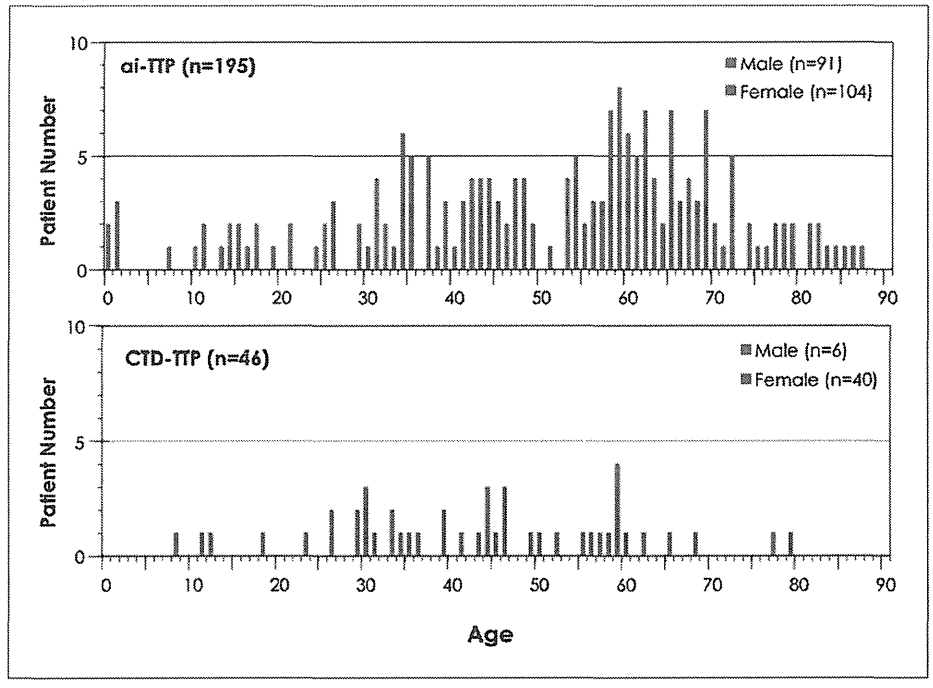

FIGURE 4

Age and gender distribution of patients with acquired idiopathic (ai)-thrombotic thrombocytopenic purpura (TTP) and connective tissue disease (CTD)-associated TTP

The upper panel shows the age and gender distribution of 195 ai-TTP patients who were registered at Nara Medical University during 1998–2008 and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. The largest population peak is found at approximately 60 years old, and we identified 14 patients (14/195, 7.2%) aged less than 15 years. Of note, five were very young children below 2 years of age.

The lower panel shows the age and gender distribution of 46 CTD-TTP patients who were registered at our institution during the same time period and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. This CTD-TTP patient population is widely distributed across ages, but is more common in patients between 30–60 years old. There is an apparent female predominance (41/46, 89%) in CTD-TTP patients in this registry, and three childhood patients were identified.

and three with connective tissue disease (CTD)-associated TTP. Of note, the former group included five patients who were less than 2 years of age and were initially misdiagnosed with other thrombocytopenic disorders, such as ITP, HUS, and hemophagocytic syndrome (HPS). It is important to examine and compare the detailed clinical features of these TTP patients to adulthood patients in order for physicians to ascertain that TTP is not a rare childhood disease. Therefore, we herein describe the clinico-laboratory features of these five young infants with ai-TTP and three childhood patients with CTD-associated TTP.

Idiopathic (ai-) thrombotic thrombocytopenic purpura

Table II summarizes the features of the 14 childhood patients with ai-TTP, including five infantile patients. Case 1 was previously reported [58] and case 5 was more recently described [59]. Interestingly, in contrast to the adulthood ai-TTP patients with a severe ADAMTS13:AC deficiency, the childhood patients had a slightly male predominance (female:male = 5:9). Five patients (5/14, 33%) had apparent prodromal illnesses, such as an upper respiratory tract infection (n = 3), Rotavirus infection (n = 1), or urinary tract infection (n = 1). Ten patients (10/14, 67%) had neurological findings, including headache (n = 5), altered mental status (n = 4), hemiparesis (n = 1), seizures (n = 1), and vision disturbance (n = 1). These patients exclusively presented with renal involvement (11/14, 73%) and fever (13/14, 93%). All the patients had hemolytic anemia (Hb, 4.5–11.3 g/dL) and thrombocytopenia (platelet, 7–38 $\times$ 10^9/L), but their serum creatinine levels (Cr, 0.19–1.0 mg/dL) remained within the normal range. Most of the childhood patients had five clinical signs that are characteristic of classic TTP ('pentad'), but six patients, including five young infants aged below 2 years, were initially misdiagnosed with other thrombocytopenic disorders, such as ITP (n = 2), HUS (n = 2), HPS (n = 1), and paroxysmal

TABLE II

Clinical features in childhood patients with acquired TTP with severe ADAMTS13 deficiency

Case	Age	Sex	Prodromal illness	Initial diagnosis	Clinical findings on admission					
					Neurological symptom	Renal involvement	Cr (mg/dL)	Fever	Hb (g/dL)	Platelet (×109/L)
1	9 m	F	Rotavirus	HUS	Altered mental status	Yes	0.3	Yes	4.5	2
2	19 m	F	URI	ITP	No	Yes	0.19	Yes	11.3	38
3	19 m	M	URI	HUS	Altered mental status	Yes	1	Yes	5.4	9
4	12 m	M	No	HPS	Hemiparesis	Yes	0.31	Yes	8.5	38
5	8 m	M	No	ITP	No	No	0.19	Yes	8.7	25
6	7 y	M	No	TTP	Altered mental status	No	0.32	Yes	5.4	7
7	10 y	M	UTI	TTP	No	Yes	0.4	No	10.5	19
8	11 y	M	No	TTP	Headache	Yes	0.7	Yes	8.8	3
9	11 y	F	URI	TTP	No	Yes	0.6	Yes	5.5	6
10	13 y	F	No	TTP	Headache	No	0.58	Yes	5.7	12
11	14 y	F	No	TTP	Altered mental status, convulsion	No	0.4	Yes	7.8	6
12	15 y	M	No	PNH	Headache	Yes	1	Yes	10.5	17
13	15 y	M	No	TTP	Headache	Yes	0.91	Yes	8.1	11
14	14 y	M	No	TTP	Headache, visual disturbance	Yes	1.05	Yes	7	28
15	8 y	F	SLE	DIC	Altered mental status, headache	Yes	0.44	Yes	8.8	38
16	11 y	F	MCTD	TTP	Headache	Yes	0.6	Yes	11.9	43
17	12 y	F	SLE	TTP	No	No	0.61	Yes	5.6	1

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
1	< 0.5	> 100	19	No	SP	Yes	No	Alive	Cerebral infarction	[58]
2	< 0.5	4.3	3	Yes	SP, PSL	Yes	Yes	Dead		
3	< 0.5	2.3	3	Yes	PSL	Yes	No	Alive		
4	< 0.5	1.7	2	No	PSL	Yes	No	Alive	Cerebral infarction	
5	< 0.5	4.8	6	No	SP, PSL	Yes	No	Alive		[59]
6	< 0.5	2.1	3	No	Rituximab	No	No	Alive		
7	< 0.5	0.5	No	No	SP, PSL, MZR	Yes	No	Alive		
8	< 0.5	1.3	No	Yes	SP	No	No	Alive		
9	< 0.5	5.6	5	No	PSL	Yes	No	Alive		
10	< 0.5	2	9	No	SP, PSL	No	No	Alive		

TABLE II (*Continued*)

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
11	< 0.5	3.2	17	No	SP, PSL, VCR	No	No	Alive		
12	< 0.5	34	39	No	PSL, VCR, CSA	No	Yes	Alive		
13	< 0.5	2.3	3	No	SP, PSL, AZT	No	No	Alive		
14	< 0.5	6.8	30	Yes	PSL	No	No	Alive		
15	< 0.5	1.2	5	Yes	SP, PSL, CY	No	No	Alive		
16	< 0.5	1.8	3	No	SP, PSL	No	No	Alive		
17	< 0.5	0.7	9	Yes	SP, PSL	No	No	Alive		

Cr: creatinine; Hb: hemoglobin; ADAMTS13:AC: ADAMTS13 activity; ADAMTS13:INH: ADAMTS13 inhibitor; PE: plasma exchange; FFP: fresh frozen plasma; URI: upper respiratory infection; UTI: urinary tract infection; SLE: systemic lupus erythematosus; MCTD: mixed connective tissue disease; TTP: thombotic thrombocytopenic purpura; ITP: idiopathic thrombocytopenic purpura; HUS: hemolytic uremic syndrome; HPS: hemophagocytic syndrome; PNH: paroxysmal noctural hemoglobinuria; SP: steroid pulse; PSL: predonosolone; MZR: mizoribine; VCR: vincristine; CSA: cyclosporine A; AZT: azathioprine; CY: cyclophosphamide.

nocturnal hemoglobinuria (PNH, $n = 1$). After analyzing ADAMTS13, they were all correctly diagnosed with ai-TTP.

