The bleeding frequency of gastric varices is lower than that of esophageal varices. However, bleeding from gastric varices is more serious than that from esophageal varices, with mortality rates of 45–55% [4, 7, 16, 17]. Kim et al. [18] reported cumulative bleeding rates from gastric varices at 1, 3 and 5 years of 16, 36 and 44%, respectively. Treatment for gastric varices in danger of bleeding is thus clinically important, but adequate therapeutic options have yet to be established. B-RTO was recently reported to be an effective new method [8–10]. Gastric varices thrombosed by B-RTO usually show marked shrinking and complete resolution, with recurrence rates of only 0–10% [2, 6, 8, 9, 11, 19, 20]. This represents an excellent outcome. Our results are consistent with those reports.

For acute bleeding, B-RTO can be performed after any hemostatic procedure, which is the main limitation of B-RTO. Some reports described that outcome of B-RTO was comparable between urgent cases and elective cases [21] and that a previous episode of bleeding was not a significant prognostic factor [9, 10, 21]. It is difficult to treat huge gastric fundal varices by endoscopic injection sclerotherapy without balloon occlusion of the gastrorenal shunt [22, 23]. All the urgent cases, including two patients who underwent endoscopic injection sclerotherapy, were in stable condition at the time of treatment and encountered no complications in this study. Because it is desirable to perform B-RTO after endoscopic hemostatic procedures with bleeding gastric varices with gastrorenal shunts, these patients should be referred to an institution in which B-RTO can be performed immediately after such transient endoscopic hemostatic procedures [21].

To establish a standard treatment for gastric varices, detailed evaluation of long-term results after B-RTO is necessary. Our study showed long-term results after B-RTO in terms of hemodynamics and liver function.

Some hemodynamic reports have shown increased portal blood flow after B-RTO, but these were short-term results within 4 weeks after B-RTO [9, 11–14]. The present findings showed increased portal blood flow 1 year after B-RTO.

In terms of survival, several reports have shown 5-year survival rates of 54–67% [9, 10, 19, 21]. Our results are consistent with those reports. Kim et al. [18] reported that most deaths occurred within 1 year after bleeding of gastric varices and that cumulative survival at 1 year was 48%. In this study, two patients died within 1 year of B-RTO with one of the patients dying from advanced HCC with portal vein tumor thrombosis and the other patient dying from hepatic failure with liver cirrhosis. Although serum albumin levels for both patients were low before their deaths, the levels were 3.3 and 2.5 g/dl, respectively, before B-RTO and 3.6 and 2.4 g/dl, respectively, 1 month after B-RTO. The Child-Pugh scores for the two patients were 7 and 8, respectively, before B-RTO and 7 and 9, respectively, 1 month after B-RTO. Accordingly, we do not believe that B-RTO contributed to a worsening of their liver functions. Although the benefit of B-RTO remains unclear in Child-Pugh class C patients [21], B-RTO can be used on patients with a poor liver function reserve or advanced HCC for emergency treatment to control bleeding from gastric varices that are among the most difficult variceal sites for treatment [9]. We think that the value of B-RTO in survival is to reduce death due to bleeding of gastric varices. Some reports have described the presence or absence of concomitant HCC and Child-Pugh classification as prognostic factors related to survival [9, 10]. However, the present study was unable to show any factors significantly associated with survival. More cases need to be accumulated.

In this study, serum albumin was significantly increased 1 year after B-RTO. Some reports have indicated that B-RTO increases portal blood flow to the liver parenchyma and contributes to improved liver function [9, 11–14]. Accumulation of more cases might reveal correlations between serum albumin parameters and portal blood flow changes in the portal trunk.

Growth of collateral veins represents one problem after B-RTO. Several reports have identified worsening of esophageal varices in 10–63% of cases [2, 6, 9, 19, 20, 24]. The worsening rate of esophageal varices in the present study was 43% at 1 year after B-RTO.

Two patients required endoscopic injection sclerotherapy within 1 year after B-RTO, as the form of esophageal varices changed from small straight to enlarged tortuous with red color sign. Esophageal varices tended to worsen about five times more frequently in patients with esophageal varices before B-RTO than in those without them [13]. Endoscopic examination appears extremely important for identifying worsening of esophageal varices after B-RTO [25].

B-RTO can be expected to elevate the portal pressure gradient because of obstruction of a large gastrorenal shunt. Ascites thus represents another problem, along with growth of collateral veins because of the elevated portal pressure gradient after B-RTO. The ascites score was significantly increased after B-RTO in this study, but this did not represent a serious problem, and control was easily achieved with pharmacotherapy. We propose that ascites should be dealt with after B-RTO. Although there was no significant change in the Child-Pugh scores reported in this study, some reports have indicated an improvement in Child-Pugh scores after B-RTO [9, 10]. An increase in portal blood flow from obliteration of large portosystemic shunts might contribute to an improvement in liver function [21]. Changes in Child-Pugh scores after B-RTO in this study seemed to depend on an increase in portal blood flow as

well as complications from the underlying chronic liver diseases such as ascites and advanced HCC.

In conclusion, we believe that B-RTO offers an effective method for treating gastric varices with gastrorenal shunt, although observation of esophageal varices and ascites may be necessary after obliteration.

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CLINICAL STUDIES

Assessment of hepatic fibrosis by analysis of the dynamic behaviour of microbubbles during contrast ultrasonography

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Abstract

Background/aims: Microbubble behaviour from the portal vein to the liver parenchyma may reflect haemodynamic changes because of hepatic fibrosis. The aim of this study was to determine the efficacy of contrast-enhanced ultrasound (US) with Sonazoid™ for the assessment of the grade of hepatic fibrosis. *Methods:* This prospective study evaluated 117 patients with chronic liver disease (chronic hepatitis 85; cirrhosis 32) and 34 controls. All subjects received both contrast-enhanced US with Sonazoid™ for 1 min after the agent injection and subsequent liver biopsy. Flow velocity and flow volume in the right portal vein, onset time of contrast enhancement in the right hepatic artery and right portal vein, maximum intensity ratio between the intrahepatic portal vein and liver parenchyma, and time interval between the onset time and the time of maximum intensity ratio were compared with the pathological findings. *Results:* Among the evaluated parameters, time interval between the onset time and the time of maximum intensity ratio showed the closest relationship with the grade of hepatic fibrosis: 4.21 ± 1.32 for controls ($n = 34$), 5.58 ± 1.39 for F1 ($n = 31$), 6.79 ± 1.77 for F2 ($n = 28$), 8.85 ± 1.97 for F3 ($n = 26$) and 14.3 ± 3.49 for cirrhosis ($n = 32$); controls vs. F2, $P = 0.0004$; F1 vs. F3, $P < 0.0001$; F2 vs. F3, $P = 0.0177$; F3 vs. cirrhosis, $P < 0.0001$. The areas under the receiver operating characteristic curves of the time interval were 0.94, 0.96 and 0.98 for the diagnosis of marked fibrosis ($\geq$F2), advanced fibrosis ($\geq$F3) and cirrhosis respectively. *Conclusions:* Contrast-enhanced US with Sonazoid™ may be a promising method for the indirect evaluation of hepatic fibrosis.

Chronic liver disease is increasing in prevalence worldwide and is one of the most important clinical problems because it is a high-risk factor for the development of portal hypertension and hepatocellular carcinoma (1–3). The severity of chronic liver disease depends on the grade of hepatic fibrosis, whose assessment supports the clinical management of these patients (4, 5). Liver biopsy remains the gold standard for the evaluation of the grade of hepatic fibrosis, in spite of its invasiveness in patients with impaired coagulation (6, 7) and the possibility of sampling error because of the heterogeneous distribution of fibrosis (8). Because patients with chronic liver disease require long-term follow-up, a repeatedly available noninvasive method is preferable for the assessment of hepatic fibrosis (9–12).

Thanks to its noninvasiveness and convenience, ultrasound (US) is one of the procedures applied most frequently for the periodic evaluation of diffuse liver disease. However, as the diagnostic accuracy of US for cirrhosis is not high, it is not regarded as a reliable method for the evaluation of the grade of hepatic fibrosis (11, 12). In recent times, the development of microbubble contrast agents has increased the diagnostic capability of US, and harmonic imaging with second-generation contrast agents confers a stable enhancement effect in the liver with improved signal-to-noise ratio (13–17). Analysis of the dynamic behaviour of microbubbles may enable a noninvasive evaluation of the severity of chronic liver disease (18–21).

Hepatic fibrosis in the downstream area may affect the inflow haemodynamics of the upstream portal vein, and the behaviour of microbubbles between the portal vein and liver parenchyma may reflect the vascular resistance, according to the grade of hepatic fibrosis. On this basis, we measured the changes of intensity ratio between the intrahepatic portal vein and liver parenchyma during contrast enhancement with Sonazoid™ (13, 15, 17) and compared the results with the histological grade of fibrosis in patients with chronic hepatitis or cirrhosis, and control subjects. The aim of this study was to determine the efficacy of contrast-enhanced US with Sonazoid™ for the assessment of the grade of hepatic fibrosis.

Patients and methods

Patients

This prospective study was carried out in our department from December 2007 to December 2009. The inclusion criteria for patients were as follows: (i) chronic liver disease patients without history or clinical signs of liver tumour, (ii) patients for whom liver biopsy was scheduled. Healthy volunteers without signs of liver disease were evaluated as controls. The exclusion criteria for all the participants included the presence of liver tumours, portal vein thrombus or vascular abnormalities such as reversed flow, arterio-portal communication or obstruction, use of vasoactive drugs, significant alcohol consumption (> 20 g/day) within 2 months, pregnancy and the presence of egg allergy, which is a contraindication of Sonazoid™ (GE Healthcare, Oslo, Norway).

