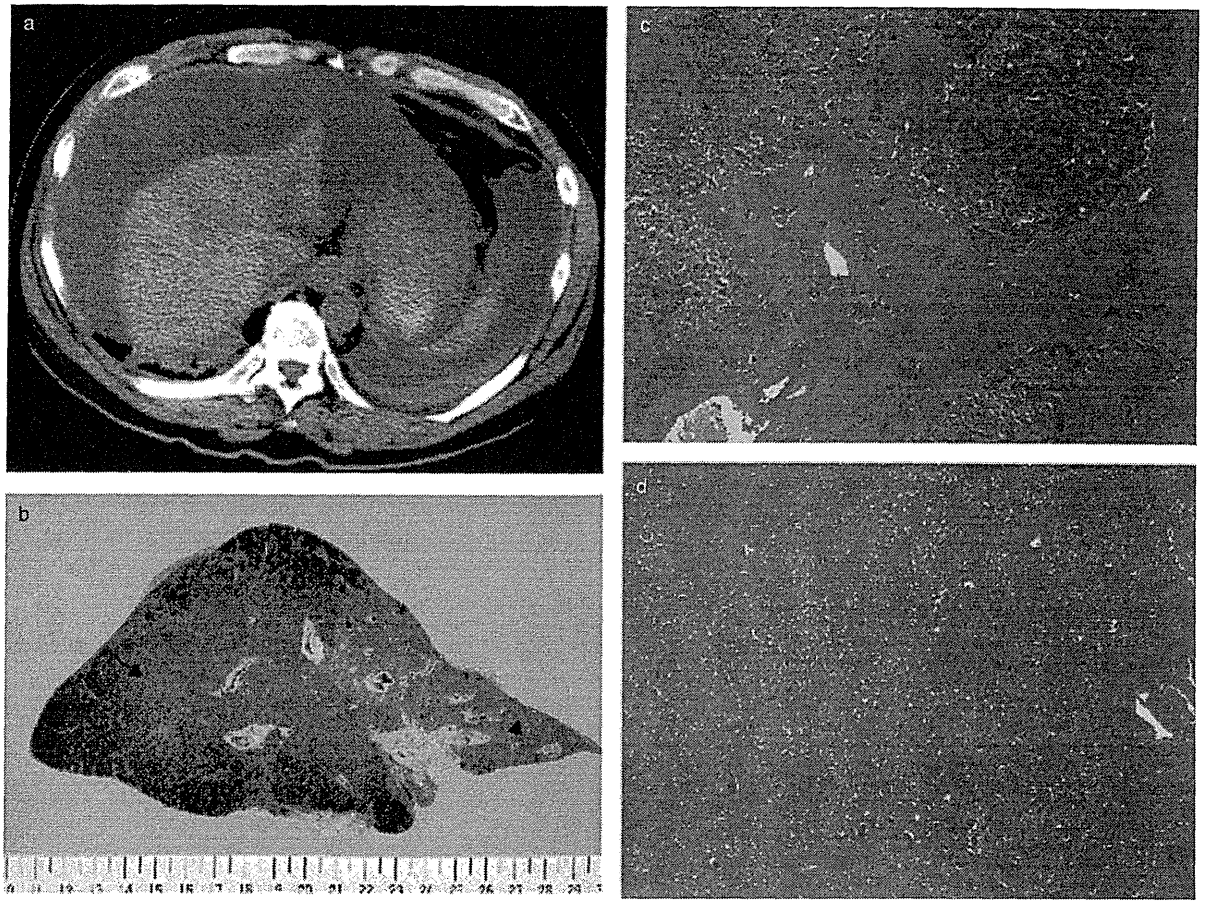

Figure 3 A 52-year-old male patient with autoimmune fulminant hepatitis. Corticosteroid was administered in combination with artificial liver support (hemodiafiltration and plasma exchange). His hepatic encephalopathy improved to grade I, but signs of liver regeneration were not found. (a) Computed tomography (CT) scan showed liver atrophy and left lobe-dominant heterogeneous hypoattenuating areas. (b) Section of extracted liver postmortem showed massive hemorrhagic necrosis in left lobe corresponding to heterogeneous hypoattenuating areas on CT. (c) Liver histology showed submassive necrosis in the right lobe (arrow), and (d) massive hemorrhagic necrosis with plasma cell accumulation in the left lobe (arrow head).

was higher in group 1 than in group 2 ($P < 0.001$), and the difference between groups 1 and 3 was not significant. Mean PT activity was higher in group 1 than in group 2 ($P = 0.028$), and the difference between groups 1 and 3 was not significant.

The imaging features of each group are shown in Table 2. The differences in presence of hypoattenuating areas on CT were not statistically significant among the three groups. Heterogeneous hypoattenuating areas on CT were present in 15 (65%) of group 1, none of group 2 and one (5%) of group 3, and the differences between groups 1 and 2 and groups 1 and 3 were significant ($P \leq 0.001$).

Comparison of characteristics between autoimmune ALF patients with and without heterogeneous hypoattenuating areas on CT

Patients with autoimmune hepatitis (group 1) were analyzed according to the presence of heterogeneous hypoattenuating areas on CT (Table 3). The differences in sex, duration from onset to admission, mean ALT, mean T-Bil, mean PT activity, mean IgG, ANA titer, AIH score and outcome were not statistically significant between patients with heterogeneous hypoattenuation and those without. Patients with heterogeneous hypoattenuation were younger than those without ($P = 0.046$).

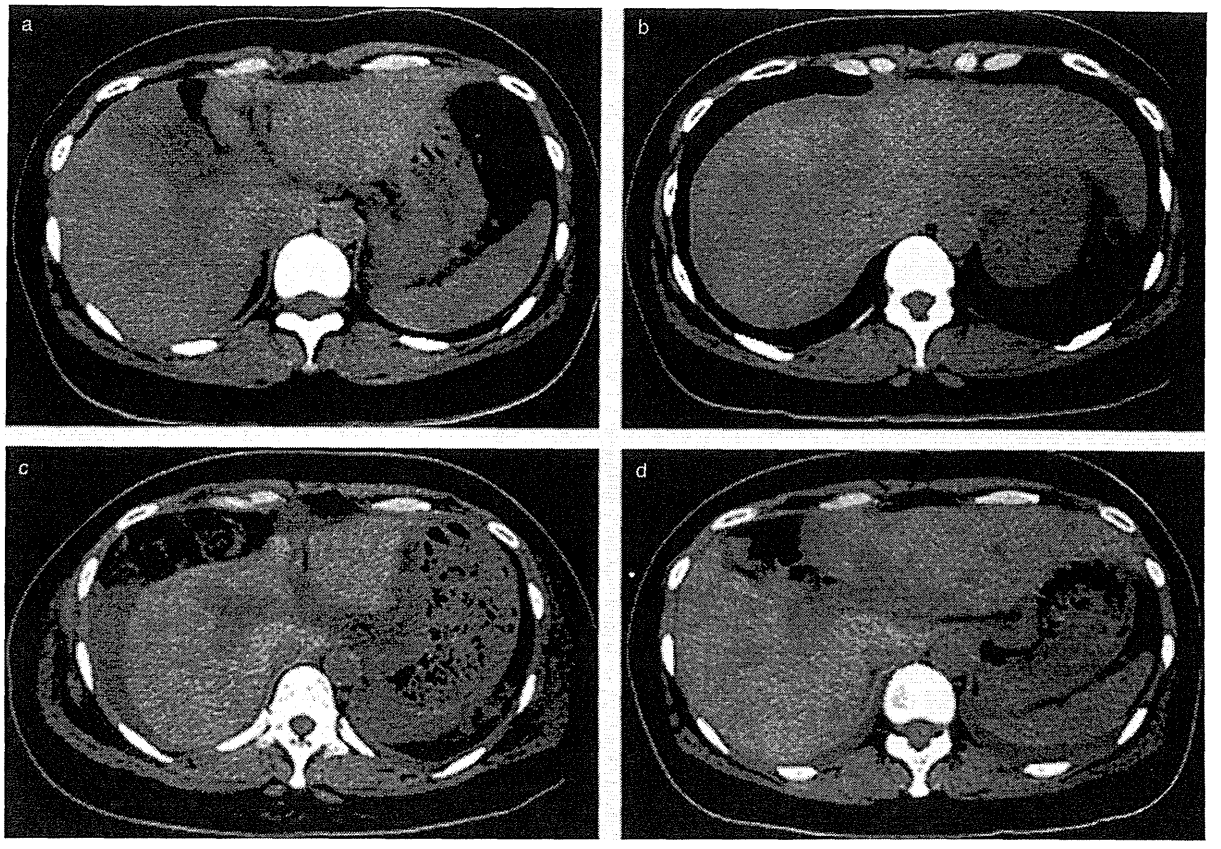

Figure 4 Computed tomography (CT) findings of a 49-year-old female patient with autoimmune fulminant hepatitis. Corticosteroid was administered and her liver function tests improved gradually. (a,b) CT scan showed heterogeneous hypoattenuating areas at admission. (c) One week after the administration of corticosteroid, her liver function tests improved gradually in response to the therapy. Nevertheless, liver atrophy of the right lobe progressed. (d) An enlargement of hyperattenuating areas and a decrease in hypoattenuating areas were found 2 weeks after.

Changes in CT findings during clinical courses of acute onset AIH

Changes in CT findings during clinical courses of acute onset AIH are shown in Figures 2 and 4. During follow-up, three of eight recovered acute onset AIH patients with heterogeneous hypoattenuating areas showed both shrinkage of hypoattenuating areas and expansion of hyperattenuating areas and this change led to "potato liver"-like deformity. None of the acute onset AIH patients without heterogeneous hypoattenuating areas and viral hepatitis showed "potato liver"-like deformity.

DISCUSSION

COMPUTED TOMOGRAPHY IS one of the best imaging modalities for evaluating ALF and predicting the prognosis by serial observations.[11,12] At an early stage of ALF, CT depicts a reduced-sized but non-deformed liver, associated with diffuse, localized, or heterogeneous hypoattenuation and localized or map-like hyperattenuation as well as dilatation of the portal vein, splenomegaly, and ascites.[13] Hypoattenuation corresponds to massive necrosis and hyperattenuation reflects preserved parenchyma at an early phase and regeneration at a late phase.[14] In acute type fulminant hepatitis, 80% of cases show diffuse type imaging pattern, whereas 57% show heterogeneous type in subacute type fulminant hepatitis.[15] During follow-up, temporal changes in the individual hypoattenuating areas are noted. A prominent deformity of the liver, known as "potato liver", occurs as a result of both shrinkage of the necrotic area and expansion of the regenerative area.[16] Similar CT findings seen in ALF liver are sometimes noted in patients with hepatitis of insidious onset.[18]

Table 1 Comparison of backgrounds of patients between autoimmune and viral hepatitis

	Group 1 (autoimmune ALF)	Group 2 (early-admission viral ALF)	Group 3 (late-admission viral ALF)	*P*-value Group 1 vs Group 2	Group 1 vs Group 3
n	23	25 (HAV 11, HBV 13, HBVex 1)	20 (HAV 2, HBV 6, HBVex 12)		
Age†	48.8 ± 15.4	45.3 ± 13.6	47.5 ± 13.1	0.401‡	0.764‡
Sex (M/F)	7/16	13/12	14/6	0.154§	0.015§
Days (onset-adm)†	39.8 ± 28.6	5.7 ± 2.3	20.5 ± 9.3	<0.001‡	0.006‡
ALT (IU/l)†	581 ± 487	6358 ± 3752	1292 ± 1759	<0.001‡	0.070‡
T-Bil (mg/dl)†	19.5 ± 11.2	6.6 ± 3.1	14.5 ± 10.4	<0.001‡	0.139‡
PT (%)†	35.5 ± 13.9	25.6 ± 16.2	32.4 ± 14.8	0.028‡	0.480‡

†mean ± standard deviation (SD).
‡Student's *t*-test.
§Fisher's exact probability test.
ALF, acute liver failure; ALT, alanine aminotransferase; ex, exacerbation; HAV, hepatitis A virus; HBV, hepatitis B virus; PT, prothrombin time; T-Bil, total bilirubin.