Of these 14 childhood patients with ai-TTP, 13 received plasma exchange (PE, 2–39 times, median 5 times), including four patients who subsequently received a FFP infusion. They also received immunosupressive therapy, including steroid pulse ($n = 7$), predonisolone ($n = 12$), vincristine ($n = 2$), cyclosporin ($n = 1$), azathioprine ($n = 1$), mizoribine ($n = 1$), and rituximab ($n = 1$). As an adjunctive therapy, the patients were given intravenous immune globulin ($n = 3$) or an antiplatelet agent ($n = 2$). Of note, seven patients received platelet transfusions before or after they were diagnosed with acquired TTP. In five of the seven patients who received platelet transfusions, there were no apparent serious complications. However, case 1 developed general convulsions soon after the platelet transfusion, and case 2 died from bleeding without appreciable hemostatic effects from the platelet transfusion. As consequence, 13 out of 14 childhood patients with ai-TTP achieved one clinical remission, but two patients relapsed, including one who died. We think that clinicians should be aware of the existence of ai-TTP during very early childhood, and herein we present a short summary for each of these five infants with ai-TTP.

Case 1

In March 2000, a 9-month-old girl presented with a fever. She subsequently showed loss of appetite, a drop in physical activity, a pale complexion, and vomiting followed by diarrhea related to a Rotavirus infection. On the following day, these symptoms continued and generalized petechiae appeared. She was taken to a family doctor who determined that she had severe anemia and thrombocytopenia. As a result, she was admitted to a nearby hospital for treatment. Upon admission, she was drowsy and her laboratory findings showed severe anemia (Hb, 4.5 g/dL), thrombocytopenia (platelet, 2.0×10^9/L), hyperbilirubinemia (total bilirubin, 2.6 mg/dL), and an elevated LDH level (2,925 IU/L). Both direct and indirect Coombs' tests were negative, and fragmented RBCs were detected in the blood film. The hemostatic tests showed the following: prothrombin time (PT, 14.0 sec), activated PTT (35.9 sec), fibrinogen (268 mg/dL), thrombin-antithrombin complex (TAT, 31.7 µg/L), D-dimer (7.14 µg/mL), and fibrin degradation product (FDP, 82.3 µg/mL). The levels of blood urea nitrogen (BUN) and creatinine were within the normal ranges (25 mg/dL and 0.3 mg/dL, respectively). Neither Shiga-like toxin nor *E. coli* O157:H7 was detected in her stool. However, she had proteinuria, hematuria, and marked petechiae on her body due to thrombocytopenia. She was tentatively diagnosed with HUS, and she received five units of platelet transfusion. Soon after completing the platelet transfusion, she developed generalized convulsions followed by right hemiplegia, and therefore, PE therapy was immediately instituted. On the following day, both CT and MRI examinations of her brain revealed a diffuse hemorrhagic infarction in the left posterior region. The PE therapy was continued for the next 37 days on a total of 19 occasions, along with steroid pulse therapy and high-dose intravenous immunogloburin (IVIG) infusions until clinical improvements were noted. The ADAMTS13:AC and ADAMTS13:INH titers measured by the VWFM assay were less than 3% and greater than 100 BU/mL, respectively (later, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasmas, and the respective data were

less than 0.5% and greater than 100 BU/mL [58]). Now, almost 10 years have passed, and the patient is apparently healthy with a minimal sequela of the right hemiplegia.

Case 2

In July 2000, a 19-month-old girl presented with a fever and cough. The next day, she was taken to a family doctor and then transferred to a nearby hospital because of thrombocytopenia (platelet, 21×10^9/L). Upon admission, her laboratory data revealed slight anemia (Hb, 11.3 g/dL), thrombocytopenia (platelet, 38×10^9/L), and an elevated LDH level (994 IU/L). An analysis of a bone marrow aspiration showed no abnormalities, and therefore she was suspected to have ITP. She was administered high-dose IVIG with steroid therapy but her platelet count did not increase. Her platelet count slightly increased soon after the platelet transfusions, while the number of schistocytes in the blood films gradually increased. This patient never had renal dysfunction or neurological signs. Thus, her physician suspected that the patient had USS but did not measure ADAMTS13:AC. The patient was given an infusion of 80 mL of FFP, but her platelet count did not increase. During this period, she was alert and no clinical deterioration was noted. Three months after admission, plasma samples from this patient were sent to our laboratory for ADAMTS13:AC and ADAMTS13:INH testing. Based on the results of the VWFM assays, the patient was diagnosed with a severe ADAMTS13:AC deficiency (< 3%) with ADAMTS13:INH (4.0 BU/mL). However, in those days we were unable to clearly determine whether this patient had USS and developed alloantibodies or acquired TTP with autoantibodies to ADAMTS13. The patient was not given PE therapy because she did not show any clinical deterioration during the subsequent 3 months. Therefore, she was discharged and then carefully observed at the outpatient clinic. However, 1 month after discharge, she was re-admitted to the hospital and received PE therapy because of exacerbated anemia and thrombocytopenia. However, her clinical signs did not improve, even after whole blood exchange therapy. Thus, she was treated with RBC and platelet transfusions, but 2 weeks later she fell into coma and died of tracheal bleeding, which was 8 months after her first hospital admission (plasma ADAMTS13:AC and ADMTS13:INH in this patient were measured only on one occasion. In recent years, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasma samples, and the data were less than 0.5% and 4.3 BU/mL, respectively).

Case 3

In July 2002, a 19-month-old boy developed a low-grade fever and cough followed by petechiae. He was taken to a family doctor because his nasal bleeding did not stop. The doctor noted thrombocytopenia and anemia and suspected HUS, and the patient was transferred to a local hospital. He had mild thrombocytopenia (platelet, 70×10^9/L) soon after birth, which spontaneously improved. Upon admission, he was drowsy and his laboratory data showed anemia (Hb, 5.4 g/dL), thrombocytopenia (platelet, 9×10^9/L), elevated LDH (1991 IU/L), and proteinuria. He was administered FFP infusions, steroid therapy, and IVIG. A platelet transfusion was performed but his platelet counts did not significantly increase. Since he was negative for DIC markers, the patient was clinically diagnosed with TTP and then administered PE therapy. After three consecutive PE therapies, he became alert, recovered, and his laboratory markers returned to normal levels. The ADAMTS13:AC and ADAMTS13:INH titers were measured by classic VWFM assays using frozen plasma that was obtained before the PE therapy was administered, and the results were less than 3% and 2.3 BU/mL, respectively (later, chromogenic act-ELISA gave values of less than 0.5% and 2.3 BU/mL, respectively). His plasma ADAMTS13:AC deficiency with ADAMTS13:INH continued for more than 6 months but without appreciable clinical manifestations. After 4 years, ADAMTS13:AC (77%) had normalized and ADAMTS13:INH (< 0.5 BU/ml) was absent.

Case 4

In June 2002, a 13-month-old boy developed a fever followed by dark urine and diarrhea. He was taken to a nearby clinic, where he was determined to have leukocytosis (WBC, 12,000/μL), anemia (Hb, 7.2 g/dL), and thrombocytopenia (platelet, 46×10^9/L). In addition, a peripheral blood film showed phagocytosis and therefore the patient was diagnosed with suspected HPS. He was transferred to a university hospital where platelet transfusions were performed twice for two consecutive days, but his platelet counts only transiently increased. Soon after the second platelet transfusion, a bone marrow aspiration was performed, but the HPS diagnosis was not confirmed. On the other hand, there was a transient increase in his platelet count (platelet, 40×10^9/L) after he was infused with a small amount of FFP, and therefore the physician suspected a diagnosis of USS. Therefore, the patient received a daily plasma infusion therapy for the next 5 days. However, hematuria developed followed by right hemiplegia. An MRI revealed a hemorrhagic infarction (3 × 4 cm) in the left parieto-occipital region. Based on the clinical course, he was eventually diagnosed with ai-TTP. After he received PE therapy for two consecutive days with orally administered prednisolone, his clinical conditions rapidly improved and his laboratory findings recovered. After his recovery, the ADAMTS13:AC and ADAMTS13:INH levels were tested using the classic VWFM assay and deep-frozen plasma samples that were obtained before the PE therapy, and the results were less than 3% and 1.9 BU/mL, respectively (later, these values were re-examined with the chromogenic act-ELISA, and the results were less than 0.5% and 1.9 BU/mL, respectively). He subsequently improved and was discharged. Three years later, his plasma

TABLE III

Comparison of clinical features and outcomes between childhood and adulthood patients with acquired idiopathic (ai)-TTP

Ai-TTP (n = 195)	Childhood patients (n = 14)	Adulthood patients (n = 181)
Age at the onset of TTP bouts (years old), Median (25, 75 percentile)	11 (1.6, 14)	57 (41, 65)
Female (%)	35.7	55.2
"Pentad"		
(1) Platelet count ($\times 10^9$/L), Median (25, 75 percentile)	15 (8, 24)	10 (7, 17)
(2) Hemoglobin (g/dL), Median (25, 75 pecentile)	8.0 (5.6, 8.8)	7.5 (6.3, 8.8)
(3) Renal involvement (%)	71.4	75.7
Serum creatinine (mg/dL), Median (25, 75 percntile)	0.5 (0.3, 0.9)	1.0 (0.7, 1.3)
(4) Central nervous system involvements (%)	71.4	79.6
(5) Fever ($\geq$ 37.0 °C) (%)	92.9	69.6
Times of plasma exchange	5.5 (3.0-17.5)	ND
Mortality in the current episode of TTP bouts (%)	7.1	15.5

ND: not determined.

ADAMTS13:AC had normalized. At present, he has fully recovered and has no residual right hemiplegia.