There were 161 participants: 127 patients with chronic liver disease (54 males, age 50.2 ± 13.7 years, 26–78; 73 females, age 56.2 ± 11.2 years, 23–76) and 34 controls (17 males, age 48.2 ± 16.9 years, 26–82; 17 females, age 53.5 ± 17.5 years, 25–85). The 127 patients underwent liver biopsy; however, the specimens were inadequate for fibrosis staging in 7 (5.5%) of them. US examination before contrast-enhanced US detected focal hepatic lesions in two patients and a portal vein thrombus in one patient. Therefore, 10 patients were excluded and the remaining 151 subjects were the participants in this study. There were 85 patients with chronic hepatitis (34 males, age 49.4 ± 15.2 years, 26–78; 51 females, age 55.9 ± 10.8 years, 23–73) and 32 patients with cirrhosis (12 males, age 55.5 ± 12.7 years, 37–75; 20 females, age 64.9 ± 8.04 years, 46–76). The mean body mass index of all subjects was 22.9 ± 3.78 kg/m^2 (16–37). Seventeen patients with cirrhosis were classified as Child–Pugh grade A and 15 were classified as grade B. The causes of chronic liver disease were as follows: viral in 90 patients (hepatitis C virus in 74 and hepatitis B virus in 16), alcohol abuse in six patients, nonalcoholic steatohepatitis in nine patients, autoimmune hepatitis in eight patients, primary sclerosing cholangitis in one patient and cryptogenic in three patients. Laboratory tests, including aspartate transaminase (AST, IU/L), alanine transaminase (ALT, IU/L) and platelet count (10^9/L), were carried out on all subjects to calculate the APRI (AST/35 × 100/platelet count) and FIB4 [age × AST/(platelet count × ALT$^{0.5}$)] as indirect markers of fibrosis (Table 1) (22).

The study protocol was in accordance with the Declaration of Helsinki and was approved by the ethics committee of our department. Informed written consent was obtained from all participants.

Ultrasound examination

The equipment was AplioXG (Toshiba, Tokyo, Japan) with a 3.75 MHz convex probe. US examinations were performed under the supine position with more than 4-h fasting.

Firstly, noncontrast grey-scale US was carried out to screen for focal hepatic lesions or portal vein thrombi. Then, colour Doppler US was performed to determine the presence or absence of vascular abnormalities. Next, a scan plane for the right lobe of the liver was selected to observe the main branch of the intrahepatic right portal vein and the right hepatic artery. Pulsed Doppler US was performed to measure the mean flow velocity and mean flow volume of the right portal vein. Sampling width was set according to the diameter of the vessel, and the angle between the US beam and the vessel was equal to or < 60° in all measurement procedures.

After that, contrast-enhanced US was performed with harmonic imaging (15 Hz) under the low mechanical index of 0.25, which has been used for Sonazoid™ in a published study (17). The depth was set to cover the entire right lobe of the liver, with a focus point 8 cm below the skin surface under right intercostal scan. Gain was adjusted at an optimal level and the dynamic range was set at 55 dB.

We injected the contrast agent Sonazoid™ (0.0075 ml/kg) manually into the antecubital vein at a rate of 1.0 ml/s, followed by a 3.0 ml flush of normal saline, and immediately started the chronometer of US equipment. The participants were asked to breathe shallowly and gently after the injection. Contrast-enhanced sonograms were taken for 1 min after the agent injection and all the cine images were recorded digitally on the hard disc of the US system.

Table 1. Clinical and biochemical data of subjects

	Controls, $n = 34$	Chronic hepatitis, $n = 85$	Cirrhosis, $n = 32$	P
Age, years	50.9 ± 17.1 (25–85)	53.3 ± 13.1 (23–78)	61.4 ± 10.9 (37–76)	0.01
Gender, male/female	17/17	34/51	12/20	0.56
Body mass index, kg/m^2	21.7 ± 2.78 (18–27)	23.2 ± 3.87 (16–34)	24.1 ± 4.45 (16–37)	0.04
HCV/HBV/alcohol/NASH/AIH/PSC/cryptogenic	–	60/15/0/5/5/0/0	14/1/6/4/3/1/3	
Presence of ascites	–	–	6	
Platelet count, 10^9/L	245 ± 49.5 (163–340)	188 ± 51.7 (97.0–335)	117 ± 91.0 (44.0–430)	< 0.01
APRI	0.23 ± 0.08 (0.11–0.52)	1.03 ± 1.00 (0.20–5.70)	1.76 ± 1.02 (0.33–5.07)	< 0.01
FIB4	1.02 ± 0.56 (0.30–2.57)	2.22 ± 1.53 (0.39–7.90)	6.40 ± 3.32 (1.04–13.2)	< 0.01
Histological staging, F0/F1/F2/F3/cirrhosis	–	0/31/28/26/0	0/0/0/0/32	
Child-Pugh grade, A/B/C	–	–	17/15/0	

AIH, autoimmune hepatitis; ALT (IU/L), alanine transaminase; APRI, (AST/35) × 100/platelet count; AST (IU/L), aspartate transaminase; FIB4, age × AST/(platelet count/ALT$^{0.5}$); HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PSC, primary sclerosing cholangitis.

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The US operators were HI in all 151 subjects and MT in 17 subjects of them, both with a 7-year experience of US examination. Inter-observer variability was examined in 17 subjects (controls five, chronic hepatitis three, cirrhosis nine), with the second US examination being performed within 7 days (3.6 ± 2.3) of the initial examination. Intra-observer variability by operator HI was examined in 10 subjects (controls two, chronic hepatitis four, cirrhosis four), with the second US examination being performed within 6 days (2.6 ± 1.9) of the initial examination. The results of the second US examination were used only to measure inter- or intra-observer variability. Clinical symptoms and vital signs of blood pressure, heart rate and oxygen saturation were checked before and after US examinations.

Liver biopsy and pathological examination

One hundred and seventeen patients (chronic hepatitis 85, cirrhosis 32) received liver biopsy within a week of the contrast-enhanced US examination. Biopsy specimens were obtained by percutaneous needle biopsy (16 G needle; BARD, Tempe, AZ, USA) in 111 patients without ascites and transjugular liver biopsy (18 G needle; Cook, Bloomington, IN, USA) in six patients with ascites. Paraffin-embedded specimens were stained with haematoxylin–eosin and Azan. Two experienced hepatologists (F. I., K. F.) evaluated the fibrosis stage according to the staging scoring system recommended by Desmet *et al.* (4) and Scheuer (7).

Data analysis

Contrast analysis was performed using an off-line personal computer with IMAGELAB-AVI software (Toshiba, Tokyo, Japan) by H. M., who was not an operator of US and was not aware of any information regarding the subjects. Firstly, we observed the cine images with frame-by-frame playback to find the first frame showing the arrival of the contrast agent in the right hepatic artery or right portal vein. The time between the agent injection and the first frame of contrast arrival in each vessel was defined as the onset time of contrast enhancement, which may reflect the extrahepatic haemodynamics of microbubbles (Fig. 1). Then, we prepared two circular regions of interest in the liver at the same depth: one for the right portal vein and the other for liver parenchyma. These two regions of interest, which were of an equal diameter, were set manually on the series of successive images for 1 min with frame-by-frame advance for analysis after the exclusion of inappropriate, blurred images (Fig. 2). Automatic calculation of the intensity ratio between the right portal vein and liver parenchyma in each frame provided a time-related intensity ratio curve featuring the intrahepatic haemodynamics of the microbubbles. The maximum intensity ratio between the right portal vein and liver parenchyma, and time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio between the right portal vein and liver parenchyma were measured on this curve (Fig. 3). Inter- and intra-observer variability of measured parameters was calculated from the coefficient of variation obtained by standard deviation/mean $\times$ 100.

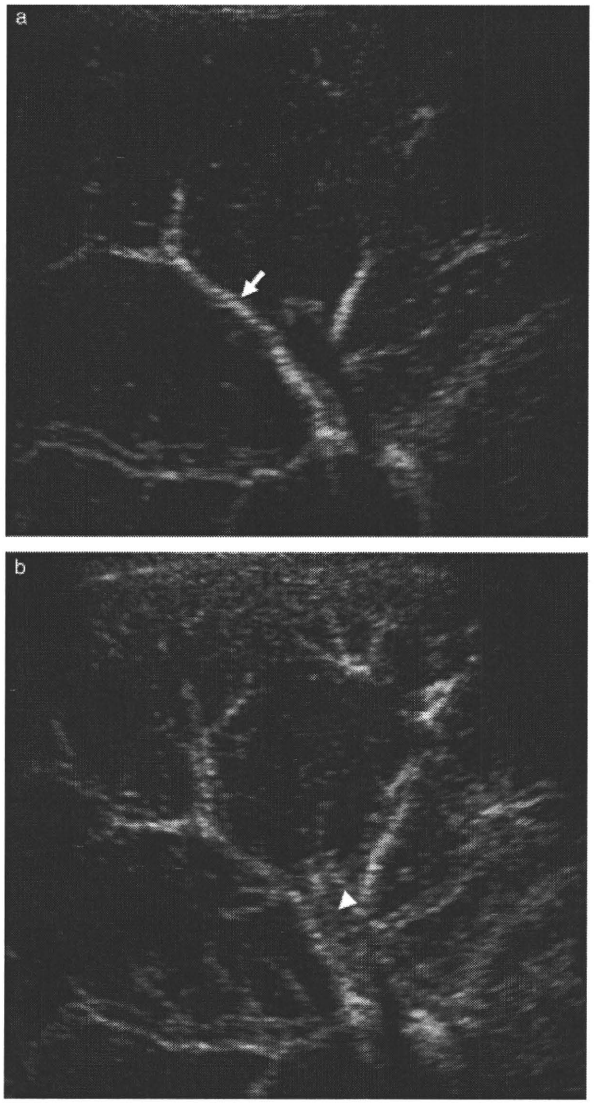

Fig. 1. Contrast-enhanced ultrasound images under right intercostal scan. (a) Onset time of contrast enhancement in the right hepatic artery: onset time of contrast enhancement was 14 s after the injection of Sonazoid™ in the right hepatic artery (arrow). (b) Onset time of contrast enhancement in the right portal vein: onset time of contrast enhancement was 16 s after the injection of Sonazoid™ in the right portal vein (arrowhead).

