Research Article

Cancer Epidemiology, Biomarkers & Prevention

Prediction of Hepatocellular Carcinoma Development by Plasma ADAMTS13 in Chronic Hepatitis B and C

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Abstract

Background: Chronic liver injury evokes a wound healing response, promoting fibrosis and finally hepatocellular carcinoma (HCC), in which hepatic stellate cells play an important role. Although a blood marker of hepatic stellate cells is not known, those cells importantly contribute to the regulation of plasma a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13) activity, a defect of which causes thrombotic thrombocytopenic purpura.

Methods: Plasma ADAMTS13 was evaluated in chronic hepatitis B or C patients with or without HCC.

Results: Plasma ADAMTS13 activity significantly correlated with serum aspartate aminotransferase and alanine aminotransferase, liver stiffness value, and aspartate aminotransferase-to-platelet ratio index, irrespective of the presence of HCC, suggesting that it may reflect hepatocellular damage and subsequent wound healing and fibrosis as a result of hepatic stellate cell action. During the three-year follow-up period for patients without HCC, it developed in 10 among 81 patients. Plasma ADAMTS13 activity was significantly higher in patients with HCC development than in those without and was a significant risk for HCC development by univariate and multivariate analyses. Furthermore, during the one-year follow-up period for patients with HCC treated with radiofrequency ablation, HCC recurred in 55 among 107 patients. Plasma ADAMTS13 activity or antigen level was significantly higher in patients with HCC recurrence than in those without and was retained as a significant risk for HCC recurrence by multivariate analysis.

Conclusions: Higher plasma ADAMTS13 activity and antigen level was a risk of HCC development in chronic liver disease.

Impact: Plasma ADAMTS13 as a potential marker of hepatic stellate cells may be useful in the prediction of hepatocarcinogenesis. *Cancer Epidemiol Biomarkers Prev; 20(10); 2204–11. ©2011 AACR.*

Introduction

It is well known that chronic wound healing generally provides a microenvironment that gives rise to cancer (1). Indeed, chronic injury in the liver evokes a perpetuating wound healing response, promoting the development of fibrosis and finally hepatocellular carcinoma (HCC; ref. 2). Among the cells in the liver, hepatic stellate cells are known as a main effector of wound healing and fibrosis following liver injury of any etiology (3), however, a useful blood marker to reflect the activity of those cells has not been found yet in the clinical setting.

In this context, we have focused on a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13), a defect of which increases unusually large multimers of von Willebrand factor in the plasma, causes platelet thrombosis under high sheer stress, and results finally in thrombotic thrombocytopenic purpura (4–6). With regard to the site of production, *ADAMTS13* mRNA expression was shown exclusively in the liver (7–9) and then both *ADAMTS13* mRNA expression and ADAMTS13 activity were determined primarily in hepatic stellate cells among the liver cells in mice (10). ADAMTS13 expression was also detected in hepatic stellate cells in human and thereby ADAMTS13 is reportedly produced in those cells (11). To elucidate a regulatory mechanism of plasma ADAMTS13 activity, we previously determined that selective hepatic stellate cell damage caused by dimethylnitrosamine in rats leads to decreased plasma ADAMTS13 activity (12). On the other hand, plasma ADAMTS13 activity was upregulated during the process of liver fibrosis due to cholestasis caused by bile duct ligation and steatohepatitis induced by a choline-deficient L-amino acid–defined diet in rats, in which hepatic stellate cells actively proliferate (13).

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These results indicate that hepatic stellate cells play an important role in the regulation of plasma ADAMTS13 activity, although other sources of ADAMTS13 were reported (14–16).

On the basis of these previous findings, we wondered whether plasma ADAMTS13 could be a blood marker of hepatic stellate cells. To examine this, plasma ADAMTS13 was evaluated in patients with chronic hepatitis B or C, in whom chronic wound healing and fibrosis are observed with a high risk of HCC development (17), in which hepatic stellate cells play an important role (3). In this study, we have found that plasma ADAMTS13 was increased in relation with serum levels of aspartate aminotransferase (AST) or alanine aminotransferase (ALT), and the markers of liver fibrosis and that higher plasma ADAMTS13 was more frequently found in patients who later developed HCC.