Case 5

In January 2005, a 9-month-old boy with generalized petechiae and a fever was referred to a local hospital, where he was determined to have thrombocytopenia (platelet, 9×10^9/L). He was admitted to a university hospital and diagnosed with acquired TTP based on ADAMTS13:AC (< 3%) and ADAMTS13:INH titers (2.8 BU/mL) that were determined using the classic VWFM assays. (Later, these values were re-examined by the chromogenic act-ELISA, and the results were less than 0.5% and 4.8 BU/mL, respectively). After he was diagnosed with ai-TTP, he was administered PE therapy for six consecutive days at a different hospital. His clinical symptoms rapidly improved, but the increase in platelet counts was only transient and his platelet count was consistently lower than 10×10^9/L. To prevent serious bleeding complications, the physician administered oral prednisolone, together with continuous low-dose platelet transfusions. Two months later, he was discharged, despite having an ADAMTS13:AC deficiency with ADAMTS13:INH that lasted for at least 8 months. Two years later, we were able to examine the plasma ADAMTS13:AC and ADAMTS13:INH in this patient, and determine that ADAMTS13 had fully normalized [59].

Table III compares the clinical features and outcomes of the childhood patients (n = 14) and adulthood patients (n = 181) with ai-TTP in our registry [13].

Connective tissue disease-associated thrombotic thrombocytopenic purpura

In 1939, Gitlow and Goldmark [60] first reported a close relationship between 'classic' TTP and systemic lupus erythematosus (SLE). In 1999, Brenner et al. [61] described five patients with childhood-onset 'classic' TTP and reviewed 30 other patients who were previously described in the literature. According to their analysis, nine (9/35, 26%) fulfilled four or more ACR criteria for SLE and eight (8/35, 23%) had incipient SLE. Interestingly, of the five patients who were initially diagnosed with 'classic' idiopathic TTP in their laboratory, three were diagnosed with SLE within 3 years, and the remaining two patients fulfilled the ACR classification criteria for SLE within 4 years of disease onset. However, at that time, ADAMTS13:AC assays were not generally available, and therefore no data on ADAMTS13 was provided in their report.

In our registry of 919 patients with TMAs, 221 had CTD-associated TMA, of which 92 had SLE-associated TMA [13]. For the 221 CTD-TMA and/or 92 SLE-TMA patients, the number of patients with a severe ADAMTS13:AC deficiency with ADAMTS13:INH was 46 and 24, respectively. Furthermore, within the 221 patients with CTD-TMA, 11 developed the disease in childhood (less than 15 years of age), including eight patients with SLE, 1 with RA (rheumatoid arthritis), and two with MCTD (mixed connective tissue disease), in whom three had a severe ADAMTS13:AC deficiency. These three patients included two SLE- and one MCTD-associated TTP patients, and they uniformly had relatively low titers of ADAMTS13:INH (0.7–1.8 BU/mL) at

the onset of TTP, which slightly differed from those with ai-TTP (table II). Here we briefly describe these three childhood patients with CTD-associated TTP (cases 15–17) due to their relevance in clinical practice.

Case 15

In April 2005, a 7-year-old girl was determined to have proteinuria and occult blood in her urine based on a school health examination. The patient was admitted to a nearby university hospital for further examination in June 2005, where she was diagnosed with SLE (Lupus nephritis) based on her clinical manifestations and the following laboratory findings: proteinuria, positive for anti-nuclear antibodies and anti-double stranded DNA antibodies, and low complementemia. She was treated with prednisolone, mizoribine, and azathioprine. Because her clinical signs significantly improved with these treatments, she was discharged in September 2005.

In April 2006 (8 years of age), this patient noticed proteinuria and hematuria based on a self-examination at home. The next day, she was admitted to the same hospital. Her laboratory data at the second admission were as follows: Hb (8.8 g/dL), LDH (1608 IU/L), platelet (2×10^9/L), PT (11.0 sec), PTT (31.1 sec), fibrinogen (355 mg/dL), antithrombin (140%), TAT (9.06 µg/L), D-dimer (5.25 ng/mL), FDP (7.9 µg/mL), and schistocytes in a peripheral blood smear. She had a DIC score of 6 according to the DIC diagnostic criteria from the International Society of Thrombosis and Haemostasis [15]. As a result, she was initially treated with nafamostat, but there were no clinical improvements. ADAMTS13:AC and ADAMTS13:INH assays were performed for a differential diagnosis of TTP, and the results were less than 0.5% and 1.2 BU/mL, respectively. Thus, she was diagnosed with SLE-associated TTP and treated with PE (five times), steroid pulse therapy, and cyclophosphamide. These treatments saved her life, and to date, she has not had a relapse of TTP.

Case 16

In July 2006, an 11-year-old girl developed general fatigue, headache, and vomiting. Two days later, she was admitted to a local hospital where laboratory tests indicated the following: Hb (11.9 g/dL), LDH (1636 IU/L), total bilirubin (9.3 mg/dL), platelet (33×10^9/L), PT (12.7 sec), PTT (34.0 sec), fibrinogen (290 mg/dL), antithrombin (> 75%), D-dimer (< 2 ng/mL), FDP (< 5 µg/mL), BUN (18.0 mg/dL), and schistocytes in a peripheral blood smear. For a differential diagnosis, her plasma ADAMTS13:AC and ADAMTS13:INH levels were determined to be less than 0.5% and 1.8 BU/mL, respectively. In addition, upon admission she simultaneously had Raynaud's phenomenon and was positive for anti-nuclear antibodies and anti-RNP antibodies. Thus, she was diagnosed with MCTD-associated TTP, and was administered PE with steroid pulse therapy starting on the third day of hospitalization. During the third PE, she had anaphylactic shock, perhaps related to the infused plasma. Thus, she stopped the PE therapy and continued the steroid pulse therapy alone. As a result of these treatments, she recovered and on the hospital day 14 her ADAMTS13:AC increased to 67% of normal and ADAMTS13:INH became negative. To date, she has had no episodes of TTP.

Case 17

In October 2007, a 12-year-old girl suddenly developed jaundice with a fever. She was admitted to a nearby university hospital, and her routine laboratory data provided the following: Hb (5.6 g/dL), platelet (1×10^9/L), PT (14.0 sec), PTT (40.1 sec), fibrinogen (333 mg/dL), FDP (20.5 µg/mL), total bilirubin (5.5 mg/dL), and schistocytes in a peripheral blood smear. Upon admission, she had low levels of complement, and was positive for anti-nuclear antibodies, anti-double stranded DNA antibodies, and anti-SS-A antibodies. Plasma ADAMTS13:AC and ADAMTS13:INH were simultaneously measured by the act-ELISA and were less than 0.5% and 0.7 BU/mL, respectively. Based on these results, she was diagnosed with SLE-associated TTP and PE therapy was initiated. After three consecutive PE treatments with steroid pulse therapy, her platelet count increased. However, on hospital day 8, her platelet count decreased again, and her ADAMTS13:INH titer increased to 2.2 BU/mL. The PE therapy was re-initiated with steroid pulse therapy. A total of nine rounds of PE therapy and two courses of steroid pulse therapy resulted in remission on hospital day 23. At this time, ADAMTS13:AC and ADAMTS13:INH were 86% and less than 0.5 BU/mL, respectively. To date, she has had no TTP relapses.

Treatment of acquired thrombotic thrombocytopenic purpura

Plasma exchange (PE) is the first line therapy that was demonstrated to be effective in randomized clinical trials for acquired TTP [62]. PE removes ADAMTS13:INH, UL-VWFM, and hazardous cytokines from the circulation in TTP patients, and replenishes ADAMTS13 without circulatory overload. Corticosteroids are often used as an adjunctive treatment. In relapsing or refractory cases, other immunosuppressive drugs such as cyclosporine, cyclophosphamide, vincristine, and rituximab are empirically used. PE therapy should be initiated as soon as possible after TTP is diagnosed, but the onset of therapy tends to be delayed in childhood patients with acquired TTP because of difficult differential diagnoses, especially with HUS, unless ADAMTS13:AC is measured.

In regard to platelet transfusions in TTP patients with a severe ADAMTS13:AC deficiency, these transfusions have consistently been viewed as a contraindication because they may enhance thrombotic complications due to platelet aggregation and thrombus formation under high shear stress generated in the microvasculature. Our experience also partially supports this concept, but such adverse effects happened only on very few occasions. We believe that prophylactic platelet

transfusions should be avoided in TTP patients with a severe ADAMTS13:AC deficiency, but that platelet transfusions must be done if patients experience overt bleeding.

In our 17 childhood patients with acquired TTP, 15 patients were promptly treated with PE and corticosteroid therapy, and 16 children (94%) achieved a first remission. Recently, McDonald et al. [63] reported that the number of PE courses to first remission was higher in children (median, 22.5; range, 10–30) than in adults (median, 15.5; range, 3–93) [64], suggesting that childhood TTP may be more resistant to treatment. By contrast, our results indicated that patients with acquired TTP and a severe ADAMTS13:AC deficiency responded well to PE (median number of PE courses, 5.5; range, 2–39), but two patients (2/17, 11.8%) relapsed and one (1/17, 5.9%) died. Furthermore, in this study, we observed that the children with a high ADAMTS13:INH titer (> 5 BU) tended to require more frequent PE courses to achieve remission.

Fakhouri et al. [65] recently reported that adulthood TTP patients with high-titer ADAMTS13:INH could be successfully treated with a combination of PE and rituximab, a chimeric monoclonal antibody to CD20. The efficacy of rituximab in such patients is apparently due to a reduction in anti-ADAMTS13 IgG antibodies by depleting the patient's B-lymphocytes [65,66]. Recently, there have been many successful cases [67–69], and to date, no significant adverse effects have been reported. In our registry, only one childhood TTP patient (7 years old) with acquired TTP with ADAMTS13:INH was successfully treated with PE followed by rituximab, as shown in table II. However, the best choice or combination in regard to immunosuppressants for treating children with acquired TTP and a severe ADAMTS13:AC deficiency needs to be carefully determined in future studies.