Statistical analysis

All data were expressed as mean $\pm$ standard deviation, range or percentage. Comparison of age, body mass

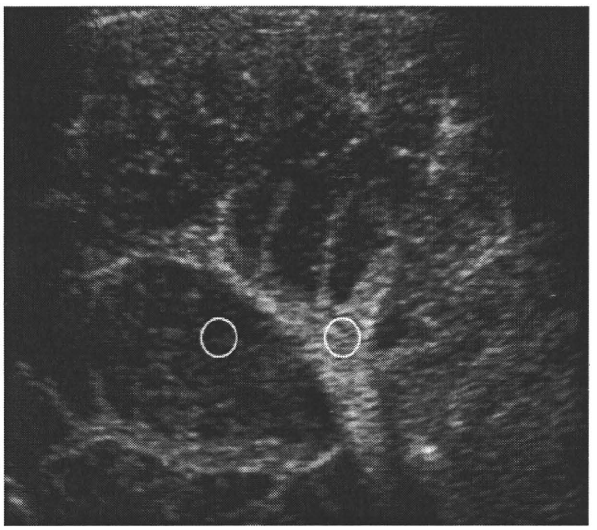

Fig. 2. Measurement of the intensity in the right portal vein and liver parenchyma. Two circular regions of interest were set on the right portal vein and adjacent liver parenchyma at the same depth.

index, platelet count, APRI, FIB4, mean flow velocity and mean flow volume in the right portal vein, onset time of contrast enhancement in the right hepatic artery and right portal vein, the maximum intensity ratio between the right portal vein and liver parenchyma, and the time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio, with the grade of fibrosis was performed by analysis of variance using the Scheffe *post hoc* test. The χ^2-test was used to compare the gender in three groups (controls, chronic hepatitis and cirrhosis). Areas under the receiver operating characteristic curves (AUC) with 95% confidence interval were calculated for the prediction of marked fibrosis ($\geq$F2), advanced fibrosis ($\geq$F3) and cirrhosis in the time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio, FIB4 and APRI. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were calculated for the best cut-off values obtained for each fibrosis stage. Probability values < 0.05 were considered to be significant. All statistical analyses were performed using the SPSS package (version 17.0J; SPSS, Chicago, IL, USA). AUC were obtained using ROCKIT1.1B2 (23).

Results

Results of liver biopsy

The mean length of the liver biopsy specimens was 21.6 ± 3.51 (15–25) mm, and the number of portal tracts was 13.5 ± 4.61 (11–30). The fibrosis stages by the consensus reading of results were F0 in 0 (0%), F1 in 31 (26.5%), F2 in 28 (23.9%), F3 in 26 (22.2%) and cirrhosis in 32 (27.4%) (Table 1).

Blood flow measurement in the right portal vein

The mean flow velocity (cm/s) of the right portal vein was 11.4 ± 2.04 (7.90–19.3) in controls, 10.4 ± 1.94 (5.90–15.1) in chronic hepatitis and 8.96 ± 1.84 (5.50–13.3) in cirrhosis. The mean flow volume (ml/min) of the right portal vein was 342 ± 101 (160–820) in controls, 318 ± 114 (100–760) in chronic hepatitis and 295 ± 136 (90.0–710) in cirrhosis. There were significant differences in mean flow velocity (controls vs. chronic hepatitis, $P = 0.0259$; controls vs. cirrhosis, $P < 0.0001$; and chronic hepatitis vs. cirrhosis, $P = 0.0113$), showing no significant differences among F1, F2 and F3. There were no significant differences in mean flow volume among controls, chronic hepatitis and cirrhosis ($P = 0.3134$). Interobserver variability was 10% for mean flow velocity and 15% for mean flow volume, and intra-observer variability was 8.7% for mean flow velocity and 14% for mean flow volume.

Relationship between the parameters of contrast enhancement and the degree of hepatic fibrosis

The onset time of contrast enhancement in the right hepatic artery was 14.3 ± 2.22 s (11–21) in controls, 15.0 ± 3.22 s (9–24) in chronic hepatitis and 14.1 ± 3.15 s (6–23) in cirrhosis and that in the right portal vein was 16.9 ± 2.44 s (13–22) in controls, 18.4 ± 3.96 s (12–29) in chronic hepatitis and 18.3 ± 3.39 s (8–26) in cirrhosis. There were no significant differences in the onset time of contrast enhancement in the right hepatic artery and right portal vein among the three groups (Fig. 4). The maximum intensity ratio between the right portal vein and liver parenchyma was 22.2 ± 5.84 dB (12–34) in controls, 19.2 ± 4.66 dB (13–32) in F1, 17.8 ± 3.73 dB (6–24) in F2, 16.1 ± 5.27 dB (8–30) in F3 and 13.8 ± 5.34 dB (3–21) in cirrhosis (controls vs. F2, $P = 0.0219$; controls vs. F3, $P = 0.0005$; controls vs. cirrhosis, $P < 0.0001$; F1 vs. cirrhosis, $P = 0.0023$) (Fig. 5). The time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio was 4.21 ± 1.32 s (1–7) for controls, 5.58 ± 1.39 s (2–10) for F1, 6.79 ± 1.77 s (4–13) for F2, 8.85 ± 1.97 s (6–14) for F3 and 14.3 ± 3.49 s (9–21) for cirrhosis, and significant differences were found between controls and F2 ($P = 0.0004$), F1 and F3 ($P < 0.0001$), F2 and F3 ($P = 0.0177$), and F3 and cirrhosis ($P < 0.0001$) (Fig. 3). Six patients with ascites were diagnosed with cirrhosis by biopsy specimens obtained and their time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio was 12.2 ± 2.40 s (10–14). Inter-/intra-observer variability was 6.1%/5.5% for onset time of contrast enhancement in the right hepatic artery, 7.1%/6.7% for onset time of contrast enhancement in the right portal vein, 7.5%/7.2% for the maximum intensity ratio between the right portal vein and liver parenchyma and 6.0%/5.7% for the time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio. No adverse effect was observed during and after the US examination.

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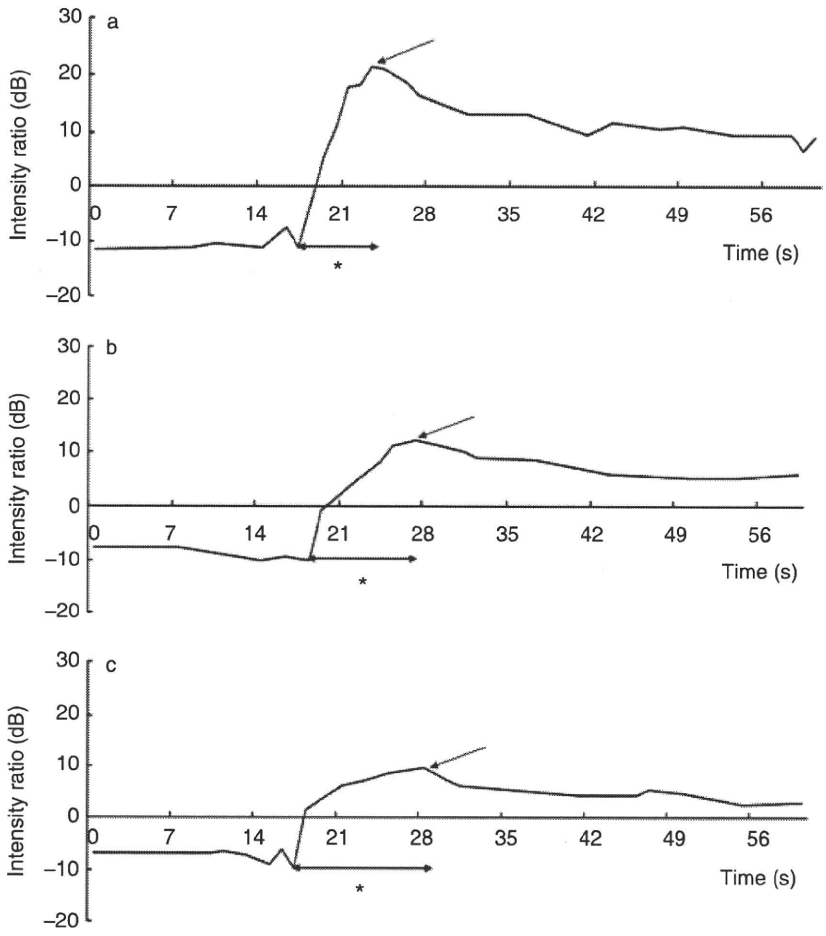

Fig. 3. Time-related changes in intensity ratio between the right portal vein and liver parenchyma. (a) Control subject (56 years old, female): the maximum intensity ratio between the right portal vein and liver parenchyma was 22 dB and time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio was 6 s. (b) Patient with chronic hepatitis (52 years old, female, hepatitis C virus, F3): the maximum intensity ratio between the right portal vein and liver parenchyma was 13 dB and time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio was 9 s. (c) Patient with cirrhosis (56 years, female, hepatitis C virus): the maximum intensity ratio between the right portal vein and liver parenchyma was 9 dB and time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio was 12 s. Arrow, maximum intensity ratio between the right portal vein and liver parenchyma.
*Time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio between the right portal vein and liver parenchyma.

Diagnostic value of time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio for the grade of fibrosis

The AUC of the time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio were 0.94 (0.89–0.97) for marked fibrosis with the best cut-off value of 6.5 s, 0.96 (0.93–0.98) for advanced fibrosis with the best cut-off value of 8 s and 0.98 (0.95–0.99) for cirrhosis with the best cut-off value of 9.5 s. Six patients with ascites had a time interval from 10 to 14 s, which ranged over the best cut-off value. These AUC values were significantly higher than those of APRI and FIB4: 0.86 (0.79–0.92) and 0.85 (0.79–0.91) for marked fibrosis, 0.85 (0.78–0.90) and 0.89 (0.82–0.94) for advanced fibrosis and 0.80 (0.71–0.86) and 0.90 (0.82–0.95) for cirrhosis respectively (Table 2, Fig. 6). Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 84, 88, 84, 88 and 87% for marked fibrosis, 83, 93, 89, 90 and 89% for advanced fibrosis and 95, 92, 77, 98 and 93% for cirrhosis respectively.