Autoimmune hepatitis has a wide variety of clinical presentations including asymptomatic state, chronic hepatitis, cirrhosis, acute hepatitis and fulminant hepatic failure. As there are no pathognomonic clinical and pathological features of AIH, a specific diagnostic scoring system has been developed that evaluates clinical, biochemical, immunological and histological features to determine the probability of the disease.[17] Although this scoring system has been used as a standard diagnostic tool in clinical practice, there have been some patients who do not show typical features and do not fulfill the criteria. AIH with clinical features of acute, severe and fulminant hepatitis (acute onset AIH) is one of these conditions. In our previous study, 23% of non-severe acute onset AIH could not reach "probable" using the scoring system in the early stage of illness.[7] Acute onset AIH is at risk of losing the timing for the initiation of immunosuppressive therapy, is sometimes resistant to immunosuppressive therapy, presents impaired hepatocellular regeneration, and has poor prognosis.[8–10]

Imaging features are not included in this diagnostic system for AIH. Although CT imaging is well established in the evaluation of liver diseases, little published data exist on the imaging appearances of AIH, especially, acute onset severe and fulminant AIH (autoimmune ALF). The aim of this study was to investigate whether CT imaging could contribute to the diagnosis and help to monitor treatment response in autoimmune ALF.

In the present study, the duration from onset to admission was longer, ALT level was lower, and T-Bil level was higher in autoimmune ALF than in viral ALF. The differences are more prominent between autoimmune ALF and early admission-viral ALF ($P < 0.001$). These findings suggest that autoimmune ALF patients have longer clinical courses and have more advanced disease than viral ALF ones. The comparison of backgrounds of autoimmune ALF and late admission-viral ALF showed no significant differences in age, mean ALT, mean T-Bil and mean PT activity. Thus, the background of both groups was almost the same except sex ($P = 0.015$) and the duration from onset to admission ($P = 0.006$). It is important to compare the two when discussing the differences of CT findings between autoimmune ALF and viral ALF, as distinguishing between atypical acute onset AIH and late admission type-unknown viral hepatitis is clinically difficult.

In CT imaging patterns of the hypoattenuating area, autoimmune ALF showed a heterogeneous pattern and viral ALF a diffuse pattern. In the comparison of autoimmune ALF and late admission-viral ALF, the former

Table 2 Imaging findings of patients with autoimmune and viral hepatitis

	Group 1 (autoimmune ALF)	Group 2 (early-admission viral ALF)	Group 3 (late-admission viral ALF)	*P*-value Group 1 vs Group 2	Group 1 vs Group 3
n	23	25	20		
CT findings					
Hypoattenuation	15	15	8	0.772†	0.131†
Diffuse	0	15	7	<0.001†	0.002†
Heterogeneous	15	0	1	<0.001†	<0.001†

†Fisher's exact probability test.
ALF, acute liver failure; CT, computed tomography.

showed a heterogeneous pattern and the latter a diffuse pattern ($P \leq 0.001$, $P = 0.002$, respectively).

During follow-up, all eight recovered acute onset AIH patients with heterogeneous hypoattenuating areas showed both shrinkage of hypoattenuating areas and expansion of hyperattenuating areas. This finding suggests a sign of recovery from massive necrosis, so we can get more information to assess treatment response by follow-up of CT.

Regarding the imaging analysis of AIH, Bilaj *et al.* reported that extensive reticular and/or confluent fibrosis is a common finding in AIH using magnetic resonance imaging (MRI).[19] Recently, Sahni *et al.* reported that there is a wide spectrum of CT and MRI imaging features in patients with AIH with no specific features.[20] They studied the correlation of imaging features and histological grade and stage among diagnosed AIH patients. We suppose that their patients perhaps included wide varieties of AIH from acute onset to liver cirrhosis, and therefore their study inevitably resulted in a wide spectrum of imaging features.

In our experiences, one of the pathological characteristics of acute onset AIH was its histological heterogeneity, especially in severe and fulminant AIH (autoimmune ALF). Histological heterogeneity leads to the radiological heterogeneity described above. Ultrasound also shows similar heterogeneity. These findings would be due to the mixture of severe centrilobular

Table 3 Comparison of characteristics between autoimmune hepatitis (AIH) patients with and without heterogenous hypoattenuating areas on computed tomography (CT)

	AIH with hypoattenuation	AIH without hypoattenuation	*P*-value
n	15	8	
Age†	44.2 ± 16.6	57.5 ± 7.8	0.046‡
Sex (M/F)	3/12	4/4	0.182§
Days (onset-adm)†	38.7 ± 32.2	42.0 ± 22.3	0.797‡
ALT (IU/L)†	517 ± 439	702 ± 579	0.399‡
T-Bil (mg/dL)†	19.2 ± 8.9	20.0 ± 15.3	0.881‡
PT (%)†	34.5 ± 13.4	37.4 ± 15.6	0.652‡
IgG (mg/dL)†	2182 ± 831	2757 ± 819	0.120‡
ANA ≦ × 40	5	1	0.369§
≧ × 80	10	7	
AIH score†	15.9 ± 2.7	15.5 ± 2.4	0.754‡
Outcome			
Recovery	8	6	0.400§
Death	5	2	1.000§
LT	2	0	0.526§

†mean ± standard deviation (SD).
‡Student's t-test.
§Fisher's exact probability test.
ALT, alanine aminotransferase; ANA, antinuclear antibody; IgG, immunoglobulin G; LT, liver transplantation; PT, prothrombin time; T-Bil, total bilirubin.

necrosis and regenerative islands usually seen in acute onset AIH. We supposed that characteristic morphological patterns of liver regeneration would exist in acute onset AIH and that their better understanding would be of help for the diagnosis.[8,9,21,22]

In summary, we found a characteristic CT imaging feature of autoimmune ALF: heterogeneous hypoattenuations on unenhanced CT. This finding could be one of the tools for diagnosing autoimmune ALF in combination with the international AIH scoring system. It would be useful especially in patients in whom histological examinations cannot be performed because of coagulopathy and ascites. Serial CT examinations depict changes in hypoattenuations and hyperattenuations, and we can evaluate the degree of liver regeneration and treatment response. CT imaging features should be included in the diagnostic tools for autoimmune ALF, of which there is no gold standard for making the diagnosis.

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REFERENCES

1 Czaja AJ. Autoimmune hepatitis: evolving concepts and treatment strategies. *Dig Dis Sci* 1995; **40**: 435–56.
2 Czaja AJ, Carpenter HA. Sensitivity, specificity and predictability of biopsy interpretations in chronic hepatitis. *Gastroenterology* 1993; **105**: 1824–32.
3 Czaja AJ, Davis GL, Ludwig J, Baggenstoss AH, Taswell HF. Autoimmune features as determinants of prognosis in steroid-treated chronic active hepatitis of uncertain etiology. *Gastroenterology* 1983; **85**: 713–17.
4 Nikias GA, Batts KP, Czaja AJ. The nature and prognostic implications of autoimmune hepatitis with an acute presentation. *J Hepatol* 1994; **21**: 866–71.
5 Porta G, Gayotto LC, Alvarez F. Anti-liver-kidney microsome antibody-positive autoimmune hepatitis presenting as fulminant liver failure. *J Pediatr Gastroenterol Nutr* 1990; **11**: 138–40.
6 Abe M, Onji M, Kawai-Ninomiya K *et al.* Clinicopathologic features of the severe form of acute type 1 autoimmune hepatitis. *Clin Gastroenterol Hepatol* 2007; **5**: 255–8.
7 Fujiwara K, Fukuda Y, Yokosuka O. Precise histological evaluation of liver biopsy specimen is indispensable for diagnosis and treatment of acute onset autoimmune hepatitis. *J Gastroenterol* 2008; **43**: 951–8.
8 Fujiwara K, Nakano M, Yasui S, Okitsu K, Yonemitsu Y, Yokosuka O. Advanced histology and impaired liver regeneration are associated with disease severity in acute onset autoimmune hepatitis. *Histopathology* 2011; **58**: 693–704.
9 Yasui S, Fujiwara K, Yonemitsu Y, Oda S, Nakano M, Yokosuka O. Clinicopathological features of severe and fulminant forms of autoimmune hepatitis. *J Gastroenterol* 2011; **46**: 378–90.
10 Fujiwara K, Yasui S, Tawada A *et al.* Autoimmune fulminant liver failure in adults. Experience in a Japanese center. *Hepatol Res* 2011; **41**: 133–41.
11 Itai Y, Sekiyama K, Ahmadi T, Obuchi M, Yoshiba M. Fulminant hepatic failure: observation with serial CT. *Radiology* 1997; **202**: 379–82.
12 Okuda K, Mitchell DG, Itai Y, Ariyama J. Acute hepatitis and acute hepatic failure. In: Okuda K, ed. *Hepatobiliary Diseases: Pathophysiology and Imaging*. London: Blackwell Science, 2001; 97–100.
13 Muto Y. Present status of fulminant hepatitis in Japan. *Gastroenterol Jpn* 1993; **28**: 120–7.
14 Murakami T, Baron RL, Peterson MS. Liver necrosis and regeneration after fulminant hepatitis: pathologic correlation with CT and MR findings. *Radiology* 1996; **198**: 239–42.
15 Muto Y. Imaging diagnosis of fulminant hepatitis. In: Muto Y, ed. *Liver Failure*. Tokyo: Nihon Iji Shimposha, 1994; 164. (In Japanese.)
16 Ishikawa N, Nakagawa S, Hirata K *et al.* Long-term follow-up of hepatitis using computed tomography. *J Comput Assist Tomogr* 1989; **13**: 645–9.
17 Alvarez F, Berg PA, Bianchi FB *et al.* International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929–38.
18 Itai Y, Ohtomo K, Kokubo T, Minami M, Yoshida H. CT and MR imaging of postnecrotic liver scars. *J Comput Assist Tomogr* 1988; **12**: 971–5.
19 Bilaj F, Hyslop WB, Rivero H *et al.* MR imaging findings in autoimmune hepatitis: correlation with clinical staging. *Radiology* 2005; **236**: 896–902.
20 Sahni VA, Raghunathan G, Mearadji B *et al.* Autoimmune hepatitis: CT and MR imaging features with histopathological correlation. *Abdom Imaging* 2010; **35**: 75–84.
21 Yasui S, Fujiwara K, Yokosuka O. Autoimmune fulminant hepatic failure in chronic hepatitis C during peg-interferon-alfa 2b plus ribavirin treatment showing histological heterogeneity. *Dig Liver Dis* 2011; **43**: 666–7.
22 Fujiwara K, Yasui S, Yokosuka O. Efforts at making the diagnosis of acute onset autoimmune hepatitis. *Hepatology* 2011; **54**: 371–2.

loss after peginterferon therapy cannot be convincingly explained by these viral mutants. Further studies are required to examine the underlying mechanisms involved in peginterferon-induced HBsAg loss.

