached 100% in patients with higher concentration more than 100 pg/ml 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.

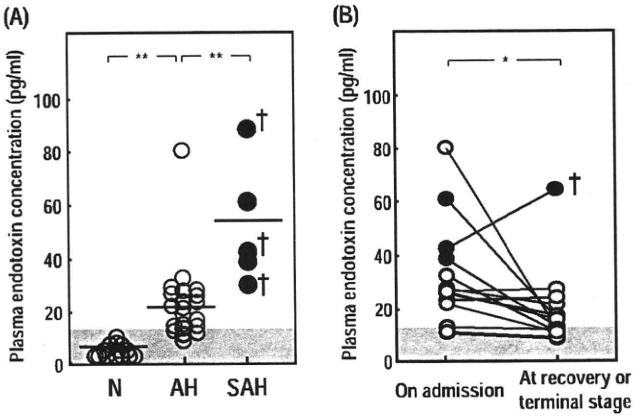

Fig. 5. Plasma endotoxin concentration in patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH). The shaded area shows the normal range. The open circles indicate AH and the closed circles indicate SAH. The crosses indicate nonsurvivors. Plasma endotoxin concentration on admission 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*),

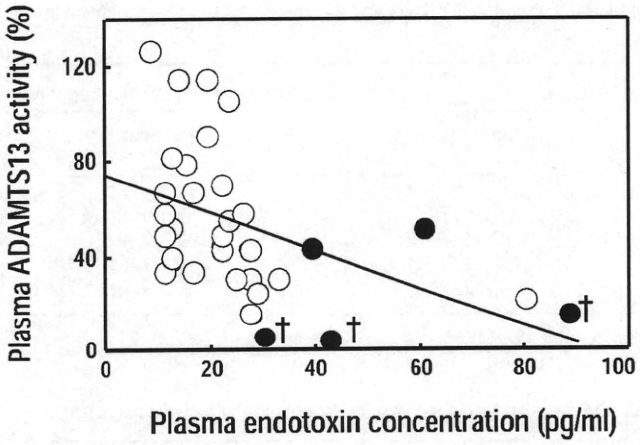

Fig. 6. Correlation between plasma endotoxin concentration and plasma ADAMTS13 activity in patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH) on admission. The open circles indicate AH and the closed circles indicate SAH. The crosses indicate nonsurvivors. The endotoxin concentration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

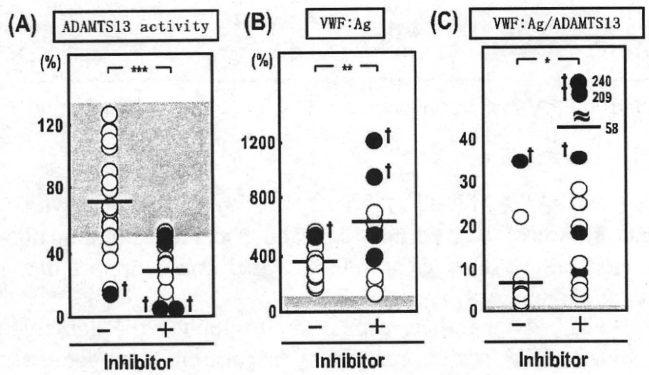

Fig. 7. Relationship of plasma inhibitor against ADAMTS13 to ADAMTS13 activity, VWF antigen (VWF:Ag), and the ratio of VWF:Ag to ADAMTS13 activity in patients with alcoholic hepatitis (AH) and severe alcoholic hepatitis (SAH) on admission. The shaded area shows the normal range. The open circles indicate 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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Regular Article

Frequency and hemostatic abnormalities in pre-DIC patients

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ABSTRACT

Disseminated intravascular coagulation (DIC) sometimes has a poor outcome, and therefore early diagnosis and treatment are required. This study prospectively evaluated the hemostatic abnormalities and the onset of DIC in 613 patients with underlying diseases to identify a useful marker for diagnosing Pre-DIC. Pre-DIC was defined as the condition of patients within a week before the onset of DIC.