Discussion

The present study revealed that the maximum intensity ratio between the right portal vein and liver parenchyma

Table 2. Value of non-invasive parameters for the diagnosis of marked fibrosis, advanced fibrosis and cirrhosis: comparison of AUC among time interval, APRI, and FIB4

	Marked fibrosis (≥F2)	Advanced fibrosis (≥F3)	Cirrhosis
Time interval	0.94 (0.89–0.97)	0.96 (0.93–0.98)	0.98 (0.95–0.99)
APRI	0.86 (0.79–0.91)	0.85 (0.78–0.90)	0.80 (0.71–0.86)
FIB4	0.85 (0.79–0.91)	0.89 (0.82–0.94)	0.90 (0.82–0.95)

AUC, areas under the receiver operating characteristic curves; marked fibrosis, ≥F2; advanced fibrosis, ≥F3; time interval, time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio between right portal vein and liver parenchyma.

decreased and the time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio was prolonged according to the progression of hepatic fibrosis, and particularly the latter, being superior to both APRI and FIB4, had the closest relationship with the degree of hepatic fibrosis. Because the flow velocity in the right portal vein in our study tended to decrease according to the progression of hepatic fibrosis, as also reported previously (10), the rate of filling the right portal vein with microbubbles may be lower. Meanwhile, an arterialization related to hepatic fibrosis may compensate the rapid parenchymal enhancement with high blood flow (24). These factors may explain the decrease of the maximum intensity ratio between the right portal vein and liver parenchyma and prolongation of time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio, according to the progression of fibrosis. However, despite the maximum intensity ratio between the right portal vein and liver parenchyma, time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio did show a close relationship with the progression of fibrosis. The former parameter represents only the peak gradient of microbubble distribution between the portal vein and liver parenchyma, while the latter parameter is coupled with time. Although the precise mechanism remains unclear, time-related haemodynamics, linked to the microbubble distribution from the upstream vessel to the periphery, might have the advantage of indicating the degree of hepatic fibrosis. In fact, it is suggested that several pathophysiological conditions, such as the presence of intrahepatic shunt and/or hyperdynamic circulatory state (11), affected the parameters measured in this study. However, we believe that time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio may represent an indirect parameter for assessing the degree of hepatic fibrosis in a comprehensive manner. Contrast-enhanced US with Sonazoid™ may have the possibility to reduce the biopsy procedure to examine the severity of hepatic fibrosis.

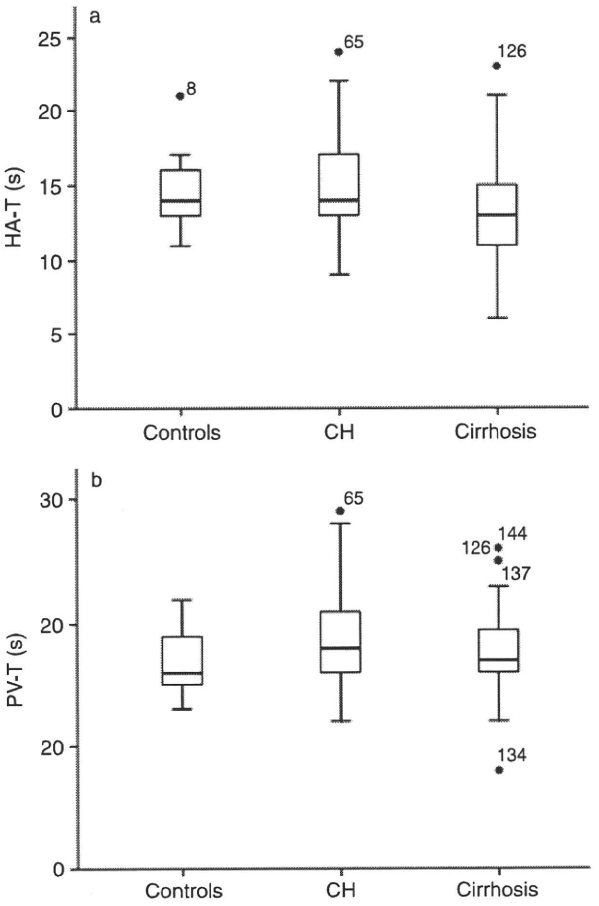

Fig. 4. Onset time of contrast enhancement in the right hepatic artery and the right portal vein. (a) Onset time of contrast enhancement in the right hepatic artery (s): there were no significant differences of the onset time of contrast enhancement in the right hepatic artery among the three groups ($P=0.0929$). (b) Onset time of contrast enhancement in the right portal vein (s): there were no significant differences of the onset time of contrast enhancement in the right portal vein among three groups ($P=0.1564$). Data are expressed by box-and-whisker plots. The top and bottom of the boxes indicate upper and lower quartiles, respectively, and the horizontal line in the bar represents the median value. The two horizontal lines outside the box (whisker) indicate the smallest and largest nonoutlier observations.

We also examined the two parameters, onset time of contrast enhancement in the right hepatic artery and right portal vein, which did not show significant correlation with the degree of hepatic fibrosis. The previous study reported similar results, in spite of the usage of different contrast agents (21). As these parameters may be affected by general conditions of systemic circulation as well as specific conditions caused by portal hypertension such as intrapulmonary shunt, hyperdynamic state, splenomegaly and development of extrahepatic collateral vessels, they may not be ideal factors to assess the degree of hepatic fibrosis.

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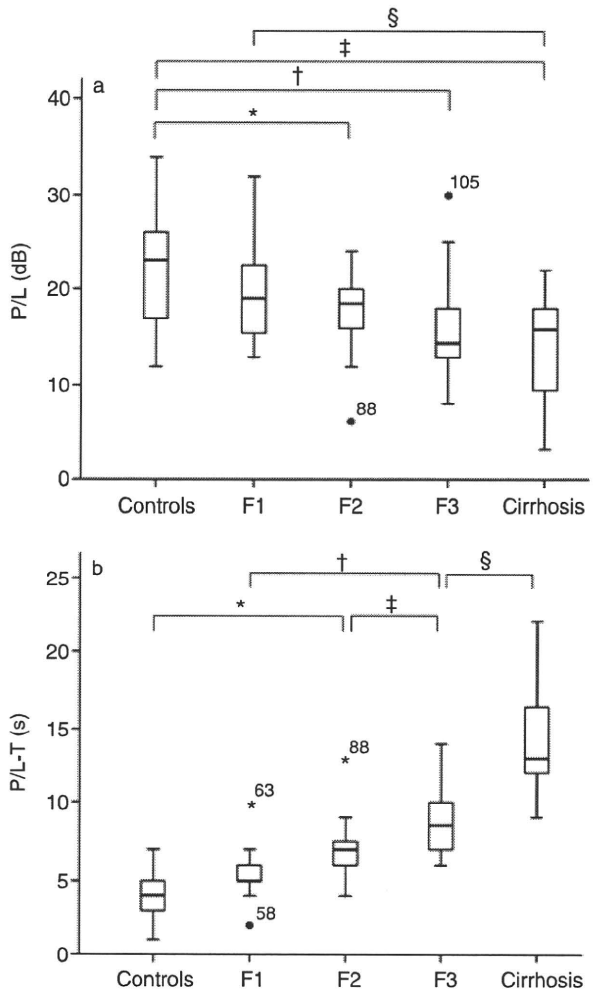

Fig. 5. Maximum intensity ratio between the right portal vein and liver parenchyma and time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio in relation to the grade of hepatic fibrosis. (a) Maximum intensity ratio between the right portal vein and liver parenchyma (dB): significant differences were found between controls and F2 (*$P = 0.0219$), controls and F3 (†$P = 0.0005$), controls and cirrhosis (‡$P < 0.0001$) and F1 and cirrhosis (§$P = 0.0023$). (b) Time Interval, time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio (s): significant differences were found between controls and F2 ($P = 0.0004$), F1 and F3 ($P < 0.0001$), F2 and F3 ($P = 0.0177$), and F3 and cirrhosis ($P < 0.0001$).

Initial study for the diagnosis of cirrhosis using a US contrast agent was performed by Albrecht *et al.* (18) as a transit time analysis with Levovist® (Schering, Berlin, Germany). Similar studies also have reported the usefulness of the second-generation contrast agent SonoVue® (Bracco, Milan, Italy) to diagnose chronic hepatitis and cirrhosis (19, 20). However, their approach was not satisfactory in differentiating the severity of fibrosis in chronic liver disease. The authors speculate that there are some limitations in transit time analysis: transit time is influenced by various changes in the intrahepatic circulation in the progress of liver disease as well as the grade of hepatic fibrosis, and transit time includes only the time factor without the distribution factor of microbubble in the liver parenchyma. Against these backgrounds, we hypothesized that increased hepatic resistance caused by fibrosis may affect the inflow haemodynamics of microbubble to the liver, and we focused on the investigation of the microbubble behaviour, from flowing into the liver to its distribution in the hepatic periphery. One of the reasons for the improved diagnostic ability for the degree of hepatic fibrosis may be that we used the parameter time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio', because it reflects microbubble distribution as well as time. There is another advantage in our technique, that is, stable observation for the main branch of the right hepatic artery and portal vein. As viral infection is the most common cause of chronic liver disease in our country, the liver becomes atrophic with deformity, which makes it difficult to observe both the hepatic vein and the hepatic artery on the same scan plane in some cirrhosis patients. Therefore, our methodology was more reasonable than transit time analysis for patients with chronic liver diseases in our country.

With a vibration-induced mechanical wave, transient elastography (FibroScan; Echosens, Paris, France) is attracting considerable attention as a noninvasive tool for the assessment of hepatic fibrosis (25, 26). A recent report by Friedrich-Rust *et al.* (25) provided several lines of evidence to suggest that FibroScan could predict the grade of hepatic fibrosis; the mean AUC for the diagnosis of marked fibrosis, severe fibrosis and cirrhosis were 0.84 (0.82–0.86), 0.89 (0.88–0.91) and 0.94 (0.93–0.95) respectively. However, because the FibroScan is specialized for the assessment of hepatic fibrosis alone, our technique may have an advantage in this regard, because patients with chronic liver disease receive regular US examination for the supervision of hepatocellular carcinoma, and the additional injection of contrast agent may not be so complicated a procedure. Furthermore, the predictive value of our results for the degree of hepatic fibrosis was almost the same as that of FibroScan. In addition, FibroScan is not suitable for patients with ascites, who are clear candidates for our technique. It is expected that the assessment of hepatic fibrosis by contrast-enhanced US could be carried out as an extension of routine US checkup. However, the assessment of liver fibrosis using noncontrast US might be a goal in the management of chronic liver disease.