Patients and Methods

Patients

Eighty-one patients with chronic hepatitis B and C, who visited the Department of Gastroenterology, the University of Tokyo Hospital, Tokyo, Japan, between April and August in 2007, were first enrolled. Chronic hepatitis B was defined as hepatitis B surface antigen (HBsAg) positivity, and chronic hepatitis C was defined as serum anti-hepatitis C virus antibody (HCVAb) positivity and a detectable HCV RNA level, having persistent liver damage for more than 6 months. Patients with HCC at the time of enrollment or with past history of HCC were excluded from this analysis.

Next, between July and September in 2009, 107 consecutive patients with chronic hepatitis B and C with HCC who were scheduled to undergo radiofrequency ablation (RFA) for HCC were enrolled.

All the studies were carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the Institutional Research Ethics Committee of the Faculty of Medicine of the University of Tokyo. Informed consent from the patients was obtained for the use of the samples in this study.

Measurement of ADAMTS13 activity

ADAMTS13 enzymatic activity was measured manually using a chromogenic ELISA kit, ADAMTS13-act-ELISA (Kainos Inc./Technoclone GmbH), which captures products cleaved by ADAMTS13 using a sandwich method, and expressed as percentage of healthy control. The very high correlation of the values measured by classical VWF multimer assay and this novel chromogenic ADAMTS13-act-ELISA was reported previously (18).

Measurement of ADAMTS13 antigen level

ADAMTS13 antigen level was measured by a latex photometric immunoassay, in which suspended polystyrene latex particles coated with polyclonal antibody F(ab′) 2 fragment against ADAMTS13 were employed. Antisera against ADAMTS13 were obtained by immunization with pCAG-ADAMTS13 plasmid DNA (donated by Dr. Soejima from The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) using electroporation. Latex agglutination was analyzed using LPIA-A700 (Mitsubishi Chemical Medience Co.), a fully automated quantitative latex photometric immunoassay instrument. ADAMTS13 antigen level in sample of each patient was expressed as the percentage of that in pooled normal human plasma.

Measurement of liver stiffness

Liver stiffness was measured by transient elastography (FibroScan 502; EchoSens) as described previously (19–21). Briefly, the measurements were done in the right lobe of the liver through the intercostal spaces, with the patient lying in the dorsal decubitus position, and were considered valid only when at least 10 acquisitions were successful, with a success rate of at least 60% and the ratio of interquartile range to the median value was larger than 30%. Liver stiffness value was expressed in kilopascals (kPa).

Patient follow-up and diagnosis of HCC

Patients without HCC were followed up at the outpatient clinic with monthly blood tests, including tumor markers and ultrasonography every 4 to 6 months. Contrast-enhanced computed tomography (CT) was done when serum alpha-fetoprotein (AFP) levels and/or plasma des-gamma-carboxy prothrombin (DCP) levels showed an abnormal rise and/or tumors were detected as possible HCC on ultrasonography. The diagnosis of HCC was based on typical findings on CT, that is, hyperattenuation in the arterial phase and hypoattenuation in the equilibrium phase (22–24).

The end points consisted of the interval between the first measurement of plasma ADAMTS13 activity and the detection of HCC development, death without HCC development, or the last examination until 30 July 2010, whichever came first. Death without HCC development was treated as censored data.

Radiofrequency ablation, patient follow-up, and analysis of HCC recurrence

The detailed procedure of RFA was meticulously described elsewhere (25). The indication criteria for RFA consisted of total bilirubin concentration less than 3.0 mg/dL and platelet count more than $5 \times 10^4/\mu L$. Patients with portal vein tumor thrombosis, massive refractory ascites, or extrahepatic metastasis were excluded. In general, RFA was done on patients with 3 or fewer lesions, each less than 3.0 cm in diameter. However, RFA was also done on patients who did not meet these criteria when complete ablation could be anticipated in all tumors without deteriorating liver function. After RFA, dynamic CT was done to evaluate treatment efficacy. Complete ablation was defined as hypoattenuation of the whole lesion together with the surrounding liver parenchyma as a safety margin.

