C-110 (LuminUltra Technologies Ltd., Canada). The method of standard additions was used to convert RLUs into plasma ATP concentration.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted using the RNAqueous-4PCR isolation kit (Ambion, USA). Reverse transcription was performed using the high-capacity cDNA reverse transcription kit (Applied Biosystems, USA). PCR was performed using the Platinum PCR SuperMix High Fidelity (Invitrogen, USA) system. The primer sets were prepared using published sequence data for 14 different P2 receptor subtypes, albumin (Alb), alpha-fetoprotein (AFP), cytokeratin 19 (CK19), tyrosine aminotransferase (TAT), glucose-6-phosphatase (G6Pase) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [26, 29]. The PCR products were separated by electrophoresis on a 1 % agarose gel, and visualized by ethidium bromide staining under ultraviolet light.

Small interfering RNA (siRNA) transfection

The siRNAs used for the knockdown of endogenous $P2Y_1$ and $P2Y_2$ proteins and the negative control siRNA were purchased from Santa Cruz Biotechnology, CA. These siRNAs were transfected into cells using Lipofectamine 2000 (Invitrogen, USA), according to manufacturer's instructions.

Measurement of cytokines in human plasma

Plasma cytokines were measured using Bio-Plex human cytokine multiplex kits (Bio-Plex human Cytokine 17-Plex Panel, Bio-Rad Laboratories, Hercules, CA, USA), according to manufacturer's instructions. The level of each cytokine is indicated by the fluorescence intensity (FI), according to the manufacturer's instructions.

Statistical analysis

The statistical analysis was carried out using the Student's *t* test. A *p*-value of less than 0.05 was considered to be significant. The results are presented as the mean ± SD.

Results

Effects of ALF-P on cell proliferation and JNK activation in OCs and primary hepatocytes

We studied the effects of ALF-P on the proliferation of primary hepatocytes and OCs because the reports describing the effects of ALF-P on the proliferation of hepatocytes present paradoxical results [8–12]. ALF-P stimulated the proliferation of normal hepatocytes about 6-fold at 24 h and 4-fold at 72 h, relative to the starting point at day 0 (Fig. 1a). In OCs, although ALF-P increased the number of cells slowly after 24 h, the increased proliferative capacity of the cells was sustained for 72 h (Fig. 1b). In addition, gene expression of Alb, AFP, and CK19, which are phenotypic markers of OC presence [26], was not affected by ALF-P treatment for 72 h. Under the same experimental condition, TAT and G6Pase, differential markers of hepatocytes, were not detectable (Supplemental data, Fig. 1). The data indicate that under the experimental conditions, ALF-P treatment stimulated the proliferation of OCs without initiating the differentiation.

ALF-P markedly stimulated the activation of JNK in OCs, which is consistent with our previous data. However, it did not induce JNK phosphorylation in primary hepatocytes. To confirm the effects of ALF-P on the activation of JNK signaling in OCs, we assessed the phosphorylation of SEK-1/MKK4 and c-Jun, which are located immediately upstream and downstream, respectively, of JNK [30–32]. Elevated SEK-1/MKK4 phosphorylation was observed after a short (30–120 min) cell culture period. However, the activation of c-Jun was not detected in the ALF-P-stimulated cells during this period. Instead, activating transcriptional factor 2 (ATF2), another target of JNK signaling in response to cellular stress [33], was activated (Fig. 1d). On the other hand, normal control plasma (NC-P) did not stimulate cell proliferation or JNK activation in either of the two types of cells (Fig. 1a–c). However, increased activation of c-Jun was observed at each indicated time point in OCs (Fig. 1d).

$P2Y_2$ receptor mediates the effects of ALF-P on the growth of OCs

Considering the importance of ATP signaling in the development of ALF and in the regulation of JNK, we sought to determine if P2 receptors contribute to the effects of ALF-P on the growth of OCs. The mRNA expression of the 14 different P2 receptor subunits, except for $P2Y_{11}$, which is absent in mice and other rodents [34], was analyzed in OCs. Our data showed that all P2Y subtypes, including $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$, were expressed in OCs. However, except for $P2X_2$, P2X receptors were not detected by RT-PCR (Fig. 2a). To dissect the role of specific P2 receptors, we firstly added P2 antagonists and ALF-P to OCs. PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid), a non-selective antagonist of P2 receptors, blocked the stimulatory effects of ALF-P on the proliferation of OCs. However, NF023, a selective and competitive antagonist of P2X receptors, did

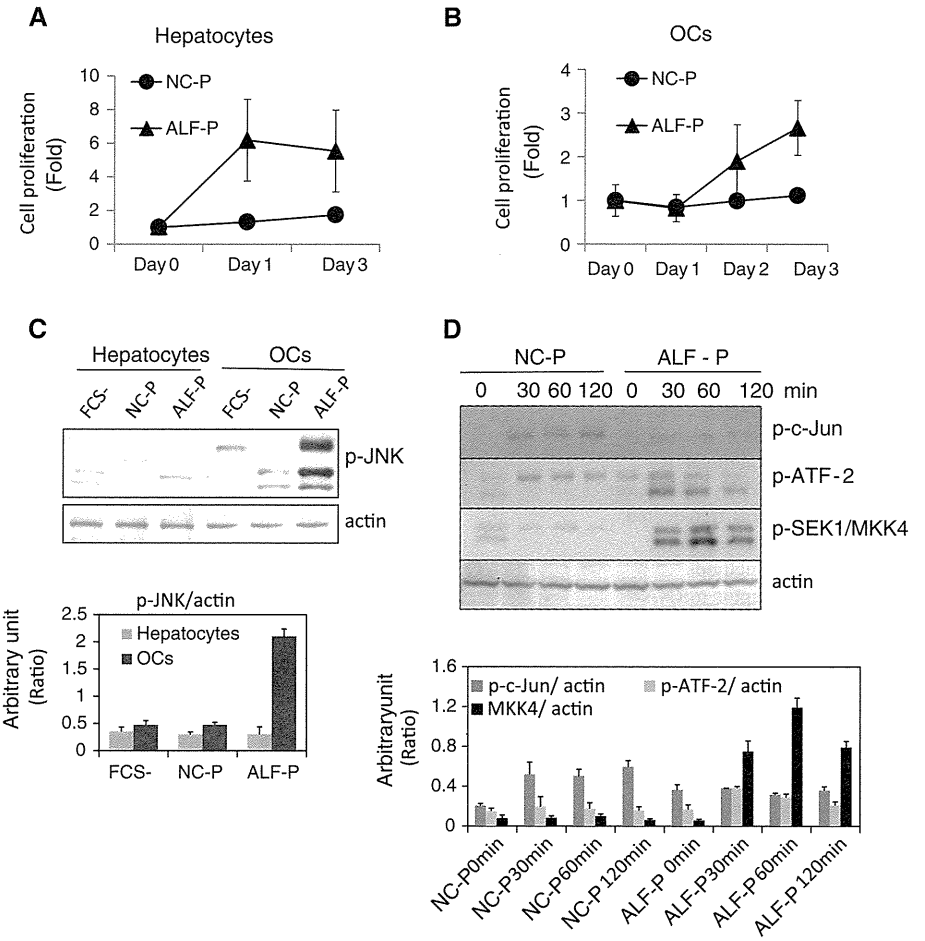

Fig. 1 Effects of ALF-P on cell proliferation and JNK phosphorylation. Mouse primary hepatocytes were prepared as described in "Materials and methods". The hepatocytes (**a**) and oval cells (OCs) (**b**) were stimulated with 30 % ALF-P or 30 % NC-P. The proliferation of the cells was measured as described in "Materials and methods". **c** OCs and primary hepatocytes received the same treatments as described above and were collected 72 h later. The phosphorylation of JNK was detected by western blot analysis. **d** OCs were treated with 30 % ALF-P and 30 % NC-P for the indicated times. Western blot analysis was performed using cell lysates and antibodies specific for p-c-Jun, p-ATF2, and p-SEK1/MKK4, as indicated. Actin was used as the internal control. Data are expressed as the mean ± SD ($n = 3$)

not block the proliferative effects of ALF-P in OCs (Fig. 2b). On the other hand, the live and dead/apoptotic cell staining was performed to investigate changes in proliferation rates in relation to the induction of apoptosis. Compared to the NC-P-treated cells, most of the cells treated with ALF-P or ALF-P and co-treated with NF023 were strongly stained by Live-dye, and quite a few of the cells were stained with DAPI or PI, indicating that either apoptosis or cell death rarely occur in the above conditions. However, co-treatment of PPADS and ALF-P increased the amounts of apoptotic or dead cells, which indicates that the reduction of proliferation in the described condition was attributable to the apoptosis-induced cell death. These results thus suggest that P2Y, but not P2X receptor, contributes to the effects of ALF-P. We next determined which subtype of P2Y receptors mediates the effects of ALF-P. Since both $P2Y_1$ and $P2Y_2$ are related to the action of extracellular ATP in regulating liver function [17], we blocked the gene expression of these two receptors using their specific siRNAs (Fig. 2d). $P2Y_2$ gene silencing reversed the effects of ALF-P on the phosphorylation of JNK and the proliferation of OCs. By contrast, $P2Y_1$ gene silencing did not alter the effects of ALF-P (Fig. 2e, f).