Several factors may affect the intrahepatic microbubble behaviours involved with hepatic haemodynamics: causes of liver diseases as aetiological factors, and steatosis, ballooning and inflammation as histological factors. At this point, our study included chronic liver diseases with different kinds of causes, viral infection, alcohol abuse, nonalcoholic steatohepatitis, autoimmune

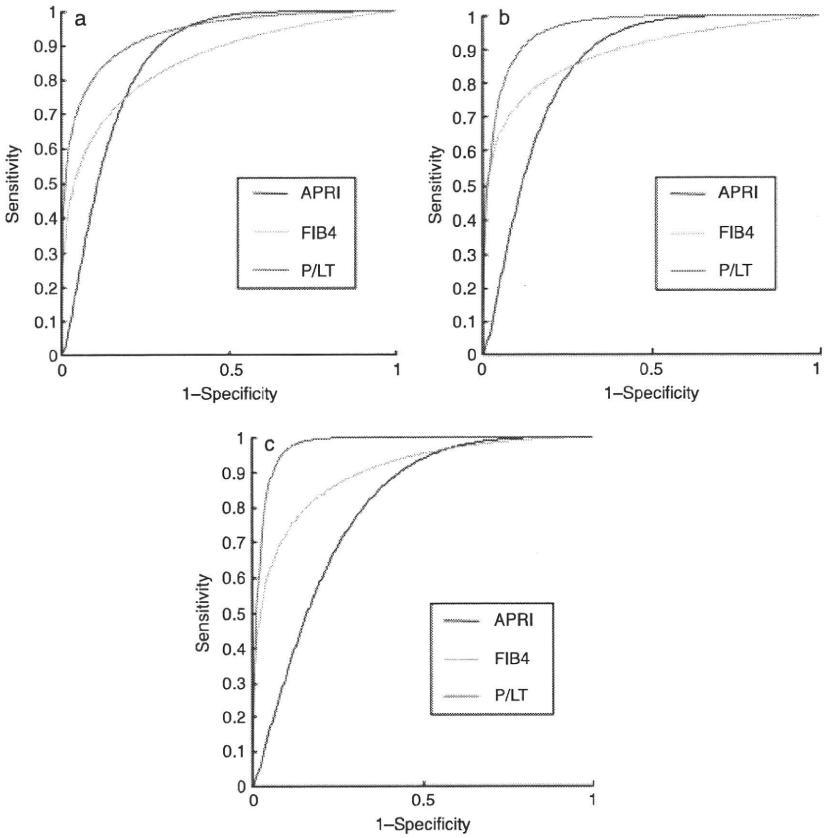

Fig. 6. Receiver operating characteristic curves of time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio, APRI and FIB4. (a) For marked fibrosis (≥F2): AUC values of time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio, APRI, and FIB4 were 0.94, 0.86 and 0.85. (b) For advanced fibrosis (≥F3): AUC values of time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio, APRI, and FIB4 were 0.96, 0.85, and 0.89. (c) For cirrhosis: AUC values of time interval from the onset of contrast enhancement in the right portal vein to the time of the maximum intensity ratio, APRI, and FIB4 were 0.98, 0.80 and 0.90. TI, time interval from the onset of contrast enhancement in the right portal vein to the time of maximum intensity ratio between right portal vein and liver parenchyma.

hepatitis, primary sclerosing cholangitis and cryptogenic, and the patients' population reflects the epidemiology of liver disease in our country. Any kind of chronic liver diseases that met our criteria were included in this study, because our technique aimed at representing the degree of hepatic fibrosis in spite of their different causes. However, as there was a potential bias of patient population in the causes of liver disease, our results presented neither cause-specific validity of contrast-enhanced US with Sonazoid™ to assess the grade of hepatic fibrosis nor relationship between the contrast-enhanced findings and the some pathological factors other than fibrosis, which remain to be solved.

There were some limitations in our study. Firstly, our result was obtained by the observation of only vascular-phase images induced by dynamic microbubble. It is well known that Sonazoid™ has a property of accumulating in the liver, and this is the most important difference between Sonazoid™ and SonoVue® (13–17, 19, 20). In fact, it has not been clarified when intrahepatic microbubble accumulation starts after the agent injection, and whether parenchymal enhancement in vascular phase might be associated with the accumulated microbubble in the liver. However, as contrast enhancement because of circulating microbubble may be dominant in the vascular phase, our study might not utilize fully the potential property of this new contrast agent. SonoVue® without accumulating property in the liver may also be acceptable in this type of study, which should be done in near future. Secondly, our study did not include cases with severe obesity that may limit the US observation, because they are in a relative minority in Japan. The results of the present study should be confirmed in different ethnic groups in different countries. Thirdly, onset time of contrast enhancement was assessed subjectively by visual investigation of recorded images. Although the intensity-based definition of onset time may be reliable, we thought positioning of the region of interest for intensity measurement was not always easy, particularly in the right hepatic artery because of their small caliber.

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Therefore, we defined the first frame showing that the arrival of the contrast agent in the vessel was the beginning of the contrast enhancement in this study. The first frame was found in the cine images with frame-by-frame playback, and interobserver variability for the onset of contrast enhancement was quite good. However, our results of the onset time should be confirmed objectively using the digital judgment method, which may be improved in the future.

In conclusion, we observed the contrast enhancement in the liver for 1 min after the injection of Sonazoid™, and found that time interval between the onset of contrast enhancement in the right portal vein and the time of maximum intensity ratio between the intrahepatic right portal vein and liver parenchyma was correlated significantly with the degree of hepatic fibrosis by the quantitative analysis of Sonazoid™-induced sonograms. Although this technique does not allow direct observation of hepatic fibrosis, it may be promising as an indirect evaluation tool for hepatic fibrosis.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinicopathological features of severe and fulminant forms of autoimmune hepatitis

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Abstract
Background and aims Diagnosis of the acute presentation of autoimmune hepatitis (AIH) is difficult because patients do not always show typical clinicopathological features of AIH. Although some of them progress to fulminant hepatitis, the survival rate of which is <20% without liver transplantation, their clinicopathological features have remained uncertain. We examined them for a better understanding and improvement of the prognosis of "life-threatening" severe and fulminant AIH.
Methods Clinical, biochemical and pathological features of 28 patients with severe or fulminant AIH and treatment responses were examined retrospectively.
Results At the time of admission, mean immunoglobulin G was 2479 ± 1170 mg/dl, with 7 (25%) patients showing normal levels. Anti-nuclear antibody was ≤1:40 in 8 (29%). Liver histology showed severe activity in 95% and acute hepatitis in 86% of the patients. Centrilobular necrosis including submassive and massive necrosis was characteristic. Of the 25 patients treated with corticosteroids, 17 responded and 8 did not. Responders to corticosteroids showed younger age and higher prothrombin time (PT) activity than non-responders at the time of corticosteroid administration. The improvement of PT activity during 2 weeks and 4 weeks and total bilirubin level during 4 weeks was statistically significant in responders, but not in non-responders.
Conclusions We should diagnose and treat acute onset AIH patients before they develop into severe and fulminant disease. Performing liver biopsy at the early stage of acute onset AIH, evaluating the biopsy specimens precisely and initiating corticosteroid therapy may be essential for improving the prognosis without liver transplantation.

Keywords Autoimmune hepatitis · Severe hepatitis · Fulminant hepatitis · Immunosuppressive therapy · Liver histology

Abbreviations
AIH Autoimmune hepatitis
FH Fulminant hepatitis
CN Centrilobular necrosis

Introduction

Autoimmune hepatitis (AIH) is regarded as a chronic hepatitis, characterized by the presence of interface hepatitis and plasma cell infiltration on histological examination, hypergammaglobulinemia and autoantibodies [1, 2]. An acute presentation of AIH is common [3–6], and severe and fulminant hepatitis is possible [7].

An AIH scoring system based on the clinicopathological features has been proposed by the international AIH group

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[8]. There have been some patients who do not show typical features of AIH. AIH with clinical features of acute, severe and fulminant hepatitis (acute onset AIH) is one of these conditions. Patients with acute onset AIH are at risk of losing the timing for the initiation of immunosuppressive therapy, and it is sometimes resistant to immunosuppressive therapy and has a poor prognosis. A nationwide survey of patients with fulminant hepatitis and late onset hepatic failure between 1998 and 2003 in Japan revealed that the prognosis was especially poor in AIH patients, whose survival rate was 17.1% without liver transplantation [9]. This is recognized everywhere around the world [10, 11].

Lefkowitch et al. [12] first reported AIH patients presenting with histologically acute hepatitis. In a Japanese nationwide survey study, 5.6% of patients with AIH were found to have a feature of acute hepatitis upon histological examination [13]. In fact, the actual number of acute onset AIH patients may possibly have been underestimated, as its diagnosis is sometimes very difficult using the AIH scoring system and because exact understanding of the pathological features of these patients is sometimes lacking. A major problem is that there is no gold standard for making the diagnosis of acute onset AIH.

Recently, we reported that histological examination was useful for an early diagnosis of acute onset AIH and that prognosis might indeed be improved by getting a head start on corticosteroid therapy in clinically non-severe cases [14]. In the present study, we examined the clinicopathological features and treatment responses of severe and fulminant forms of AIH patients, and attempted to determine the exact requirements for a more precise diagnosis and the correct time point for switching to liver transplantation after the administration of immunosuppressive therapy in order to improve their very poor prognoses.

Patients and methods

Selection criteria of patients

Patients with severe and fulminant AIH were enrolled between 1990 and 2009. A diagnosis of AIH was made based on the presence of anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA), as well as on the criteria defined by the International Autoimmune Hepatitis Group reaching the score for probable or definite AIH [8].

Eligibility criteria of clinically "acute onset" AIH were as follows, in addition to the AIH criteria described above: (1) acute onset liver injury, (2) no history of chronic liver injury, (3) negativity of active viral markers such as hepatitis A, B, C and E viruses, Epstein–Barr virus (EBV), cytomegalovirus (CMV) and herpes simplex virus (HSV), drug-induced liver injury, toxic and metabolic disorders, and (4) no signs of chronicity on the basis of physical examination, laboratory data and abdominal ultrasound findings.

Eligibility criteria of severe and fulminant AIH, in addition to the criteria described above, were as follows: patients with prothrombin time (PT) activity <50% of control or total bilirubin level more than 20 mg/dl during the disease course were defined as having severe AIH, and patients with PT activity <40% of control and hepatic encephalopathy were defined as having fulminant AIH. Informed consent was obtained from all patients or appropriate family members.

Clinical, biochemical and immunoserologic analysis

Data obtained from patients were as follows: sex; age at diagnosis; time of onset, severe disease and fulminant disease; complications; serum levels of alanine aminotransferase (ALT), total bilirubin (T-Bil), alkaline phosphatase (ALP), PT activity, immunoglobulin G (IgG), immunoglobulin M (IgM), ANA, anti-smooth muscle antibody (ASMA), liver kidney microsomal antibody-1 (LKM-1) and anti-mitochondrial antibody (AMA); human leukocyte antigen (HLA); types of therapy; and response to therapy. They were also examined for any history of recent exposure to drugs and chemical agents as well as heavy alcohol consumption (>50 g/day). ANA and ASMA were examined by a fluorescent antibody method, and AMA was examined by a fluorescent antibody method or an enzyme linked immunosorbent assay (ELISA), and LKM-1 was examined by ELISA.