Patients received additional RFA until complete ablation was confirmed for each HCC nodule.

The follow-up consisted of monthly blood tests and monitoring of tumor markers at the outpatient clinic, with ultrasonography and dynamic CT scan done every 4 months. HCC recurrence was diagnosed on the basis of the criteria as described earlier.

The end points consisted of the interval between the first ablation and the detection of HCC recurrence, death without recurrence, or the last examination until 30 September 2010, whichever came first. Death without recurrence was treated as censored data.

Statistical analysis

Comparisons between groups were made using Student's t test or χ^2 test. The correlation between 2 groups, in which the data points were distribution free, was analyzed using Spearman's rank correlation coefficient (ρs). The cumulative incidence of HCC was estimated using the Kaplan–Meier method. In the analysis of risk factors for hepatocarcinogenesis, we tested the following variables obtained at the time of entry in univariate and multivariate Cox proportional hazard regression analyses: age, sex, positivity for HBsAg and HCVAb, albumin, total bilirubin, AST, ALT, prothrombin time, platelet counts, liver stiffness value, APRI, AFP, DCP, and either plasma ADAMTS13 activity or antigen level. Multichotomous categorical variables were represented by corresponding binary dummy variables. Factors that had a $P < 0.2$ in univariate analysis were subsequently included in a multivariate Cox proportional hazard regression model, with stepwise selection of variables based on the Akaike information criterion (AIC). Data processing and analysis were done by using the S-plus Ver. 7 (TIBCO Software Inc.).

Results

Characteristics of the patients without HCC and correlation between plasma ADAMTS13 activity and clinical variables

The characteristics of the patients, who were first enrolled for the measurement of plasma ADAMTS13 activity, are summarized in Table 1. There were 21 patients with chronic hepatitis B and 60 patients with chronic hepatitis C. All the patients were outpatients without HCC at the time of enrollment and past history of HCC.

Plasma ADAMTS13 activity in these patients was 114.0 ± 45.4% (mean ± SD) of control, ranged from 28.0% to 221.5%, as shown in Table 1. Relationships between plasma ADAMTS13 activity and clinical variables are shown in Table 2. The significant correlations were determined between plasma ADAMTS13 activity and serum AST and ALT levels ($P < 0.001$). On the other hand, the significant correlations were also determined between plasma ADAMTS13 activity and the variables predicting the stage of liver fibrosis, liver stiffness value ($P < 0.001$), and aspartate aminotransferase-to-platelet ratio index (APRI; $P = 0.027$). Of note is the finding that plasma ADAMTS13 activity significantly correlated with serum AFP level ($P < 0.001$).

HCC development and risk analysis

Next, a potential link between plasma ADAMTS13 activity and HCC was examined. During the mean follow-up period of 35.4 months, one patient had been lost to follow-up evaluation and one patient died before HCC was identified. By the end of the follow-up, HCC developed in 10 patients, among whom 2 patients died of HCC. The cumulative incidence rates of HCC at

Table 1. Characteristics of patients without HCC or with HCC

Variables	Patients without HCC	Patients with HCC
Age (y)	63 ± 12 (23–85)	68.9 ± 8.5 (43–86)
Man/Woman	49/32	68/39
HBV/HCV	21/60	15/92
Albumin (g/dL)	4.1 ± 0.4 (3.1–4.9)	3.7 ± 0.6 (2.0–5.1)
AST (U/L)	48 ± 35 (3–270)	61.4 ± 39.2 (16–289)
ALT (U/L)	53 ± 66 (11–542)	54.1 ± 38.6 (11–276)
Platelet count (× 10^4/μL)	15.2 ± 6.3 (3.4–30.8)	10.8 ± 4.7 (3.4–25.2)
Prothrombin time (%)	87.7 ± 11.6 (49.2–100.0)	98.3 ± 5.2 (73.0–100.0)
Plasma ADAMTS13 activity (%)	114.0 ± 45.4 (28.0–221.5)	125.0 ± 32.4 (62.0–223.0)
Plasma ADAMTS13 antigen level (%)	Not measured	128.6 ± 39.6 (48.9–258.3)
Liver stiffness (kPa)	11.4 ± 9.2 (3.1–48.0)	28.5 ± 17.9 (6.1–75.0)
APRI	1.07 ± 1.00 (0.08–5.92)	1.89 ± 1.44 (0.22–7.81)
AFP (ng/mL)	12.6 ± 38.2 (1–319)	99.4 ± 361.2 (1–3,399)
DCP (mAu/mL)	18.1 ± 17.6 (10–165)	70.7 ± 194.3 (8–1,462)
Maximum size of HCC (mm)	Not available	17.8 ± 6.0 (6.0–33.0)