Extracellular ATP does not contribute to the ALF-P-induced proliferation of OCs

We next sought to identify whether the effects of ALF-P are due to the main ligand of P2 receptors, extracellular ATP, which increases inflammatory processes in the liver. Unexpectedly, we found that ATP levels in ALF-P were significantly lower than those in NC-P (Fig. 3a). Although ATP stimulated the activation of JNK in OCs (Fig. 3b), it did not promote cellular proliferation, unlike two other P2 receptor agonists, ATPγS and 2MeSATP (Fig. 3c). Moreover, when cells were treated with apyrase, an ATP diphosphohydrolase that catalyzes the hydrolysis of ATP to yield AMP and inorganic phosphate, the ALF-P-stimulated activation of JNK was not inhibited, whereas it was inhibited when cells were treated with PPADS (Fig. 3d).

TNF receptor signaling is involved in the ALF-P-stimulated proliferation of OCs in a P2Y-dependent manner

We therefore focused on other molecules that could mediate the proliferatory effects of ALF-P, such as

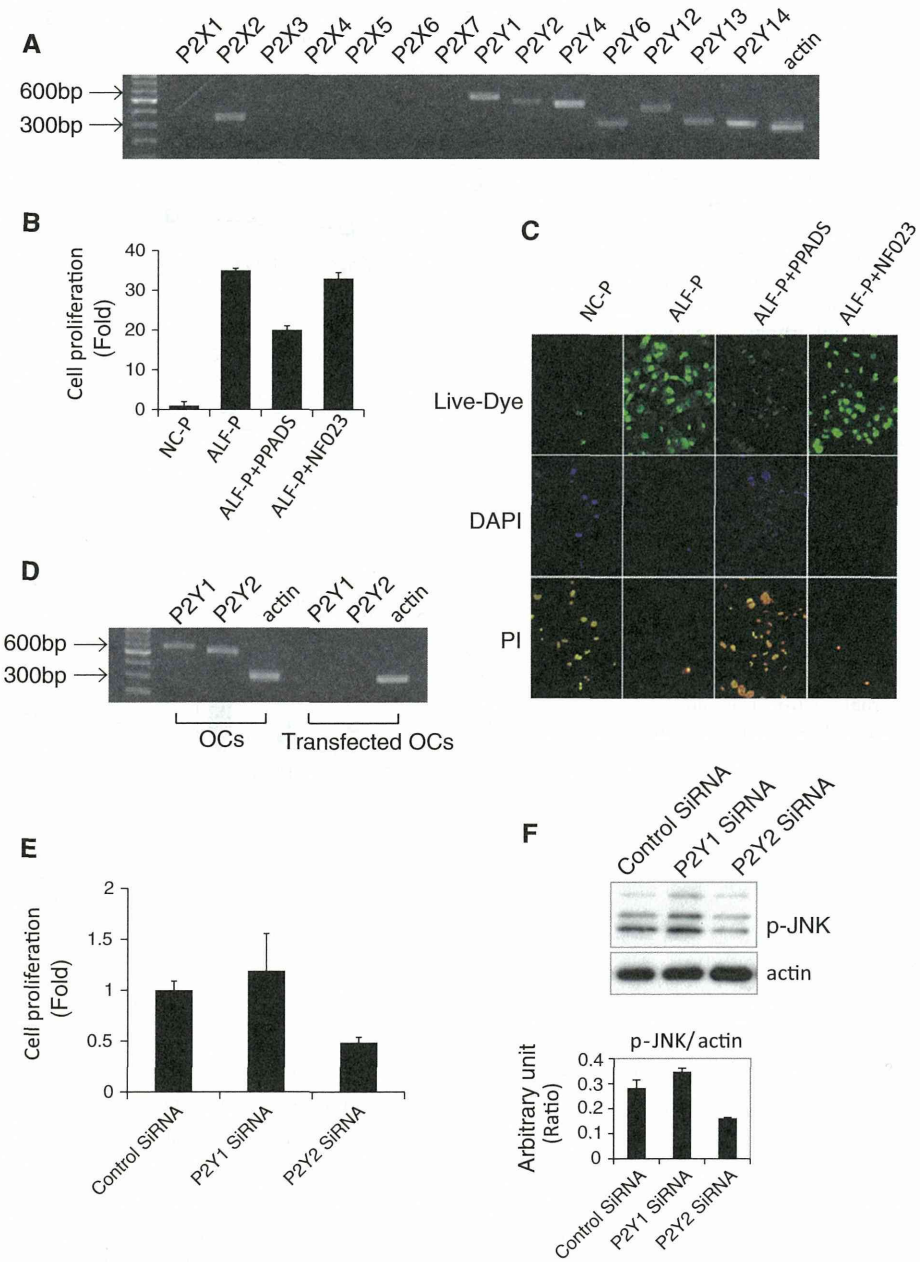

Fig. 2 ALF-P regulates the growth of OCs through $P2Y_2$ receptor. **a** The expression of P2 receptors in OCs was detected using RT-PCR analysis as described in "Materials and methods". **b** OCs were incubated with 30 % NC-P or 30 % ALF-P with or without 250 μM PPADS or 200 μM NF023. The proliferation of the cells was evaluated after 72 h. Data are expressed as the mean ± SD ($n = 3$). **c** OCs were treated as described in **b**. Apoptotic and dead cells were evaluated as described in "Materials and methods". **d** $P2Y_1$ siRNA, $P2Y_2$ siRNA, or control siRNA was transfected into OCs as described in "Materials and methods". Blockage of receptor mRNA expression was confirmed by RT-PCR analysis. **e**, **f** The control cells and the cells with knockdown of endogenous $P2Y_1$ or $P2Y_2$ were treated with ALF-P for 3 days. The proliferation of the cells (**e**) and the phosphorylation of JNK (**f**) were examined subsequently. Data are expressed as the mean ± SD ($n = 3$)

hepatocyte growth factor (HGF), tauroursodeoxycholate (TUDC), insulin, epidermal growth factor (EGF), interferon (IFN) γ, transforming growth factor beta (TGFβ), and tumor necrosis factor (TNF) α. Similar to ALF-P, EGF, TGFβ1/2, TGFβ3, and TNFα stimulated the phosphorylation of JNK, whereas the other reagents, including HGF, TUDC, insulin, and IFNγ, did not have an effect (Fig. 4a). Furthermore, when we used SB-431542, AG1478, and R-7050, specific inhibitors of EGF, TGFβ, and TNFα, respectively, we found that SB-431542 and AG147 had no significant effect on ALF-P-stimulated cell proliferation or JNK activation, whereas R-7050 markedly suppressed the effects of ALF-P. PPADS inhibited the effects of ALF-P to a greater degree than R-7050 (Fig. 4b, c). Treatment with TNFα for 72 h stimulated the proliferation of OCs and protected the cells from apoptosis, and this effect was abolished by co-treatment with PPADS, but not NF023 (Figs. 4d, e). However, our data showed no significant difference in plasma TNFα levels between the NC-P and the ALF-P samples (Fig. 4f). By contrast, Interleukin 8 (IL-8) levels increased, whereas IL-5 and IL-17 levels decreased in the ALF-P compared with those in NC-P (Supplemental data, Table 1).