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References

1. Hsu CW, Yeh CT. Emergence of hepatitis B virus S gene mutants in patients experiencing hepatitis B surface antigen seroconversion after peginterferon therapy. HEPATOLOGY 2011;54:101-108.
2. Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. HEPATOLOGY 2009;49:1141-1150.
3. Block TM, Guo H, Guo JT. Molecular virology of hepatitis B virus for clinicians. Clin Liver Dis 2007;11:685-706, vii.

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Histological Discrimination Between Autoimmune Hepatitis and Drug-Induced Liver Injury

To the Editor:

We read with interest the article by Suzuki et al.[1] We were surprised that only 1 (4%) of the autoimmune hepatitis (AIH) cases whose diagnoses were made by experienced hepatologists in Mayo Clinic showed "typical" histology, and that complete agreement on histological diagnosis among four experienced hepatopathologists was less than 50%, if biopsy slides were evaluated blinded to the clinical information. We realized that we should renew our awareness of evaluating liver histology.

In daily clinical practice, it is often very difficult in distinguishing drug-induced liver injury (DILI) from AIH with acute presentation of the disease (i.e., acute AIH) as a cause of acute hepatitis. As the investigators described, there is no pathognomonic feature for AIH or DILI, so the evaluation of liver histology in determining AIH versus DILI is important.

The diagnosis of AIH is challenging and that of acute onset AIH is even more challenging and difficult, because patients show acute presentation, such as acute hepatitis, and may not have typical clinicopathological features of AIH, and because there is no gold standard for it. Some acute AIH cases are at risk of losing the timing of starting immunosuppressive therapy, develop into severe or fulminant form, and are sometimes resistant to immunosuppressive therapy and have a poor prognosis. It is most important to exclude other causes systematically and apply the International AIH Group original revised scoring system,[2] rather than simplified the scoring system.[3,4] Especially, precise pathological evaluation plays an important role in the differential diagnosis.[5]

As the investigators commented in the Discussion, the sample size was too small and there was a possibility that some of the observed histological features may have been influenced by clinical presentation of AIH (i.e., acute versus chronic presentation). Therefore, it is important to show how many patients of the examined 28 AIH cases were clinically and histologically "acute AIH" who usually present atypical clinicopathological features and may have influenced the histological findings of their study.

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References

1. Suzuki A, Brunt EM, Kleiner DE, Miquel R, Smyrk TC, Andrade RJ, et al. The use of liver biopsy evaluation in discrimination of idiopathic autoimmune hepatitis versus drug-induced liver injury. HEPATOLOGY 2011;54:931-939.
2. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929-938.
3. Hennes EM, Zeniya M, Czaja AJ, Pares A, Dalekos GN, Krawitt EL, et al. Simplified diagnostic criteria for autoimmune hepatitis. HEPATOLOGY 2008;48:169-176.
4. Fujiwara K, Yasui S, Tawada A, Fukuda Y, Nakano M, Yokosuka O. Diagnostic value and utility of the simplified International Autoimmune Hepatitis Group criteria in acute onset autoimmune hepatitis. Liver Int 2011;31:1013-1020.
5. Fujiwara K, Fukuda Y, Yokosuka O. Precise histological evaluation of liver biopsy specimen is indispensable for diagnosis and treatment of acute-onset autoimmune hepatitis. J Gastroenterol 2008;43:951-958.

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Research Paper

Efficacy of Lamivudine or Entecavir on Acute Exacerbation of Chronic Hepatitis B

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Abstract

Background/Aims: Spontaneous acute exacerbation of chronic hepatitis B virus (HBV) infection occasionally occurs in its natural history, sometimes leading rapidly to fatal hepatic failure. We compared the effects of lamivudine (LAM) with those of entecavir (ETV) treatments in acute exacerbation of chronic hepatitis B with 500 IU/L or higher alanine aminotransferase (ALT) levels.

Methods: Thirty-four patients with acute exacerbation were consecutively treated with LAM /ETV. Their clinical improvements were compared.

Results: Among LAM-treated and ETV-treated patients, none showed a reduction of <1 log IU/mL in HBV DNA after 1 or 3 months of treatment. Initial virological response, defined as a reduction of 4 log IU/mL in HBV DNA at 6 months, with LAM and ETV, respectively, was 83.3% and 100%. One LAM patient developed hepatic encephalopathy, but all patients in both groups survived. Twelve months after treatment, 41.6% of 24 LAM group patients switched to another drug or added adefovir to their treatment due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change drugs.

Conclusions: ETV appears to be as effective as LAM in the treatment of patients with acute exacerbation of chronic hepatitis B. Clinicians should carefully start to treat these patients as soon as possible.

Key words: acute exacerbation, ALT, entecavir, HBV, lamivudine

INTRODUCTION

Chronic hepatitis B infection is associated with the development of hepatocellular carcinoma [1]. Infection with hepatitis B virus (HBV) also leads to wide a spectrum of liver injury, including acute, self-limited infection, fulminant hepatitis, and chronic hepatitis with progression to cirrhosis and liver failure, as well as to an asymptomatic chronic carrier state [2, 3].

Reactivation of hepatitis B is a well-characterized syndrome marked by the abrupt reappearance or rise of HBV DNA in the serum of a patient with previously inactivated or resolved HBV infection [4]. Reac-

tivation is often spontaneous, but can also be triggered by cancer chemotherapy and immune suppression. Spontaneous acute exacerbation of chronic hepatitis B infection is seen with a cumulative probability of 15-37% after 4 years of follow-up [5]. Prognosis is generally poor in HBV carriers with spontaneous acute exacerbation together with high alanine aminotransferase (ALT) levels, jaundice, and liver failure [4, 6, 7]. This condition has been defined as acute-on-chronic liver failure according to a recent Asia-Pacific consensus recommendation [8]. Acute exacerbation occasionally leads to a critical scenario, meaning that clinicians need to treat this condition immediately.

Lamivudine (LAM) is a reverse-transcriptase inhibitor of viral DNA polymerase with an excellent profile of safety and tolerability, causing inhibition of viral replication, and it is approved for antiviral treatment of hepatitis B patients [9, 10]. LAM suppresses serum HBV DNA values in up to 98% of patients within a median period of 4 weeks, leading to aminotransferase normalization, increased hepatitis B e antigen (HBeAg) seroconversion rate, and improvement of histological parameters [11, 12]. A study from Taiwan showed that LAM had a survival benefit and was effective for patients with baseline bilirubin levels below 20 mg/dL [7].

Entecavir (ETV), a deoxyguanosine analogue, is a potent and selective inhibitor of HBV replication; its *in vitro* potency is 100- to 1,000-fold greater than that of LAM, and it has a selectivity index (concentration of drug reducing the viable cell number by 50% [CC_{50}]/concentration of drug reducing viral replication by 50% [EC_{50}]) of ~8,000 [13, 14]. At present, the Japanese national health insurance system approves ETV as the first-line therapy for chronic hepatitis B, although some patients are treated with standard interferon-alfa. ETV is a nucleoside analogue (NUC) belonging to a new subgroup, cyclopentane [15], and it has been shown to be highly effective in suppressing HBV replication to an undetectable level and normalizing ALT, although NUCs do not eradicate the virus. ETV develops less resistance than LAM.

We undertook a retrospective study to compare the efficacy of LAM with that of ETV in the reduction of HBV DNA levels and associated improvement in disease severity and biochemical recovery in patients with acute exacerbation together with higher ALT levels due to HBV reactivation.

MATERIALS AND METHODS

Patients

A retrospective analysis of LAM/ETV-treated chronic hepatitis B patients at Chiba University Hospital and Numazu City Hospital, Japan, between May 2003 and December 2009 was performed. The inclusion criteria were: acute exacerbation of chronic hepatitis B characterized by an elevation of ALT level ≥ 500 IU/L along with HBV DNA ≥ 4.5 log IU/mL presenting in a patient with diagnosed chronic liver disease. The exclusion criteria were: acute hepatitis B, superinfection with other viruses (hepatitis E, A, D, or C), other causes of chronic liver failure [16, 17], coexistent hepatocellular carcinoma, portal thrombosis, coexistent renal impairment, pregnancy, coinfection with human immunodeficiency virus (HIV), or patients who had received a previous course of NUC treatment. This retrospective study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committee of Chiba University, Graduate School of Medicine [18].

Baseline assessment of patients

Retrospectively collected data included patient demographics, clinical findings, all laboratory variables including virological tests and abdominal ultrasound. HBsAg, HBeAg, anti-HBe antibody and immunoglobulin M (IgM) anti-HBc antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [19]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al [20]. HBV DNA was measured by Roche Amplicor™ PCR assay (detection limits: 2.6 log IU/mL; Roche Diagnostics, Tokyo, Japan).

Definitions

Primary antiviral treatment failure was defined as a reduction of < 1 log IU/mL in HBV DNA after 3 months of therapy. Initial virological response (IVR) was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

Follow-up

Clinical assessment and routine investigations were done every 15 days or every month for at least 6 months. HBV DNA measurements were repeated monthly.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 for Windows™ 7 and StatView 5 (SAS Institute Inc, Cary, NC). Continuous variables were expressed as mean ± standard deviation and were compared by two-factor analysis of variance (ANOVA) and two-way repeated measures ANOVA. Categorical variables were compared by Chi-square

test. Baseline was taken as the date when the first dose of LAM/ETV was administered. Statistical significance was considered at a *P*-value < 0.05.

RESULTS

Patients

Between May 2003 and December 2009, 34 patients with spontaneous acute exacerbation of chronic hepatitis B, with ALT levels ≥ 500 IU/mL and treated with LAM or ETV, were consecutively enrolled and retrospectively analyzed. 24 (70.5%) were treated with LAM at 100 mg daily and 10 (29.4%) were treated with ETV at 0.5 mg daily. All patients were followed for at least 6 months. Mean follow-up in the LAM and ETV groups was 55.5 ± 25.4 and 16.5 ± 9.9 months, respectively.

Baseline characteristics

Baseline characteristics in the two patient groups were similar **(Table 1)**. Median age was 37 (21-73) years and 79.4% were men. One patient of the LAM group developed hepatic encephalopathy, but recovered. All patients in both groups survived. At admission, the serological profile showed HBsAg positivity in all 34 (100%); 22 (64.7%) were HBeAg positive. The median HBV DNA level was 7.4 log IU/mL in the LAM group and 7.9 log IU/mL in the ETV group **(Table 1)**.

Table 1 Demographic, Clinical, and Laboratory Variables of Patients at Entry.

Parameters	Total Patients (N=34)	LAM (N=24)	ETV (N=10)	*P*-value
Age (years)	37 (21-73)	37 (21-73)	39 (24-67)	NS
Male (%)	27 (79.4)	18 (75)	9 (90)	NS
Cirrhosis (+/-)	2/32	2/22	0/10	NS
ALT (IU/L)	986 (523-2,450)	995 (523-2,450)	1,046 (523-2,140)	NS
T. Bil (mg/dL)	2.0 (0.8-22.0)	2.4 (0.8-20.6)	1.6 (1.9-22.0)	NS
PT (%)	83 (24-121)	81.5 (24-119)	83.6 (35-121)	NS
HBeAg (+/-)	22/12	18/6	4/6	NS
HBV DNA (log IU/mL)	7.6 (4.8-8.7)	7.4 (5.2-8.7)	7.9 (4.8-8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in HBV DNA of total patients

LAM significantly reduced HBV DNA levels from baseline 7.24 log IU/mL to 3.27 log IU/mL at 1 month ($P < 0.001$), to 2.21 log IU/mL at 3 months ($P < 0.001$), and to 1.53 log IU/mL at 6 months ($P < 0.001$). ETV also significantly reduced HBV DNA levels from baseline 7.56 log IU/mL to 3.12 log IU/mL at 1 month ($P < 0.001$), to 2.14 log IU/mL at 3 months ($P < 0.001$), and to 1.77 log IU/mL at 6 months ($P < 0.001$). There were no differences in HBV DNA levels from baseline to 6 months between the two groups. None with primary antiviral treatment failure was identified in either group. There were no significant differences in IVR between the two groups **(Figure 1)**.