In acute onset AIH, early symptoms including fever, general malaise, fatigue, nausea, vomiting and right upper quadrant discomfort are frequently observed, so we defined the beginning of these symptoms as clinical onset.

Virological analysis

Patients were examined for viral markers such as IgM anti-hepatitis A virus antibody (IgM-HA), IgM anti-HBc antibody (IgM-HBc), HBsAg, anti-HCV antibody, HCV RNA, HEV RNA, IgM anti-Epstein-Barr virus (EBV) antibody (IgM-EBV), IgM anti-herpes simplex virus (HSV) antibody (IgM-HSV) and IgM anti-cytomegalovirus (CMV) antibody (IgM-CMV). None of the patients had clinical or laboratory evidence of acquired immune deficiency syndrome.

Histological analysis

Histological examination was performed by a percutaneous or transjugular approach, explanted liver or post mortem.

Twelve were percutaneous needle biopsy, 2 transjugular needle biopsy, 2 explanted liver, and 7 post mortem. Three specialists (M.N., K.F. and O.Y.) independently reviewed the histopathological changes by evaluating the degrees of portal and lobular changes and plasma cell infiltrations on hematoxylin-eosin stained sections. Staging and grading were evaluated based on the classification of Desmet et al. [15]. (−), (±), (+), (++) and (+++) represent absent, very mild, mild, moderate and severe in interface hepatitis, zonal necrosis, plasma cell infiltration and collapse. (−), (±), (+) and (++) represent absent, slightly present, present and prominent in rosette formation and cobblestone appearance. The scores were averaged and presented in Table 3.

Treatment response

In this study, we defined responders and non-responders according to the recovery of liver function (regeneration), not to the control of liver inflammation, and we judged them at 2 weeks after the starting of corticosteroid therapy. We used PT as a marker of liver regeneration, which is generally used in acute liver failure. We also defined recovery of liver regeneration and normalization of liver inflammation as complete response (CR) and non-recovery of liver regeneration as no response (NR).

Statistical analysis

Differences in proportions among the groups were compared by Fisher's exact probability test, Student's t test and Welch's t test ($p < 0.05$ was considered significant).

Results

Clinical and biochemical features

Twenty-eight patients, 7 men and 21 women, were enrolled in the study. Fourteen were cases of fulminant hepatitis and 14 severe hepatitis. The clinical and biochemical features of all patients at admission are provided in Tables 1 and 2. Mean age at the time of diagnosis was 46.9 ± 15.8 years. Mean ALT was 527 ± 458 IU/l, mean highest T-Bil 23.3 ± 11.6 mg/dl and mean lowest PT activity 28 ± 15%.

Mean IgG was 2479 ± 1170 mg/dl. The IgG level was normal (<1.0 × upper normal value: UNV) in 7 of 28 (25%), 1.0–1.5 × UNV in 12 (43%), 1.5–2.0 × UNV in 5 (18%) and >2.0 × UNV in 4 (14%). ANA was positive (≥1:40) in 25 of 28 (89%) patients, <1:40 in 3 (11%), 1:40 in 5 (18%), 1:80 in 6 (21%) and >1:80 in 14 (50%). ASMA was positive (≥1:40) in 8 of 27 (30%). One patient was positive for LKM-1.

The duration from initial symptoms to the admission to our unit was 48.4 ± 39.9 days (11–176 days). Twelve patients (43%) had primary complications and histories of medications, five with hypertension, three with Hashimoto disease, one with hyperuricemia, one with ischemic heart disease, one with Sjögren syndrome and one with neurosis.

No patients were positive for HBs Ag, and one patient was positive for HCV Ab. Although 43% of the patients had primary complications and histories of medications as described above, suspected hepatotoxic drugs were excluded according to the drug-induced liver injury diagnostic scale of Maria and Victorino [16] in this study.

In AIH, there are two forms according to HLA-DR differences. In Japan, almost all AIH patients do not have HLA-DR 3. This suggests the possible benefit of examining the HLA-DR backgrounds, although we could perform this analysis in only 13 of the patients because this procedure is not covered by the Japanese national health insurance plan. None of the 13 had HLA-DR 3, but 6 had HLA-DR 4.

Histological features

The pathological characteristics of the patients are summarized in Table 3. Histological examination was performed in 23 of 28 patients, and 22 were appropriate for evaluation. Nineteen (86%) of 22 showed acute hepatitis, exhibiting zonal, submassive and massive necrosis with or without plasma cell accumulation in portal and centrilobular areas. Three (14%) showed chronic hepatitis.

Twenty-one of 22 (95%) patients showed severe activity, 10 with massive necrosis, 2 with submassive necrosis, 7 with severe acute hepatitis and 2 with severe activity with fibrosis stage 2–3. Only one showed moderate activity with fibrosis stage 2.

The duration from onset to histological examination was 85.6 ± 47.6 days. That in massive necrosis, submassive necrosis, severe acute hepatitis and chronic hepatitis was 61.0 ± 31.4, 135.0 ± 79.2, 85.7 ± 17.6 and 134.3 ± 79.2, respectively. The difference between the acute and chronic form was not statistically significant ($p = 0.35$).

AIH scoring system

The provisional scoring system (AIH score) proposed by the International Autoimmune Hepatitis Group [8] was used to score all patients (Table 4). The AIH score ranged from 12 to 22 (16.3 ± 2.8) before the treatment. Fourteen of 28 patients (50%) were diagnosed as having 'definite' AIH and 14 (50%) as having 'probable.'

Table 1 Clinical characteristics of 28 patients

Patients	Diagnosis	Onset (year)	Age	Sex	ALT (IU/l)	ALP (IU/l)	Highest T-Bil (mg/dl)	PT activity on admission (%)	Lowest PT activity (%)	IgG (mg/dl)
1	FH	1989	26	F	189	415	20.5	25	19	2630
2	FH	1990	59	F	916	304	33.7	10	10	3325
3	AHs	1991	45	F	812	261	49.9	31	31	2057
4	FH	1993	27	F	427	285	18.3	35	5	1654
5	AHs	1993	31	F	317	362	32.1	42	42	1193
6	AHs	1994	30	F	673	172	22.2	53	53	2192
7	AHs	1994	40	F	900	185	32.5	48	42	2155
8	AHs	1995	66	M	244	163	15.8	58	41	2676
9	AHs	2000	44	F	122	568	20.4	49	48	1320
10	AHs	2000	51	M	513	578	3.6	48	46	1870
11	FH	2002	17	F	496	802	14.8	29	29	2400
12	FH	2003	56	M	49	280	38.4	15	15	3053
13	FH	2004	64	M	1998	513	22.9	40	33	2377
14	AHs	2005	37	F	498	499	11.2	49	49	6424
15	AHs	2006	61	M	333	341	12.0	36	35	2662
16	AHs	2007	39	F	424	543	19.8	49	44	1249
17	AHs	2007	72	F	964	651	22.8	45	43	2295
18	FH	2007	58	F	230	367	32.0	16	15	1957
19	FH	2007	56	M	355	427	26.3	28	23	2123
20	AHs	2007	26	F	183	377	5.8	48	35	1233
21	FH	2007	52	M	329	989	33.8	34	8	1274
22	AHs	2007	23	F	185	646	7.9	52	33	4127
23	FH	2007	70	F	148	396	45.4	25	9	4178
24	FH	2008	71	F	607	445	33.8	21	9	1990
25	FH	2008	55	F	395	472	28.3	18	7	4322
26	FH	2009	49	F	1789	379	23.6	31	29	2868
27	FH	2009	38	F	321	469	12.9	16	16	2546
28	AHs	2009	49	F	349	369	12.3	33	26	1272

FH fulminant hepatitis, *AHs* acute hepatitis severe type, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *T-Bil* total bilirubin, *PT* prothrombin time, *IgG* immunoglobulin G

Treatment response and outcome

In 25 of 28 (89%) patients, an initial dose of 40–60 mg prednisolone or 1000 mg methylprednisolone daily was administered. Patients with marked elevation of ALT and prolongation of PT were treated with 1000 mg of methylprednisolone pulse therapy followed by prednisolone therapy. Seventeen (61%) survived without liver transplantation, one (4%) survived with liver transplantation and seven (25%) died without liver transplantation because of liver failure. One (4%) received a liver transplantation and died. Two patients (7%) were already in terminal stage liver failure and died (Table 4).

Changes in PT activities, ALT levels and T-Bil levels during the initial 4 weeks after the introduction of corticosteroid therapy are shown in Fig. 1. Improvement of PT activities was found in all of the responders. In non-responders, PT activities did not improve, although some of them showed a transient rise in the first 2 weeks by infusions of fresh frozen plasma. The elevations of PT activities during 2 weeks and 4 weeks were statistically significant ($p = 0.001$ and $p < 0.001$, respectively) in responders, but not in non-responders. The ALT levels fell during the course, with the declines in the first 2 weeks and 4 weeks being statistically significant in responders ($p = 0.003$ and $p < 0.001$, respectively) and non-responders ($p = 0.004$ and $p = 0.033$, respectively). T-Bil levels fell during the course, with the declines in the first 4 weeks being statistically significant ($p = 0.031$) in responders, but they did not fall in non-responders.