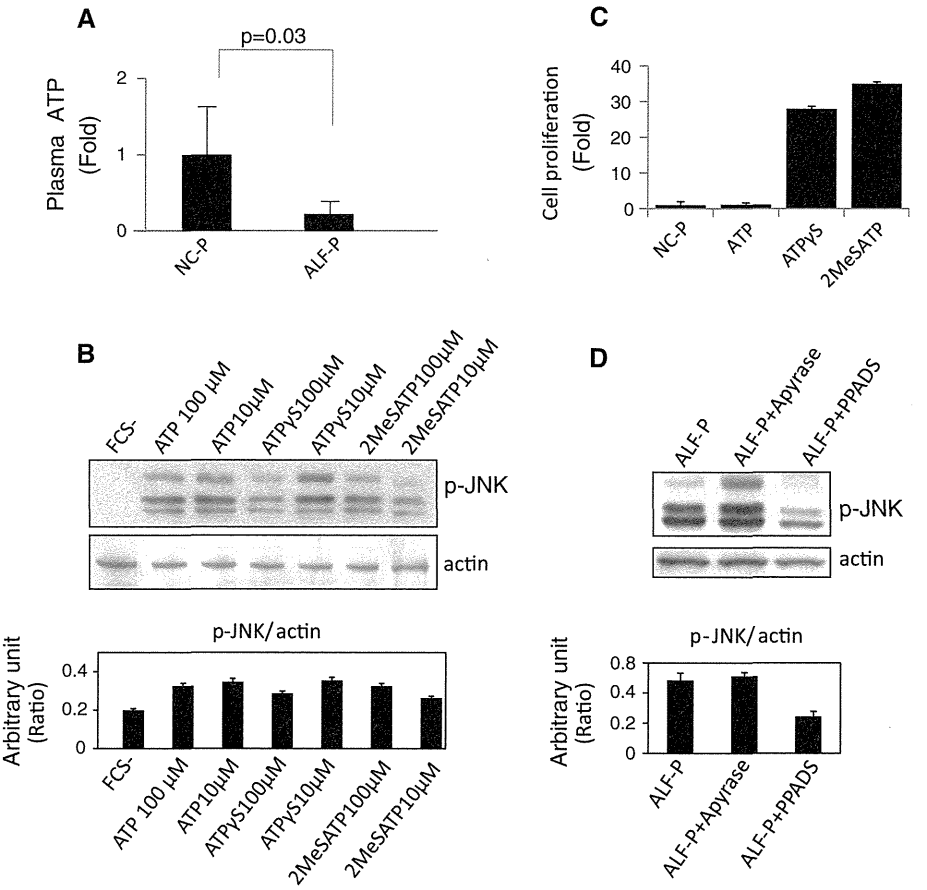

Fig. 3 Extracellular ATP does not contribute to the effects of ALF-P on the growth of OCs. **a** Plasma ATP was measured using samples from patients with ALF and normal control subjects. Data are expressed as the mean ± SD ($n = 3$). **b** OCs were treated with ATP or two other P2 receptor agonists at the indicated concentrations. The proliferation of the cells was evaluated after 72 h. **c** The cells were treated as described in **b**. The phosphorylation of JNK was determined by western blot analysis. **d** OCs were treated with ALF-P with or without the indicated inhibitors for 72 h. The phosphorylation of JNK was determined by western blot analysis. Actin was used as the internal control. Data in **b**–**d** are expressed as the mean ± SD ($n = 3$)

Discussion

Total plasma exchange (TPE) was significantly effective for correcting coagulopathy and improving liver tests in the treatment of ALF [35], indicating that some hepatotoxins/cytokines in ALF plasma can pathologically affect the growth of hepatocytes and OCs. The results of the present study showed that ALF-P promoted the proliferation of primary hepatocytes and OCs. However, in contrast to the sustained increase in cell proliferation and JNK activation in OCs, the proliferation of primary hepatocytes peaked during the first 24 h of stimulation and declined thereafter. Moreover, no activation of JNK was observed in these cells. The data suggest that although ALF plasma stimulates the proliferation of primary hepatocytes, it exerts its effect early and transiently through a JNK-independent signaling pathway, which is considerably different from the action of ALF-P in OCs. Clinically, liver progenitor cells may therefore proliferate much more than normal hepatocytes in response to sustained stimulation with ALF-P. Normal control plasma did not stimulate JNK activation in OCs or primary hepatocytes. JNK activation was observed in ALF-P-stimulated OCs, but not in primary hepatocytes. The findings demonstrate the pathologic roles of the JNK pathway in regulating the proliferation of OCs, which may be related to the impairment of liver regeneration in ALF.

The transcription factor c-Jun is required for hepatocyte survival and proliferation [36–39]. c-Jun phosphorylation is usually activated by JNK but sometimes is JNK-independent [40]. The present study showed that NC-P stimulated the phosphorylation of c-Jun in OCs in a JNK-independent manner. In addition, NC-P negatively regulated the proliferation of OCs by inducing apoptosis. Since Harrington has demonstrated the negative effect of serum on the proliferation of primary hepatocytes [41], which was also shown in the present study using NC-P, our results demonstrate the physiological significance of c-Jun in the growth of OCs. Contrary to NC-P, ALF-P activated ATF2 and JNK but not c-Jun. To date, although the functional properties of ATF2 remain poorly understood, significant in vitro and in situ experiments demonstrate mainly a proliferative role for ATF2 in several types of cancer, including hepatic cancer [42]. Taken together, the data suggest that the activation of different JNK downstream target genes may result in different outcomes for the growth of OCs.

The present study addressed the role of P2 receptors in the ALF-P-stimulated proliferation of OCs. It has been