Reduction in ALT levels of total patients

LAM significantly reduced ALT levels from baseline 1,130 IU/mL to 102 ($P < 0.001$) at 1 month, to 28.6 ($P < 0.001$) at 3 months, and to 23.1 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,210 IU/mL to 117 ($P < 0.001$) at 1 month, to 25 ($P < 0.001$) at 3 months, and to 24.4 ($P < 0.001$) at 6 months. There were no differences in ALT levels from baseline to 6 months between the two groups **(Figure 2)**.

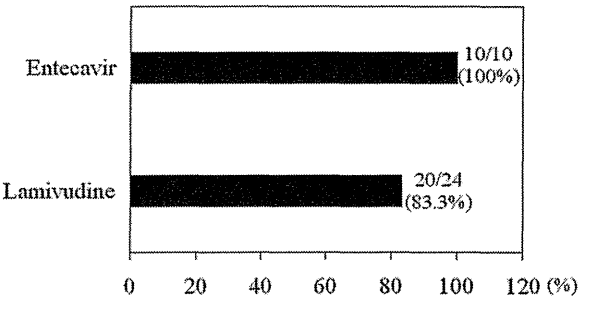

Figure 1 Initial virological response (IVR). IVR was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

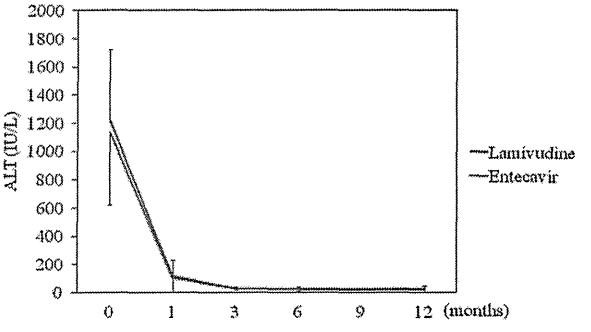

Figure 2 Efficacy of lamivudine and entecavir for ALT levels. Lamivudine (N=24) vs. entecavir (N=10); data are shown as mean ± SD.

Reduction in HBV DNA of HBeAg-positive patients

It has been demonstrated that the levels of HBV DNA in the HBeAg-positive phase were generally higher than those in the ant-HBe-positive phase [19, 22]. HBeAg positivity is also associated with HBV viremia and increased ALT levels in HIV/HBV co-infected patients [23]. Next, we compared the response to LAM or ETV in 18 or 4 HBeAg-positive patients, respectively **(Table 2)**. LAM significantly reduced HBV DNA levels from baseline 7.52 log IU/mL to 3.35 log IU/mL ($P < 0.001$) at 1 month, to 2.38 log IU/mL ($P < 0.001$) at 3 months, and to 1.55 log IU/mL ($P < 0.001$) at 6 months. ETV also significantly reduced HBV DNA levels from baseline 8.42 log IU/mL to 3.87 log IU/mL ($P < 0.001$) at 1 month, to 2.90 log IU/mL ($P < 0.001$) at 3 months, and to 2.22 log IU/mL ($P < 0.001$) at 6 months. There were no differences in HBV DNA levels from baseline to 6 months between the two groups. Primary antiviral treatment failure was not observed in either group. Four patients in the LAM group did not achieve IVR.

Table 2 Demographic, Clinical, and Laboratory Variables of HBeAg-positive Patients at Entry.

Parameters	Total Patients (N=22)	LAM (N=18)	ETV (N=4)	*P*-value
Age (years)	34.5 (21-51)	36.5 (21-51)	30 (24-33)	NS
Male (%)	18 (81.8)	14 (77.7)	4 (100)	NS
Cirrhosis (+/-)	1/21	1/17	0/4	NS
ALT (IU/L)	1,030 (523-2,450)	1,990 (523-2,450)	1,363 (980-1,620)	NS
T. Bil (mg/dL)	1.75 (0.8-20.6)	2.0 (0.8-20.6)	1.5 (1.0-18.7)	NS
PT (%)	77 (24-119)	73.6 (24-119)	95.0 (44.1-113)	NS
HBeAg (+)	22	18	4	
HBV DNA (log IU/mL)	7.6 (5.5- 8.8)	7.6 (5.5- 8.7)	8.6 (7.6- 8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in ALT levels of HBeAg-positive patients

LAM significantly reduced ALT levels from baseline 1,150 IU/mL to 84 ($P < 0.001$) at 1 month, to 27.5 ($P < 0.001$) at 3 months, and to 22.0 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,460 IU/mL to 230 ($P = 0.0038$) at 1 month, to 22.2 ($P = 0.0016$) at 3 months, and to 24.0 ($P = 0.0016$) at 6 months. At 1 month after treatment, the ALT levels of the LAM groups were lower than those of the ETV group ($P < 0.0001$) **(Figure 3)**. During follow-up periods, 10 and 1 sero-converters of HBeAg to anti-HBe antibody phase were seen in 18 LAM-treated and in 4 ETV-treated patients, respectively.

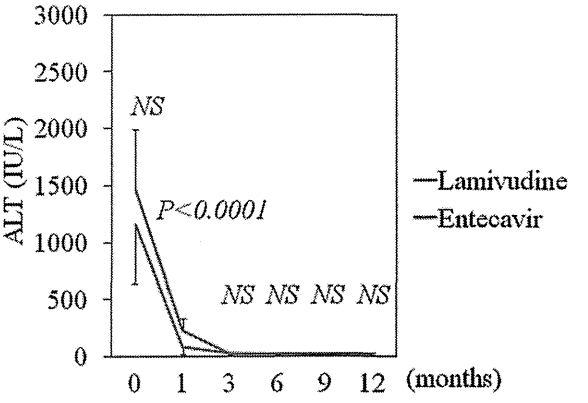

Figure 3 Efficacy of lamivudine and entecavir for ALT levels in HBeAg-positive patients. Lamivudine (N=18) vs. entecavir (N=4); data are shown as mean ± SD.

Safety

No patient stopped taking medications. Twelve months after treatment, 10 of 24 patients (41.6%) in the LAM group switched from LAM to ETV (n=4) or added adefovir (n=6) due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change their medication.

DISCUSSION

The present study compared the use of NUCs, LAM and ETV, for the treatment of acute exacerbation of chronic hepatitis B. The results clearly showed significant benefits of a rapid reduction of HBV DNA levels, compared with untreated patients in a previous report [4].

It was reported that ETV treatment is associated with increased short-term mortality in patients with severe acute exacerbation of chronic hepatitis B, but that it achieves better virological response in the long run [24]. We used LAM or ETV for patients with acute exacerbation of chronic hepatitis B presenting with ALT ≥ 500 IU/L in the present study. The effects of LAM on HBV DNA levels were the same as those of ETV (Figure 1). But the effects of LAM on ALT levels after 1 month were stronger than those of ETV in HBeAg-positive patients **(Figure 3)**. In spite of the limited number of these patients, the effects were possibly related to immunomodulating activities of LAM [25]. The patients' prognoses were more favorable than in the previous report [4]. This might have

depended on the fact that, in the present study, treatment was begun as soon as possible, and some patients may have had a milder grade of acute exacerbation of chronic hepatitis B than those in the previous report [4]. We believe that patients with acute exacerbation of chronic hepatitis B need to be subjected to treatment as promptly as possible.

The major routes of HBV infection in our country have been mother-to-child transmission and blood transfusion. However, cases with HBV transmitted through sexual contact are increasing, especially among HIV-1-seropositive patients [26]. One should bear in mind that knowledge about interactions between ETV and anti-HIV nucleoside analogues is limited [27]. Because long-term use of LAM induces LAM-resistant mutants [28], we can only use LAM for short-term treatment of patients with acute exacerbation of chronic hepatitis B. On the other hand, the present study also revealed that patients receiving ETV did not need to change drugs.

Recently, there have been several reports that reactivation of HBV is a fatal complication following systemic chemotherapy or other immunosuppressive therapy including rituximab and steroid therapies mainly in HBsAg-positive and -negative lymphoma patients. It is important to enable early diagnosis of HBV reactivation as well as initiation of antiviral therapy [29, 30].

In conclusion, ETV appears to be as effective as LAM in the treatment of patients with acute exacerbation of chronic hepatitis B. Clinicians should start to treat these patients with NUCs as soon as possible.

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ABBREVIATIONS

ETV: Entecavir; HIV: Human immunodeficiency virus; IVR: Initial virological response; LAM: Lamivudine; NUC: Nucleoside analogue.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

REFERENCES

1. Di Bisceglie AM. Hepatitis B and Hepatocellular Carcinoma. Hepatology. 2009; 49 (5 Suppl): S56-S60.
2. Omata M, Ehata T, Yokosuka O, et al. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med. 1991; 324: 1699-1704.
3. Liang TJ, Hasegawa K, Rimon N, et al. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. N Engl J Med. 1991; 324: 1705-1709.
4. Garg H, Sarin SK, Kumar M, et al. Tenofovir improves the outcome in patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure. Hepatology. 2011; 53: 774-780.
5. Lok AS, Lai CL. Acute exacerbation in Chinese patients with chronic hepatitis B virus (HBV) infection. Incidence, predisposing factors and etiology. J Hepatol. 1990; 10: 29-34.
6. Oketani M, Ido A, Tsubouchi H. Changing etiologies and outcomes of acute liver failure: A perspective from Japan. J Gastroenterol Hepatol. 2011; 26 (Suppl 1): 65-71.
7. Chien RN, Lin CH, Liaw YF. The effect of lamivudine therapy in hepatic decompensation during acute exacerbation of chronic hepatitis B. J Hepatol. 2003; 38: 322-327.
8. Sarin SK, Kumar A, Almeida JA, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). Hepatol Int. 2009; 3: 269-282.
9. Dienstag JL, Perrillo R, Schiff ER, et al. A preliminary trial of lamivudine for chronic hepatitis B infection. N Engl J Med. 1999; 333: 1657-1661.
10. Lai CL, Chien RN, Leung NW, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med. 1998; 339: 61-68.
11. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999; 341: 1256-1263.
12. Potthoff A, Tillmann HL, Bara C, et al. Improved outcome of chronic hepatitis B after heart transplantation by long-term antiviral therapy. J Viral Hepat. 2006; 13: 734-741.
13. Innaimo SF, Seifer M, Bisacchi GS, et al. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. Antimicrob Agents Chemother. 1997; 41: 1444-1448.
14. Ono SK, Kato N, Shiratori Y, et al. The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. J Clin Invest. 2001; 107: 449-455.
15. Yuen MF, Lai CL. Treatment of chronic hepatitis B: evolution over two decades. J Gastroenterol Hepatol. 2011; 26 (Suppl 1): S138-S143.
16. Kanda T, Yokosuka O, Ehata T, et al. Detection of GBV-C RNA in patients with non-A-E fulminant hepatitis by reverse-transcription polymerase chain reaction. Hepatology. 1997; 25: 1261-1265.
17. Kanda T, Yokosuka O, Ikeuchi T, et al. The role of TT virus infection in acute viral hepatitis. Hepatology. 1999; 29: 1905-1908.
18. Kamezaki H, Kanda T, Wu S, et al. Emergence of entecavir-resistant mutations in nucleos(t)ide-naïve Japanese patients infected with hepatitis B virus: Virological breakthrough is also dependent on adherence to medication. Scand J Gastroenterol. 2011; 46: 1111-1117.