NOTE: Values are expressed as the mean ± SD (range).

Table 2. Relation between plasma ADAMTS13 activity and clinical variables in patients without HCC or with HCC

Variables	Patients without HCC		Patients with HCC	
	ρs[a]	*P*	ρs[a]	*P*
Age	−0.067	0.554	−0.030	0.760
AST (U/L)	0.360	<0.001	0.531	<0.001
ALT (U/L)	0.426	<0.001	0.519	<0.001
Albumin (g/dL)	−0.114	0.309	−0.146	0.133
Platelet count ($\times 10^4$/μL)	−0.091	0.418	−0.129	0.185
Prothrombin time (%)	−0.343	<0.005	−0.029	0.764
Liver stiffness (kPa)	0.379	<0.001	0.216	0.026
APRI	0.245	0.027	0.403	<0.001
AFP (ng/mL)	0.465	<0.001	0.554	<0.001
DCP (mAu/mL)	0.135	0.230	−0.281	0.003
Size of HCC (mm)[b]	Not available		−0.075	0.571

[a]Spearman's rank correlation coefficient.
[b]Analyzed in patients with single nodule of HCC.

1, 2, and 3 years estimated by the Kaplan–Meier method were 4.9%, 9.1%, and 11.1%, respectively, as shown in Figure 1A. In these patients who developed HCC, plasma ADAMTS13 activity was significantly higher than that in patients who did not develop HCC ($P < 0.001$), as depicted in Table 3; plasma ADAMTS13 activity was 161.9 ± 33.8% in patients who developed HCC and 108.8 ± 42.2% in patients who did not develop HCC. Liver stiffness value was also significantly higher in patients with HCC development, and serum albumin level and prothrombin time (%) were significantly lower in those patients. Then, univariate analyses showed that the higher plasma ADAMTS13 activity was a risk for HCC development ($P < 0.001$; Table 4). Other significant risk factors for HCC included lower albumin level, higher ALT level, lower prothrombin time (%), and higher liver stiffness value. Next, stepwise variable selection with AIC was used to find the best model in multivariate analysis (Table 4), which revealed that the higher plasma ADAMTS13 activity ($P = 0.03$) and the higher liver stiffness value ($P = 0.03$) were the significant risk factors for HCC. These results suggest that plasma ADAMTS13 activity may predict HCC development in patients with chronic hepatitis B or C.

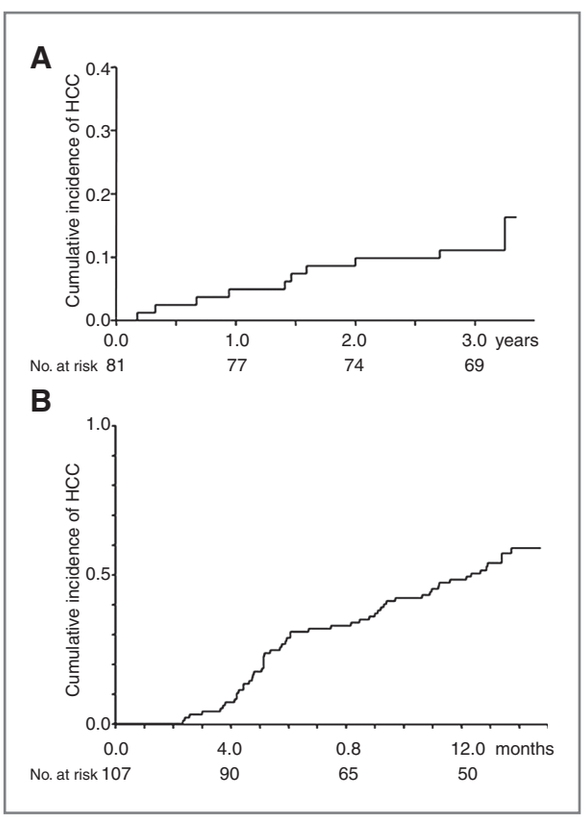

Figure 1. Cumulative incidence of HCC development (A) and recurrence (B).