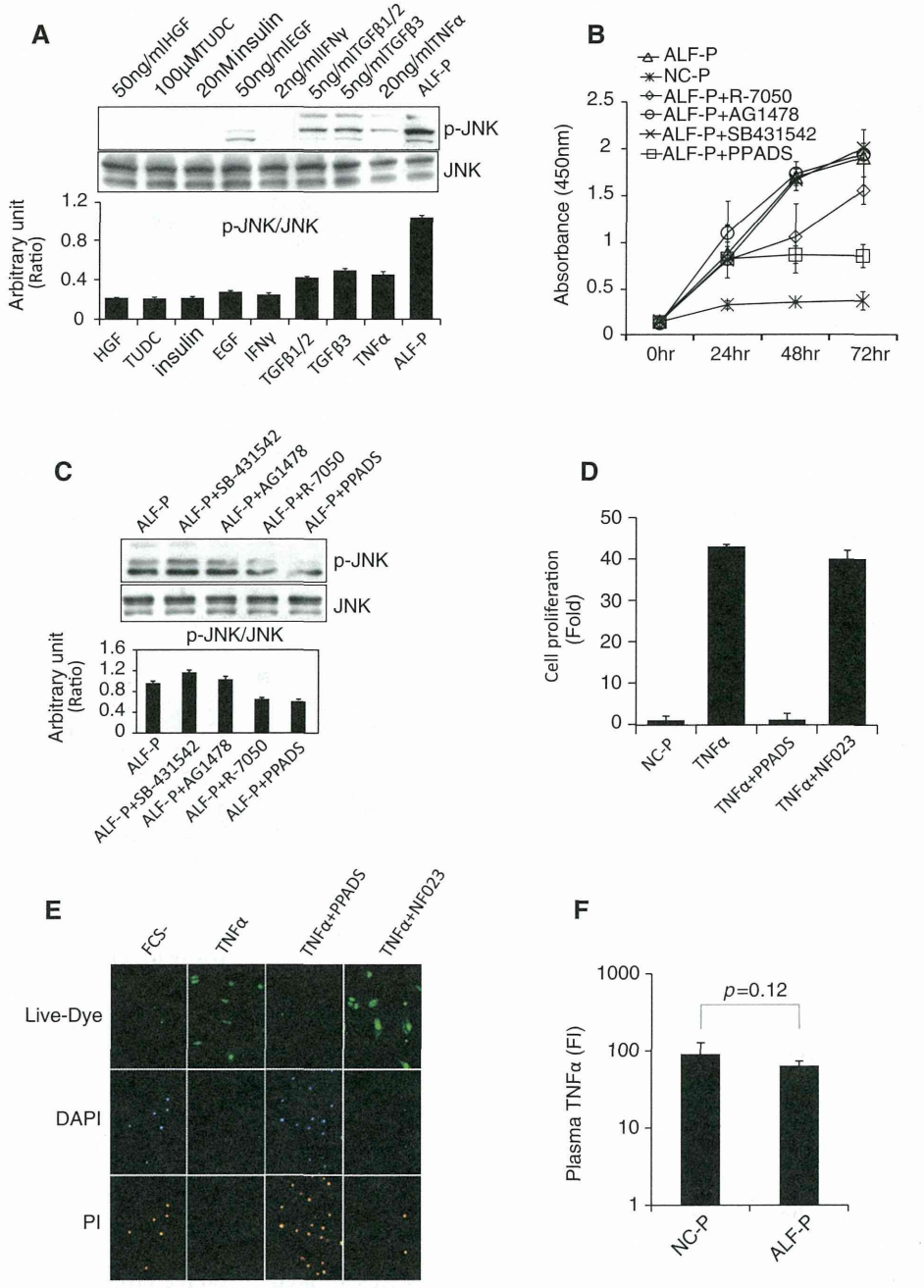

Fig. 4 TNFα receptor signaling mediates the function of ALF-P in a P2 receptor-dependent manner. **a** Cells were incubated with 30 % ALF-P and the other indicated reagents. The phosphorylation of JNK was detected after 3 days of stimulation. JNK was also detected as an internal control. Data are expressed as the mean ± SD ($n = 3$). **b** Cells were incubated with 30 % NC-P or 30 % ALF-P with or without the indicated inhibitors. The proliferation of the cells was measured daily by using cell count reagent SF. In addition, the phosphorylation of JNK (**c**) was detected after 72 h of stimulation. Data are expressed as the mean ± SD ($n = 3$). **d** OCs were treated with 10 μg/mL TNFα with or without 250 μM PPADS or 200 μM NF023. Cells proliferation was determined after 3 days of stimulation. Data are expressed as the mean ± SD ($n = 3$). **e** OCs were treated as described in **d**. Apoptotic and dead cells were evaluated as described in "Materials and methods". **f** Plasma TNFα levels were measured as described in "Materials and methods" using samples from patients with ALF and normal control subjects. Data are expressed as the mean ± SD ($n = 3$). $^*p < 0.05$ versus control

reported that $P2X_4$ and $P2X_7$ receptors are expressed predominantly in hepatocytes, and the former contributes to ATP-dependent calcium signaling and glucose release [43]. However, our results showed that P2X receptor transcripts other than $P2X_2$ were not detectable. Moreover, a P2X-specific inhibitor did not block the effects of ALF-P on the proliferation of OCs, suggesting that P2X receptors are not involved in the function of ALF-P. On the other hand, pharmacological and RNA interference (RNAi) approaches provided evidence that $P2Y_2$ rather than $P2Y_1$ participates in the regulation of OCs by ALF-P. Notably, although the expression of $P2Y_2$ mRNA was blocked by siRNA transfection, neither the proliferation of OCs nor JNK activation was completely abolished in response to stimulation with ALF-P, as shown in Fig. 2. Since in addition to $P2Y_2$, $P2Y_4$, and $P2Y_6$ have roles in the proliferation of cell lines other than hepatocytes [44, 45], it is reasonable to assume, in combination with our findings, that P2Y receptors other than $P2Y_2$ may participate in the effects of ALF-P.

Interestingly, despite the importance of P2Y receptors demonstrated in the present study, our findings showed that extracellular ATP does not participate in the function of ALF-P, suggesting that P2 agonists other than ATP may be

involved. Although all P2Y receptors are activated by ATP, at $P2Y_1$, ADP is reported to be equipotent or more potent than ATP, and at $P2Y_2$, UTP and ATP are equipotent [46]. In addition, UTP levels are critical for efficient hepatitis C virus replication, and UTP depletion contributes to the development of liver injury [47, 48]. Whether ADP or UTP contributes to the effects of ALF-P on the growth of OCs is now under investigation.

Given the evidence on the importance of TNFα in the development of ALF and the negative correlation between ALF prognosis and TNFα levels, the present study suggests that a TNFα receptor signal is involved in the effects of ALF-P. Moreover, P2 receptor inhibition completely abolished the effects of TNFα on the proliferation and apoptosis of OCs, indicating that the TNFα pathway is P2 receptor-dependent. Our data showed no significant difference in plasma TNFα levels between the NC-P and the ALF-P samples. Although the plasma levels of other cytokines, including IL-5, IL-8, and IL-17, were significantly changed in the ALF-P compared with those in NC-P, because these interleukins do not interact with ATP or TNFα receptors, we can therefore predict that all the above cytokines do not contribute to the ALF-P-induced action of the TNFα receptors. On the other hand, several members of the TNF family besides TNFα serve as ligands of the TNF receptors [49]. There is another possibility that some unknown TNF ligands may contribute to the effects of ALF-P through TNF receptor signaling. These factors should thus be clarified in future investigations.

The components of normal human plasma have been reported [50, 51]. However, the exact nature of toxic molecules in the plasma during liver failure is unknown and the toxicity effects may vary among different organ systems [52]. In the present study, we focused on the effects of ATP, TNFα, and their related signals on the proliferation of oval cells based on the fact that both ATP and TNFα play essential roles in the development of fulminant hepatitis and in regulating the proliferation of hepatocytes. Although our data did not support that either ATP or TNFα should be the target molecule in the ALF plasma, the importance of $P2Y_2$ receptor crosstalk with the TNFα signaling pathway has been clearly addressed and subsequently will be valuable for our later investigations.

In conclusion, the present study demonstrated the specific involvement of JNK activation, the important roles of ATP receptor $P2Y_2$, and the crosstalk of $P2Y_2$ with TNFα receptor signaling in mediating the effects ALF-P on the regulation of OC growth. The data also suggested that targeting the JNK pathway could selectively inhibit the abnormal proliferation of OCs in ALF without affecting the growth of normal hepatocytes, which may be of clinical significance in the treatment of ALF.

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Conflict of interest The authors declare that they have no conflicts of interest.

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Original Article

Liver stiffness measured by acoustic radiation force impulse elastography reflects the severity of liver damage and prognosis in patients with acute liver failure

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Aim: We measured liver stiffness (LS) in patients with acute liver failure (ALF) using acoustic radiation force impulse (ARFI) elastography and investigated the usefulness of measuring LS for predicting the prognosis of ALF patients.

Methods: From April 2010 to December 2013, we evaluated 63 patients with acute liver disease. The subjects included 41 patients with acute hepatitis (AH), 16 patients with severe AH (SAH), who had no hepatic encephalopathy despite plasma prothrombin time of 40% or less, and six patients with fulminant hepatitis (FH) diagnosed according to the criteria of the Japanese Study Group. The relationships among shear wave velocity (SWV), clinical diagnosis, liver function tests and prognosis were evaluated. Receiver–operator curve (ROC) analysis was performed to investigate whether ARFI elastography exhibits potential usefulness for the prediction of FH.

Results: The mean SWV on admission were 1.98 ± 0.55, 2.61 ± 0.58 and 3.66 ± 0.86 m/s in the AH, SAH and FH groups, respectively. The SWV was significantly higher in the FH group than in the other groups ($P < 0.001$), and in the SAH group than in the AH group ($P = 0.002$). The area under the ROC for predicting FH was 0.924 (sensitivity, 83.3%; specificity, 93.0%). The SWV was significantly increased in non-survivors, while remaining decreased in survivors ($P = 0.002$).

Conclusion: The SWV measured by ARFI elastography reflects severity of liver damage, and serial changes in SWV predict the prognosis of ALF patients. The SWV is an early and precise biomarker of FH.