Initially, 34.4% of patients were diagnosed with DIC, and about 8.5% of the patients without DIC were diagnosed as DIC within a week after registration (pre-DIC). The mortality of DIC, Pre-DIC and "without DIC" was 35.3%, 32.4% and 17.2%, respectively. All hemostatic parameters were significantly worse in "DIC" than "without DIC" and the values of the prothrombin time ratio, platelet count and fibrin monomer complex could classify the three groups; "DIC", "pre-DIC" and "without DIC". No useful marker was identified that provided an adequate cutoff value to differentiate "pre-DIC" from "without DIC". A multivariate analysis identified clinical symptoms that were related to poor outcome.

DIC must be treated immediately; there is no specific marker to identify pre-DIC.

Introduction

Disseminated intravascular coagulation (DIC) is a frequent complication of hematopoietic disorders, solid cancers and inflammatory diseases and trauma [1–4]. Recent clinical trials for severe sepsis [5–7] showed that the mortality in severe sepsis was about 35 – 45% and it was higher in those that experience DIC than in those without. The frequency of DIC in patients with severe sepsis was 40.7% in the KyberSept trial on antithrombin (AT) [5] and 22.4% in PROWESS study on recombinant activated protein C (APC) [6]. Such patients tend to have a poor outcome because the patients with severe sepsis frequently experience DIC.

Several diagnostic criteria for DIC have been proposed by the Japanese Ministry Health and Welfare (JMHW) [8], the International Society of Thrombosis and Haemostasis (ISTH) [3] and The Japanese Association for Acute Medicine (JAAM) [9]. These diagnostic criteria use global coagulation tests such as prothrombin time (PT), platelet count, fibrinogen and fibrin and fibrinogen degradation products (FDP) or D-dimer in scoring for hemostatic abnormalities.

The efficacy of DIC treatment in relation to the JMHW DIC score when the treatment was begun showed that greater efficacy was achieved in pre-DIC than in DIC patients [10]. The outcome was poorer with an increasing DIC score, thus suggesting that both an early diagnosis and early treatment for DIC are important.

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Therefore, diagnostic criteria for non-overt DIC [3] were proposed by the ISTH/SSC subcommittee in order to diagnose the early phase of DIC but these criteria were not established [11–14]. Both the JAAM DIC criteria [9] and non-overt-DIC diagnostic criteria established by ISTH [3] have adopted the rate of change in global coagulation tests. Some non-overt-DIC criteria [3,13] also adopted both AT and hemostatic molecular markers. However, several hemostatic molecular markers such as thrombin AT complex (TAT), soluble fibrin (SF) and plasmin-plasmin inhibitor complex (PPIC) etc. have been reported to detect the early phase of DIC [15,16].

This study prospectively evaluated global coagulation tests, hemostatic molecular markers and the onset of DIC within a week after registration; in order to define the pre-DIC state.

Materials and Methods

A total of 613 patients with diseases associated with DIC at nine institutes were registered for this prospective study for DIC diagnostic criteria from January 1, 2005 to December 31, 2008. The inclusion criteria were; more than one abnormal finding was observed according to laboratory tests (platelet count; less than 120/ $x10^3/\mu l$, FDP; more than 10 μg/ml, fibrinogen; less than 1 g/l, PT ratio; more than 1.25) in addition to diseases associated DIC. Any associations with heparin induced thrombocytopenia (HIT), thrombotic thrombocytopenic purpura (TTP), antiphospholipid syndrome (APS) or severe liver injuries were excluded in this study. APS was diagnosed according to the Sapporo criteria [17]. Organ failure and inflammatory conditions were evaluated by the sepsis-related organ failure assessment (SOFA) [18] and the systemic inflammatory response syndrome (SIRS, [19]) score, respectively. The nine institutes were the Department of Molecular and Laboratory Medicine and Emergency Medicine, Mie University School of Medicine, First Department of Surgery, University of Occupational and Environmental Health School of Medicine, Department of Internal Medicine, Takasaki National Hospital, Takasaki, Department of Internal Medicine, Teikyo University School of Medicine, Department of Emergency Medicine and Intensive Care, Graduate School of Medicine, Nagoya University, Department of Anesthesiology and Critical Care Medicine, Hokkaido University Graduate School of Medicine, Department of Emergency and Critical Care Medicine, Nippon Medical School, Department of Internal Medicine, Shibata Hospital-Niigata Prefectural Hospital, Research Division of Cell and Molecular Medicine, Center for Molecular Medicine and Jichi Medical University School of Medicine. The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine and a signed consent form was obtained from each subject.