Then, the relation between plasma ADAMTS13 activity and HCC development was analyzed separately in patients with chronic hepatitis B and with chronic hepatitis C. In patients with chronic hepatitis B ($n = 20$), plasma ADAMTS13 activity was significantly higher in patients who developed HCC than that in patients who did not develop HCC ($P < 0.005$); plasma ADAMTS13 activity was 158.9 ± 36.7% in patients who developed HCC and 95.3 ± 35.0% in patients who did not develop HCC. Then, univariate analyses showed that the higher plasma ADAMTS13 activity was a risk for HCC development ($P < 0.001$), and further multivariate analysis revealed that the higher plasma ADAMTS13 activity was a significant risk factor for HCC ($P = 0.03$) in these patients. In patients

Table 3. Characteristics of patients according to HCC development and recurrence

Variables	Development (−)	Development (+)	*P*	Recurrence (−)	Recurrence (+)	*P*
Age (y)	63.0 ± 12.4	61.4 ± 12.4	0.695	70.2 ± 7.5	68.3 ± 9.2	0.267
Man/Woman	40/29	6/4	0.82	23/19	36/19	0.39
HBV/HCV	16/53	4/6	0.45	7/35	7/48	0.80
Albumin (g/dL)	4.1 ± 0.3	3.8 ± 0.6	0.024	3.8 ± 0.5	3.6 ± 0.6	0.296
AST (IU/L)	46.9 ± 36.6	55.1 ± 23.0	0.494	61.0 ± 46.5	60.5 ± 33.3	0.951
ALT (IU/L)	52.0 ± 68.8	63.2 ± 46.9	0.620	56.9 ± 46.7	49.7 ± 29.5	0.359
Platelet count ($\times 10^4/\mu L$)	15.7 ± 6.5	12.2 ± 3.9	0.097	10.9 ± 5.5	10.5 ± 4.3	0.667
Prothrombin time (%)	89.5 ± 10.5	74.7 ± 11.6	<0.001	98.9 ± 3.5	97.5 ± 6.5	0.188
Plasma ADAMTS13 activity (%)	108.8 ± 42.2	161.9 ± 33.8	<0.001	116.8 ± 28.5	130.0 ± 30.8	0.039
Plasma ADAMTS13 antigen (%)	Not measured	Not measured		118.9 ± 35.4	134.3 ± 36.1	0.037
Liver stiffness (kPa)	9.2 ± 5.7	22.6 ± 13.7	<0.001	23.4 ± 15.0	30.6 ± 18.4	0.053
APRI	1.03 ± 1.03	1.35 ± 0.80	0.35	1.57 ± 0.81	1.47 ± 0.77	0.517
AFP (ng/mL)	10.7 ± 38.1	27.4 ± 41.4	0.203	131.5 ± 546.0	81.2 ± 158.1	0.521
DCP (mAu/mL)	17.9 ± 18.8	19.7 ± 8.2	0.766	114.0 ± 297.6	41.7 ± 64.3	0.082

NOTE: Values are expressed as the mean ± SD (range).

with chronic hepatitis C ($n = 59$), plasma ADAMTS13 activity was significantly higher in patients who develop HCC than that in patients who did not develop HCC ($P < 0.01$); plasma ADAMTS13 activity was 163.9 ± 35.2% in patients who developed HCC and 112.9 ± 43.6% in patients who did not develop HCC. Then, univariate analyses showed that the higher plasma ADAMTS13 activity was a risk for HCC development ($P < 0.001$), and multivariate analysis revealed that the higher plasma ADAMTS13 activity was a significant risk factor for HCC ($P = 0.02$) in these patients.