Key words: acoustic radiation force impulse elastography, acute liver failure, liver stiffness, prognosis, shear wave velocity

INTRODUCTION

MOST PATIENTS WITH conventional acute liver disease recover without specific therapy such as liver transplantation or artificial liver support, but not all.[1,2] Among patients with acute liver disease, acute liver failure (ALF) is a devastating clinical syndrome in which rapid deterioration of liver function results in altered mentation and coagulopathy in previously normal individuals, leading to multiple organ failure and often death.[3,4] The mortality rate of ALF is still often more than 50% in the absence of emergency liver transplantation.[5,6] The severity of liver tissue damage and the prompt reconstruction of liver tissue in ALF are critical factors that impact the prognosis.[7,8] Therefore, the establishment of an early and non-invasive method for predicting the prognosis of ALF patients is urgently needed for determining the necessity for liver transplantation. The Model for End-Stage Liver Disease (MELD) score, the King's College Hospital (KCH) criteria and Japan hepatic encephalopathy (HE) prediction model are useful prognostic models for assessing the risk of poor outcome in a patient with ALF.[9–11] These prognostic models obtain their data from hematological examination and the patient's background. On the other hand, histological examination of liver biopsy specimens obtained through transvenous biopsy and various

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imaging methods such as computed tomography (CT) could be used to assess liver damage and reconstruction.[12] However, ALF patients are often too sick to undergo these examinations frequently and continuously. In contrast, ultrasonography can be performed on a seriously ill patient at any time, because it is non-invasive and can be performed at the bedside.

Recently, with regard to elastography that can be used to assess liver stiffness (LS), several previous studies using transient elastography (TE) have revealed that the degree of LS increased transiently during the acute phase of ALF.[13–17] Increased LS in ALF patients has been reported to be caused by a combination of hepatic necrosis, inflammation, hepatocyte edema, bilirubin elevation and intrahepatic collagen deposition.[13–19] However, there has been no study to compare disease severity or prognosis using LS as a marker in patients with ALF.

Acoustic radiation force impulse (ARFI) elastography is a reproducible technology using ultrasonography for quantitative assessment of tissue stiffness through measurement of shear wave velocity (SWV). ARFI elastography has been reported to be a reliable method for evaluating the extent of fibrosis in chronic liver disease (CLD).[20–23] In our previous study using ARFI elastography, we reported that LS was increased in patients with ALF.[24]

In the present study, we used ARFI elastography to measure SWV in patients with ALF on admission, and compared the results with the patients' disease severity. Additionally, we investigated the usefulness of measuring SWV for an early prediction of fulminant hepatitis (FH) and in predicting the prognosis of patients with ALF.

METHODS

Patients

THIS STUDY WAS conducted with the approval of the ethics committee of Iwate Medical University, and written informed consent from the study subjects was obtained. The study was retrospective, and included 63 consecutive patients with acute liver disease (25 men and 38 women; mean age, 51.1 ± 16.8 years; age range, 18–79 years) admitted to our institution between April 2010 and December 2013. The subjects included 41 patients with acute hepatitis (AH), 16 patients with severe AH (SAH), who had no HE despite a plasma prothrombin time (PT) of 40% or less, and six patients with FH diagnosed according to the criteria of the Japanese Study Group, with a PT of 40% or less, in whom severe HE of grade II or more developed within 8 weeks of the onset of symptoms.[25,26] The diagnostic criteria of ALF in Japan are different from those in the USA and Europe, where ALF is diagnosed when patients show coagulopathy with PT international normalized ratio of 1.5 or more and any degree of HE within 24 weeks after the onset of disease.[27] ALF is a clinical syndrome defined as an acute liver disease complicated by HE, and it includes non-hepatitis.[3,26] All patients in the present study had hepatitis. We excluded CLD and liver fibrosis clinically from the patient's laboratory data and medical history, but hepatitis B virus carriers and autoimmune hepatitis patients showing acute exacerbation of hepatitis in the normal liver are included. Moreover, the patients with alcoholic hepatitis caused by habitual alcohol consumption were excluded from the study group. The etiology of hepatitis is based on the diagnostic criteria for ALF in Japan.[26] These patients had no cirrhosis. The background of the subjects and their laboratory data on admission are summarized in Table 1.

LS measurements

Liver stiffness was measured using ARFI elastography.[20–23,28] All measurements of LS were performed by a single operator. ARFI elastography has been incorporated into a conventional ultrasonographic device (Acuson S2000 with a Siemens 4C1 curved array, 4.00 MHz for B-mode, 2.67 MHz for push pulses and 3.08 MHz for detection pulses; Siemens Medical Solutions, Mountain View, CA, USA). An actual ARFI elastography session is shown in Figure 1. The region of interest (ROI) was set in the area 2 cm from the surface of the hepatic anterior segment, through the intercostal space. The measurement of LS was defined as the mean value of 10 SWV measurements using ARFI elastography. The measure was considered incorrect by motion artifact when it was "xxx". The SWV was measured until recovery or death since hospital admission at 7-day intervals.

Serum markers of liver function and CT imaging

In all patients, biochemical tests, CT imaging and ARFI elastography were performed on the first hospital day. Liver atrophy is considered to be a prognostic factor for poor outcome in a patient with ALF.[29,30] Sekiyama *et al.* demonstrated that the CT-derived liver volume (CTLV) of survivors of ALF was significantly greater than that of non-survivors.[31] Serial abdominal transverse CT was taken at 10-mm intervals. Volumetric measurement of

Table 1 The clinical features and laboratory data on admission

Parameters	AH	SAH	FH
No. of patients	41	16	6
Sex (male/female)	17/24	5/11	3/3
Mean age, years (range)	49.9 (18–79)	50.4 (23–76)	61.2 (52–70)
Etiology			
HBV	9	1	3
HEV	4	0	0
EBV	3	0	0
CMV	1	0	0
PB19	0	1	0
Drug	10	2	0
AIH	6	3	0
Unknown	8	9	3
T.Bil (mg/dL)	7.1 (4.1–13.0)	7.6 (3.6–16.3)	11.7 (4.7–26.1)*,***
AST (IU/L)	1011 (429–1635)	795 (478–1894)	419 (157–1489)
ALT (IU/L)	1199 (661–2104)	1078 (386–2572)	543 (129–1665)
PT (%)	71.3 (54.1–83.1)	36.8 (33.0–38.6)*	24.9 (19.1–34.2)*
CTLV/SLV ratio	1.07 (0.98–1.25)	1.06 (0.87–1.13)	0.79 (0.76–0.82)*,***
MELD score (score)	18.0 (14.0–20.0)	24.0 (20.5–26.5)*	30.5 (26.0–39.5)*,**
KCH criteria met (%)	2/41 (4.9)	3/16 (18.8)	4/6 (66.7)*,**
HE prediction model (%)	4.9 (0.9–14.1)	34.8 (24.0–48.9)*	81.2 (77.2–89.2)*
Survival (%)	41/41 (100)	16/16 (100)	1/6 (16.7)*,**

*$P < 0.01$ (compared with AH), **$P < 0.01$ (compared with SAH), ***$P < 0.05$ (compared with SAH). Values were expressed as median (25–75th percentile).
AH, acute hepatitis; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMV, cytomegalovirus; CTLV, computed tomography-derived liver volume; EBV, Epstein–Barr virus; FH, fulminant hepatitis; HBV, hepatitis B virus; HE, hepatic encephalopathy; HEV, hepatitis E virus; KCH, King's College Hospital; MELD, Model for End-Stage Liver Disease; PB19, parvovirus B19; PT, prothrombin time; SAH, severe acute hepatitis; SLV, standardized liver volume; T.Bil, total bilirubin.