DIC was diagnosed on the day of registration, using the modified DIC diagnostic criteria established by the Japanese Ministry of Health and Welfare (Table 1) [8]. Pre-DIC was defined as the state within a week before the onset of DIC; therefore, these patients were not diagnosed with DIC on the registration day, but they developed DIC within a week after registration [16]. The DIC score using platelet count, FDP, fibrinogen and PT was thereafter checked in all patients without DIC every day after registration. Hemostatic molecular markers such TAT, FMC, D-dimer, PPIC thrombomodulin (TM) and AT were measured at registaration. No DIC treatment was administered prior to the diagnosis of DIC.

PT, fibrinogen, platelet count and FDP were measured in each institutes based on numerous previous reports [20–22]. TAT, FMC, D-dimer, PPIC, thrombomodulin (TM) and AT activity were measured in SRL Inc. (Tokyo, Japan). TAT and TM was measured by enzyme immunoassay (EIA) using a TAT [S] (TFB, Tokyo, Japan) and TM Banasera (Fujirebio, Tokyo, Japan), respectively. Fibrin monomer complex (FMC), D-dimer and PPIC were measured by latex immune agglutination test using Auto LIA FM (Roshe Diagnostic, Tokyo, Japan), LATECLE D-dimer (Kainos, Tokyo, Japan) and LPIA-ACE PPI II (Mitsubishi Chemical Medicine Corporation, Tokyo, Japan) respectively. AT activity was measured by heparin cofactor activity using Testchyme S ATIII (Sekisui Medical, Tokyo, Japan).

The platelet count, FDP, fibrinogen and PT were compared using the cutoff values established by the JMHW diagnostic criteria for the analysis of hemostatic parameters between the two groups, A comparison of the D-dimer, FMC, TAT, PPIC, TM and AT levels used the cutoff values with the highest odds ratio from an ROC analysis.

Statistical analysis

The data are expressed as the median (25% -75% percentile). The differences between the groups were examined for statistical significance using the Mann-Whitney U test. A *P* value of less than 0.05 was considered to be significant. A statistical analysis demonstrated an odds' ratio of 95% CI for the mortality, resolution rate from DIC, and the cut off value of hemostatic parameters. A multivariate analysis for mortality and resolution from DIC was performed. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

Results

Two hundred and nineteen of the registered patients presented with infectious disease [female ; male = 78 ; 141, median (25 – 75 percentile); 70.0 years old (60.0 – 77.0 years old)], 142 with solid cancer [47 ; 95, 63.0 years old (52.0 – 72.0 years old)], 115 with hematopoietic tumors [50 ; 65, 62.0 years old (45.0 – 73.0 years old)], 29 with an aneurysm [11 ; 18, 74.0 years old (69.5 – 84.6 years old)], 10 with obstetrics disease [10 ; 0, 33.0 years old (29.0 – 34.0 years old)], 23 with trauma [12 ; 11, 67.0 years old (48.8 – 74.0 years old), 5 with liver disease [2 ; 3, 53.0 years old [53.0 years old (50.0 – 70.3 years old), 70 with other disease [30 ; 40, 66.0 years old (43.0 -76.0 years old)].

The frequency of DIC was 34.4% in all patients with diseases associated with DIC (Table 2). The highest absolute number of DIC cases was observed in patients with infectious disease, solid cancer and hematopoietic disorders. Thirty-four of the 432 patients without

Table 1
Modified diagnostic criteria for DIC established by the Japanese Ministry of Health and Welfare.

	With hematopoietic disorders	Without hematopoietic disorders
Underlying disease	1 point	1 point
Clinical symptoms	bleeding 0 point organ failure 1point	bleeding 1 point organ failure 1point
Platelet counts ($x10^3/\mu l$)	0 point	>80 but < 120; 1 point, >50 but <80; 2 points <50; 3 points
Fibrin-related marker	FDP (μg/ml) >10 but < 20; 1 point, >20 but <40; 2 points, >40; 3 points	FDP (μg/ml) >10 but < 20; 1 point, >20 but <40; 2 points, >40; 3 points
Fibrinogen (g/l)	>1 but < 1.5; 1 point, <1; 2 points	>1 but < 1.5; 1 point, <1; 2 points
PT (PT ratio)	>1.25 but <1.67; 1 point, >1.67; 2 points	>1.25 but <1.67; 1 point, >1.67; 2 points
Diagnosis of DIC	≥4 points	≥7 points

Table 2
Frequency of patients without DIC, with overt-DIC and pre-DIC.