19. Wu S, Kanda T, Imazeki F, et al. Hepatitis B virus e antigen downregulates cytokine production in human hepatoma cell lines. Viral Immunol. 2010; 23: 467-476.
20. Usuda S, Okamoto H, Iwanari H, et al. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. J Virol Methods. 1999; 80: 97-112.
21. Gallego A, Sheldon J, Garcia-Samaniego J, et al. Evaluation of initial virological response to adefovir and development of adefovir-resistant mutations in patients with chronic hepatitis B. J Viral Hepat. 2008; 15: 392-398.
22. Fujiwara K, Yokosuka O, Ehata T, et al. The two different states of hepatitis B virus DNA in asymptomatic carriers: HBe-antigen-positive versus anti-HBe-positive asymptomatic carriers. Dig Dis Sci. 1998; 43: 368-376.
23. Mendes-Correa MC, Pinho JR, Gomes-Gouvea MS, et al. Predictors of HBeAg status and hepatitis B viremia in HIV-infected patients with chronic hepatitis B in the HAART era in Brazil. BMC Infect Dis. 2011; 11: 247.
24. Wong VW, Wong GL, Yiu KK, et al. Entecavir treatment in patients with severe acute exacerbation of chronic hepatitis B. J Hepatol. 2011; 54: 236-242.
25. Sarin SK, Sandhu BS, Sharma BC, et al. Beneficial effects of 'lamivudine pulse' therapy in HBeAg-positive patients with normal ALT. J Viral Hepat. 2004; 11: 552-558.
26. Fujisaki S, Yokomaku Y, Shiino T, et al. Outbreak of infections by hepatitis B virus genotype A and transmission of genetic drug resistance in patients coinfected with HIV-1 in Japan. J Clin Microbiol. 2011; 49: 1017-1024.
27. Lindh M, Uhnoo I, Blackberg J, et al. Treatment of chronic hepatitis B infection: An update of Swedish recommendations. Scand J Infect Dis. 2008; 40: 436-450.
28. Seta T, Yokosuka O, Imazeki F, et al. Emergence of YMDD motif mutants of hepatitis B virus during lamivudine treatment of immunocompetent type B hepatitis patients. J Med Virol. 2000; 60: 8-16.
29. Kusumoto S, Tanaka Y, Ueda R, et al. Reactivation of hepatitis B virus following rituximab-plus-steroid combination chemotherapy. J Gastroenterol. 2011; 46: 9-16.
30. Yeo W, Johnson PJ. Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy. Hepatology. 2006; 43: 209-220.

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Review Article

Determination of ADAMTS13 and Its Clinical Significance for ADAMTS13 Supplementation Therapy to Improve the Survival of Patients with Decompensated Liver Cirrhosis

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The liver plays a central role in hemostasis by synthesizing clotting factors, coagulation inhibitors, and fibrinolytic proteins. Liver cirrhosis (LC), therefore, impacts on both primary and secondary hemostatic mechanisms. ADAMTS13 is a metalloproteinase, produced exclusively in hepatic stellate cells, and specifically cleaves unusually large von Willebrand factor multimers (UL-VWFM). Deficiency of ADAMTS13 results in accumulation of UL-VWFM, which induces platelet clumping or thrombi under high shear stress, followed by sinusoidal microcirculatory disturbances and subsequent progression of liver injuries, eventually leading to multiorgan failure. The marked imbalance between decreased ADAMTS13 activity (ADAMTS13 : AC) and increased production of UL-VWFM indicating a high-risk state of platelet microthrombi formation was closely related to functional liver capacity, hepatic encephalopathy, hepatorenal syndrome, and intractable ascites in advanced LC. Some end-stage LC patients with extremely low ADAMTS13 : AC and its IgG inhibitor may reflect conditions similar to thrombotic thrombocytopenic purpura (TTP) or may reflect "subclinical TTP." Hence, cirrhotic patients with severe to moderate deficiency of ADAMTS13 : AC may be candidates for FFP infusion as a source of ADAMTS13 or for recombinant ADAMTS13 supplementation. Such treatments may improve the survival of patients with decompensated LC.

1. Introduction

The liver is a major source of clotting and fibrinolytic proteins and plays a central role in thromboregulation [1–4]. Liver diseases, hence, impact on both primary and secondary hemostatic mechanisms. Because the hemostatic system is normally in a delicate balance between pro-hemostatic and antihemostatic processes, advanced liver cirrhosis (LC) patients experience multiple changes in the hemostatic system that may lead to either bleeding or thrombosis [1–4]. Despite clinical evidence of increasing bleeding tendency in LC patients, many facts indicate local and systemic hypercoagulability including portal or hepatic vein thrombosis, pulmonary embolism, and deep vein thrombosis, which are closely related to microcirculatory disturbances [4]. Deficiency of anticoagulant proteins and high levels of several procoagulant factors may favor hypercoagulability [4], but the mechanisms underlying this disorder have not been fully elucidated.

ADAMTS13 (**a** **d**isintegrin-like **a**nd **m**etalloproteinase with thrombospondin type-1 motifs **13**) is a metalloproteinase that specifically cleaves multimeric von Willebrand factor (VWF) between Tyr1605 and Met1606 residues in the A2 domain [5, 6]. In the absence of ADAMTS13 activity (ADAMTS13 : AC), unusually large VWF multimers (UL-VWFMs) are released from vascular endothelial cells (ECs) and improperly cleaved, causing them to accumulate and to induce the formation of platelet thrombi in the microvasculature under conditions of high shear stress. Currently, a severe deficiency in ADAMTS13 : AC, which results either

from genetic mutations in the *ADAMTS13* gene (Upshaw-Schulman syndrome, (USS)) [5–8] or acquired autoantibodies against ADAMTS13 [9, 10], is thought to be a specific feature of thrombotic thrombocytopenic purpura (TTP) [5–12].

In 2000, we demonstrated that a decreased plasma ADAMTS13 : AC in patients with cirrhotic biliary atresia can be fully restored after liver transplantation, indicating that the liver is the main organ producing ADAMTS13 [13]. One year later, northern blot analysis showed that the 4.6-kilobase ADAMTS13 mRNA was highly expressed in the liver [7, 14, 15], and subsequently both *in situ* hybridization and immunohistochemistry clearly indicated that ADAMTS13 is produced exclusively in hepatic stellate cells (HSCs) [16]. Platelets [17], vascular ECs [18], and kidney podocytes [19] have also been implicated as ADAMTS13-producing cells, but the amount produced by these cell types in the liver appears to be far less than that produced by HSC.

Mannucci et al. [20] originally reported a reduction of the ADAMTS13 : AC in advanced LC. Since HSCs were shown to be the major producing cells in the liver [16], much attention has been paid to the potential role of ADAMTS13 in the pathophysiology of liver diseases associated with sinusoidal and/or systemic microcirculatory disturbance [21–35]. ADAMTS13 : AC significantly decreased in patients with hepatic veno-occlusive disease (VOD) [22, 23], alcoholic hepatitis [24–27], liver cirrhosis [29, 30], and those undergoing living-donor-related liver transplantation [31–33] and partial hepatectomy [34]. Furthermore, hepatitis C virus- (HCV-) related LC patients with ADAMTS13 inhibitor (ADAMTS13 : INH) typically developed TTP [35]. Once patients with LC develop a decompensated condition, the risk of early mortality sharply increases for specific life-threatening complications such as ascites, hepatic encephalopathy, sepsis, hepatorenal syndrome, or hepatopulmonary syndrome [36].

In this paper, we will focus on the importance of ADAMTS13 determination for a better understanding of pathophysiology and/or for possible therapeutic approaches of ADAMTS13 supplementation to improve survival in patients with advanced LC.

2. Hepatic Microcirculation and Hypercoagulable State in LC

Hepatic microcirculation compromises a unique system of capillaries, called sinusoids, which are lined by three different cell types: sinusoidal endothelial cells (SECs), HSC, and Kupffer cells [37]. The SEC modulates microcirculation between hepatocytes and the sinusoidal space through the sinusoidal endothelial fenestration. The SEC has tremendous endocytic capacity, including VWF and the extracellular matrix, and secretes many vasoactive substances [37]. The HSC is located in the space of Disse adjacent to the SEC and regulates sinusoidal blood flow by contraction or relaxation induced by vasoactive substances [38]. Kupffer cells are intrasinusoidally located tissue macrophages and secrete potent inflammatory mediators during the early phase of liver inflammation [37]. Intimate cell-to-cell interaction has been found between these sinusoidal cells and hepatocytes [37, 38]. In LC, a sinusoidal microcirculatory disturbance occurs when the normal hepatic structure is disrupted by fibrin deposition [39] or by impaired balance between the action of vasoconstrictors and vasodilators in hepatic vascular circulation [37]. Studies have shown that cirrhotic liver exhibits a hyperresponse to vasoconstrictors, including catecholamine, endothelin, and leukotrienes D_4 [37].

Vascular endothelial cells play a pivotal role in hemostasis and thrombosis [5, 6]. VWF is a marker of endothelial cell activation (damage) and plays an essential role in hemostasis [5, 6]. In the normal state, VWF immunostaining is usually positive in large vessels but negative in the SEC [40]. On the occurrence of liver injury accompanied by a necroinflammatory process, the SEC becomes positive for VWF, presumably in association with the capillarization of hepatic sinusoids [39]. Subsequently, platelets adhere to subendothelial tissue mediated by UL-VWFM [5, 6]. ADAMTS13 then cleaves UL-VWFM into smaller VWF multimers [5, 6]. This interaction of ADAMTS13 and UL-VWFM is, indeed, the initial step in hemostasis [5, 6].

In patients with LC, circulating plasma VWF levels are extremely high [41, 42]. In liver tissue from cirrhotics [43] and even from the early stages of alcoholic liver diseases [44], VWF immunostaining shows positive cells predominantly at the scar-parenchyma interface, within the septum, and in the sinusoidal lining cells. Actually, portal or hepatic vein thrombosis is often observed in advanced LC routinely screened with Doppler ultrasound [45], and, in cirrhotic liver removed at transplantation, intimal fibrosis suggesting hepatic and portal vein thrombosis was frequently observed [46]. An autopsy series revealed microthrombi in one or multiple organs in one-half of cirrhotics [47]. Such a hypercoagulable state in liver diseases may be involved in hepatic parenchymal destruction, the acceleration of liver fibrosis and disease progression [4], leading to hepatorenal syndrome, portopulmonary hypertension, and spontaneous bacterial peritonitis [48].

Systemically, deficiency of anticoagulant proteins (antithrombin, protein C, and protein S) and the high levels of several procoagulant factors (factor VIII and VWF) may contribute to hypercoagulability in patients with LC [4]. Locally, the SEC dysfunction could lead to the development of a hypercoagulable state at the hepatic sinusoids corresponding to the site of liver injury, even in the face of a systemic hypocoagulable state [4]. Considering that ADAMTS13 is synthesized in HSC and its substrate, UL-VWFM, is produced in transformed SEC during liver injury, decreased plasma ADAMTS13 : AC may involve not only sinusoidal microcirculatory disturbances, but also subsequent progression of liver diseases, finally leading to multiorgan failure. Based on these findings, it is of particular interest to evaluate the activity of plasma ADAMTS13 : AC in LC patients.