Conclusion

The discovery of ADAMTS13 provided a breakthrough in our understanding of the mechanism of platelet thrombus formation under high shear stress and directly linked this enzyme to TTP pathogenesis in humans. Subsequently, the recent development of rapid and sensitive ADAMTS13 assays and their utilization in clinical practice have shown that the early- and late-onset phenotypes of USS are not different diseases and are likely affected by both acquired endogenous and exogenous circumstances. Furthermore, we have presented a novel category of ai-TTP that occurs during very early childhood (less than 2 years of age), which was perhaps totally overlooked or misdiagnosed before 2002 [39]. Thus, TTP should be recognized as a life-threathening generalized disease that not only occurs in adulthood, but also in childhood, causing a paradigm shift in our clinical understanding of TTP since the first discovery by Moschcowitz in 1924.

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Original Article

ADAMTS13 activity may predict the cumulative survival of patients with liver cirrhosis in comparison with the Child-Turcotte-Pugh score and the Model for End-Stage Liver Disease score

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Aim: Decreased plasma ADAMTS13 activity (ADAMTS13:AC) results in accumulation of unusually large von Willebrand factor multimers and platelet thrombi formation. Our aim was to evaluate whether ADAMTS13:AC is a prognostic marker in patients with liver cirrhosis.

Methods: Plasma ADAMTS13:AC and its related parameters were examined in 108 cirrhotic patients.

Results: ADAMTS13:AC decreased as the severity of liver disease increased (means: controls 100%, Child A-cirrhotics 79%, Child B-cirrhotics 63%, and Child C-cirrhotics 31%). ADAMTS13:AC markedly decreased in the cirrhotics with hepatorenal syndrome, refractory ascites and hepatic encephalopathy. The cumulative survival time was the shortest (median: 4.5 months) in the cirrhotics with severe to moderate ADAMTS13:AC deficiency (<3–25%), followed by those with mild ADAMTS13:AC deficiency (25–50%), and was the longest in those with normal activity (>50%). In contrast, based on the Child-Turcotte-Pugh (CTP) score, Child C-cirrhotics had the worst survival, but the survival probabilities did not differ between Child A and B cirrhotics. Based on the Model for End-Stage Liver Disease (MELD) score, the survival was the worst for the cirrhotics in the fourth quartile, but it was not different among cirrhotics in the first three quartiles. Cox proportional-hazards regression analysis showed that ADAMTS13:AC and serum albumin were independent factors affecting the survival.

Conclusions: ADAMTS13:AC concomitantly decreases as the functional liver capacity decreases. This activity may be a useful prognostic marker that is equal or superior to the CTP score and the MELD score to predict not only the short-term prognosis but also the long-term survival of the cirrhotic patients.

Key words: ADAMTS13 activity, Child-Turcotte-Pugh score, liver cirrhosis, Model for End-Stage Liver Disease score, prognosis

INTRODUCTION

ONCE PATIENTS WITH liver cirrhosis (LC) develop a decompensated condition, the risk of early mortality sharply increases.[1] Any patient with LC is at risk for specific life threatening complications such as variceal bleeding, sepsis, hepatorenal syndrome, and hepatopulmonary syndrome. Many studies examined the factors that predict the survival of patients with LC.[1–7] The Child score was originally designed to assess the prognosis of cirrhotic patients undergoing surgical treatment for portal hypertension in 1964,[2] and thereafter its modified form, the Child-Turcotte-Pugh (CTP) score,[3] has been widely used to prognosticate the patients with LC.[1] However, this score includes some subjective components and does not estimate other factors, such as renal dysfunction and pulmonary dysfunction, that are commonly associated with decompensated cirrhosis.[2,3] Furthermore, the CTP score is not always sufficient, particularly when predicting the short-term prognosis of

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patients.[4] The Model for End-Stage Liver Disease (MELD) score was designed to assess the prognosis of cirrhotics who receive transjugular intrahepatic portosystemic shunt (TIPS), and has been used as a disease severity index and a new liver organ allocation system for liver transplantation since 2002.[5] However, the main causes of death, such as variceal bleeding, ascites, hepatorenal syndrome and hepatopulmonary syndrome, in the advanced cirrhotics are not included in the MELD score.[6] Patients with advanced liver diseases tend to bleed because of reduced plasma levels of several clotting factors and thrombocytopenia, but they also exhibit thrombotic complications.[7] Portal or hepatic vein thrombosis is often observed in advanced cirrhosis,[8,9] and microthrombi formation was found in one or multiple organs in half of the autopsied cirrhotics.[10] This hypercoagulable state may not only affect hepatic parenchymal extinction, the acceleration of liver fibrosis, and disease progression but also influence other organs and potentially lead to multi-organ failure.

ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) is a metalloproteinase that specifically cleaves the multimeric von Willebrand factor (VWF) between the Tyr1605 and Met1606 residues in the A2 domain.[11,12] ADAMTS13 deficiency, caused either by mutations in the *ADAMTS13* gene[11–14] or by inhibitory autoantibodies against ADAMTS13,[15,16] results in accumulation of "unusually large" VWF multimers (UL-VWFM) in the plasma. This accumulation leads to platelet clumping and/or thrombi under high shear stress and subsequent microcirculatory disturbances. ADAMTS13 is produced exclusively in the hepatic stellate cells (HSC),[17] although platelets,[18] vascular endothelial cells,[19] and kidney podocytes[20] have been implicated as ADAMTS13-producing cells. The plasma levels of von Willebrand factor antigen (VWF: Ag), the substrate for ADAMTS13, substantially increases as the liver disease progresses,[21,22] and thrombocytopenia is commonly seen in patients with advanced LC.[23–25] Previous studies revealed a significant reduction in the ADAMTS13 activity (ADAMTS13:AC) in advanced LC,[26,27] while the ADAMTS13 activity was unchanged in another study.[28] Subsequently, we demonstrated that both the plasma ADAMTS13 activity and antigen levels decreased as the severity of cirrhosis increased, and an imbalance between the decreased ADAMTS13:AC and the increased levels of its substrate may reflect a state that predisposes the patients with advanced LC to platelet thrombus formation.[29] In addition, we have shown that ADAMTS13:AC is reduced in the patients with hepatic veno-occlusive disease,[30] alcoholic hepatitis,[31] and those undergoing living-donor-related liver transplantation.[32] Thus, ADAMTS13:AC likely decreases as the functional liver capacity also declines in advanced liver diseases.

In this study, we investigated the relationship between ADAMTS13:AC and the prognosis of patients with LC, and examined whether the ADAMTS13:AC is a useful prognostic factor for cirrhotic patients as compared to the CTP score and the MELD score.

METHODS

Patients

IN THIS STUDY, we examined a total of 108 LC patients, including one patient with thrombotic thrombocytopenic purpura (TTP).[33] Patients with a known history of coagulopathies, platelet disorders, or liver transplantation at basal evaluation were excluded. The origin of liver disease was hepatitis C virus (HCV) in 67 patients, hepatitis B virus (HBV) in 16, alcohol abuse in 10, primary biliary cirrhosis (PBC) in four, and cryptogenic in 11. Cirrhosis was diagnosed based on physical findings and laboratory tests, and in many cases was confirmed by histological criteria. The cirrhotic patients were classified into subgroups, according to the CTP score (Table 1), the quartiles of the MELD risk score (RS) (first quartile, RS: 0–3; second quartile, RS: 4–7; third quartile, RS: 8–13; and fourth quartile, RS: 14–43) (Table 2), or the ADAMTS13:AC (severe to moderate deficiency: <3–25% of the healthy control, mild deficiency: 25–50%, and the normal range: >50%) (Table 3). The MELD RS was calculated according to the following formula: $RS = 3.8 \times \log_e$ (bilirubin mg/dL) $+ 11.2 \times \log_e$ (INR) $+ 9.6 \times \log_e$ (creatinine mg/dL) $+ 6.4 \times$ (cause of cirrhosis) in which the value for the cause of cirrhosis was 0 for an alcoholic or cholestatic etiology and one for viral or other etiologies.[5] The spleen volume was determined by computed tomography (CT) scanning.[34] The diagnosis of ascites, spontaneous bacterial peritonitis (SBP), and hepatorenal syndrome was made according to previously described criteria.[35] Hepatic encephalopathy grade II or higher was evaluated by the classification of Trey *et al.*[36] Endoscopic signs of an impending variceal rupture were classified according to the criteria of the Japanese Research Society for Portal Hypertension.[37] The Japan Integrated Staging (JIS) score was obtained by adding the tumor stage score of hepato-

Table 1 Clinical data of the patients with liver cirrhosis according to the Child-Turcotte-Pugh (CTP) score

	Child A ($n = 35$)	Child B ($n = 33$)	Child C ($n = 40$)
Age (years)	66.4 ± 7.8	63.6 ± 8.3	64.7 ± 15.1
Sex (male/female)	25/10	17/16	22/18
Cause of liver disease			
HCV/HBV/Alcohol/PBC/Cryptogenic	24/4/4/0/3	20/7/2/0/4	23/5/4/4/4
Child–Pugh score	5.5 ± 0.5	7.9 ± 1.0**	11.4 ± 1.5**,***
MELD score	6 ± 5	9 ± 5*	16 ± 8**,****
Platelet count ($\times 10^4/mm^3$)	9.6 ± 4.6	6.9 ± 2.4*	5.9 ± 3.6*
Spleen volume (mm^3)	323 ± 181	399 ± 250	551 ± 243**,***
Ascites (−/easily mobilized/refractory)	0	21/10/2	8/6/26
Spontaneous bacterial peritonitis	0	0	10**,***
Hepatorenal syndrome (+)	0	0	10**,***
Encephalopathy (+)	0	9*	32**,****
Esophageal varices (−/mild/severe)†	10/12/13	3/7/23*	3/6/31**
Each incidence (−/mild/severe)†	29%/34%/37%	9%/21%/70%*	7%/15%/78%**
Hepatocellular carcinoma (+)	22	16	19
JIS score‡	1.4 ± 0.9	2.8 ± 1.0**	3.7 ± 1.1**,***
Portal thrombosis	0	3	3
Outcome (alive/died)	30/5	27/6	9/31
Cause of death			
Hepatocellular carcinoma	4	5	17
Hepatic failure	0	0	7
Hepatorenal syndrome	0	0	6
Gastrointestinal bleeding	0	1	0
Thrombotic thrombocytopenic purpura	0	0	1
Acute myocardial infarction	1	0	0

*$P < 0.01$ and **$P < 0.001$ vs. cirrhotics with Child A, respectively. ***$P < 0.01$ and ****$P < 0.001$ vs. cirrhotics with Child B, respectively.