	Without DIC	With DIC	Pre-DIC	total
Infectious disease	142	71	6	219
Solid cancer	81	50	11	142
Hematopoietic tumor	54	49	11	114
Aneurysm	14	14	1	29
Obstetrics disease	4	6	0	10
Trauma	16	8	2	26
Digestive disease	13	5	0	18
Collagen disease	9	1	0	10
Other disease	35	7	3	45
Total	368	211	34	613

DIC (8.5%) progressed to DIC within a week; these patients were classified as pre-DIC. The average time from registration to the onset of DIC was 3.4 ± 2.2 days. Finally the frequency of DIC was about 40% in one week after registration. The development of DIC within one week after registration was frequently observed in patients with hematopoietic tumors, with solid cancer or with infectious diseases.

The mortality was higher in the patients with DIC (35.3%) or pre-DIC (32.4%) than in those without DIC (17.2%); DIC vs. without DIC (2.63: 1.78 – 3.91) and pre-DIC vs. without DIC (2.31: 1.09 – 4.90; Table 3). No significant difference was observed in the mortality between solid cancer (28.9%) and infections (26.0%), but the mortality associated with hematological malignancy (15.7%) did tend to be low. The resolution rate from DIC tended to be higher in the patients with Pre-DIC than in the patients with DIC (Table 3; 67.9% vs. 57.6, 1.56: 0.56 – 2.50). The median value of all hemostatic parameters, such as the platelet count, PT ratio, FDP, fibrinogen, D-dimer, FMC, TAT, PPIC, AT and TM were significantly worse in the patients with DIC than those without DIC (Table 4). The values of PT ratio and platelet count were significantly worse ($p<0.05$ and $p<0.001$, respectively) in the patients with DIC than those with pre-DIC and those were significantly worse ($p<0.01$, respectively) in the patients with pre-DIC than those without DIC. The values of FDP and fibrinogen were also significantly worse ($p<0.001$ and $p<0.05$, respectively) in the patients with DIC than those with pre-DIC but there was no significant difference between the patients with Pre-DIC and without DIC. The values of D-dimer and FMC were significantly worse ($p<0.001$ and $p<0.05$, respectively) in the patients with pre-DIC than those without DIC, but there was no significant difference of TAT, PPIC, AT and TM between the patients with Pre-DIC and without DIC. The appropriate cutoff value of hemostatic markers, the platelet count, FDP and FMC all had a high odds ratio for differentiating "DIC" from "without DIC", but only fibrinogen and D-dimer had a relatively high odds ratio for differentiating "pre-DIC" from "without DIC" (Table 5). A multivariate analysis showed the mortality to be related to the SOFA score ($p<0.01$), SIRS score ($p<0.01$), bleeding symptoms ($p<0.05$), TAT ($p<0.05$), PPIC ($p<0.05$) and PAI-I ($p<0.01$), and that the resolution rate from DIC was related to the SOFA ($p<0.05$) and SIRS score ($p<0.05$), and that DIC state was related to SIRS score ($p<0.05$), D-dimer ($p<0.01$), PAI-1($p<0.05$), platelet counts ($p<0.01$) and SFMC ($p<0.01$).

Table 4
Hemostatic parameters in the patients with DIC, those with pre-DIC and those without DIC.