3. Cleavage of UL-VWFM by ADAMTS13

Although the mechanism by which TTP develops in the absence of ADAMTS13 : AC has not been fully elucidated,

accumulating evidence has provided a hypothesis as illustrated in Figure 1 [49]. UL-VWFMs are produced exclusively in vascular ECs and stored in an intracellular organelle termed Weidel-palade bodies (WPBs) and then released into the circulation upon stimulation. Under physiological conditions, epinephrine acts as an endogenous stimulus, but under nonphysiological conditions, DDAVP (1-deamino-8-D-arginine vasopressin), hypoxia, and several cytokines such as interleukin IL-2, IL-6, IL-8, and tumor necrosis factor- (TNF-) α act as stimuli that upregulate VWF release. Once ECs are stimulated, UL-VWFMs and P-selectin, both stored in WPBs, move to the membrane surface of ECs, where P-selectin anchors UL-VWFMs on the ECs surface [50]. Under these circumstances, high shear stress generated in the microvasculature induces a change in the UL-VWFM from a globular to an extended form [51]. The ADAMTS13 protease efficiently cleaves the active extended form of UL-VWFM between the Tyr1605 and Met1606 residues in the A2 domain [52]. In this context, it has been postulated that multiple exocites within the disintegrin-like/TSP1/cysteine-rich/spacer (DTCS) domains of ADAMTS13 play an important role in interacting with the unfolded VWF-A2 domain [53]. ADAMTS13 may more efficiently cleave newly released UL-VWFMs that exist as solid-phase enzymes anchored to the vascular EC surface by binding to CD36, because CD36 is a receptor for TSP1, which is a repeated domain within the ADAMTS13 molecule [54]. When ADAMTS13 activity is reduced, UL-VWFM interacts more intensively with platelet GPIb and generates signals that further accelerate platelet activation [5, 6]. A series of these reactions leads to platelet microaggregates and thrombocytopenia. However, little information has been available on the cleavage of the UL-VWFMs by ADAMTS13 in the sinusoidal microcirculation in LC.

4. Assays for Plasma ADAMTS13 : AC and ADAMTS13 : INH

ADAMTS13 : AC was determined with a classic VWFM assay in the presence of 1.5 mol/L urea using purified plasma-derived VWF as a substrate according to the method described by Furlan et al. [55], and the detection limit of this assay was 3% of the normal control in our laboratory [56]. In 2005, we developed a novel chromogenic ADAMTS13-act-ELISA using both an N- and C-terminal tagged recombinant VWF substrate (termed GST-VWF73-His). This assay was highly sensitive, and the detection limit was 0.5% of the normal control [57]. Plasma ADAMTS13 : AC levels highly correlated between VWFM assay and ADAMTS13-act-ELISA (mean $\pm$ SD, 102 $\pm$ 23% versus 99.1 $\pm$ 21.5%, $r^2 = 0.72$, $P < .01$) [57]. No interference of the ADAMTS13-act-ELISA occurred even in the presence of hemoglobin, bilirubin, or chylomicrons in the samples, thus enabling distinction from the FRETS-VWF73 assay [58]. Because of its high sensitivity, easy handling, and lack of interference from plasma components, the ADAMTS13-act-ELISA would be recommended for routine laboratory use.

The ADAMTS13 : INH has also been evaluated with the chromogenic act-ELISA by means of the Bethesda method [59]. Prior to the assay, the test samples were heat-treated at 56°C for 60 min to eliminate endogenous enzyme activity, mixed with an equal volume of intact normal pooled plasma, and incubated for 2 hours at 37°C. The residual enzyme activity is measured after incubation. One Bethesda unit is defined as the amount of inhibitor that reduces activity by 50% of the control value, and values greater than 0.5 U/mL are significant.

5. Thrombocytopenia, Determination of ADAMTS13 : AC, and Its Clinical Significance in LC

5.1. Thrombocytopenia. It is well accepted that thrombocytopenia gradually progresses as functional liver capacity decreases [30, 60] (Figure 2(a)). The pathogenesis of thrombocytopenia in LC includes splenic sequestration in portal hypertension [61], impaired platelet production due to decreased synthesis of thrombopoietin in the liver [62] or due to myelosupression resulting from HCV infection [63], folic acid deficiency, or ethanol chronic consumption [64], which has a negative effect on megacaryocytopoiesis. However, our recent studies have provided evidence that in patients with advanced LC, elevated plasma levels of UL-VWFM enhance high-shear stress-induced platelet aggregation, resulting in thrombocytopenia [30].

5.2. ADAMTS13 : AC. Our study showed that ADAMTS13 : AC decreased with increasing severity of cirrhosis [30] (Figure 2(b)). The values determined by act-ELISA correlated well with those of the classical VWFM assay and also closely correlated with ADAMTS13 antigen de-termined by the antigen-ELISA. These results confirmed that both ADAMTS13 activity and antigen decreased with increasing cirrhosis severity [30] (Figures 2(b) and 2(c)), which are consistent with findings described by Feys et al. [29]. In contrast, Lisman et al. showed that both ADAMTS13 activity and antigen levels were highly variable; however, they did not distinguish between patients with varying degrees of cirrhosis [28]. It is unclear why they reached different conclusions from ours. One possible explanation relates to different etiologies: a majority of our patients developed cirrhosis secondary to HCV infection, whereas in their study one-half of the patients suffered from alcohol abuse-related cirrhosis. Further, the techniques used to determine ADAMTS13 : AC differed between our study [55–57] and theirs [65]. It is assumed that the collagen binding assay they used can be highly influenced by the increased amount of VWF : Ag in tested cirrhotic plasmas [29], because the substrate in this assay is intact multimeric VWF. In this regard, our act-ELISA is performed using VWF73-based fusion protein, termed GST-VWF73-His, which is readily cleaved by ADAMTS13 without any protein denaturant, and therefore the increased amount of VWF : Ag in tested plasmas does not interfere with the assays [57].

As shown in Figure 3, ADAMTS13 : ACs were significantly lower in LC patients with hepatic encephalopathy (Figure 3(a)), hepatorenal syndrome (Figure 3(b)), and

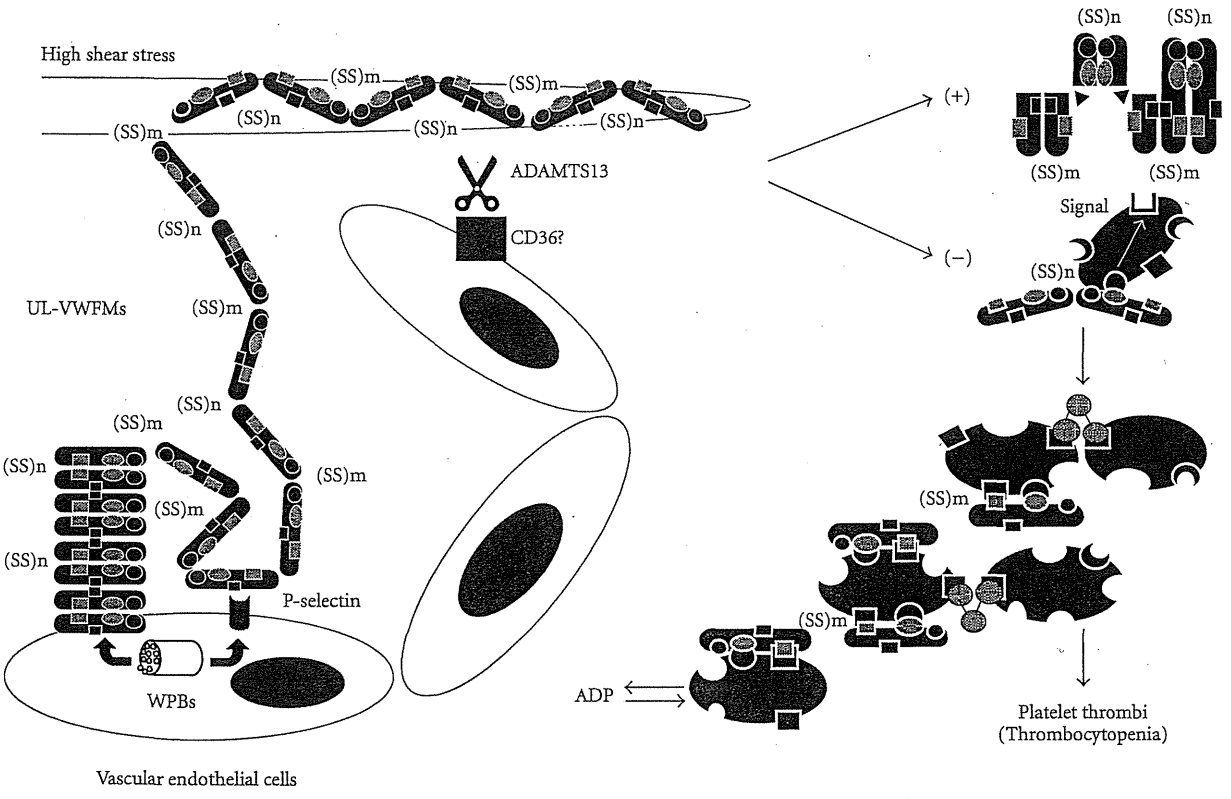

FIGURE 1: Proposed mechanism of platelet thrombi under high shear stress in the absence of ADAMTS13 : AC. Unusually large von Willebrand factor multimers (UL-VWFMs) are produced in vascular endothelial cells (ECs) and stored in Weidel-palade bodies (WPBs). UL-VWFMs are released from WPBs into the circulation upon stimulation by cytokines, hypoxia, DDAVP, and epinephrine. P-selectin that comigrates from WPBs anchors UL-VWFMs on the vascular EC surface. Under these circumstances, high shear stress changed the molecular conformation of UL-VWFMs from a globular to an extended form, allowing ADAMTS13 to access this molecule. In the absence of ADAMTS13 : AC, UL-VWFMs remain uncleaved, allowing them to excessively interact with platelet glycoprotein (GP)Ibα and activate platelets via intraplatelet signaling, which result in the formation of platelet thrombi. (Partially modified from Fujimura et al., [49]).

severe esophageal varices than those without [30]. Moreover, patients with refractory ascites had lower ADAMTS13 : AC levels than patients without ascites or those with easily mobilized ascites (Figure 3(c)). A multivariate analysis using all significant baseline parameters determined by the univariate analysis, excluding the Child-Pugh score, showed spleen volume, blood ammonia, and serum creatinine independently correlated with ADAMTS13 : AC. As a second step, the three parameters that contribute to the Child-Pugh classification (total bilirubin, albumin, and prothrombin time) were replaced by the Child-Pugh score. As a result, the Child-Pugh score and spleen volume were independently selected, indicating that ADAMTS13 : AC is closely related to the severity of liver disease and splenomegaly in cirrhotic patients [30].