Patients were analyzed according to their responses to corticosteroid therapies—responders or non-responders

Table 2 Immunoserological and virological analysis of 28 patients

Patient	ANA (fold)	ASMA (fold)	AMA	LKM-1	HLA-DR	IgM-HA	HBsAg/ IgM-HBc	HCV-Ab/ RNA	HEV-RNA	IgM-EBV/ HSV/CMV
1	320	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
2	<40	40	–	ND	ND	–	–/–	–/–	–	–/–/–
3	40	80	–	ND	4	–	–/–	–/–	–	–/–/–
4	1280	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
5	<40	40	–	–	8, 9	–	–/–	–/–	–	–/–/–
6	160	<40	–	–	ND	–	–/–	–/–	–	–/–/–
7	<40	40	–	–	ND	–	–/–	–/–	–	–/–/–
8	40	320	–	–	8, 9	–	–/–	–/–	–	–/–/–
9	40	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
10	80	40	–	ND	4, 8	–	–/–	–/–	–	–/–/–
11	320	<40	–	–	ND	–	–/–	–/–	–	–/–/–
12	>1280	80	–	–	ND	–	–/–	–/–	–	–/–/–
13	640	ND	–	+	ND	–	–/–	–/–	–	–/–/–
14	1280	<40	–	–	ND	–	–/–	–/–	–	–/–/–
15	640	<40	–	–	ND	–	–/–	–/–	–	–/–/–
16	40	<40	–	–	4, 12	–	–/–	–/–	–	–/–/–
17	80	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
18	80	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
19	80	<40	–	–	ND	–	–/–	–/–	–	–/–/–
20	80	<40	–	ND	14, 15	–	–/–	–/–	–	–/–/–
21	80	<40	–	ND	12, 13	–	–/–	–/–	–	–/–/–
22	1280	<40	–	–	9, 13	–	–/–	–/–	–	–/–/–
23	>1280	<40	–	ND	ND	–	–/–	–/–	–	–/–/–
24	40	<40	–	–	4, 8	–	–/–	–/–	–	–/–/–
25	640	<40	–	–	8, 12	–	–/–	–/–	–	–/–/–
26	640	40	–	–	13, 15	–	–/–	–/–	–	–/–/–
27	320	<40	–	–	4	–	–/–	–/–	–	–/–/–
28	160	<40	–	–	4, 12	–	–/–	–/–	–	–/–/–

ANA anti-nuclear antibody, *ASMA* anti-smooth muscle antibody, *AMA* anti-mitochondrial antibody, *LKM-1* liver kidney microsomal antibody-1, *HLA* human leukocyte antigen, *IgM-HA* IgM anti-hepatitis A virus antibody, *IgM-HBc* IgM anti-hepatitis B virus core antibody, *IgM-EBV* IgM anti-Epstein–Barr virus antibody, *IgM-HSV* IgM anti-herpes simplex virus antibody, *IgM-CMV* IgM anti-cytomegalovirus antibody, *ND* not done

(Table 4). The differences in sex, mean ALT, mean T-Bil, mean IgG, ANA titer and AIH score were not statistically significant. Mean age was higher in non-responders than in responders ($p < 0.05$). PT activity was lower in non-responders than in responders ($p < 0.001$). All responders survived, 7 of non-responders died ($p < 0.001$) and one non-responder survived with liver transplantation. Interestingly, the duration from initial symptoms to the administration of corticosteroids was not different between responders and non-responders (Table 5; Fig. 2).

Long-term changes in ALT levels after the start of treatment in all the patients are shown in Fig. 3. ALT levels remained normal except for two patients with some transient exacerbations.

Discussion

After the establishment of the criteria of the International Autoimmune Hepatitis Group [8] and the recognition of acute onset AIH [6], the diagnosis of severe and fulminant type of AIH came to be made, and most of those thusly diagnosed would have been diagnosed as cryptogenic hepatitis. But some patients have no autoantibodies and/or no hypergammaglobulinemia, and at present they are being diagnosed with cryptogenic hepatitis. Kaymakoglu et al. [17] reported that severe cryptogenic chronic hepatitis was similar to AIH in clinical, biochemical and histological features as well as responsiveness to immunosuppressive therapy, and severe cryptogenic chronic hepatitis patients might have an autoimmune liver disease with no identified

Table 3 Pathological characteristics of patients

Patient	Histological diagnosis	Interface hepatitis	Zonal necrosis	Plasma cell infiltration	Rosette formation	Collapse	Cobblestone appearance	Duration from onset to exam. (days)	Method
1	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Massive necrosis	+++	+++	+	−	−	−	45	Post mortem
3	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	Inappropriate for evaluation	×	×	×	×	×	×	40	Percutaneous
5	Severe acute hepatitis	+	++	+	−−	±	−	95	Percutaneous
6	CH (F3, severe)	+++	+++	++	+	+	+	43	Percutaneous
7	Submassive necrosis	+	+++	++	±	+	±	79	Percutaneous
8	CH (F3, severe)	+++	++	++	+	+	+	176	Percutaneous
9	Severe acute hepatitis	+	+	++	+	−	+	107	Percutaneous
10	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	Massive necrosis	+++	+++	−	×	×	×	25	Explanted
12	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	CH (F2, moderate)	+++	+	−	−	+	±	184	Percutaneous
14	Severe acute hepatitis	++	++	+	−	+	−	80	Percutaneous
15	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	Massive necrosis	+++	+	−	−	−	−	36	Transjugular
17	Severe acute hepatitis	+	+	−	−	+	+	107	Percutaneous
18	Massive necrosis	+++	+++	++	−	−	−	52	Post mortem
19	Submassive necrosis	++	++	++	−	−	−	191	Post mortem
20	Massive necrosis	+++	+++	±	×	×	×	51	Transjugular
21	Massive necrosis	+++	++	−	×	×	×	68	Post mortem
22	Severe acute hepatitis	++	+	+	+	−	++	71	Percutaneous
23	Massive necrosis	+++	+++	+++	−	−	−	121	Post mortem
24	Massive necrosis	+++	+++	+++	×	×	×	39	Post mortem
25	Massive necrosis	+++	+++	+	−	−	−	110	Post mortem
26	Severe acute hepatitis	++	++	+	+	++	+	78	Percutaneous
27	Massive necrosis	+++	+++	++	−	−	−	63	Explanted
28	Severe acute hepatitis	++	++	+	−	++	−	62	Percutaneous

CH chronic hepatitis, *ND* not done, × inappropriate for evaluation, *percutaneous* percutaneous needle biopsy, *transjugular* transjugular needle biopsy

immunoserologic marker. Potthoff et al. [18] suggested that steroids have to be considered in the therapy for severe acute cryptogenic hepatitis, and the response to steroid treatment may indicate an autoimmune genesis of the disease. On the other hand, Bernal et al. reported that autoantibodies were present in 30% of patients with acute liver failure and that significantly higher international AIH scores were found in patients with cryptogenic disease as compared to other etiological ones. They suggested it is difficult to evaluate whether primary autoimmune processes are responsible for the condition, although cryptogenic cases have features of autoimmune pathogenesis [19].

The duration from onset to admission to our unit was 48.4 (11–176) days in the present study. In most patients, the severity of hepatitis was not severe at onset, but they advanced to severe diseases without precise diagnosis and treatment, and were referred to our unit. This means that the diagnosis of acute onset AIH is still difficult in Japan (Figs. 4, 5, 6, 7).

In our study, ANA was $\leq$1:40 in 29% of the patients, ASMA was negative (<1:40) in 68%, and none was negative for both. In the Italian study of acute onset AIH, 27% of the patients were negative for ANA, 18% were negative for ASMA, and 9% were negative for both [20]. In the US study of acute onset AIH, 31% were negative for ANA, 15% were negative for ASMA, and 4% were negative for both [21]. Thus, the negativity of ANA was about the same as other reports of acute onset AIH, but the negativity of ASMA was higher in our patients. In another Japanese study of acute AIH, 56% were negative for ASMA [22].

It was reported that the period of initial symptoms to the diagnosis of fulminant or severe acute hepatitis is occasionally longer than that of acute hepatitis based on their

Table 4 Treatment and outcome of all patients

Patients	Treatment	Loading dose of corticosteroid (mg/day)	Days from onset to corticosteroid therapy	Response to corticosteroid	Outcome	AIH score	Simplified AIH score
1	PSL	60	43	CR	Recovery	15	6
2	Dex	10	19	NR	Death	17	6
3	mPSL	1000	124	CR	Recovery	14	6
4	SNMC				Death	14	5
5	PSL	60	22	CR	Recovery	14	4
6	PSL	60	24	CR	Recovery	18	8
7	PSL	60	18	CR	Recovery	16	6
8	PSL	30	161	CR	Recovery	17	8
9	PSL	60	31	CR	Recovery	15	5
10	PSL	60	96	CR	Recovery	12	6
11	LT				Death after LT	17	7
12	PSL	60	62	NR	Death	13	6
13	PSL	50	134	CR	Recovery	15	7
14	PSL	40	36	CR	Recovery	22	7
15	PSL	60	86	CR	Recovery	12	6
16	PSL	60	30	CR	Recovery	15	4
17	mPSL	1000	54	CR	Recovery	16	7
18	SNMC				Death	17	7
19	mPSL	1000	134	NR	Death	15	7
20	PSL	40	45	CR	Recovery	15	5
21	mPSL	1000	25	NR	Death	13	5
22	mPSL	1000	24	CR	Recovery	22	8
23	mPSL	500	45	NR	Death	20	7
24	mPSL	1000	17	NR	Death	19	5
25	mPSL	1000	85	NR	Death	20	6
26	mPSL	1000	28	CR	Recovery	20	8
27	mPSL	1000	46	NR	Recovery after LT	15	5
28	mPSL	1000	10	CR	Recovery	18	5

PSL prednisolone, *Dex* dexamethasone, *mPSL* methylprednisolone, *SNMC* stronger neominophagen C, *LT* liver transplantation, *CR* complete response, *NR* no response

observation that severe or fulminant patients had a higher titer of ANA and higher levels of IgG than non-severe ones [23, 24]. In our present study, the IgG level was higher than normal in 75% of the patients and was significantly higher than that of our non-severe acute onset AIH patients [14] ($p = 0.002$), indicating that our severe and fulminant patients might also have longer clinical courses than non-severe patients.

The mean AIH score was the same as in non-severe patients, but all patients were diagnosed as 'probable' or 'definite,' although 77% of non-severe patients were diagnosed as 'probable' or 'definite' in our previous study [14].

In our previous study of non-severe acute onset AIH [14], liver histology showed severe activity with zonal necrosis in 95% of the patients, despite PT activity being maintained, suggesting that AIH with low PT activity must have very severe and advanced histology (submassive to massive necrosis) and present impaired hepatocellular regeneration, a condition that may be associated with resistance to immunosuppressive therapy. This was confirmed in the present study.