Characteristics of the patients with HCC and correlation between plasma ADAMTS13 activity or antigen level and clinical variables

To further examine a potential link between plasma ADAMTS13 and HCC, plasma ADAMTS13 activity and antigen level were measured in 107 patients with HCC. Their characteristics are summarized in Table 1. There were 15 patients with chronic hepatitis B and 92 patients with chronic hepatitis C.

Plasma ADAMTS13 activity in these patients was 124.9% ± 32.3% (mean ± SD) of control, ranged from 62.0% to 223.0%, and plasma ADAMTS13 antigen level, 128.3% ± 39.3% (mean ± SD) of control, ranged from 48.9% to 258.3%, respectively (Table 1). Of note, the strong correlation between plasma ADAMTS13 activity and plasma ADAMTS13 antigen level was observed (Spearman's rank; $\rho s = 0.803$, $P < 0.00001$, $n = 107$). Relationships between plasma ADAMTS13 activity and clinical variables are shown in Table 2. Same as in patients without HCC, the significant correlations were determined between plasma ADAMTS13 activity and serum AST and ALT levels ($P < 0.001$), liver stiffness value ($P = 0.026$), APRI ($P < 0.001$), and serum AFP level ($P < 0.001$). Of note, there was no significant correlation between plasma ADAMTS13 activity and maximum tumor size in patients with single nodule, suggesting that plasma ADAMTS13 activity is not a tumor marker of HCC.

Table 4. Risk factors for HCC development–univariate and multivariate analyses

Variable	HR (95% CI)	*P*
Univariate analysis		
ADAMTS13 (per 10% increase)	1.29 (1.11–1.50)	<0.001
Age (per 1 year increase)	0.990 (0.943–1.04)	0.68
Sex (male vs. female)	1.07 (0.563–2.02)	0.84
Hepatitis virus (HCV vs. HBV)	0.718 (0.380–1.36)	0.31
Albumin (per 1 g/dL increase)	0.208 (0.0477–0.905)	0.04
AST >40 U/L	1.73 (0.881–3.41)	0.11
ALT >40 U/L	2.06 (1.05–4.07)	0.04
PLT $<15 \times 10^4/\mu L$	1.97 (0.908–4.29)	0.09
Prothrombin time (%; per 10% increase)	0.490 (0.324–0.743)	<0.001
Liver stiffness (per 10% increase)	1.16 (1.07–1.26)	<0.001
APRI (per 10% increase)	1.05 (0.983–1.13)	0.14
AFP >20 ng/mL	1.71 (0.785–3.71)	0.18
DCP >40 mAU/mL[a]	NA	
Multivariate analysis		
ADAMTS13 (per 10% increase)	1.20 (1.02–1.40)	0.03
Liver stiffness (per 10% increase)	1.12 (1.01–1.23)	0.03

[a]Not accessed as only DCP was more than 40 mAU/mL in only 1 patient.

HCC recurrence and risk analysis

During the follow-up period of 12 months, 1 patient died without HCC. Two patients who developed extrahepatic recurrence and 3 patients who developed recurrence at a site adjacent to the treated site were excluded from the analysis. Four patients who were treated with IFN were not analyzed because IFN is known to reduce the risk of HCC development in chronic hepatitis B and C (26, 27). By the end of the follow-up, HCC recurrence was determined in 55 patients. The cumulative recurrence rates of HCC by the Kaplan–Meier method are shown in Figure 1B. The characteristics of patients with or without HCC recurrence are shown in Table 3. Among the various parameters, plasma ADAMTS13 activity ($P = 0.039$) and antigen level ($P = 0.037$) were significantly higher in patients with HCC recurrence than those in patients without HCC recurrence (Table 3). No significant differences were determined in other parameters between patients with and without HCC recurrence. Although there was no significant risk factor for HCC recurrence in univariate analyses (Table 5), plasma ADAMTS13 activity was retained as a significant risk factor of HCC recurrence ($P = 0.028$) in the multivariate Cox proportional hazard model, as shown in Table 5. When plasma ADAMTS13 antigen level was analyzed instead of plasma ADAMTS13 activity level, plasma ADAMTS13 antigen level was also a significant risk factor of HCC recurrence ($P = 0.007$) in multivariate analysis. These results suggest that plasma ADAMTS13 activity may predict HCC recurrence in patients with chronic hepatitis B or C.