5.3. VWF : Ag and VWF Multimer Patterns. Plasma levels of VWF : Ag substantially increase as liver diseases progress (Figure 2(d)) [30], as previously reported [41, 42]. This is presumably attributed to sinusoidal and/or extrahepatic endothelial damage induced by endotoxin and cytokines [41, 42, 66, 67]. The VWF : RCo was higher (Figure 2(e)) [30], but the ratio of VWF : RCo/VWF : Ag was lower in LC patients than that in healthy subjects. These findings suggest that increased VWF : Ag appears less functional in LC patients [30], which are consistent with previous reports [28]. Nevertheless, our study has clearly shown that the ratio of VWF : RCo/ADAMTS13 : AC progressively increases with the worsening of chronic liver diseases (Figure 2(f)), further intensifying an enhanced thrombogenesis with the progression of liver dysfunction and thrombocytopenia [30].

With regard to VWF multimers, the higher molecular weight multimer showed greater degradation than in healthy controls, thus maintaining normal enzyme-to-substrate (ADAMTS13/UL-VWFMs) ratio to maintain blood fluidity [29]. We showed that there were three different VWFM patterns in LC patients with lower ADAMTS13 : AC (<50 % of controls): normal-VWFM was detected in 53%, degraded-VWFM in 31%, and UL-VWFM in 16% (Table 1) [30]. UL-VWFM-positive patients showed the lowest ADAMTS13 : AC and the highest values of serum creatinine, blood urea nitrogen, and blood ammonia. In addition, LC patients with UL- and normal-VWFM had higher levels of VWF : RCo

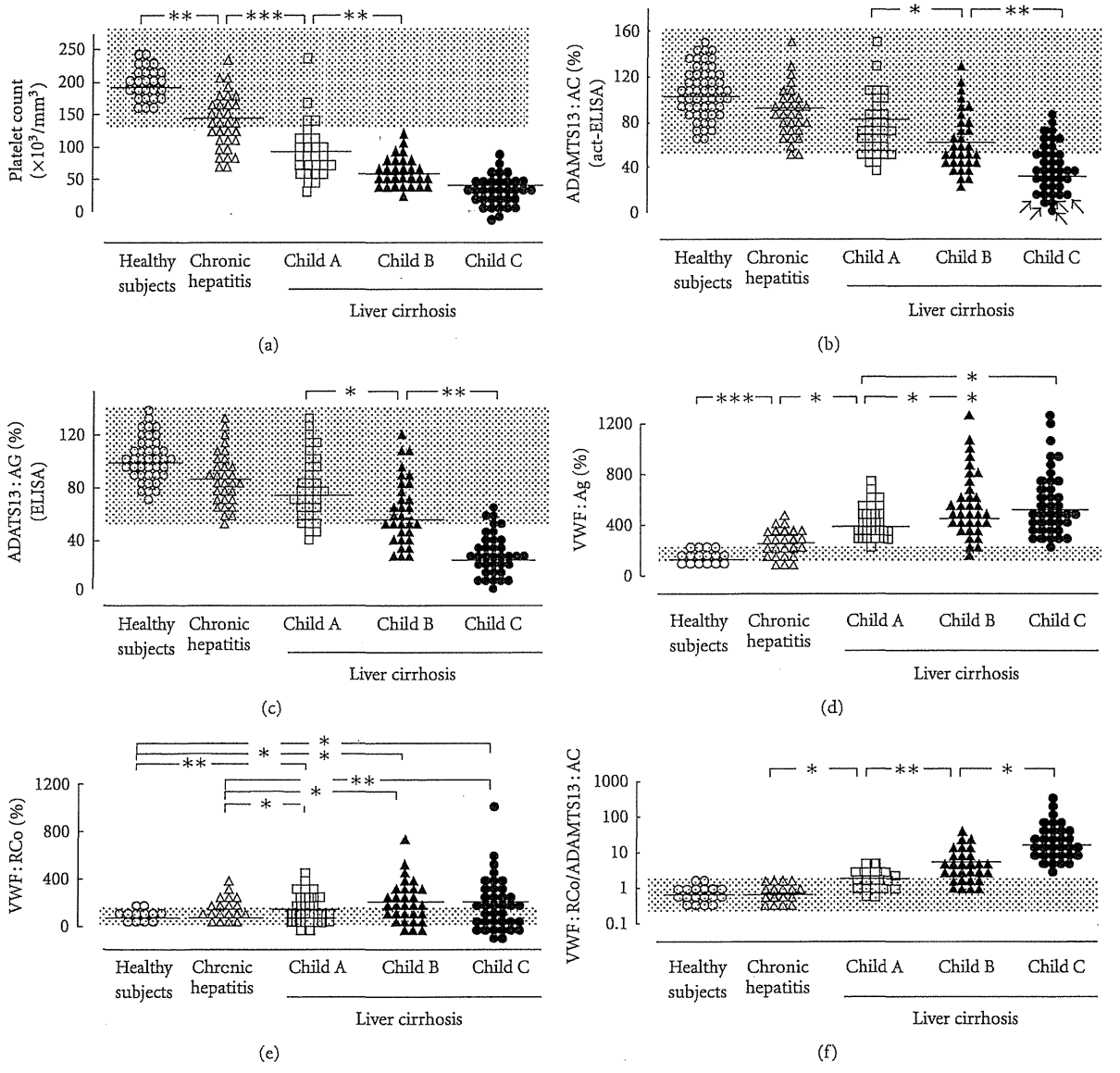

FIGURE 2: Platelet counts and plasma levels of ADAMTS13 : AC and its related parameters in patients with chronic liver diseases. Platelet counts decreased with the severity of chronic liver diseases, but no difference was found between Child B and C (a). Plasma ADAMTS13 : AC determined by ELISA progressively decreased with worsening cirrhosis (b). Arrows indicate patients whose plasma ADAMTS13 : AC was extremely low (< 3% of normal control by VWFM assay). The ADAMTS13 : AG levels determined by ELISA also decreased with increasing cirrhosis severity (c), which highly correlated with ADAMTS13 : AC measured by the act-ELISA ($r = 0.715$, $P < .001$). The VWF : Ag increased with the progression of chronic liver diseases, but the difference between Child B and C did not reach statistical significance (d). The VWF : RCo is higher in liver cirrhosis patients than that in patients with chronic hepatitis and healthy subjects, but it did not differ among subgroups within liver cirrhosis (e). The VWF : RCo relative to ADAMTS13 : AC progressively increased with worsening chronic liver disease (f). Open circles: normal controls; open triangles: chronic hepatitis; open squares: cirrhosis with Child A; closed triangles: cirrhosis with Child B; closed circles: cirrhosis with Child C. Shaded area shows normal range. ADAMTS13 : AC : ADAMTS13 activity, ADAMTS13 : AG = ADAMTS13 antigen. VWF : Ag = von Willebrand factor antigen, VWF : RCo = von Willebrand factor ristocetin cofactor activity; $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ significantly different between the two groups. (Partially modified from Uemura et al., [30]).

and Child-Pugh score and lower values of cholinesterase and hemoglobin than those with degraded-VWFM [30] (Table 1). The pattern, therefore, appears to shift from degraded- to normal-VWFM, and finally to UL-VWFM as functional liver capacity and renal function deteriorates, indicating that advanced LC may be a predisposing state toward platelet microthrombi formation, even in the absence of clinically overt thrombotic events [30].

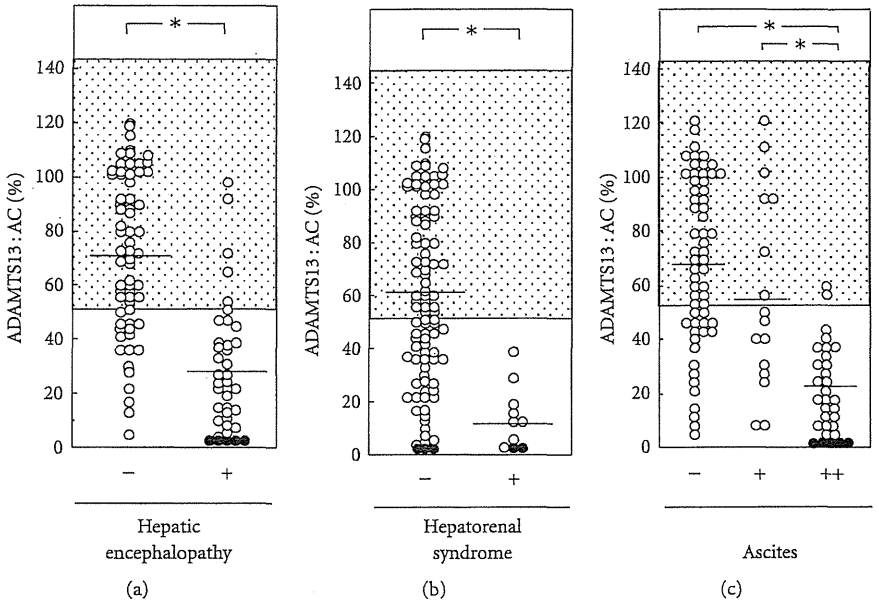

FIGURE 3: Relationship of ADAMTS13 : AC to the presence or absence of hepatic encephalopathy, hepatorenal syndrome, and ascites in patients with liver cirrhosis. The ADAMTS13 : AC was significantly lower in LC patients with hepatic encephalopathy (a) and hepatorenal syndrome (b) than that those without. Moreover, patients with refractory ascites had lower ADAMTS13 : AC than those without ascites or those with easily mobilized ascites (c). Closed circles indicate patients whose plasma ADAMTS13 : AC was extremely low (< 3% of normal control by VWFM assay). ADAMTS13 : AC: ADAMTS13 activity; $*P < .001$ significantly different between the two groups. (Partially modified from Uemura et al., [30]).

TABLE 1: Comparison of clinical parameters among cirrhotic patients according to VWF multimer patterns.

Variables	VWF multimer pattrens			a versus b	a versus c	b versus c
	Degraded[a] ($n = 15$)	Normal[b] ($n = 26$)	Unusually large[c] ($n = 8$)			
ADAMTS13 : AC (%) (ELISA)	47 ± 24	44 ± 13	26 ± 14	n.s.	$P < .05$	$P < .01$
VWF : RCo (%)	110 ± 92	196 ± 134	216 ± 110	$P < .05$	$P < .05$	n.s.
Child-Pugh score	8.6 ± 2.5	10.9 ± 2.1	12.4 ± 1.7	$P < .01$	$P < .005$	n.s.
Serum albumin (g/dL)	3.07 ± 0.54	2.85 ± 0.54	2.59 ± 0.25	n.s.	$P < .05$	n.s.
Cholinesterase (IU/L)	126 ± 62	78 ± 64	60 ± 36	$P < .05$	$P < .02$	n.s.
Total cholesterol (mg/dL)	142 ± 51	93 ± 45	88 ± 40	$P < .01$	$P < .03$	n.s.
Hemoglobin (g/dL)	11.0 ± 1.7	9.3 ± 2.0	8.9 ± 1.7	$P < .02$	$P < .02$	n.s.
Serum creatinine (mg/dL)	1.06 ± 0.72	1.11 ± 0.79	2.43 ± 2.16	n.s.	$P < .05$	$P < .03$
Blood urea nitrogen (mg/dL)	22 ± 17	30 ± 21	74 ± 62	n.s.	$P < .01$	$P < .01$
Blood ammonia (μg/dL)	87 ± 50	100 ± 39	144 ± 53	n.s.	$P < .05$	$P < .05$

VWF: von Willebrand factor; ADAMTS13 : AC : ADAMTS13 activity; ELISA : enzyme-linked immunosorbent assay; VWF : RCo: VWF ristocetin cofactor activity; n.s.: not significant. (Partially modified from Uemura et al., [30]).