Liver histology showed acute hepatitis (massive necrosis, submassive necrosis and severe acute hepatitis) in 86% of our patients and chronic hepatitis in 14%. The duration from onset to histological examination was not different between acute hepatitis and chronic hepatitis ($p = 0.35$). The differences were not statistically significant among the four histological forms described above, which means that the difference in histological findings did not depend on the timing of histological examination. That in non-severe acute onset AIH patients was 32.4 ± 24.0 days in our previous study [14], and the difference was significant between non-severe and severe/fulminant ones ($p < 0.001$). This also

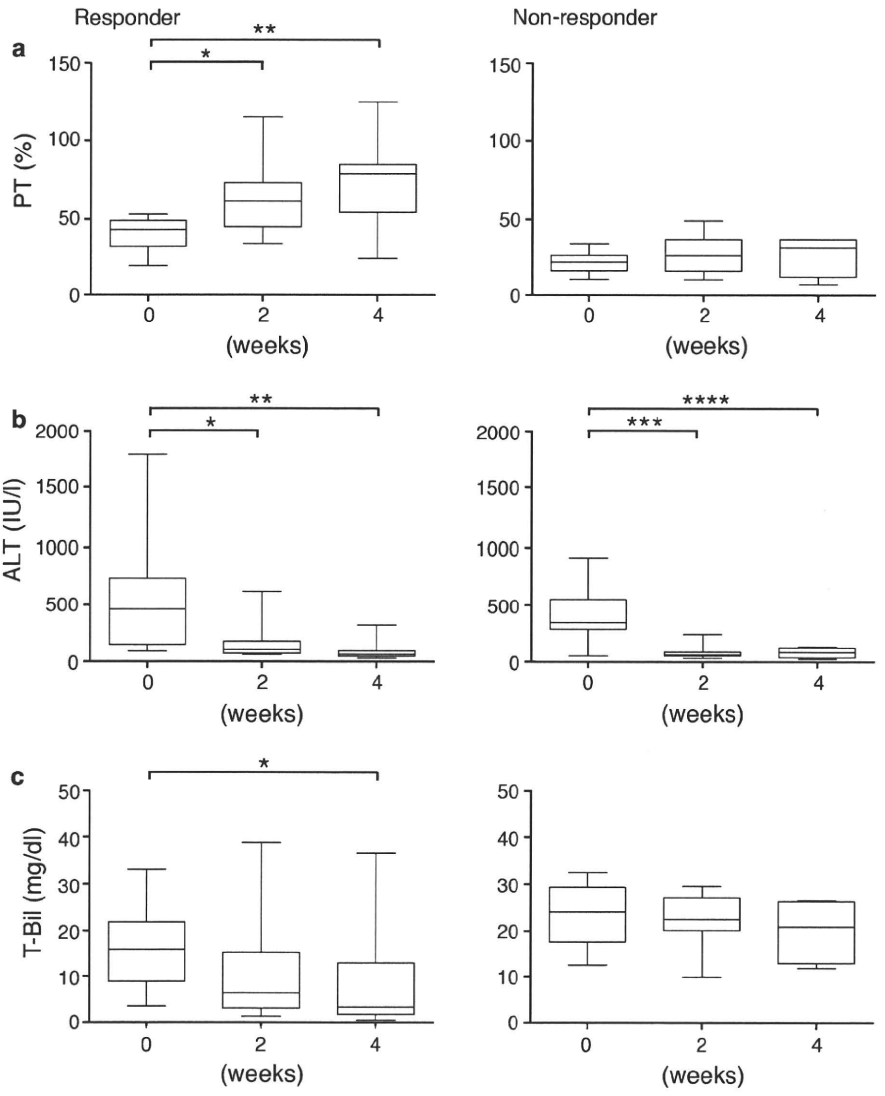

Fig. 1 Prothrombin time (PT) activities, alanine transaminase (ALT) levels and total bilirubin (T-Bil) levels before, 2 weeks after and 4 weeks after the administration of corticosteroid in 17 responders and 8 non-responders. **a** The mean PT activity at each point was 40 ± 10, 62 ± 22 and 74 ± 26% in responders and 21 ± 7, 27 ± 13 and 27 ± 14% in non-responders, respectively (*$p = 0.001$, **$p < 0.001$). **b** The mean ALT level at each point was 552 ± 489, 152 ± 142 and 81 ± 66 IU/l in responders and 404 ± 257, 79 ± 65 and 78 ± 43 IU/l in non-responders, respectively (*$p = 0.003$, **$p < 0.001$, ***$p = 0.004$, ****$p = 0.033$). **c** The mean T-Bil level at each point was 16.1 ± 8.9, 10.7 ± 10.6 and 8.5 ± 10.7 mg/dl in responders and 23.4 ± 6.8, 22.3 ± 6.0 and 20.1 ± 7.3 mg/dl in non-responders, respectively (*$p = 0.031$)

means that our severe and fulminant patients have longer clinical courses than non-severe patients.

Regarding three patients showing histologically chronic hepatitis, the fibrosis stage of patient 6 was F3, and the duration from clinical onset to histological examination was 43 days. Therefore, we speculate that this patient might have had mild-moderate fibrosis before the severe exacerbation and progressed during the relatively short period. In contrast, the fibrosis stage of patient 8 and 13 was F3 and F2, and the duration was 176 and 184 days, respectively. We speculate that they might have less than mild-moderate fibrosis before the exacerbation and progressed during the long period.

In the majority of patients, severe centrizonal necrosis with or without plasma cell accumulation was found. There are no morphological features pathognomonic of AIH, but the characteristic histological picture is that of an interface hepatitis with predominantly lymphoplasmacytic necroinflammatory infiltrates, with or without lobular involvement and bridging necrosis, often with the formation of liver cell rosettes [8]. Moreover, there are only a few reports on the histological features of acute onset AIH. Abe et al. [22] reported that the histological findings are very useful for differentiating between acute AIH and acute hepatitis resulting from other causes, because the former showed plasma cell infiltration, zonal necrosis and early cell infiltration into portal areas, features absent in the latter, and that early histological diagnosis and treatment might be important for patients with acute AIH. Centrilobular necrosis (CN) is associated with an acute clinical presentation and might reflect an early lesion preceding portal involvement, although CN with sparing of the portal areas represents a rare histological pattern in AIH. Recognition of this particular histological appearance enables an early diagnosis of AIH and a timely initiation of immunosuppressive therapy [6, 24–27].

Table 5 Comparison of responders and non-responders

	Responder	Non-responder	*p* value
N	17	8	
Age (years)[a]	44.3 ± 15.0	57.1 ± 10.4	0.004*
Sex (M/F)	4/13	3/5	0.640**
ALT (IU/l)[a,b]	552 ± 489	404 ± 257	0.435*
T-Bil (mg/dl)[a,b]	16.1 ± 8.9	23.4 ± 6.8	0.054*
PT activity (%)[a,b]	40.4 ± 9.9	21.4 ± 7.3	<0.001*
IgG (mg/dl)[a]	2388 ± 1291	2851 ± 1072	0.388*
ANA ≤×40	6	2	1.000**
ANA ≥×80	11	6	
AIH score[a]	16.2 ± 2.7	16.5 ± 2.9	0.823*
Simplified AIH score[a]	6.3 ± 1.4	6.6 ± 1.0	0.654*
Days from onset to corticosteroid therapy (days)[a]	56.8 ± 45.9	54.1 ± 39.7	0.888*
Survivor/non-survivor	17/0	1/7	<0.001**

$p < 0.05$ was considered significant

[a] Mean ± SD

[b] Day 1, the day when corticosteroid was administered

* Unpaired *t* test

** Fisher's exact test

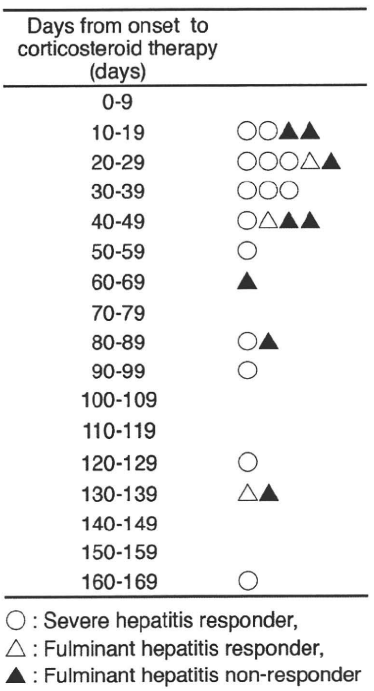

Fig. 2 The duration from initial symptoms to the administration of corticosteroids and outcome

One of the pathological characteristics of severe and fulminant AIH is its heterogeneity. Unenhanced CT scan shows both hypoattenuation and hyperattenuation areas. The former reflect massive hepatic necrosis and the latter regenerative islands [28–30]. Ultrasound also shows the same heterogeneity. These findings would be of help for the diagnosis of severe and fulminant AIH.

In our study, non-responders to corticosteroid treatment, with significance, showed higher age than non-responders. They also showed lower PT activity at the time of corticosteroid administration than responders. This might be due to the impaired hepatocyte regeneration of non-responders, because considerably large numbers of hepatocytes would already have been destroyed, and inhibition of inflammatory reaction might not be effective enough to allow regeneration. The T-Bil level has been considered a critical prognostic factor in fulminant hepatic failure [31] and also in fulminant AIH [32]. Miyake et al. [32] reported that patients whose T-Bil levels worsen during days 8–15 after the diagnosis of fulminant hepatic failure should be considered for liver transplantation. Czaja et al. [33] reported that mortality has uniformly occurred in those whose histology showed multilobular collapse and whose laboratory data did not improve within 2 weeks of corticosteroid treatment, and that such patients are in need of urgent transplantation. In our present study, the improvement of PT activity, a marker of liver regeneration, during the first 2 weeks after the corticosteroid treatment was statistically significant in responders, but not in non-responders. Even in the responders, the improvement of liver function, especially T-Bil, was slow in our patients. This means that the time limit for switching to liver transplantation to avoid infectious complications is 2 weeks after the administration of corticosteroids, and we agree with Czaja's report. Czaja et al. [34] also reported that treatment failure in AIH tends to develop in patients with early age onset, HLA DRB1*03 or variant syndromes, especially in those with primary sclerosing cholangitis, but these were not found in our patients.

As described above, the disease severity of non-responders at the time of starting corticosteroid therapy was more advanced than in responders. The duration from initial symptoms to the administration of corticosteroids was not different between responders and non-responders. This means that response to corticosteroid therapy is not associated with the time duration from clinical onset but with the disease severity. Therefore, we should diagnose