	DIC	Pre-DIC	Without DIC
Platelet ($X10^4/\mu l$)	4.3 (2.7 – 7.0) *** ###	7.0 (4.5 – 7.0) **	11.2 (6.8 – 40.5)
PT ratio	1.37 (1.15 – 1.65) *** #	1.22 (1.14 – 1.38) **	1.14 (1.04 – 1.27)
FDP (μg/ml)	41.8 (22.7 – 69.2) *** ###	20.2 (13.6 – 25.4) NS*	17.0 (10.2 – 31.4)
Fibrinogen (mg/dl)	188 (115 – 358) *** #	340 (187 – 414) NS*	350 (248 – 485)
D-dimer (μg/ml)	21.9 (11.3 – 45.0) *** NS#	19.3 (8.1 – 28.4) ***	8.7 (4.5 – 17.0)
FMC (μg/ml)	139 (22.9 – 350) *** ##	56.1 (12.1 – 151) *	14.1 (6.2 – 91.8)
TAT (ng/ml)	31.7 (15.0 – 90.0) *** NS#	19.7 (11.7 – 40.6) NS*	14.5 (7.4 – 29.4)
PPIC (μg/ml)	2.95 (1.20 – 7.80) *** NS#	2.00 (1.00 – 3.20) NS*	1.90 (1.10 – 15.90)
AT (%)	63.6 (44.7 – 84.2) * NS#	66.5 (46.2 – 85.3) NS*	67.3 (52.8 – 88.0)
TM (ng/ml)	5.60 (3.60 – 8.33) *** NS#	3.65 (2.40 – 4.10) NS*	3.00 (1.90 – 4.20)

Data are represent the median (25%tile – 75% tile).
***, ** or *; $p<0.001$, $p<0.01$ or $p<0.05$ in comparison to without DIC.
###, ## or #; $p<0.001$, $p<0.01$ or $p<0.05$ in comparison to pre-DIC.
NS* or NS#; not significant in comparison to without DIC or pre-DIC.

Discussion

Clotting activity tends to be activated in many physiological and pathological conditions, such as pregnancy, tissue remodeling, inflammation, neuronal or vascular growth and others [23,24]. However, the JMHW, cannot differentiate those conditions due to various underlying diseases; however it is possible to differentiate DIC state from non DIC. This study demonstrated a higher frequency of DIC and pre-DIC in patients with solid cancers, hematological diseases and infection. However, an increase in the D-dimer levels [25] or a decrease in the platelet count is frequently observed in patients with either a hematological malignancy or solid cancer; however, this study did not determine the platelet counts in hematological malignancy and it used the FDP score as a fibrin related marker (FRMs). Patients with cancer, infections or hematological diseases produce antiphospholipid antibodies [26]. Although APS was excluded in this study, accurately differenting DIC from APS is important. Furthermore this study carefully determined the hemostatic markers on a daily basis for one week. There are several definitions of pre-DIC; such as early phase of DIC which is within a week before the onset of DIC [16], 6 points in non-hematopoietic disorders or 3 points in hematopoietic disorders on the JMHW DIC scores or diseases associated with DIC.

About 8.5% of the patients were diagnosed with DIC (pre-DIC) within one week after a negative diagnosis of DIC. It is important to regularly check for the presence of any hemostatic abnormalities in patients with diseases associated with DIC. The mortality of patients with DIC or pre-DIC was significantly high in comparison to those without DIC. No marked difference in the mortality and resolution

Table 3
Mortality of the patients with DIC, without DIC and the Pre-DIC and Resolution rate from DIC.

	Without DIC vs With DIC	Without DIC vs Pre-DIC
Mortality (odd's ratio: 95% CI)	17.2% vs 35.3% (2.63: 1.78 – 3.91, p<0.01)	17.2% vs 32.4% (2.31: 1.09 – 4.90, P<0.01)
Resolution rate from DIC (odd's ratio: 95% CI)	Pre-DIC vs DIC 67.9% vs 57.6% (1.56: 0.56 – 2.50, p<0.05)	

Table 5
Significant difference in the hemostatic parameters between the 2groups among DIC, Pre-DIC and without DIC.