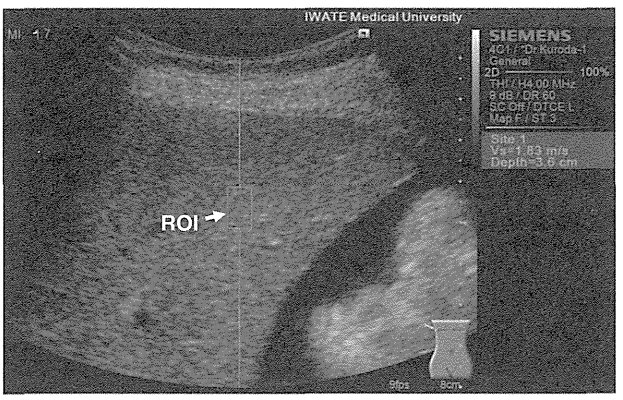

Figure 1 Acoustic radiation force impulse elastography. Sonogram shows the placement of the region of interest (ROI) for measurement of shear wave velocity at a depth of 2 cm from the surface of hepatic anterior segment, through the intercostal space.

the liver was performed essentially according to the method by Henderson *et al.*[32] The margin of each slice of liver was outlined by a cursor to measure the area. These areas were then multiplied by the height (10 mm) and summed to estimate whole liver volume (mL). Bodyweight and body height were recorded and used to calculate body surface area (BSA). The standardized liver volume (SLV) was calculated from the BSA using the formula of Urata *et al.* on the first hospital day.[33] The present study evaluated the relationships among the SWV values, clinical diagnosis, results of clinical liver function tests and CTLV/SLV ratio.

Prognostic model for ALF

The MELD score, the KCH criteria and Japan HE prediction model were calculated for each patient based on the results of hematological examination and the patient's background on admission.[9–11] The early predictive performance of FH of ARFI elastography, MELD score, KCH criteria, HE prediction model and laboratory data were assessed by receiver–operator curves (ROC). The cut-off

values for early prediction of FH were estimated using the area under the ROC (AUROC). Serial changes in the SWV values between the survivors ($n = 58$) and non-survivors ($n = 5$) were compared.

Statistical analysis

Statistical tests were performed by using SPSS version 12.0 software (SPSS, Chicago, IL, USA). The values are shown as the means ± standard deviation, or medians (range) according to the distribution of values. A statistical analysis of the differences in SWV values among patients with AH, SAH and FH was performed using the Tukey–Kramer method. The correlations between SWV and other parameters were assessed using Spearman's rank correlation coefficient. ROC analysis was performed to investigate whether ARFI elastography exhibits its potential usefulness for the early prediction of FH. The sensitivity and specificity were calculated to determine the cut-off point. Moreover, repeated measures ANOVA was used to assess changes in SWV during 2 weeks. A *P*-value of less than 0.05 was considered statistically significant.

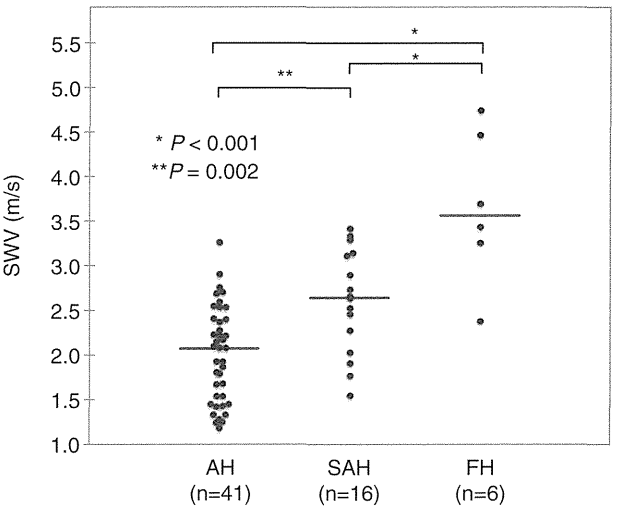

Figure 2 The shear wave velocity (SWV) value for each patients in three groups. The mean SWV value (± standard deviation) on admission was 1.98 ± 0.55, 2.61 ± 0.58 and 3.66 ± 0.86 m/s in the acute hepatitis (AH), severe AH (SAH) and fulminant hepatitis (FH) groups, respectively. *$P < 0.001$; **$P = 0.002$.

RESULTS

THE MEAN SWV on admission were 1.98 ± 0.55, 2.61 ± 0.58 and 3.66 ± 0.86 m/s in the AH, SAH and FH groups, respectively. The SWV was significantly higher in the FH group than in the other groups ($P < 0.001$), and in the SAH group than in the AH group ($P = 0.002$) (Fig. 2).

The relationships between the SWV and the clinical parameters in the patients upon admission are shown in Table 2. The SWV showed a significant negative correlation with PT ($P = 0.0003$), and a significant positive correlation with serum total bilirubin (T.Bil) ($P = 0.0011$) and hepatocyte growth factor (HGF) ($P = 0.0001$). The SWV correlated significantly with the CTLV/SLV ratio ($P = 0.0002$).

The AUROC in early prediction of FH were 0.971 for the HE prediction model, 0.924 for ARFI elastography, 0.914 for the MELD score, 0.865 for HGF and 0.789 for the KCH criteria (Fig. 3). The differences between ARFI elastography and the HE prediction model, the MELD score or HGF did not reach statistical significance ($P = 0.160$, $P = 0.810$, $P = 0.619$, respectively). On the other hand, the difference between ARFI elastography and the KCH criteria was statistically significant ($P = 0.028$). The most appropriate cut-off value of SWV for predicting FH was 3.14 m/s, and the sensitivity, specificity, positive predictive value and negative predictive value were 83.3%, 93.0%, 55.6% and 98.1%, respectively.

During convalescence, the SWV in all patients with AH and SAH showed a significant decrease. On the other

Table 2 Correlation between the SWV value and various biochemical parameters or CTLV/SLV ratio

Parameters	Correlation coefficient	Significance (*P*-value)
T.Bil	0.417	0.0011*
AST	−0.021	0.8689
ALT	−0.078	0.5385
Alb	−0.228	0.1232
PT	−0.535	0.0003*
HGF	0.653	0.0001*
WBC	−0.084	0.5143
CRP	0.014	0.9144
CTLV/SLV ratio	−0.453	0.0002*

*$P < 0.01$.
Alb, aluminum; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CTLV, computed tomography-derived liver volume; HGF, hepatocyte growth factor; PT, prothrombin time; SLV, standardized liver volume; SWV, shear wave velocity; T.Bil, total bilirubin; WBC, white blood cell.

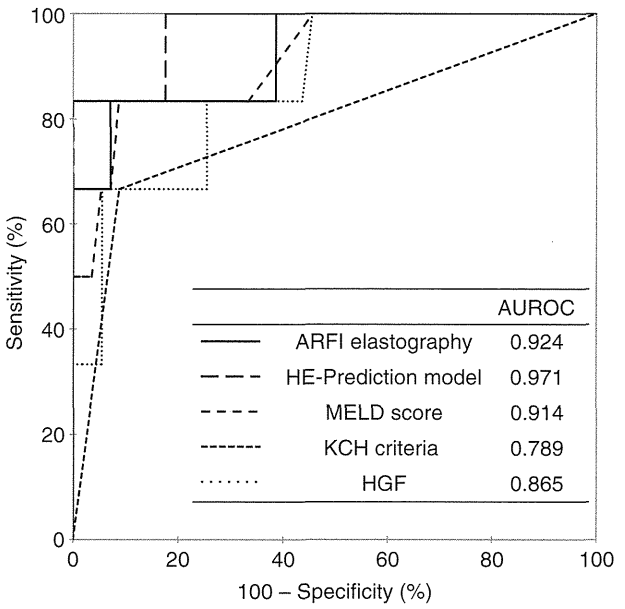

Figure 3 Receiver–operator curve (ROC) analysis for the prediction of fulminant hepatitis (FH). The area under the ROC (AUROC) in early prediction of FH were 0.971 for the hepatic encephalopathy (HE) prediction model, 0.924 for acoustic radiation force impulse (ARFI) elastography, 0.914 for the Model for End-Stage Liver Disease (MELD) score, 0.865 for hepatocyte growth factor (HGF) and 0.789 for the King's College Hospital (KCH) criteria. ——, ARFI elastography; — –, HE prediction model; – – , MELD score; ----, KCH criteria; ····, HGF.

hand, among the patients with FH, the SWV decreased in the one survivor, while it significantly increased to 4.23 ± 0.45 m/s over 2 weeks from 3.66 ± 0.76 m/s in the five non-survivors ($P = 0.002$) (Fig. 4).

