Hepatology Research
JSH

The ADAMTS13 activity may predict the cumulative survival of patients with liver cirrhosis – in comparison with the Child-Turcotte-Pugh score and the MELD score

Journal:	*Hepatology Research*
Manuscript ID:	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Takaya, Hiroaki; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE Uemura, Masahito; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE Fujimura, Yoshihiro; NARA MEDICAL UNIVERSITY, DEPARTMENT of BLOOD TRANSFUSIOIN MEDICINE Matsumoto, Masanori; NARA MEDICAL UNIVERSITY, DEPARTMENT of BLOOD TRANSFUSIOIN MEDICINE Matsuyama, Tomomi; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE Kato, Seiji; NARA MEDICAL UNIVERSITY, DEPARTMENT of BLOOD TRANSFUSIOIN MEDICINE Morioka, Chie; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE Ishizashi, Hiromichi; NARA MEDICAL UNIVERSITY, DEPARTMENT of BLOOD TRANSFUSIOIN MEDICINE Hori, Yuji; NARA MEDICAL UNIVERSITY, DEPARTMENT of BLOOD TRANSFUSIOIN MEDICINE Fujimoto, Masao; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE Tsujimoto, Tatsuhiro; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE Kawaratani, Hideto; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE Kurumatani, Norio; NARA MEDICAL UNIVERSITY, DEPARTMENT OF HEALTH AND EPIDEMIOLOGY Fukui, Hiroshi; NARA MEDICAL UNIVERSITY, 3RD DEPT OF INTERNAL MEDICINE
Category:	10. Liver failure, 8. Fibrosis and cirrhosis

The ADAMTS13 activity may predict the cumulative survival of patients with liver cirrhosis – in comparison with the Child-Turcotte-Pugh score and the MELD score

Running title: ADAMTS13 and prognosis of liver cirrhosis

Hiroaki Takaya[1], Masahito Uemura[1], Yoshihiro Fujimura[2], Masanori Matsumoto[2], Tomomi Matsuyama[1], Seiji Kato[2], Chie Morioka[1], Hiromichi Ishizashi[2], Yuji Hori[2], Masao Fujimoto[1], Tatsuhiro Tsujimoto[1], Hideto Kawaratani[1], Norio Kurumatani[3], Hiroshi Fukui[1]

[1]Third Department of Internal Medicine, [2]Department of Blood Transfusion Medicine, [3]Department of Community Health and Epidemiology, Nara Medical University, Kashihara, Nara, 634-8522, Japan

Address correspondence to Masahito Uemura, M.D. Third Department of Internal Medicine, Nara Medical University, Kashihara, Nara, 634-8522, Japan. Tel +81-744-22-3051, ext. 3415; Fax: +81-744-24-7122; E-mail:muemura@naramed-u.ac.jp

Abbreviations used in this paper: LC, liver cirrhosis; CTP score, Child-Turcotte-Pugh score, MELD score, Model for End-Stage Liver Disease score; TIPS, transjugular intrahepatic portosystemic shunt; ADAMTS, a disintegrin-like and metalloproteinase domain with thrombospondin type-1 motif; VWF, von Willebrand factor; UL-VWFM, unusually large von Willebrand factor multimer; HSC, hepatic stellate cells; VWF:AG, von Willebrand factor antigen; ADAMTS13:AC, ADAMTS13 activity; TTP, thrombotic thrombocytopenic purpura; HCV, hepatitis C virus; HBV, hepatitis B virus; PBC, primary biliary cirrhosis; RS, risk score; SBP, spontaneous bacterial peritonitis; HCC, hepatocellular carcinoma; JIS, Japan Integrated Staging; VWFM, VWF multimer; VWF:RCo, VWF ristocetin cofactor activity; ELISA, enzyme-linked immunosorbent assay; ADAMTS13:INH, ADAMTS13 inhibitor.