6. Mechanism of Decreased ADAMTS13 : AC in LC Patients

The mechanism responsible for the decrease in ADAMTS13 : AC in advanced LC may include enhanced consumption due to the degradation of large quantities of VWF : AG [20], inflammatory cytokines [68, 69], and/or ADAMTS13 plasma inhibitor [9, 10]. It is controversial whether ADAMTS13 deficiency is caused by decreased production in the liver; Kume et al. reported that HSC apoptosis plays an essential role in decreased ADAMTS13 : AC using dimethylnitrosamine-treated rats, but not carbon tetrachloride- (CCl_4-) treated

animals [70], whereas Niiya et al. found upregulation of ADAMTS13 antigen and proteolytic activity in liver tissue using rats with CCl_4-induced liver fibrosis [71]. We observed the inhibitor of ADAMTS13 in 83% of patients with severe to moderate ADAMTS13 deficiency, but its inhibitory activity was in a marginal zone between 0.5 and 1.0 BU/mL in most cases except in cases of a TTP patient (2.0 BU/mL) and a patient with severe ADAMTS13 deficiency (3.0 BU/mL) [30]. Interestingly, IgG-type autoantibodies specific to purified plasma derived-ADAMTS13 were detected by Western blotting only in five end-stage cirrhotics with severe ADAMTS13 deficiency (<3%) corresponding to TTP [30]. One patient showed an apparent TTP [35], while the other four cirrhotics did not show apparent clinical features of TTP but had complications of hepatorenal syndrome, spontaneous bacterial peritonitis (SBP), marked inflammation together with cytokinemia, and advanced hepatocellular carcinoma (HCC) [30]. Various clinical conditions, including infection, malignancies, and certain drugs, can lead to acquired TTP [72]. In advanced LC patients, endotoxemia is frequently detected [42, 73], and SBP sometimes occurs [74]. HCC is highly complicated as the cirrhotic stage progresses [75], suggesting a high-risk state of platelet microthrombi formation. Some end-stage LC patients with extremely low ADAMTS13 : AC and its IgG inhibitor may reflect conditions similar to TTP or may reflect "subclinical TTP" [21]. Further studies will be necessary to clarify whether inhibitors other than the IgG inhibitor might be involved in cirrhotics with lower ADAMTS13 : AC.

Alternatively, cytokinemia [25, 68, 69, 76] and endotoxemia [25, 77] are additional potential candidates for decreasing plasma ADAMTS13 : AC. Recent investigations demonstrated that IL-6 inhibited the action of ADAMTS13 under flow conditions and both IL-8 and TNF-α stimulated the release of UL-VWFM in human umbilical vein endothelial cells *in vitro* [68]. It remains to be clarified whether IL-6 directly hampers the cleavage of UL-VWFM or downregulates gene expression of ADAMTS13 with modification of promoter activity. IFN-γ, IL-4, and TNF-α also inhibit ADAMTS13 synthesis and activity in rat primary HSC [69]. In addition, ADAMTS13 deficiency associated with inflammation promoted formation of UL-VWFM [78], and intravenous infusion of endotoxin to healthy volunteers caused a decrease in plasma ADAMTS13 : AC together with the appearance of UL-VWFM [77]. In patients with alcoholic hepatitis, especially in severe cases complicated by LC, ADAMTS13 : AC concomitantly decreased, and VWF : Ag progressively increased with increasing concentrations of these cytokines from normal range to over 100 pg/mL [25]. Plasma endotoxin concentration inversely correlated with ADAMTS13 activity and was higher in patients with UL-VWFM than that those without [25]. From these results as well as our own, marked cytokinemia and/or enhanced endotoxemia may be closely related to decreased ADAMTS13 : AC and the appearance of UL-VWFM [25]. It will be necessary to clarify what types of inhibitor may be involved in association with inflammatory cytokines and endotoxin.

7. Typical TTP in Patients with Liver Diseases

We previously encountered a patient with HCV-related LC who was compromised by fatal TTP [35]. This case showed advanced LC and rigid ascites. As reported in the literature, since 1979, there have been 13 patients with liver diseases who developed TTP [35, 79–90]. Five of them were treated with IFN therapy, but the remaining 8 were not. Three of them showed evidence of autoimmune hepatitis, one of which was complicated by systemic lupus erythematosus (SLE). The remaining 4 patients had HCV-related LC, hepatitis B virus- (HBV-) related LC, alcoholic LC, or haemochromatosis. IFN may be able to induce autoimmune reactions, resulting in the generation of autoantibodies against ADAMTS13, although this phenomenon has yet to be confirmed. On the other hand, irrespective of IFN therapy, HCV infection and/or advanced LC itself may contribute to the development of TTP.

There is general consensus that the overall prevalence of serum non-organ-specific autoantibodies is significantly higher in patients with HCV (about one third of all cases) than that in both healthy subjects and patients with HBV [91–93], but not alcoholic liver injury. In addition, HCV infection was confirmed in five of 10 patients (50%) who developed thrombotic microangiopathy (TMA) after living-donor liver transplantation [94]. In our study, the etiology of our five end-stage LC patients with IgG-type autoantibodies was HCV in 2, HBV in 1, PBC in 1, and cryptogenic in 1, but none of the patients displayed alcohol-abuse-related cirrhosis [30]. Nevertheless, the diagnosis of TTP may be hampered by clinical features accompanying hepatic failure similar to the pentad of typical TTP (fever, thrombocytopenia, renal failure, fluctuating neurological signs, and microangiopathic hemolytic anemia) [11, 12].

8. Possible Therapeutic Approaches of ADAMTS13 Supplementation for Patients with Decompensated LC

Fresh frozen plasma (FFP) infusion is commonly used to correct the prolonged prothrombin time in patients with advanced chronic liver disease, but exact indication for its use has not been clearly defined [95]. The aim of FFP infusions is usually either to improve the coagulopathy before invasive procedures or to control ongoing bleeding from various sites in patients with vitamin K-unresponsiveness prolonged prothrombin time. The mean prothrombin time was improved by the infusion of 2–6 units of FFP, but only 12.5% of the retrospective study group and 10% of the prospective study groups showed reversal of their coagulopathy, and higher volume (6 or more units) may be more effective but rarely is employed [96]. However, attention should be directed to complications including the risk of infection, allergic reaction, and acute volume expansion leading to heart failure or pulmonary edema [95, 96].

With regard to FFP infusion as a unique source of ADAMTS13, we clearly showed that preexisting UL-VWFMs

in the plasma of USS patients began to diminish within 1 hour and completely diminished 24 hours after ADAMTS13 was replenished with infusions of FFP [97]. Retrospectively, these results indicated that exogenous ADAMTS13 could efficiently cleave both UL-VWFMs that preexisted in the circulation and the newly produced molecules at the ECs surface. Advanced LC is known to be a predisposing state toward platelet microthrombi formation, even in the absence of clinically overt thrombi [30]. In our study, UL-VWFM-positive patients showed the lowest ADAMTS13 : AC and the highest values of serum creatinine, blood urea nitrogen, and blood ammonia, and the VWFM patterns appeared to shift from degraded to normal VWFM and finally to UL-VWFM as functional liver capacity and renal function deteriorated (Table 1). From these results, it may be reasonable to assume that advanced LC patients with severe to moderate deficiency of ADAMTS13 : AC (<3% to ~25% of normal control) could be candidates for FFP infusion as a source of ADAMTS13. It is necessary to evaluate the effectiveness of FFP administration to patients with ADAMTS13 : AC levels from 25% to 50%.

Alternatively, our recent study demonstrated that plasma ADAMTS13 : AC is reduced in VOD patients after stem cell transplantation (SCT) (12–32% of normal) compared to non-VOD patients (57–78% of normal), even before any conditioning regimen and throughout SCT, and that the activity might thus be a predictor for the development of hepatic VOD [22]. A multicenter, prospective, randomized controlled study revealed that prophylactic FFP infusion may be instrumental in preventing the development of hepatic VOD after SCT [23]. The imbalance caused by decreased ADAMTS13 : AC versus increased production of VWF : Ag before and during the early stage after SCT would contribute to a microcirculatory disturbance that could ultimately lead to VOD [23]. The supplementation of ADAMTS13 by prophylactic FFP infusion may suppress the increase in VWF : AG that is extensively released from damaged SEC. Furthermore, we first reported in 2006 that a significant reduction of ADAMTS13 : AC with a concomitant appearance of UL-VWFM was consistently observed in patient plasma soon after liver transplantation [31]. These changes were closely related to liver-graft dysfunction, ischemia-reperfusion injury, and acute rejection. The ADAMTS13 : AC often decreased to less than 10% of normal controls, concurrent with severe thrombocytopenia. The organ dysfunction appeared to be restricted to the liver graft, indicating that a decrease of plasma ADAMTS13 : AC coupled with the appearance of UL-VWFM was attributed to a mechanism of "local TTP" within the liver graft [21, 31]. It is, therefore, extremely important to monitor plasma ADAMTS13 : AC in the treatment of thrombocytopenia associated with allograft dysfunction after liver transplantation. This is because the infusions of platelet concentrate under conditions of an imbalance of decreased ADAMTS13 : AC to enhanced UL-VWFM production might further exacerbate the formation of platelet aggregates mediated by uncleaved UL-VWFM, leading to graft failure via the "local TTP" mechanism [21, 31]. FFP infusion as ADAMTS13 replacement therapy may improve both liver dysfunction and thrombocytopenia in liver transplant patients. From this point of view, we are particularly interested in conducting clinical trials with recombinant ADAMTS13 preparations not only in patients with advanced LC but also in patients with VOD and liver transplantations.

9. Conclusion and Future Perspectives

The introduction of ADAMTS13 to the field of hepatology not only enabled us to confirm the diagnosis of TTP early but also provided novel insight into the pathophysiology of liver diseases. Some diseases were shown to be TTP itself, but others did not show any apparent clinical features of TTP, even in the presence of extremely decreased ADAMTS13 : AC and increased UL-VWFM corresponding to TTP. Such TTP-like states, but without disseminated intravascular coagulation, might be "subclinical TTP" as seen in advanced liver cirrhotics [30] and SAH patients [24–27] or "local TTP" as shown in patients with hepatic VOD after SCT [22, 23] and patients with adverse events after living-donor liver transplantation [31, 32]. Essentially, one would be unable to detect such TTP-like phenomena without the determination of ADAMTS13 : AC, because the interaction of ADAMTS13 and UL-VWFM is the initial step in hemostasis, and their abnormalities do occur in the absence of apparent imbalance in other hemostatic factors and/or irrespective of the presence or absence of abnormal conventional hemostatic factors. The origin of VWF, the substrate of ADAMS13, indeed may be transformed hepatic sinusoidal and/or extrahepatic endothelial cells, but not hepatocytes. The procoagulant and anticoagulant proteins synthesized in hepatocytes decrease as liver disease progresses, whereas VWF markedly increases. Under such circumstances, ADAMTS13 deficiency may lead to a microcirculatory disturbance not only in the liver, but also in the systemic circulation. The determination of ADAMTS13 and its related parameters thus will be quite useful for improved understanding of the pathophysiology and for providing appropriate treatments especially in severe liver disease patients. It will be necessary to measure ADAMTS13 : AC when patients with unexplained thrombocytopenia are encountered in the course of liver disease. When "subclinical or local TTP" status would be confirmed, FFP infusion as ADAMTS13 replacement therapy may improve both liver dysfunction and thrombocytopenia. Further investigation will be necessary to define candidates for ADAMTS13 supplementation therapy and to evaluate its potential therapeutic efficacy in advanced LC patients.

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