DISCUSSION

MANY REPORTS HAVE demonstrated that the diagnostic accuracy of LS measured using ultrasound-based elastographic methods such as TE and ARFI elastography is comparable to that obtained in the prediction of severe fibrosis and cirrhosis in CLD.[20–23,34] Recently, the performance of liver elastography is expected not only in the evaluation of fibrosis for CLD, but also in the non-invasive assessment of esophageal varices, hepatocellular carcinoma and portal hypertension in patients with CLD.[35–37] On the other hand, several previous studies using elastography have revealed that the degree of LS increased transiently during the acute phase of ALF.[13–19] Our previous study presented the case of a patient who survived hyperacute-type ALF and who showed a dramatic change in the SWV as measured by ARFI elastography.[24] However, there has been no investigation of the usefulness of measuring LS in predicting the prognosis of patients with ALF. To the best of our knowledge, the present study provides the first data in comparing LS with disease severity in patients with acute liver disease.

The present study demonstrated that SWV was significantly higher in the FH group than in the other groups, and it was also significantly higher in the SAH group than in the AH group. In addition, the AUROC for early prediction of FH was 0.924 for ARFI elastography, and it was determined with high accuracy. The MELD score, KCH criteria and Japan HE prediction model are well known, very useful criteria for predicting the prognosis of ALF.[9–11] However, a hepatic morphological marker such as LS is not included in the aforementioned criteria. Based on the data of this study, measurement of SWV may have a use in the early detection of FH.

The mechanism of increased LS in ALF is thought to derive from the combination of hepatic necrosis, inflammation, hepatocyte edema, bilirubin elevation, congestion and intrahepatic collagen deposition.[13–19,38] In addition, it has been reported that change in liver viscoelasticity influences SWV.[39] In a recent study,

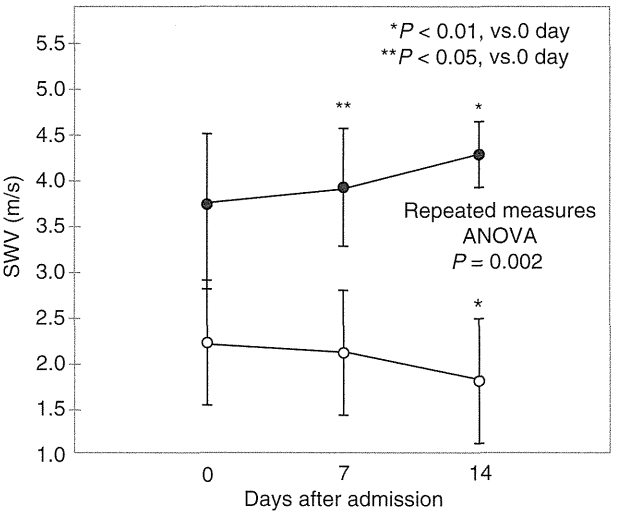

Figure 4 Serial changes in the shear wave velocity (SWV) of survivors ($n = 58$) and non-survivors ($n = 5$). Data represent means ± standard error of the mean. In patients with survivors (open circle) and non-survivors (closed circle). –●–, non-survivors ($n = 5$); –○–, survivors ($n = 58$). *$P < 0.01$, vs 0 days; **$P < 0.05$, vs 0 days.

Dechêne *et al.* reported that LS in patients with ALF showed positive correlations with the degree of liver cell damage and the intensity of hepatic stellate cell activation.[19] The results of the Dechêne study share certain similarities with our data in that LS was found to be significantly higher in the patients with severe ALF. A weakness of our study is the lack of histological correlation, and it is a subject for future analysis.

The present study has shown that SWV correlates significantly with T.Bil, PT and HGF. SWV was observed to strongly correlate with serum levels of HGF, which is a possible product of non-parenchymal cells. HGF is considered to be a reliable indicator for assessing the prognosis of ALF, along with several other serum biochemical parameters that indicate hepatocellular dysfunction, such as T.Bil and PT.[40]

We also observed that SWV correlated significantly with the CTLV/SLV ratio. Small liver volume has been reported to be one of the factors in poor prognosis in patients with ALF.[29,30] However, CT scanning requires transfer of the patient to the radiology department, which can exacerbate a patient's hemodynamic instability and intracranial hypertension associated with ALF. In contrast, the advantage of ultrasonography-based ARFI elastography over CT is that measurements can be performed at the bedside non-invasively without transfer of the patient. ARFI elastography uses software integrated into a conventional ultrasound machine. Therefore, the measurement of LS can be accomplished in the same session and with the same machine that is used for the conventional ultrasound examination. Moreover, ARFI elastography can be performed in patients with ascites, because the ROI can be set under real-time ultrasound guidance, which is not possible with TE.

In the present study, we found that SWV decreased gradually during convalescence in all patients with AH and SAH. In the one patient with FH who survived, SWV decreased, while SWV remained increased in the five FH non-survivors. It is conjectured that SWV does peak after the inflammation that occurs during the acute phase of ALF subsides. Therefore, serial measurements of SWV may be useful in evaluating the prognosis of FH. However, it should be noted that a small number of non-survivors were included in our study because the present study was undertaken at a single institution.

In conclusion, the findings of the present study suggest that SWV measured by ARFI elastography reflects the severity of liver damage, and serial changes in SWV may be a useful indicator for evaluating the prognosis of patients with ALF. From examining the findings, SWV is found to be an early and precise biomarker of FH.

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Special Report

Classification of the etiologies of acute liver failure in Japan: A report by the Intractable Hepato-Biliary Diseases Study Group of Japan

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The Intractable Liver Diseases Study Group of Japan, supported by the Ministry of Health, Labor and Welfare, established novel diagnostic criteria for "acute liver failure" in 2011. In these criteria, patients without histological findings of hepatitis are included in the disease entity of "acute liver failure", as in Europe and the USA. In this report, classification criteria for the etiologies of "acute liver failure" in Japan are proposed.

Key words: autoimmune hepatitis, drug-induced liver injury, fulminant hepatic failure, fulminant hepatitis, HBV

Hepatitis Viral Infection is the most important and common cause of acute liver failure in Japan. Acute liver failure is typically represented by fulminant hepatitis in Japan, and the diagnostic criteria for "fulminant hepatitis" were established by the Inuyama Symposium in 1981.[1] The etiology of fulminant hepatitis includes viral infection, autoimmune hepatitis, drug allergy-induced liver injury and hepatitis of indeterminate etiologies.[2] In contrast, in the USA, Trey and Davidson proposed criteria for the diagnosis of "fulminant hepatic failure" in 1970,[3] which includes liver failure caused by drug toxicity, circulatory disturbances, metabolic diseases, acute fatty liver of pregnancy and postoperative liver damage, none of which is included in the etiological factors of the disease entity of "fulminant hepatitis" in Japan. Then, Polson and Lee published an American Association for the Study of Liver Diseases position paper in 2005,[4] and "fulminant hepatic failure" was replaced by "acute liver failure", although the etiological factors of the disease entity of "acute liver failure" have not been changed until now, either in Europe or in the USA.

The diagnostic criteria for "fulminant hepatitis" in Japan need to be revised to correspond to those for "acute liver failure" in Europe and the USA. Thus, the Intractable Liver Diseases Study Group of Japan, supported by the Ministry of Health, Labor and Welfare, established novel diagnostic criteria for "acute liver failure", which include the disease entity of "fulminant hepatitis" in 2011.[5,6] According to these criteria, patients showing prothrombin time values of 40% or less of the standardized values or international normalized ratios of 1.5 or more caused by severe liver damage developing within 8 weeks of the onset of symptoms are diagnosed as having "acute liver failure", with the liver function prior to the current onset of liver damage being

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Table 1 Classification of the etiologies of acute liver failure

I. Viral infection: Those satisfying the following criteria for laboratory data, showing clinical features consistent with viral infection.

I-① HAV: Positive test result for anti-HAV (IgM).

I-② HBV: Positive test result for either HBsAg or anti-HBc (IgM), but care should be exercised in rare cases in which the test result for serum HBV DNA is positive whereas all of the serum markers for HBV are negative.*

I-②-1 Transient HBV infection; when any of the following three situations is satisfied:

- Negative test result for HBsAg preceding the onset of liver injury in the absence of immunosuppressive and/or anticancer therapies in the previous 12 months.
- High levels of anti-HBc (IgM).
- Low levels of anti-HBc (IgG).