Total word count; in abstract 250; and text 3549 (excluding the figures, legends and references).
The number of tables; 2, The number of figures; 3

Abstract

Aims: Decreased plasma ADAMTS13 activity (ADAMTS13:AC) results in the 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 from 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 cirrhotics with a severe to moderate ADAMTS13:AC deficiency (<3~25%), followed by those with a mild ADAMTS13:AC deficiency (25%~50%), and was the longest in those with a normal range of 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 cirrhotics in the fourth quartile, while it was not different among cirrhotics in the first three quartiles. In cirrhotics in the sequential study, ADAMTS13:AC significantly decreased with the progression of liver disturbance.

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 cirrhotic patients.

Key words: ADAMTS13 activity, CTP score, liver cirrhosis, MELD 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, or hepatorenal syndrome, and hepatopulmonary syndrome. Many studies have examined 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 patients with LC[1]. However, this score includes some subjective components and does not estimate other factors, such as renal 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 patients[4]. The Model for End-Stage Liver Disease (MELD) score was designed to assess the prognosis of cirrhotics who receive a 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, including variceal bleeding, ascites, hepatorenal syndrome and hepatopulmonary syndrome, in 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 autopsied cirrhotics[10]. This hypercoagulable state may not only affect hepatic parenchymal extinction, the acceleration of liver fibrosis, and disease progression but also impact 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 multimeric von Willebrand factor (VWF) between the Tyr1605 and Met1606 residues in the A2 domain[11, 12]. An ADAMTS13 deficiency, caused either by mutations in the *ADAMTS13* gene[11-14] or by inhibitory autoantibodies against ADAMTS13[15, 16], results in the 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 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 VWF:Ag, the substrate for ADAMTS13, substantially increases as liver disease progresses[21-22], and thrombocytopenia is commonly seen in patients with advanced LC[23-25]. Previous studies reported a significant reduction in ADAMTS13 activity (ADAMTS13:AC) in advanced LC[26, 27], while 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 increased levels of its substrate may reflect a state that predisposes patients with advanced LC to platelet thrombus formation[29]. In addition, we have shown that ADAMTS13:AC is reduced in 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 compared to the CTP score and the MELD score.

MATERIALS AND METHODS

Patients

This study examined a total of 108 LC patients, including one patient with thrombotic thrombocytopenic purpura (TTP)[33] (Table 1). 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 cases, hepatitis B virus (HBV) in 16 patients, alcohol abuse in 10 patients, primary biliary cirrhosis (PBC) in 4 cases, and cryptogenic in 11 patients. Cirrhosis was diagnosed based on physical findings and laboratory tests, and in many cases was confirmed by histological criteria. Of the LC patients, 35 were Child A, 33 were Child B, and 40 were Child C according to Child–Pugh's criteria[3]. The MELD risk score (RS) was calculated according to the following formula: RS=0.957 x $\log_e$ (creatinine mg/dl) + 0.378 x $\log_e$ (bilirubin