†Mild or severe esophageal varices indicate lesions without or with endoscopic signs of impending variceal rupture, respectively.

‡The Japan Integrated Staging score obtained via the summation of Child–Pugh score and tumor stage score.[38]

The data are expressed as mean ± standard deviation (SD).

HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis.

cellular carcinoma (HCC) and the CTP score.[38] The portal thrombosis was examined by Doppler-ultrasonography (US). Once patients were discharged, they were carefully followed up by conventional liver function tests and tumor markers including α-fetoprotein and des-γ-carboxy prothrombin every month as outpatients until death, which was the primary end-point to estimate survival probabilities by the CTP score, the MELD score and ADAMTS13:AC. Patients with liver transplantation were, therefore, excluded. Image diagnosis by US, dynamic enhanced CT, or dynamic enhanced magnetic resonance imaging (MRI) was performed every 3 months to evaluate the presence or absence of HCC or ascites. All subjects provided informed consent to participate in the study. The study protocol was approved by the Nara Medical University Hospital Ethics Committee.

Determination of ADAMTS13:AC, VWF: Ag, VWF: RCo, UL-VWFMs and ADAMTS13:INH

Blood samples were taken from patients at the time of admission or during their hospital stay. These samples were stored in plastic tubes containing 1/10th the volume of 3.8% sodium citrate. Platelet-poor plasma was prepared by centrifuging at 3000 *g* at 4°C for 15 min and stored in aliquots at −80°C until analysis. For seven patients with LC, a second plasma sample was taken between days 120 and 630 (mean: 345 days) during their second hospitalization because of hepatic encephalopathy (three patients), ascites augmentation (three patients), and variceal rupture (one patient). Plasma ADAMTS13:AC was determined by the classic VWFM assay[39,40] and the sensitive chromogenic enzyme-linked immunosorbent assay (ELISA, ADAMTS13-act-

Table 2 Clinical data of the patients with liver cirrhosis according to the Model for End-Stage Liver Disease (MELD) score

	First quartile ($n = 12$)	Second quartile ($n = 30$)	Third quartile ($n = 33$)	Fourth quartile ($n = 33$)
Age (years)	63.8 ± 7.9	68.0 ± 8.8	65.1 ± 10.4	66.7 ± 9.5
Sex (male/female)	6/6	16/14	21/12	24/9
Cause of liver disease				
HCV/HBV/Alcohol/PBC/Cryptogenic	6/2/3/0/1	21/3/4/1/1	20/7/0/3/3	20/4/3/0/6
Child–Pugh score	6.6 ± 1.7	6.8 ± 2.0	7.9 ± 1.9***	11.1 ± 2.5**,*****,******
ADMTS13 activity (%)	69 ± 25	74 ± 34	68 ± 29	32 ± 21**,*****,******
MELD score	2 ± 3	6 ± 1**	10 ± 2**,*****	19 ± 6**,*****,******
Platelet count ($\times 10^4/mm^3$)	9.2 ± 6.0	10.7 ± 8.7	7.8 ± 4.2	6.8 ± 4.1*,***
Spleen volume (mm^3)	285 ± 75	397 ± 146	386 ± 99	567 ± 162*,****,*****
Ascites (−/easily mobilized/refractory)	11/1/0	24/4/2	24/5/4	5/6/22**,*****,******
Spontaneous bacterial peritonitis	0	1	1	8****,******
Hepatorenal syndrome (+)	0	1	0	9*,****,******
Encephalopathy (−/+)	1	4	9	27**,****,*******
Esophageal varices (−/mild/severe)†	4/3/5	6/9/15	4/9/20	2/4/27
Each incidence (−/mild/severe)†	33%/25%/42%	20%/30%/50%	12%/27%/61%	6%/12%/82%**,*****
Hepatocellular carcinoma (+)	5	16	16	20
JIS score‡	1.8 ± 1.3	1.7 ± 1.0	2.2 ± 1.2	3.6 ± 1.3*,*****,*******
Portal thrombosis	0	3	2	1
Outcome (alive/died)	9/3	25/5	24/9	8/25
Cause of death				
Hepatocellular carcinoma	0	3	6	17
Hepatic failure	1	1	3	2
Hepatorenal syndrome	0	1	0	5
Gastrointestinal bleeding	1	0	0	0
Thrombotic thrombocytopenic purpura	0	0	0	1
Acute myocardial infarction	1	0	0	0

*$P < 0.05$ and **$P < 0.001$ vs. cirrhotics with the first quartile, respectively. ***$P < 0.05$ and ****$P < 0.001$ vs. cirrhotics with the second quartile, respectively.
*****$P < 0.05$ and ******$P < 0.001$ vs. cirrhotics with the third quartile, respectively.
†Mild or severe esophageal varices indicate lesions without or with endoscopic signs of impending variceal rupture, respectively.
‡The Japan Integrated Staging score obtained via the summation of Child–Pugh score and tumor stage score.[38]
The data are expressed as mean ± standard deviation (SD).
HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis.

Table 3 Clinical data of the patients with liver cirrhosis according to the ADAMTS13 activity

	Normal range (>50%) ($n = 56$)	Mild deficiency (25–50%) ($n = 27$)	Severe to moderate deficiency (<25%) ($n = 25$)
Age (years)	64.9 ± 10.8	66.8 ± 7.6	67.8 ± 9.4
Sex (male/female)	32/24	15/12	20/5
Cause of liver disease			
HCV/HBV/Alcohol/PBC/Cryptogenic	34/9/7/2/4	19/3/1/0/4	14/4/2/2/3
ADMTS13 activity (%)	83 ± 22	43 ± 12**	20 ± 18**,****
Child–Pugh score	7.0 ± 1.9	9.5 ± 3.0**	10.7 ± 2.3**
MELD score	7 ± 5	13 ± 9**	16 ± 6**
Platelet count ($\times 10^4$/mm^3)	9.5 ± 5.6	8.2 ± 7.9	6.5 ± 3.2*
Spleen volume (mm^3)	324 ± 85	559 ± 104**	534 ± 198**
Ascites (−/easily mobilized/refractory)	46/8/2	11/4/12**	7/4/14**
Spontaneous bacterial peritonitis	0	2*	8**,***
Hepatorenal syndrome (+)	0	2*	8**,***
Encephalopathy (−/+)	6	13**	22**,***
Esophageal varices (−/mild/severe)†	9/18/29	5/4/18	2/3/20
Each incidence (−/mild/severe)†	16%/32%/52%	19%/15%/66%	8%/12%/80%
Hepatocellular carcinoma (+)	27	15	15
JIS score‡	1.9 ± 1.3	2.5 ± 1.5	3.6 ± 0.9**,***
Portal thrombosis	0	3	3
Outcome (alive/died)	45/11	15/12	6/19
Cause of death			
Hepatocellular carcinoma	6	7	13
Hepatic failure	3	3	1
Hepatorenal syndrome	0	2	4
Gastrointestinal bleeding	1	0	0
Thrombotic thrombocytopenic purpura	0	0	1
Acute myocardial infarction	1	0	0

*$P < 0.05$ and **$P < 0.001$ vs. cirrhotics with >50% of ADAMTS13 activity, respectively. ***$P < 0.05$ and ****$P < 0.001$ vs. cirrhotics with 25% to 50% of ADAMTS13, respectively.
†Mild or severe esophageal varices indicate lesions without or with endoscopic signs of impending variceal rupture, respectively.
‡The Japan Integrated Staging score obtained via the summation of Child–Pugh score and tumor stage score.[38]
The data are expressed as mean ± standard deviation (SD).
HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis.

ELISA: Kainos Laboratories Inc., Tokyo, Japan).[41] The normal values were 102 ± 23% in the VWFM assay[39] and 99 ± 22% in the act-ELISA.[41] Plasma VWF: Ag was measured using a rabbit polyclonal sandwich ELISA (Dako, Denmark), and its normal level was 100 ± 53% ($n = 60$, age: 20–39 years). VWF ristocetin cofactor activity (VWF: RCo) was determined as previously described,[42] and its normal value was 100 ± 15%. In 49 LC patients with lower ADAMTS13:AC (<50% of the normal control), the plasma UL-VWFMs were analyzed by a vertical sodium dodecyl sulfate (SDS)–1.0% agarose gel electrophoresis system[43] and evaluated using NIH image J. ADAMTS13 inhibitor (ADAMTS13: INH) was evaluated using plasma that was heat-inactivated at 56°C for 30 min.[15,16] One Bethesda unit (BU) of inhibitor was defined as the amount of plasma that reduces ADAMTS13:AC to 50% of the control,[44] and its titer was considered significant at >0.5 BU/mL.