		DIC vs without DIC			Pre-DIC vs without DIC		
	cutoff	Sensitivity	Specificity	odd's ratio (95% CI, p value)	Sensitivity	Specificity	odd's ratio (95% CI, p value)
Platelet ($X10^4/\mu l$)	< 12.0	94.3%	44.8%	13.55 (8.073~22.73, p<0.001)	72.7%	44.3%	2.120 (0.974~4.618, p<0.06)
	< 8.0	84.0%	67.9%	11.09 (7.497~16.410, p<0.001)	54.6	67.7%	2.511 (1.247~5.057, p<0.01)
	< 5.0	57.1%	86.7%	8.656 (3.492~5.261, p<0.001)	27.3%	86.7%	2.441 (1.096~5.439, p<0.05)
PT ratio	1.25<	63.2%	74.9%	5.106 (3.585~7.273, p<0.001)	42.4%	73.5%	2.038 (0.994~4.178, NS)
	1.67<	24.4%	94.1%	5.118 (3.090~8.479, p<0.01)	12.1%	94.1%	2.187 (0.722~6.629, NS)
FDP (μg/ml)	10 <	97.6%	23.1%	12.39 (5.881~26.10, p<0.01)	90.6%	18.4%	2.177 (0.662~7.162, NS)
	20 <	82.5%	56.8%	6.189 (4.192~9.139, p<0.001)	46.9%	43.5%	0.678 (0.330~1.395, NS)
	40 <	54.5%	81.9	5.418 (3.754~7.820, p<0.001)	12.5%	77.7%	0.498 (0.173~1.434, NS)
Fibrinogen (mg/dl)	< 150	40.3%	94.7%	12.07 (7.564~19.27, p<0.001)	18.8%	94.7%	4.130 (1.626~10.49, p<0.01)
	< 100	19.0%	98.3%	13.76 (6.818~27.78, p<0.001)	9.4%	98.3%	6.086 (1.707~21.70, p<0.01)
D-dimer (μg/ml)	6.0 <	87.3%	43.9%	5.364 (3.500~8.221, p<0.001)	91.2%	36.5%	5.930 (2.028~17.345, p<0.005)
	13.0 <	66.2%	68.2%	4.202 (2.945~5.996, p<0.001)	58.8%	68.5%	3.108 (1.559~6.197, p<0.005)
FMC (μg/ml)	10.0 <	91.9%	41.9%	8.199 (4.987~13.48, p<0.001)	80.0%	41.0%	2.780 (1.144~6.754, p<0.05)
TAT (ng/ml)	16.0 <	70.5%	56.0%	3.042 (2.003~4.621, p<0.01)	63.6%	54.6%	2.103 (0.868~5.092, NS)
PPIC (μg/ml)	3.8 <	43.6%	79.0%	2.902 (2.004~4.204, p<0.01)	83.3%	21.9%	1.403 (0.522~3.769, NS)
AT (%)	70 >	57.3%	46.9%	1.187 (0.809~1.372, NS)	58.3%	45.5%	1.168 (0.502~2.718, NS)
TM (ng/ml)	5 <	59.3%	69.2%	3.267 (2.170~4.918, p<0.01)	47.4%	69.2%	2.017 (0.804~5.061, NS)

Cut off value in platelet count, PT ratio, FDP and fibrinogen was used diagnostic criteria by JMHW and it in D-dimer, FMC, TAT, PPIC, AT and TM was used it at highest odd's ratio.

rate from DIC was observed between the DIC or pre-DIC populations because no treatments were administered for pre-DIC patients until the progression to DIC, A previous report [10] shows that early treatment of DIC may improve the outcome, suggesting that early diagnosis and treatment of DIC might be required.

All of the hemostatic parameters were significantly worse in the patients with DIC in comparison to those without DIC. An analysis of the cutoff value to differentiate DIC from "without DIC" show those cutoff values included in the JMHW diagnostic criteria to be both adequate and useful. The increase in the platelet count in solid cancer and the increase in fibrinogen observed during infection may also cause a decrease in the sensitivity of the DIC diagnostic criteria as established by JMHW, and more sensitive diagnostic criteria that can detect the early stage of DIC (Pre-DIC) are therefore required. An analysis of the median value showed that the PT ratio, platelet count, FMC and D-dimer were useful for the diagnosis of pre-DIC in this prospective study. Previous reports have shown hemostatic molecular markers to be more sensitive for the diagnosis of pre-DIC than global coagulation tests [16,22].

Only fibrinogen and D-dimer are considered to potentially be useful markers, to differentiate "pre-DIC" from "without DIC". Fibrinogen, PT ratio, AT, PPIC and TM have low sensitivity for pre-DIC. On the other hand, FDP, platelet count, D-dimer, FMC and TAT are all too sensitivity for pre-DIC but lack an adequate cut off value for differentiating "Pre-DIC" from "without DIC". An algorithm of non-overt-DIC [3] might be required to diagnose a pre-DIC state.