mg/dl) + 1.120 x $\log_e$ (INR) + 0.643 x (cause of cirrhosis) in which the value for the cause of cirrhosis was 0 for an alcoholic or cholestatic etiology and 1 for viral or other etiologies[5]. The MELD score progressively increased from Child A to C. The platelet counts decreased with the severity of chronic liver diseases. The spleen volume determined by computed tomography scans[34] increased as liver disease progressed. The diagnosis of ascites, spontaneous bacterial peritonitis (SBP), and hepatorenal syndrome was made according to the criteria previously described[35]. 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 7 patients. Forty-one LC patients developed hepatic encephalopathy grade II or higher as described by the classification of Trey et al..[37] Sixty-seven LC patients had endoscopic signs of an impending variceal rupture according to the criteria of Japanese Research Society for Portal Hypertension[36]. The incidence of endoscopic signs of an impending variceal rupture was higher in LC patients with Child B and Child C than in Child A. Hepatocellular carcinoma (HCC) was detected in 22 patients (62.9%) with Child A, 16 patients (48.5%) with Child B, and 19 patients (47.5%) with in Child C. The incidence of HCC did not differ among three groups, but the Japan Integrated Staging (JIS) score[38] obtained by adding the tumor stage score of HCC and the CTP score progressively increased from Child A to C. The portal thrombosis examined by Doppler-ultrasonography (US) was detected in 3 patients with Child B and 3 with Child C (Table 1). 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 computed tomography (CT), or dynamic enhanced magnetic resonance imaging (MRI) was performed every three months to evaluate 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 was obtained from patients at the time of admission or during their hospital stay. 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 x g at 4°C for 15 minutes and stored in aliquots at –80°C until analysis. For 7 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 in 3 patients, ascites augmentation in 4 patients, and variceal rupture in 1 patient. Plasma ADAMTS13:AC was determined by both a classic VWFM assay[39, 40] and a sensitive chromogenic enzyme-linked immunosorbent assay (ELISA, ADAMTS13-act-ELISA: Kainos Inc., Tokyo)[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, 20–39 years of age). VWF ristocetin cofactor activity (VWF:RCo) was determined as described[42], and its normal value was 100±15%. In 49 LC patients with lower ADAMTS13:AC (less than 50% of the normal control), plasma UL-VWFMs were analyzed by a vertical 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 minutes[15, 16]. One Bethesda unit of inhibitor was defined as the amount of plasma that reduces ADAMTS13:AC to 50% of the control[44], and its titer was defined to be significant at >0.5 Bethesda Units (BU)/ml.

Statistical Analysis

Differences among cirrhotics with Child A, B and C, and healthy subjects were analyzed with the Kruskal-Wallis rank test before pair-wise comparisons (Table 1 & Table 2). If the Kruskal-Wallis rank test showed significant differences in each parameter among groups, pair-wise comparison in each parameter was evaluated by Mann-Whitney U test for continuous variable. The χ^2- test was used for categorical data. 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 Sperman rank test. The analyses were carried out using Statview statistical software (version 5.0; SAS Institute, Cary, NC, USA). The Kaplan–Meier analysis was used to evaluate the prognosis of cirrhotic patients according to the degree of the plasma ADAMTS13 activity, the CTP score, and the MELD score (Figure 1, 2, and 3) by a log-rank test using StatMate IV for Windows (AT0484, Advanced Technology for Medicine & Science, Tokyo, Japan). A two-tailed p-value less than 0.05 was considered significant. All data are presented as the mean ± standard deviation (SD).

RESULTS

Clinical outcome (Table 1)

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, 5 cirrhotic patients were classified as Child A, 6 as Child B, and 31 as Child C (Table 1). The cause of death in Child A patients was HCC in 4 patients and acute myocardial infarction in 1 patient, that in Child B patients was HCC in 5 patients and gastrointestinal bleeding in 1 patient, and that in Child C patients was HCC in 17 patients, hepatic failure in 7 patients, hepatorenal syndrome in 6 patients, and TTP in 1 patient (Table 1).

Determination of ADAMTS13:AC and its related parameters (Table 2)

Based on the VWFM assay, the plasma ADAMTS13:AC was 56±34% in LC patients, which was significantly lower than that in healthy subjects ($p<0.001$). This activity progressively decreased as cirrhosis advanced from Child A to C (Table 2). 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 LC patients than in healthy subjects, and was higher in 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 LC patients than in 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 patients with LC (0.54±0.45, $p<0.001$) than in healthy subjects, but there were no differences among 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, 2 Child A cirrhotics had only degraded-VWFM, 8 and 5 Child B cirrhotics had degraded-VWFM and normal-VWFM, respectively, and 6, 20, and 8 Child C cirrhotics had degraded-VWFM, normal-VWFM, and UL-VWFM, respectively. The incidence of ADAMTS13:INH was increased from cirrhotic patients with Child A to C (Table 2). The inhibitory activity was 2.0 BU/ml[33] in one LC patient with TTP and 3.0 BU/ml in a patient with a severe ADAMTS13:AC deficiency, but was in the marginal zone between 0.5 and 1.0 BU/ml in the remaining patients.