Statistical analysis

The differences among cirrhotics with the CTP score, the MELD score, and the ADAMTS13:AC, and healthy subjects were analyzed with the Kruskal–Wallis rank test before pair-wise comparisons (Tables 1–4). If the Kruskal–Wallis rank test showed significant differences in each parameter among groups, pairwise comparison in each parameter was evaluated by Mann–Whitney *U*-test for continuous variables. The χ^2-test was used for

Table 4 The plasma values of ADAMTS13 activity and its related parameters

Variable	Liver cirrhosis			Healthy subjects ($n = 60$)
	Child A ($n = 35$)	Child B ($n = 33$)	Child C ($n = 40$)	
ADAMTS13:AC (%) (VWFM assay)	79 ± 25**	63 ± 34**,***	31 ± 22**,***,******	102 ± 23
ADAMTS13:AC (%) (ELISA)	80 ± 24**	65 ± 31**,***	40 ± 22**,***,******	99 ± 22
VWF: Ag (%)	320 ± 174**	436 ± 267**,***	486 ± 254**,*****	100 ± 53
VWF: RCo (%)	186 ± 137*	198 ± 172*	227 ± 187*	100 ± 15
VWF: RCo/VWF: Ag ratio	0.63 ± 0.49**	0.50 ± 0.46**	0.51 ± 0.40**	1.1 ± 0.42
VWF: RCo/ADAMTS13 ratio	1.6 ± 1.7**	5.0 ± 5.7**,***	16.8 ± 28.2**,***,*****	0.9 ± 0.2
VWFM patterns† (degraded/normal/unusually-large)	2/0/0	8***/5***/0	6/20*****/8***,******	
Inhibitor against ADAMTS13† (number of positive cases)	1	9***	19*****	absent

*$P < 0.05$ and **$P < 0.001$ vs. healthy subjects, respectively. ***$P < 0.05$, and ****$P < 0.001$ vs. cirrhotics with Child A, respectively. *****$P < 0.05$ and ******$P < 0.001$ vs. cirrhotics with Child B, respectively.

†The VWFM patterns and ADAMTS13 inhibitor were analyzed in 49 cirrhotic patients with lower ADAMTS13:AC (less than 50% of the healthy control).

The data are expressed as mean ± standard deviation (SD).

ADAMTS13: AC, ADAMTS13 activity; ELISA, enzyme-linked immunosorbent assay; VWF: Ag, von Willebrand factor antigen; VWF: RCo, von Willebrand factor ristocetin cofactor activity; VWFM, von Willebrand factor multimer.

categorical data. The differences in the ADAMTS13:AC and CTP scores obtained by the sequential study in identical patients were estimated by Wilcoxon signed-ranks test. Correlations were calculated by the Spearman rank test. The analyses were carried out using Statview statistical software (version 5.0; SAS Institute Inc., Cary, NC, USA). The Kaplan–Meier analysis was used to evaluate the prognosis of cirrhotic patients according to the degree of the CTP score, the MELD score and plasma ADAMTS13:AC (Figs 1–3) by the log-rank test using StatMate IV for Windows (AT0484, Advanced Technology for Medicine & Science, Tokyo, Japan). Cox proportional-hazard model was used to evaluate the hazard ratio of each class in the CTP score, the MELD score, and the ADAMTS13:AC (Table 5). In addition, Cox proportional-hazards regression analysis was applied to determine independent prognostic markers including the ADAMTS13:AC. The following eight variables were analyzed for potential covariates to predict the survival at the time of sample collection: age, sex, ADAMTS13:AC, albumin, total bilirubin, prothrombin time, blood ammonia, and platelet count. Although continuous variables without data conversion were used at first, some multivariate analyses could not be performed because of the multi-collinearity among parameters, and then, several variables were transformed into categorical data consisting of two or three simple ordinal numbers to obtain each hazard ratio (Table 6).

To further compare the overall accuracy of these three models including the CTP score, the MELD score, and the ADAMTS13:AC, the areas under the curves (AUCs) were determined by receiver operating characteristic (ROC) curves for 1-year and 2-year survival (Fig. 4). The

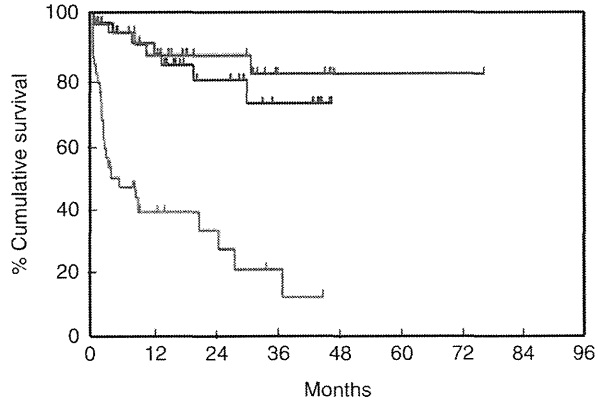

Figure 1 The cumulative survival rate of 108 patients with liver cirrhosis according to the Child classification. Child C patients had worse survival than Child A and B patients, but the survival probabilities were not different between Child A and Child B patients (Log rank test among the three groups, $P < 0.0001$; Child C vs. Child A, $P < 0.0001$; Child C vs. Child B, $P < 0.0001$). The red, blue, and green lines indicate cirrhotic patients with Child A, Child B, and Child C, respectively.

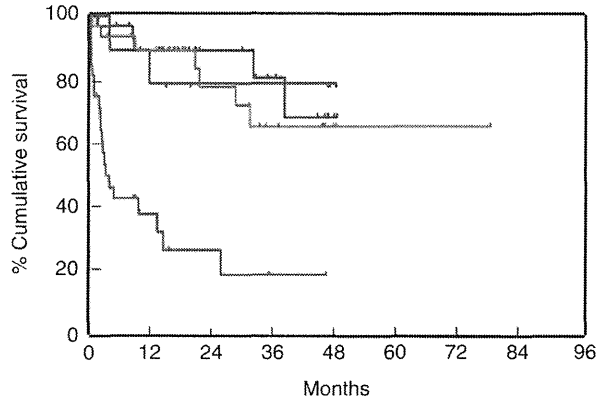

Figure 2 The cumulative survival rate of 108 patients with liver cirrhosis according to the Model for End-Stage Liver Disease (MELD) score. The survival of patients in the fourth quartile of the MELD risk score (RS) calculated by the formula[5] was worse than that of the patients in the first three RS quartiles, while the survival of the patients in the first three RS quartiles did not differ among them (Log rank test among the four groups, $P < 0.0001$; fourth quartile vs. others, $P < 0.0001$). The red, blue, green, and pink lines indicate cirrhotic patients with the first RS quartile, second RS quartile, third RS quartile, and fourth RS quartile, respectively.

ROC and Cox proportional-hazard analysis were performed using a StatFlex ver. 6 for Windows. A two-tailed *P*-value less than 0.05 was considered significant. All data are presented as the mean ± standard deviation (SD).

RESULTS

Clinical characteristics of patients with liver cirrhosis according to the CTP score, the MELD score, and the ADAMTS13:AC

OF THE LC patients, 35 were Child A, 33 were Child B, and 40 were Child C according to Child–Pugh's criteria[3] (Table 1). The MELD score progressively increased from Child A to C. The platelet counts decreased with the severity of chronic liver diseases. The spleen volume increased as liver disease progressed. Ascites was easily mobilized in 16 patients and refractory in 28. Ten out of 26 Child C-LC with refractory ascites finally progressed to hepatorenal syndrome. SBP occurred in 10 Child C-LC with refractory ascites, and this was complicated by hepatorenal syndrome in seven patients. Forty-one LC patients developed hepatic encephalopathy grade II or higher. Sixty-seven LC patients had endoscopic signs of impending variceal rupture, and its incidence was higher in LC patients with Child B and Child C than in Child A patients. HCC was detected in 22 patients (62.9%) with Child A, 16 patients (48.5%) with Child B, and 19 patients (47.5%) with Child C. The incidence of HCC did not differ among the three groups, but the JIS score progressively increased from Child A to C. Portal thrombosis was detected in three patients with Child B and in three with Child C. During a median follow-up of 475 days (range: 5 to 2406 days), 42 LC patients died within 5 to 1161 days after they had provided samples. Of these, five cirrhotic patients were classified as Child A, six as Child B, and 31 as Child C. The cause of death in Child A patients was HCC in four patients and acute myocardial infarction in one, that in Child B patients was HCC in five and gastrointestinal bleeding in one, and that in Child C patients was HCC in 17, hepatic failure in seven, hepatorenal syndrome in six, and TTP in one (Table 1).