A multivariate analysis for the mortality or resolution rate from DIC suggested that clinical symptoms might be important. Hemostatic markers, such as TAT, PPIC and PAI-I might be related to the outcome in the patients with DIC. Other researchers have also reported that a scoring system that includes the platelet count and PT has a prognostic value in severe sepsis [27]. The values of TM and AT are reported to be worse in patients with poor outcome than those without [28], thus suggesting that TM and AT may therefore be useful as injured vascular endothelial cell markers.

In conclusion, about 8.5% of high risk patients without DIC developed DIC within one week and this state was designated to therefore be pre-DIC. Although the early treatment of DIC is crucial, this study revealed no hemostatic marker to diagnose pre-DIC.

Conflict of interest statement

Authors have no conflict of interest.

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Prospective evaluation of three different diagnostic criteria for disseminated intravascular coagulation

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Summary

There are three different diagnostic score systems for disseminated intravascular coagulation (DIC) established by the Japanese Ministry Health and Welfare (JMHW), the International Society on Thrombosis and Haemostasis (ISTH) and the Japanese Association for Acute Medicine (JAAM). The JMHW criteria are still used in Japan. In the present study, all three diagnostic criteria were used to prospectively evaluate 413 patients with different underlying diseases of DIC who were treated at the Mie University Hospital (JMHW, n= 166; ISTH, n=143; JAAM, n=291). The odds ratio (95% confidence interval) for death was 1.88 (1.22 – 2.90) in JMHW, 2.55 (1.65 – 3.95) in ISHT and 1.99 (1.19 – 3.32) in JAAM. The platelet count, prothrombin time, fibrin and fibrinogen degradation products and fibrinogen were significantly important for diagnosis of DIC by all three diagnostic criteria. Haemostatic molecular markers were significantly high in all patients and were useful for the diagnosis of DIC. The JAAM diagnostic criteria displayed a high sensitivity for DIC and the ISTH overt-DIC diagnostic criteria displayed a high specificity for DIC. All three diagnostic criteria for DIC were related to a poor patient outcome.

Keywords

DIC, Japanese Ministry Health and Welfare, ISTH, haemostatic markers, mortality, resolution rate

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Introduction

Disseminated intravascular coagulation (DIC) is a life-threatening disease that is often associated with severe organ failure and a bleeding tendency (1–4). Recent clinical trials for severe sepsis (5–7) revealed a high mortality rate in the patients with severe sepsis. The frequency of DIC in patients with severe sepsis was reported to be 40.7% in the KyberSept trial for antithrombin (AT) (5) and 22.4% in the PROWESS study for activated protein C (APC) (6). Such patients tend to have a poor outcome.

Several diagnostic criteria for DIC have been established. These include those of the Japanese Ministry Health and Welfare (JMHW) (8), the International Society on Thrombosis and Haemostasis (ISTH) (3), and The Japanese Association for Acute Medicine (JAAM) (9). These diagnostic criteria adopt global coagulation tests such as prothrombin time (PT), platelet count, fibrinogen and fibrin and fibrinogen degradation products (FDP) or D-dimer in scoring for haemostatic abnormalities. Both the JAAM DIC criteria (9) and non-overt-DIC diagnostic criteria established by ISTH (3) have adopted the rate of change in global coagulation tests.

A study in which the efficacy of DIC treatment in relation to the JMHW DIC score was compared at the beginning of treatment showed that greater efficacy was achieved in late-onset DIC patients than in DIC patients (10). The outcome was poorer with an increasing DIC score, thus suggesting that both an early diagnosis and early treatment for DIC are important. The late-onset DIC is considered to be within a week before the onset of DIC (11) or non-overt DIC (3, 12).

This study prospectively evaluated the JMHW, ISTH and JAAM diagnostic criteria for DIC and examined the usefulness of haemostatic molecular markers for the diagnosis of DIC.

Materials and methods

A total of 413 patients [female: male; 173: 242, age (median; 64.0 years old, 25%-75% tile; 49.0–73.0 years old)] with diseases associated with DIC who were treated from January 1, 2005 to December 31, 2008 at Mie University Hospital and associated hospitals were