Relationship of plasma ADAMTS13:AC to clinical variables

Regarding the influence of HCC complicated with 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 both in LC patients with Child B and Child C. Furthermore, the plasma ADAMTS13:AC levels were lower in patients with a JIS score of 4 (32±28%, $p<0.05$) and JIS score of 5 (26± 29%, $p<0.05$) than in patients with a 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 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, 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 cirrhotic patients with portal thrombosis than those without (18±19% vs. 58±34%, $p<0.005$). In 7 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 to the CTP score, MELD score and plasma ADAMTS13:AC (Fig. 1,2 & 3)

Figures 1 to 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 (first quartile, RS: 0–0.36, second quartile, RS: 0.37–0.73, third quartile, RS: 0.74–1.37, and fourth quartile, RS: 1.38–4.32), or the three ADAMTS13:AC classes (severe to moderate deficiency: <25% of the normal control, mild deficiency: 25–50% of the normal control, and normal range: > 50%). Child C patients had worse survival than Child A and B patients (Figure 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 patients corresponding to the fourth quartile of the MELD RS was worse than that of patients corresponding to 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, $p<0.0001$). In contrast, the cumulative survival was clearly different among patients with a 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, $p<0.0001$)(Figure 3). The median survival time was the lowest (4.5 months) in cirrhotic patients with a severe to moderate ADAMTS13:AC deficiency.

DISCUSSION

In this study, we demonstrated that cumulative survival differed according to the levels of plasma ADAMTS13:AC in patients with LC; the survival time was the shortest in cirrhotics with a severe to moderate ADAMTS13:AC deficiency, followed by those with a mild ADAMTS13:AC deficiency, and was the longest in those with a normal range of activity (Figure 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 (Figure 1). Based on the MELD score, survival was the poorest in cirrhotics in the fourth quartile, while it did not differ among cirrhotics in the first three quartiles

(Figure 2). The CTP score is not always sufficient to predict short-term prognosis[4], and the MELD score has been applied as a disease severity index to perform organ allocation for liver transplantation in patients with significantly advanced liver diseases. Remarkably, the median survival time was the lowest (4.5 months) in cirrhotics with a severe to moderate ADAMTS13:AC deficiency, and the actuarial curve was very similar or almost identical to that of patients belonging to the fourth quartile the MELD score (Figures 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 (from 5 to 2406 days). Based on previous reports and ours, the degree of decrease in plasma ADAMTS13:AC may be a useful marker to predict not only the short-term prognosis, but also the long-term survival of patients with LC. 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 7 LC patients with the sequential study. These patients were admitted to the hospital for a second time because of hepatic encephalopathy in 3 patients, ascites augmentation in 4 patients, and variceal rupture in 1 patient. We previously demonstrated that plasma ADAMTS13:AC was remarkably low in 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 is closely related to the severity of hepatic encephalopathy, renal dysfunction and splenomegaly in LC patients[29]. These selected parameters are intimately related to factors that comprise the CTP score or the MELD score and predict the prognosis of cirrhotic patients. Alternatively, the ADAMTS13:AC was lower in 3 Child B and 3 Child C LC patients with portal thrombosis than those without, but further studies will be needed to elucidate the significance of lower ADAMTS13:AC associated with portal thrombus formation 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 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 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 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 and 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 ultrasound[8], and in cirrhotic liver tissue removed at transplantation[9]. Moreover, microthrombi were found in one or more organs in half of cirrhotic livers at autopsy[10].