With respect to the classification by the MELD score, the patients belonging to the fourth quartile showed the

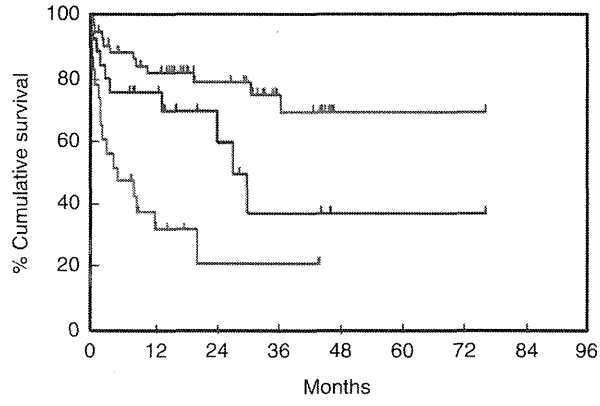

Figure 3 The cumulative survival rate of 108 patients with liver cirrhosis according to ADAMTS13 activity (ADAMTS13:AC). The survival rate was remarkably different among the patients with severe to moderate ADAMTS13:AC deficiency, mild ADAMTS13:AC deficiency, and normal range of ADAMTS13:AC (Log rank test among the three groups, $P < 0.0001$; severe to moderate deficiency vs. mild deficiency, $P = 0.0219$; mild deficiency vs. normal range, $P = 0.0287$; severe to moderate deficiency vs. normal range, $P < 0.0001$). The median survival time was the lowest (4.5 months) in the cirrhotic patients with severe to moderate ADAMTS13:AC deficiency. The red, blue, and green lines indicate cirrhotic patients with normal range of ADAMTS13:AC (>50%), mild ADAMTS13:AC deficiency (25–50% of the normal control), severe to moderate ADAMTS13:AC deficiency (<25% of the normal control), respectively.

Table 5 Hazard ratios for predicting the survival of the patients with liver cirrhosis

Factors	Category	Hazard ratio (95% confidence interval)	*P*-value
Child score	1: Child A	1.0	
	2: Child B	1.04 (0.71–1.50)	0.8453
	3: Child C	4.00 (2.35–6.80)	<0.001
MELD score	1: first quartile	1.0	
	2: second quartile	0.73 (0.44–1.19)	0.2095
	3: third quartile	0.85 (0.52–1.41)	0.5391
	4: fourth quartile	5.16 (2.70–9.86)	<0.001
ADAMTS13 activity	1: normal range (>50%)	1.0	
	2: mild deficiency (25–50%)	1.51 (1.05–2.17)	0.0248
	3: severe to moderate deficiency (<25%)	2.50 (1.68–3.72)	<0.001

MELD, Model for End-Stage Liver Disease.

highest values of CTP score, spleen volume, and JIS score, and the lowest levels of ADAMTS13:AC and platelet count (Table 2). The incidence of patients with refractory ascites, SBP, hepatorenal syndrome, and hepatic encephalopathy was higher in the fourth quartile group than in the other three quartile groups. The number of patient deaths was three in the first quartile, five in the second quartile, nine in the third quartile and 25 in the fourth quartile (Table 2).

According to the classification of ADAMTS13:AC, the incidence of patients with SBP, hepatorenal syndrome, and hepatic encephalopathy progressively increased from patients with normal range to those with severe to moderate deficiency of the activity (Table 3). The CTP score, MELD score, spleen volume, and the incidence of refractory ascites were higher in the patients with mild and severe to moderate ADAMTS13:AC deficiency than in those with normal activity, but did not differ between patients with mild and severe to moderate deficiency of the activity. The JIS score was the lowest in patients with severe to moderate ADAMTS13:AC deficiency. The number of patient deaths was 11 in the group with normal activity, 12 in those with mild deficiency, and 19 in those with severe to moderate deficiency of the activ-

Table 6 Factors affecting the survival of the patients with liver cirrhosis in the multivariate analysis

Factors	Category	Hazard ratio (95% confidence interval)		*P*-value
ADAMTS13 activity	1: ≥50%	1.0		0.0059
	2: 25–50%	2.25	(1.27–3.99)	
	3: <25%	5.08	(1.62–15.97)	
Albumin	1: ≥2.8 mg/dL	1.0		0.0101
	2: <2.8 mg/dL	3.39	(1.34–8.61)	
Total bilirubin	1: <3.0 mg/dL	1.0		
	2: ≥3.0 mg/dL	1.20	(0.38–3.77)	0.7573
Prothrombin time	1: ≥40%	1.0		
	2: <40%	2.24	(0.55–9.09)	0.2602
Blood ammonia	1: <80 μg/dl†	1.0		
	2: ≥80 μg/dl	1.45	(0.57–3.71)	0.4389
Platelet count	1: ≥50 × 10^9/L	1.0		
	2: <50 × 10^9/L	0.56	(0.17–1.82)	0.3329
Age (years)	1: <65	1.0		
	2: ≥65	0.45	(0.19–1.06)	0.0683
Sex	1: male	1.0		
	2: female	1.808	(0.65–4.98)	0.2552

†Normal range of blood ammonia.

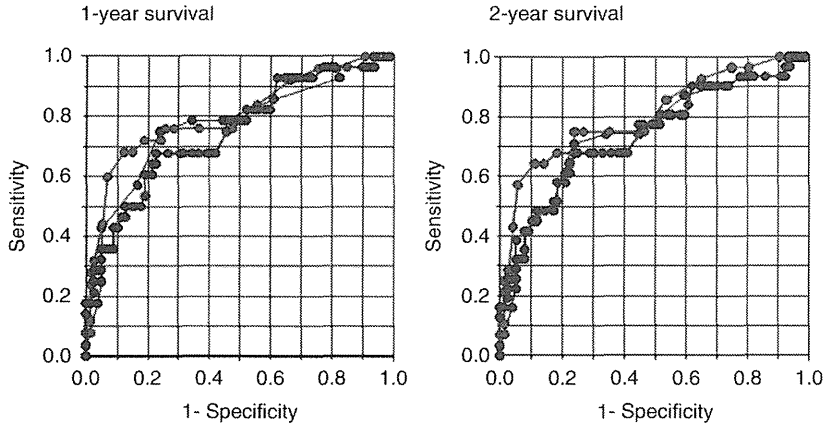

Figure 4 Receiver operating characteristic curves for the Child-Turcotte-Pugh (CTP) score, the Model for End-Stage Liver Disease (MELD) score, and the ADAMTS13 activity in predicting the 1-year and 2-year survival. The CTP score is represented by the black line, the MELD score by the red line, and the ADAMTS13 activity by the blue line. The areas under the curves (AUCs) for the CTP score, the MELD score, and the ADAMTS13 activity were 0.769, 0.805, and 0.752 in predicting the 1-year survival (left), and were 0.752, 0.805, and 0.739 in predicting the 2-year survival (right), respectively. The AUCs for the ADAMTS13:AC tended to be the lowest in the 1-year and 2-year survival, but the differences were not significant among the CTP score, the MELD score, and the ADAMTS13 activity.

ity. The mortality rate was higher in the patients with mild deficiency of ADAMTS13:AC (44.4%, $P < 0.03$) (Table 3) than in those with Child B (18.2%) according to the CTP score (Table 1) and the second quartile (16.7%) according to the MELD score (Table 2).

Determination of ADAMTS13:AC and its related parameters

Based on the VWFM assay, the plasma ADAMTS13:AC was 56 ± 34% in the LC patients, which was significantly lower than that in the healthy subjects ($P < 0.001$). This activity progressively decreased as cirrhosis advanced from Child A to C (Table 4). The ADAMTS13:AC determined by the act-ELISA also decreased as the cirrhosis severity increased and was consistent with the results of the VWFM assay ($r = 0.831$, $P < 0.001$). VWF: Ag was significantly higher in the LC patients than in the healthy subjects, and was higher in the patients with Child B and C than in those with Child A, but there was no difference between Child B and Child C patients. The VWF: RCo values were higher in the LC patients than in the healthy subjects but did not differ among the subgroups of LC patients from Child A to C. The ratio of VWF: RCo to VWF: Ag was lower in the LC patients (0.54 ± 0.45, $P < 0.001$) than in the healthy subjects, but there were no differences among the LC patients with Child A, B, and C. In contrast, the ratio of VWF: RCo to ADAMTS13:AC significantly increased with the progression of liver disease. With respect to the VWFM analyses, two Child A cirrhotics had only degraded-VWFM, eight and five Child B cirrhotics had degraded-VWFM and normal-VWFM, respectively, and six, 20, and eight Child C cirrhotics had degraded-VWFM, normal-VWFM, and UL-VWFM, respectively. The incidence of ADAMTS13:INH increased in the cirrhotic patients from Child A to C (Table 4). The inhibitory activity was 2.0 BU/mL[33] in one LC patient with TTP and 3.0 BU/mL in a patient with severe ADAMTS13:AC deficiency, but was in the marginal zone between 0.5 and 1.0 BU/mL in the remaining patients.

Relationship of the plasma ADAMTS13:AC with the clinical variables

Regarding the influence of HCC complicating LC on the plasma levels of ADAMTS13:AC, Child A LC patients with HCC had a significantly lower ADAMTS13:AC than those without HCC (69 ± 22% vs. 103 ± 11%, $P < 0.001$), but there were no differences between with and without HCC in the LC patients with Child B and Child C. Furthermore, the plasma ADAMTS13:AC levels were lower in the patients with JIS score of 4 (32 ± 28%, $P < 0.05$) and JIS score of 5 (26 ± 29%, $P < 0.05$) than in the patients with JIS score of 0 (67 ± 24%), score of 1 (65 ± 37%), and score of 2 (69 ± 22%). In addition, the ADAMTS13:AC levels were significantly lower in the LC patients with the following clinical conditions

than in those without; hepatic encephalopathy (28 ± 24% vs. 71 ± 29%, $P < 0.001$), hepatorenal syndrome (13 ± 12% vs. 61 ± 33%, $P < 0.001$), and severe esophageal varices (46 ± 33% vs. 75 ± 34%, $P < 0.05$). Moreover, the patients with refractory ascites had a lower ADAMTS13:AC (25 ± 17%) than those without ascites (70 ± 30%, $P < 0.001$) or those with easily mobilized ascites (56 ± 37%, $P < 0.001$). The ADAMTS13:AC was lower in the cirrhotic patients with portal thrombosis than those without (18 ± 19% vs. 58 ± 34%, $P < 0.005$). In seven cirrhotics in the sequential study, the ADAMTS13:AC significantly decreased with the progression of liver disturbance (ADAMTS13:AC 67 ± 23% → 47 ± 29%, $P < 0.02$, Child score 7.7 ± 2.3 → 8.9 ± 2.7).