Table 5. Risk factors for HCC recurrence–univariate and multivariate analyses

Variable	HR (95%CI)	*P*
Univariate analysis		
ADAMTS13 activity (per 10% increase)	1.106 (0.997–1.228)	0.052
Age (per 1 year increase)	0.982 (0.953–1.013)	0.25
Sex (male vs. female)	1.22 (0.70–2.13)	0.49
Hepatitis virus (HCV vs. HBV)	1.10 (0.50–2.44)	0.81
Albumin (per 1g/dL increase)	0.723 (0.432–1.210)	0.22
AST > 40 IU/L	1.35 (0.75–2.42)	0.31
ALT > 40 IU/L	1.00 (0.58–1.71)	0.99
PLT < 15 × 10^4/μL	1.25 (0.65–2.43)	0.51
Prothrombin Activity (per 10% increase)	0.88 (0.42–1.07)	0.20
Liver stiffness (per 10% increase)	1.12 (0.98–1.28)	0.11
APRI (per 10% increase)	1.00 (0.973–1.04)	0.81
AFP > 20 ng/mL	1.24 (0.73–2.11)	0.42
DCP > 40 mAU/mL	1.17 (0.64–2.14)	0.62
Multivariate analysis		
ADAMTS13 activity (per 10% increase)	1.14 (1.01–1.29)	0.028

The relation between plasma ADAMTS13 activity and HCC recurrence was also analyzed separately in patients with chronic hepatitis B and with chronic hepatitis C. In patients with chronic hepatitis B ($n = 14$), plasma ADAMTS13 activity or antigen level was not different between patients with ($105.0 \pm 34.0\%$ or $97.3 \pm 24.7\%$) and without HCC recurrence ($104.0 \pm 16.3\%$ or $98.9 \pm 14.4\%$), possibly because the number of patients analyzed was small. On the other hand, in patients with chronic hepatitis C ($n = 83$), plasma ADAMTS13 activity or antigen level was significantly higher in patients with HCC recurrence ($133.2 \pm 28.9\%$ or $139.7 \pm 34.4\%$) than that in patients without HCC recurrence ($119.4 \pm 29.8\%$ or 122.9 ± 37.1; $P = 0.037$ or $P = 0.036$). Multivariate analysis revealed that the higher plasma ADAMTS13 activity or antigen level was a significant risk factor for HCC ($P = 0.024$ or $P = 0.005$) in these patients.

Discussion

In the current study, plasma ADAMTS13 activity or antigen level significantly correlated with serum AST and ALT levels and also the variables predicting the stage of liver fibrosis, liver stiffness value, and APRI in patients with chronic hepatitis B or C, irrespective of the presence of HCC. Serum levels of AST and ALT reflect hepatocellular damage, and higher hepatocellular damage generally induces a higher wound healing response. Thus, our current findings may be in line with our speculation that plasma ADAMTS13 activity or antigen level reflects the activity of hepatic stellate cells as a main effector of wound healing and fibrosis in the liver.

Major finding of this study is that the higher plasma ADAMTS13 activity or antigen level was a significant risk factor for HCC development. With regard to HCC development among patients with chronic hepatitis B or C without the past history of HCC, plasma ADAMTS13 activity was higher in the patients who developed HCC than in those who did not develop HCC. Among the various clinical parameters, univariate analysis revealed that the higher plasma ADAMTS13 activity was a significant risk factor for HCC development. Then, multivariate analysis showed that the higher plasma ADAMTS13 activity was a significantly predicting factor for hepatocarcinogenesis, independent of other significant risk factors for HCC development, including the variables predicting the stage of liver fibrosis. This potential link between plasma ADAMTS13 activity and HCC development was further observed in the analysis of HCC recurrence: the patients who had HCC recurrence during the 1-year follow-up period had also significantly higher plasma ADAMTS13 activity or antigen level than those who did not have HCC recurrence. Then, only plasma ADAMTS13 activity or antigen level was retained in the multivariate Cox proportional hazard model as a significant risk factor of recurrence.