Plasma ADAMTS13:AC may decrease in advanced cirrhosis due to reduced ADAMTS13 production in HSCs[47], enhanced consumption to degrade large quantities of VWF:Ag[26], and/or its plasma inhibitor[15, 16]. We detected plasma ADAMTS13:INH in 83% of patients with a severe to moderate ADAMTS13:AC deficiency, but the ADAMTS13 inhibitory activity was in the marginal zone between 0.5 and 1.0 BU/ml in most cases except one TTP patient (2.0 BU/ml)[33] and one patient with a severe ADAMTS13:AC deficiency (3.0 BU/ml)[29]. We could detect IgG-type autoantibodies in 5 end-stage cirrhotic patients with a severe ADAMTS13:AC deficiency (<3%)[29]. One patient had characteristic clinical features of TTP. However, the remaining 4 patients did not show any apparent clinical features of TTP, but were indistinguishable from the typical TTP patient based on ADAMTS13:AC and the presence of anti-ADAMTS13 autoantibodies. These results indicate that some end-stage cirrhotic patients with extremely low ADAMTS13:AC and the IgG ADAMTS13 inhibitor might have a condition similar to TTP or have "subclinical" TTP. Together with our present results, these findings suggest that the degree of decrease in plasma ADAMTS13:AC may be a useful predictor that is closely related to the pathogenesis of hepatic failure, including encephalopathy and/or renal disturbance, in patients with advanced LC.

Alternatively, various clinical conditions, including infection, malignancies, and certain drugs, can lead to acquired TTP[48]. In addition, some patients with congenital TTP whose ADAMTS13:AC is extremely low (<0.5%) due to mutations in the *ADAMTS13* gene showed no apparent clinical features during their childhood except mild to moderate thrombocytopenia during stressful conditions such as infection, but apparently developed "TTP" during the third trimester of pregnancy when VWF production from placental endothelial cells markedly increases[49]. This condition is called "masqueraded TTP in congenital Upshaw–Schulman syndrome". Furthermore, recently, sepsis-induced multi-organ failure was shown to be closely related to the decrease in ADAMTS13:AC and increase in VWF:Ag[50]. This finding indicates that the enzyme to substrate ratio is extremely important in the formation of platelet microthrombi and subsequent microcirculatory disturbances that lead to multi-organ failure. In advanced cirrhotics, endotoxemia is frequently detected[21], and SBP sometimes occurs[35]. HCC becomes highly complicated as the cirrhotic stage progresses[51], which suggests that these patients are at high risk for platelet microthrombi formation. Therefore, LC patients with a moderate ADAMTS13:AC deficiency (3–25%) who have no apparent IgG ADAMTS13 inhibitor and whose inhibitory activity is in the marginal zone (0.5–1.0 BU/ml) may be especially prone to a poorer prognosis when infection and endotoxemia to augment VWF production from endothelial cell precipitates, ultimately resulting in the shortest survival.

In summary, the plasma ADAMTS13:AC concomitantly decreases as the functional liver capacity declines, and the degree of activity is closely related to the prognosis of patients with LC. Therefore, ADAMTS13 activity may be a useful marker to predict the clinical outcome in advanced cirrhosis.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (MU, YF) and from the Ministry of Health and Welfare of Japan for Blood Coagulation Abnormalities (YF). The authors sincerely thank Ayami Isonishi for her great help in the assay of ADAMTS13 activity and VWF antigen.

REFERENCES

1. Durand F, Valla D. Assessment of prognosis of cirrhosis. Semin Liver Dis 2008; 28:110-22.
2. Child CG, Turcotte JG. The liver and portal hypertension. In Child CI, ed. Surgery and portal Hypertension. Philadelphia, USA: W.B. Saunders, 1964; 50-8.
3. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the esophagus for bleeding oesophageal varices. Br J Surg 1973; 60:646-9.
4. Salerno F, Merli M, Cazzaniga M, et al. MELD score is better than Child-Pugh score in predicting 3-month survival of patients undergoing transjugular intrahepatic portosystemic shunt. J Hepatol 2002; 36: 494- 500.
5. Kamath PS, Wiesner RH, Malinchoc M, et al. A model to predict survival in patients with end-stage liver disease. Hepatology 2001; 33:464-70.
6. Yoo HY, Edwin D, Thuluvath PJ. Relationship of the model for end-stage liver disease (MELD) scale to hepatic encephalopathy, as defined by electroencephalography and neuropsychometric testing, and ascites. Am J Gastroenterol 2003; 98:1395-9.
7. Northup PG, Sundaram V, Fallon MB, et al. Coagulation in Liver Disease Group. Hypercoagulation and thrombophilia in liver disease. J Thromb Haemost 2008; 6:2-9.
8. Amitrano L, Guardascione MA, Brancaccio V, et al. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. J Hepatol 2004; 40:736-41.
9. Wanless IR, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. Hepatology 1995; 21:1238-47.
10. Oka K, Tanaka K. Intravascular coagulation in autopsy cases with liver diseases. Thromb Haemost 1979; 42:564-70.
11. Moake JL. Thrombotic microangiopathies. N Engl J Med 2002; 347:589-99.
12. Fujimura Y, Matsumoto M, Yagi H, Yoshioka A, Matsui T, Titani K. Von Willebrand factor-cleaving protease and Upshaw-Schulman syndrome. Int J Hematol 2002; 75:25-34.
13. Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS13 gene family cause thrombotic thrombocytopenic purpura. Nature 2001; 413: 488-94.