Relationship of prognosis with the CTP score, MELD score and the plasma ADAMTS13:AC

Figures 1–3 show the actuarial curves calculated for the survival of the different patient subgroups, according to the three CTP classes, the quartiles of the MELD risk score, or the three ADAMTS13:AC classes. Child C patients had worse survival than Child A and B patients (Fig. 1) (Log rank test among the three groups, $P < 0.0001$; Child C vs. Child A, $P < 0.0001$; Child C vs. Child B, $P < 0.0001$), but the survival probabilities were not different between Child A and Child B patients. In addition, the survival of the patients in the fourth quartile of the MELD RS was worse than that of the patients in the first three RS quartiles (Fig. 2), while the survival of the patients in the first three RS quartiles did not differ among them (Log rank test among the four groups, $P < 0.0001$; fourth quartile vs. others, $P < 0.0001$). In contrast, the cumulative survival was clearly different among patients with severe to moderate ADAMTS13:AC deficiency, mild ADAMTS13:AC deficiency, and normal range of ADAMTS13:AC (Fig. 3) (Log rank test among the three groups, $P < 0.0001$; severe to moderate deficiency vs. mild deficiency, $P = 0.0219$; mild deficiency vs. normal range, $P = 0.0287$; severe to moderate deficiency vs. normal range, $P < 0.0001$). The median survival time was the lowest (4.5 months) in the cirrhotic patients with severe to moderate ADAMTS13:AC deficiency.

The hazard ratios for predicting the survival of cirrhotic patients were the highest in those with Child C (4.00 as compared to Child A) in the CTP score and the fourth quartile (5.16 as compared with the first quartile) in the MELD score, but did not differ between the patients with Child A and B, and among the patients with the first, second, and third quartiles (Table 5). In contrast, the hazard ratios for the ADAMTS13:AC progressively increased from lowest in patients with normal activity (1.0), to those with mild deficiency (1.51), and to highest in those with severe to moderate deficiency of the activity (2.50) (Table 5). The AUCs for the CTP score, the MELD score, and the ADAMTS13:AC were 0.769 (95% confidence interval (CI) = 0.658–0.881), 0.805 (95% CI = 0.695–0.915), and 0.752 (95% CI = 0.643–0.861) in predicting the 1-year survival, and were 0.752 (95% CI = 0.645–0.859), 0.805 (95% CI = 0.702–0.907), and 0.739 (95% CI = 0.629–0.849) in predicting the 2-year survival, respectively (Fig. 4). The AUC for the ADAMTS13:AC tended to be the lowest in the 1-year and 2-year survival, but the differences were not significant among the CTP score, the MELD score, and the ADAMTS13:AC. The cut-off point for the ADAMTS13:AC was 44% for 1-year survival with the values of sensitivity and specificity being 67.9% and 77.2%, respectively.

Furthermore, to evaluate whether the ADAMTS13:AC is a significant predictor for survival of the cirrhotic patients, Cox proportional-hazards regression analysis was conducted using eight variables (age, sex, ADAMTS13:AC, albumin, total bilirubin, prothrombin time, blood ammonia, and platelet count) for potential covariates to predict the survival. In this analysis, ADAMTS13:AC and albumin were independently selected (Table 6). The hazard ratios of the patients with 25–50% and <25% of ADAMTS13:AC were 2.25 and 5.08, respectively ($P < 0.006$), and that of low serum albumin (<2.8 mg/dL) was 3.39 ($P < 0.01$) (Table 6).

DISCUSSION

IN THIS STUDY, we demonstrated that the cumulative survival differed according to the levels of plasma ADAMTS13:AC in the LC patients; the survival time was the shortest in the cirrhotics with severe to moderate ADAMTS13:AC deficiency, followed by those with mild ADAMTS13:AC deficiency, and was the longest in those with a normal range of activity (Fig. 3). In contrast, based on the CTP score, Child C patients had the worst survival, but the survival probabilities of Child A and Child B patients did not differ (Fig. 1). Based on the MELD score, the survival was the poorest in the cirrhotics in the fourth quartile, but it did not differ among the cirrhotics in the first three quartiles (Fig. 2). The CTP score is not always sufficient to predict the short-term prognosis,[4] and the MELD score has been applied as a disease severity index to perform organ allocation for

liver transplantation in the patients with significantly advanced liver diseases. Remarkably, the median survival time was the lowest (4.5 months) in the cirrhotics with severe to moderate ADAMTS13:AC deficiency, and the actuarial curve was very similar or almost identical to that of the patients belonging to the fourth quartile of the MELD score (Figs 2,3).

Moreover, the three cumulative survival curves stratified by the degree of decrease in the ADAMTS13:AC were clearly different during a median follow-up of 475 days (range: 5 to 2406 days). This may be attributed to the higher mortality rate in the patients with mild deficiency of ADAMTS13:AC (44.4%, $P < 0.03$) (Table 3) than in those with Child B (18.2%) (Table 1) according to the CTP score and the second quartile (16.7%) according to the MELD score (Table 2). The hazard ratios for predicting the survival of cirrhotic patients were the highest in those with Child C in the CTP score and the fourth quartile in the MELD score, but did not differ between the patients with Child A and B, and among the patients with the first, second, and third quartiles (Table 5). In contrast, the hazard ratios for the ADAMTS13:AC progressively increased from lowest in patients with normal activity, to those with mild deficiency, and to highest in those with severe to moderate deficiency of the activity (Table 5). The AUCs for the ADAMTS13:AC tended to be the lowest in the 1-year and 2-year survival, but the differences did not differ among the CTP score, the MELD score, and the ADAMTS13:AC (Fig. 4), indicating that the change in the plasma ADAMTS13:AC is sufficient to evaluate the prognosis of the cirrhotic patients similar to the Child score and the MELD score. Furthermore, multivariate analysis using several potential covariates to predict the survival showed that ADAMTS13:AC and albumin were independent predictors for survival of the patients with liver cirrhosis (Table 6). Based on previous reports and our present study, the degree of decrease in the plasma ADAMTS13:AC may be a useful marker to predict not only the short-term prognosis, but also the long-term survival of the LC patients. It is important to be able to evaluate the prognosis of cirrhotic patients using only one parameter, such as ADAMTS13:AC, because both the CTP score and the MELD score are calculated based on a scoring system that encompasses several parameters (bilirubin, albumin, prothrombin time, ascites, and encephalopathy in the former, and creatinine, bilirubin, prothrombin time, and cause of cirrhosis in the latter).

Interestingly, ADAMTS13:AC significantly decreased with the progression of liver disturbance in the seven LC patients with the sequential study. These patients were admitted into the hospital for a second time because of hepatic encephalopathy in three patients, ascites augmentation in three, and variceal rupture in one. We previously demonstrated that the plasma ADAMTS13:AC was remarkably low in the LC patients with hepatic encephalopathy, hepatorenal syndrome and refractory ascites.[29] A multivariate analysis identified blood ammonia, serum creatinine and spleen volume as independent factors that contribute to the decrease in ADAMTS13:AC, indicating that ADAMTS13:AC closely correlated with the severity of hepatic encephalopathy, renal dysfunction and splenomegaly in the LC patients.[29] Actually, the incidence of patients with SBP, hepatorenal syndrome, and hepatic encephalopathy progressively increased from the patients with normal range to those with severe to moderate deficiency of the ADAMTS13:AC (Table 3). These selected parameters are intimately related to the CTP score and the MELD score that predict the prognosis of the cirrhotic patients. On the other hand, the ADAMTS13:AC was lower in three Child B and three Child C LC patients with portal thrombosis than in those without, but further studies are needed to elucidate the significance of lower ADAMTS13:AC associated with portal thrombosis from the point of decreased functional liver capacity in more LC patients with portal thrombosis.

The levels of VWF: Ag, the substrate of ADAMTS13, progressively increased as the functional liver capacity decreased, probably due to the neocapilarization of the hepatic endothelial cells that leads to liver fibrosis, increased endothelial production induced by endotoxin[21,45] and/or increased synthesis by extrahepatic endothelial cells.[46] VWF: RCo relative to ADAMTS13:AC increased as the chronic liver disease progressed, and VWF multimers appeared to shift from a degraded- to normal-VWFM and finally to UL-VWFM as the functional liver capacity and renal function deteriorated, indicating that advanced cirrhosis may predispose the patients to platelet microthrombi formation.[29] The marked impairment in the enzyme to substrate ratio; i.e., decreased ADAMTS13 to increased VWF: Ag, may lead to platelet hyperaggregability with subsequent microcirculatory disturbances not only in the liver but also in other organs, leading to multi-organ failure. Indeed, portal or hepatic vein thrombosis is often observed in advanced LC patients routinely screened with Doppler US,[8] and in cirrhotic liver tissue removed at transplantation.[9] Moreover, microthrombi were found in one or more organs in half of the cirrhotic livers at autopsy.[10]