Then, we wondered how HCC development might be predictable by the activity or antigen level of plasma ADAMTS13, whose source is mainly hepatic stellate cells, as a key player of liver fibrosis. To explain this, the notion that advanced liver fibrosis is the strong risk factor for HCC development (17) may be important. Furthermore, the recent evidence suggests a potential direct link between hepatic stellate cells and HCC (3), as follows.

It is well known that HCC usually develops in the liver already suffering from chronic liver disease (2). In particular, HCV-related cirrhosis is associated with an extremely high risk of HCC development, with a reported annual incidence ranging between 3% and 8% (28–30). Thus, advanced liver fibrosis is one of the strongest risk factors for HCC development. In fact, the higher liver stiffness value is reportedly a strong risk for HCC development (21). In this study, a significant correlation was observed between plasma ADAMTS13 activity or antigen level and the variables predicting the stage of liver fibrosis such as liver stiffness value. Thus, we have first speculated that plasma ADAMTS13 activity is retained as a risk factor for HCC development by univariate analysis because plasma ADAMTS13 activity may reflect liver fibrosis. However, the higher plasma ADAMTS13 activity was a significant risk factor for HCC development, independent of liver stiffness value by multivariate analysis. Furthermore, in the analysis of HCC recurrence, plasma ADAMTS13 activity or antigen level was retained as a significant risk for HCC development, but not liver stiffness value, by multivariate analysis. The current finding that plasma ADAMTS13 activity or antigen level significantly correlated with serum AST and ALT levels may explain this. Of note, it was previously shown that the higher serum ALT is associated with the higher rate of incidence of HCC development (31) and HCC recurrence after the surgical treatment (32) in HCV-related cirrhosis, suggesting that more hepatocellular damage increases a risk for HCC development in the liver of the same stage of fibrosis. Because plasma ADAMTS13 activity or antigen level reflect hepatocellular damage and subsequent wound healing as well as liver fibrosis stage, plasma ADAMTS13 activity, or antigen level may act distinctly from liver stiffness value in the risk analysis of HCC development.

Alternatively, the prediction of HCC development by plasma ADAMTS13 activity or antigen level may be explained by a potential direct link between hepatic stellate cells and HCC, which has been recently reported (3). This concept is suggested based on the findings that hepatic stellate cells express the stem cell marker of CD133 (33) and both hedgehog (34, 35) and Wnt signaling (36) are found in hepatic stellate cells, two pathways implicated in stem cell differentiation and cancer (37). Furthermore, the direct promotion of tumorigenicity of HCC by hepatic stellate cells has been reported (38).

In human studies, the alteration of plasma ADAMTS13 activity in chronic liver disease has already been reported (39–44). In patients with liver cirrhosis, plasma ADAMTS13 activity was shown to be decreased (39) in relation to the severity of cirrhosis (44), although the wide range of values were detected compared with normal controls (43). In contrast, Lisman and colleagues showed that plasma ADAMTS13 activity in patients with liver cirrhosis was highly variable and not significantly different from that in normal controls (42). In line with the latter report, plasma ADAMTS13 activity in chronic hepatitis B and C was variable in the current study. We speculate that these distinct results of plasma ADAMTS13 activity in chronic liver disease may be caused by the characteristics of the patients enrolled in the analysis. The patients with reduced plasma ADAMTS13 activity in the previous reports (39, 43, 44) might have minimal hepatitis activity, that is, minimal wound healing response. Highly variable activity of plasma ADAMTS13 in liver cirrhosis (42) might also be explained by the variable hepatitis activity in those patients. This issue should be further clarified.

In conclusion, the higher plasma ADAMTS13 activity or antigen level was a significantly independent risk factor for HCC development in chronic hepatitis B or C, suggesting that plasma ADAMTS13 activity and antigen level may be useful in the prediction of hepatocarcinogenesis in chronic liver disease. It should be further evaluated whether plasma ADAMTS13 activity and antigen level could be useful as a predictor of HCC development with a larger sample size and also with other etiology of underlying chronic liver disease such as NASH.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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