14. Kokame K, Matsumoto M, Soejima K, et al. Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity. Proc Natl Acad Sci USA 2002; 99: 11902-7.

15. Furlan M, Robles R, Galbusera M, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med 1998; 339:1578-84.

16. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med 1998; 339:1585-94.

17. Uemura M, Tatsumi K, Matsumoto M, et al. Localization of ADAMTS13 to the stellate cells of human liver. Blood 2005; 106:922-4.

18. Suzuki M, Murata M, Matsubara Y, et al. Detection of von Willebrand factor-cleaving protease (ADAMTS-13) in human platelets. Biochem Biophys Res Commun 2004; 313:212-6.

19. Turner N, Nolasco L, Tao Z, Dong JF, Moake J. Human endothelial cells synthesize and release ADAMTS-13. J Thromb Haemost 2006; 4:1396-404.

20. Manea M, Kristoffersson A, Schneppenheim R, et al. Podocytes express ADAMTS13 in normal renal cortex and in patients with thrombotic thrombocytopenic purpura. Br J Haematol 2007; 138:651-62.

21. Ferro D, Quintarelli C, Lattuada A, et al. High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia. Hepatology 1996; 23:1377-83.

22. Albornoz L, Alvarez D, Otaso JC, et al. Von Willebrand factor could be an index of endothelial dysfunction in patients with cirrhosis: relationship to degree of liver failure and nitric oxide levels. J Hepatol 1999; 30:451-5.

23. Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. J Clin Invest 1966; 45:645-57.

24. Peck-Radosavljevic M, Wichlas M, Zacherl J, et al. Thrombopoietin induces rapid resolution of thrombocytopenia after orthotopic liver transplantation through increased platelet production. Blood 2000; 95: 795-801.

25. Schhffski P, Tacke F, Trautwein C, et al. Thrombopoietin serum levels are elevated in patients hepatitis B/C infection compared to other causes of chronic liver diseases. Liver 2002; 22:114-20.

26. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloproteinase that cleaves von Willebrand factor. Blood 2001; 98:2730-5.

27. Feys HB, Canciani MT, Peyvandi F, Deckmyn H, Vanhoorelbeke K, Mannucci PM. ADAMTS13 activity to antigen ratio in physiological and pathological conditions associated with an increased risk of thrombosis. Br J Haematol 2007; 138:534-40.

28. Lisman T, Bongers TN, Adelmeijer J, et al. Elevated levels of von Willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity. Hepatology 2006; 44:53-61.

29. Uemura M, Fujimura Y, Matsumoto M, et al. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. Thromb Haemost 2008 ; 99:1019-29.

30. Park YD, Yoshioka A, Kawa K, et al. Impaired activity of plasma von Willebrand factor-cleaving protease may predict the occurrence of hepatic veno-occlusive disease after stem cell transplantation. Bone Marrow Transplant 2002; 29:789-94.

31. Uemura M, Matsuyama T, Ishikawa M, et al. Decreased Activity of plasma ADAMTS13 may contribute to the development of liver disturbance and multiorgan failure in patients with alcoholic hepatitis. Alcohol Clin Exp Res 2005; 29: 264S-71S.

32. Ko S, Okano E, Kanehiro H, et al. Plasma ADAMTS13 activity may predict early adverse events in living donor liver transplantation: observations in 3 cases. Liver Transpl 2006; 12: 859-69.

33. Yagita M, Uemura M, Nakamura T, Kunitomi A, Matsumoto M, Fujimura Y. Development of ADAMTS-13 inhibitor in a patient with hepatitis C virus-related liver cirrhosis causes thrombotic thrombocytopenic purpura. J Hepatol 2005; 42:420-1.

34. Henderson JM, Heymsfield SB, Horowitz J, Kutner MH. Measurement of liver and spleen volume by computed tomography. Radiology 1981; 141:525-7.

35. Arroyo V, GinPERL, Gerbes AL, et al. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International ascites club. Hepatology 1996; 23: 164-76.

36. Japanese Research Society for Portal Hypertension. The general rules for recording endoscopic findings on esophageal varices. Jpn J Surg 1980; 10:329-37.

37. Trey C, Burns DG, and Saunders SJ. Treatment of hepatic coma by exchange blood transfusion. N Engl J Med 1966; 274:473-81.

38. Kudo M, Chung H, Haji S, et al. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. Hepatology 2004; 40:1396-1405.

39. Mori Y, Wada H, Gabazza EC, et al. Predicting response to plasma exchange in patients with thrombotic thrombocytopenic purpura with measurement of vWF-cleaving protease activity. Transfusion 2002; 42: 572-80.

40. Furlan M, Robles R, Solenthaler M, Wassmer M, Sandoz P, Lämmle B. Deficiency activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. Blood 1997; 89:3097-103.

41. Kato S, Matsumoto M, Matsuyama T, Isonishi A, Hiura H, Fujimura Y. Novel monoclonal antibody-based enzyme immunoassay for determinig plasma levels of ADAMTS13 activity. Transfusion 2006; 46:1444-52.

42. Macfarlane DE, Stibbe J, Kirby EP, Zucker MB, Grant RA, McPherson J. A method for assaying von Willebrand factor (ristocetin cofactor). Thromb Diath Haemorrh 1975; 34:306-8.

43. Warren CM, Krzesinski PR, Greaser ML. Vertical agarose gel electrophoresis and electroblotting of high-molecular-weight proteins. Electrophoresis 2003; 24:1695-1702.

44. Kasper CK, Aledort L, Aronson D, et al. A more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh 1975; 34:869-72.

45. Schorer AE, Moldow CF, Rick ME. Interleukin 1 or endotoxin increases the release of von Willebrand factor from human endothelial cells. Brit J Haematol 1987; 67:193-7.

46. Tornai I, HIrsfalvi J, Boda Z, Udvardy M, Pfliegler G, Rak K. Endothelium releases more von Willebrand factor and tissue-type plasmionogen activator upon venous occlusion in patients with liver cirrhosis than in normals. Haemostasis 1993; 23: 58-64.

47. Kume Y, Ikeda H, Inoue M, et al. Hepatic stellate cell damage may lead to decreased plasma ADAMTS13 activity in rats. *FEBS Lett.* 2007; 581:1631-4.

48. Matsumoto M, Yagi H, Ishizashi H, Wada H, Fujimura Y. The Japanese experience with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Semin Hematol 2004; 41:68-74.

49. Fujimura Y, Matsumoto M, Kokame K, et al. Pregnancy-induced thrombocytopenia and TTP, and the risk of fetal death, in Upshaw-Schulman syndrome: a series of 15 pregnancies in 9 genotyped patients. Br J Haematol 2009; 144:742-54.

50. Claus RA, Bockmeyer CL, Budde U, et al. Variations in the ratio between von Willebrand factor and its cleaving protease during systemic inflammation and association with severity and prognosis of organ failure. Thromb Haemost 2009; 101:239-47.

51. Sala M, Forner A, Varela M, Bruix J. Prognostic prediction in patients with hepatocellular carcinoma. Semin Liver Dis 2005; 25:171-80.

Figure legends

Figure 1 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 Cumulative survival rate of 108 patients with liver cirrhosis according to the Model for End-Stage Liver Disease (MELD) score. The survival of patients corresponding to the fourth quartile of the MELD risk score (RS) calculated by the formula[5] was worse than that of patients corresponding to 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 first RS quartile, second RS quartile, third RS quartile, and fourth RS quartile, respectively.

Figure 3 Cumulative survival rate of 108 patients with liver cirrhosis according to ADAMTS13 activity (ADAMTS13:AC). The survival rate was clearly differentiated among patients with a 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 cirrhotic patients with a 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) , a severe to moderate ADAMTS13:AC deficiency (<25% of the normal control), respectively.

Table 1. Clinical data of patients with liver cirrhosis

	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	0.59 ± 0.48	0.91 ± 0.45*	1.61 ± 0.75**, ****
Platelet count (x10^4/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 thrombicytopenic 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 ± SD. HCV, hepatitis C virus; HBV, hepatitis B virus; PBC, primary biliary cirrhosis.

Table 2. 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 normal control).

The data are expressed as mean ± SD. ADAMTS13:AC, ADAMTS13 activity; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor activity; VWFM, von Willebrand factor multmer, ELISA, Enzyme-Linked ImmunoSorbent Assay

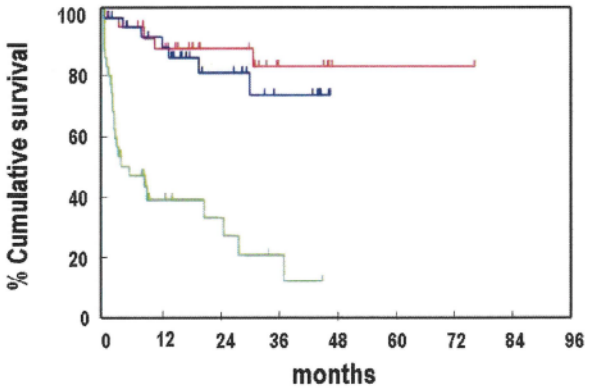

Figure 1

275x190mm (96 x 96 DPI)

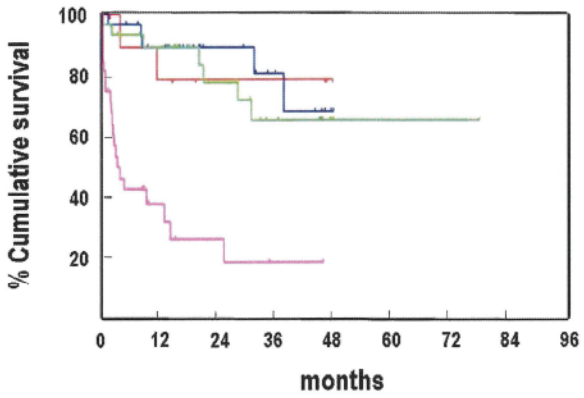

Figure 2

275x190mm (96 x 96 DPI)

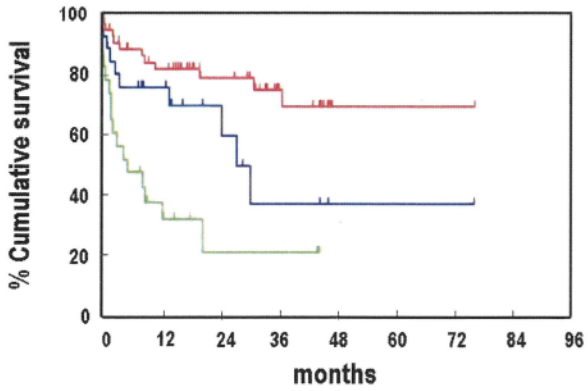

Figure 3

275x190mm (96 x 96 DPI)