I-②-2 Acute exacerbation in HBV carriers; when any of the following four situations is satisfied:

- Positive test result for HBsAg preceding the onset of liver injury (A).
- Low levels of anti-HBc (IgM) (B).
- High levels of anti-HBc (IgG) (C).
- Negative test result for HBs antigen, but positive test results for anti-HBc or anti-HBs preceding the onset of liver injury, in cases with a history of immunosuppressive and/or anticancer therapies in the previous recent 12 months (D).

I-②-2-i Asymptomatic or inactive HBV carriers without drug exposure; those satisfying A, B or C above in the absence of immunosuppressive and/or anticancer therapies in the previous 12 months.

I-②-2-ii Reactivation in asymptomatic or inactive HBV carriers receiving immunosuppressive and/or anticancer drugs; those with a history of immunosuppressive and/or anticancer therapies in the previous 12 months satisfying A, B or C above.

I-②-2-iii Reactivation by immunosuppressants and/or anticancer drugs in patients with resolved HBV (de novo HBV hepatitis); those satisfying D.

I-②-3 Indeterminate HBV infection; those with HBV infection, but not fulfilling the criteria shown in I-②-1 and I-②-2.

*To bear in mind that in general, hepatitis due to HBV is associated with high levels of serum HBV DNA, except in HBeAg positive asymptomatic carriers.

I-③ HCV: Positive for anti-HCV and/or HCV RNA.

I-④ HEV: Positive for anti-HEV (IgA) and/or HEV RNA.

I-⑤ Other viruses: demonstration of transient infection or reactivation of EBV, cytomegalovirus and other viruses through measurements of serological markers and viral genomes.

II. Autoimmune hepatitis; those satisfying "Criteria for Diagnosis of Autoimmune Hepatitis" proposed by the International Autoimmune Hepatitis Group, or those positive for antinuclear antibody or serum IgG concentrations 1.1-times the upper limit of the normal range at each institution or greater.**

**To bear in mind that patients with autoimmune hepatitis may be confused with those having drug-induced liver injuries or hepatitis of indeterminate etiology. Patients with the possibility of this condition should be treated as soon as possible as cases for autoimmune hepatitis.

III. Drug-induced liver injuries; those consistent with drug-induced liver injurybased on their clinical courses.

III-① Drug allergy-induced hepatitis.***

III-② Drug toxicity-induced liver injury (excluded from hepatitis).***

***Differential diagnosis between drug allergy-induced hepatitis and drug toxicity-induced liver injuries is based on the types and doses of the drugs and the clinical features of the patients.

IV. Liver injuries without the histological findings of hepatitis; diagnosis is based on the clinical features of the patients.

IV-① Circulatory disturbance.****

IV-② Metabolic diseases; Wilson's disease, anorexia nervosa, acute fatty liver of pregnancy, Reye's syndrome and others.

IV-③ Infiltration of the liver by malignant cells.

IV-④ Liver injuries after liver resection and transplantation.

IV-⑤ Miscellaneous etiologies.

****Liver injuries after operation other than liver resection and transplantation; those due to bacterial infection, DIC and heat stroke are in general classified as being caused by circulatory disturbance

V. Indeterminate etiology despite adequate examinations.

VI. Unclassified due to inadequate examinations.

DIC, disseminated intravascular coagulation; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; Ig, immunoglobulin.

estimated to be normal. Patients without histological findings of hepatitis are included in the disease entity of "acute liver failure", as in Europe and the USA. In this report, classification criteria for the etiologies of "acute liver failure" in Japan are proposed (Table 1).

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＜原　著＞

薬物性肝障害診断スコアリングにおけるE型肝炎の診断マーカー追加の必要性についての検討

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要旨：薬物性肝障害診断時の項目にE型肝炎の除外は含まれていないが，IgA-HE抗体測定系保険収載後の2012年よりE型肝炎届出数は増加している現状がある．2003年～2012年の期間に当院で経験したE型肝炎13例で，薬物性肝障害診断時のE型肝炎除外の必要性について検討した．E型肝炎12例の感染源は不明であった．E型肝炎マーカー未測定時，8例が薬物性肝障害と誤診され，薬物性肝障害例の11.6％を占めた．E型肝炎例はスコアリングシステムを使用時全例が5点以上となり，点数分布を薬物性肝障害例と比較しても有意差を認めなかった．従来指摘されている畜肉の摂取は13例中1例でのみであり喫食歴からE型肝炎感染を推測することは困難であった．正確な薬物性肝障害診断のため，現行の薬物性肝障害診断基準にE型肝炎マーカー，特に保険収載されたIgA-HE抗体価測定を加え，スコアリングの段階でE型肝炎の除外診断を行う必要があると考えられる．

索引用語：　E型肝炎　　薬物性肝障害　　スコアリングシステム

はじめに

薬物性肝障害（drug-induced liver injury：DILI）は日常診療で経験する代表的な急性肝障害の原因の一つである．現在本邦でのDILIの診断には，「DDW-J 2004薬物性肝障害ワークショップ」において提案された診断基準[1)]が使用されることが一般的であるが，この診断基準は，その後に行われたValidity studyでも高い確率でDILIの診断が可能であることが示されており[2)]，臨床現場でDILIの診断を行うにあたり極めて有用なツールであるといえる．DILIの診断には，薬物以外の原因による肝障害の除外が重要である．現行の診断基準では項目4（カテゴリー1及び2）として，A型肝炎ウイルス（HAV），B型肝炎ウイルス（HBV），C型肝炎ウイルス（HCV），胆道系疾患，アルコール，ショック，EBウイルス（EBV）やサイトメガロウイルス（CMV）による肝障害の除外を求めている[1)]．これら以外にも肝障害を生じうる原因はまだ多数存在すると思われるが，日常診療で遭遇することはまれであり，肝臓専門医以外が日常診療で使用することを目的として作成された診断基準でもあることから，特にウイルスマーカーについては比較的発生頻度が多いと考えられる項目を採用するに留められている[1)3)]．

E型肝炎は，現行のDILI診断基準で採用が見送られた項目の1つである．E型肝炎は従来，本邦においては東南アジアや南アジアなど流行地域からの輸入感染症として認識されていたが，2001年に初めて国内感染患者が報告され，また飼育ブタから日本土着のE型肝炎ウイルス（HEV）株が分離され報告されて[4)5)]以後，国内各地から我が国土着のHEV株の感染が原因と考えられる急性肝炎症例発生の報告が相次ぎ，本邦におけるE型肝炎に対する認識は大きく変容した[6)7)]．その一方でDILI診断に際してのE型肝炎マーカーの測定については，現行の診断基準が作成された時点及びValidity studyが行われた時点で，本邦でのE型肝炎の発生頻度は他のウイルス性肝炎に比して低いこと，またIgM-HE抗体やHEV RNAを使用した測定系が保険未収載であることから，肝臓専門医が必要と判断した時に測定を考慮するのみに留められ[1)~3)]，その後2013年6月現在に至るまで，E型肝炎の取扱いについてDILI診断基

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Table 1 The number of cases tested for hepatitis E virus markers among 199 cases with acute liver injury during 2003-2012 in our hospital

HEV markers*		Number of cases			
		Hepatitis E	DILI	Others**	total
HEV RNA		7	19	34	60
IgM-HE Ab		2	1	1	4
IgA-HE Ab		4	16	19	39
	Subtotal	13 (12)	36 (31)	54 (48)	103 (91)
NT***		0	25	71	96
	Total	13	61	125	199

*: The HEV markers were tested as the initial screening in a commercial laboratory (SRL, Tokyo, Japan and Mitsubishi Chemical Medience Corporation, Tokyo, Japan) and the presence of HEV markers was confirmed in stored serum samples indicated in parentheses in our research laboratory in Jichi Medical University.
**: Others include all acute liver injury cases except for those with hepatitis E or drug-induced liver injury (DILI).
***: NT, not tested.

準では変更されていない.

しかし,最近になってDILIを診断する際にE型肝炎との鑑別の必要性に言及している論文が本邦及び海外からも報告されてきている[8)~15)]. これらの論文では,E型肝炎の抗体やHEV RNAの検出を行なわずに,従来通りの診断方法のみを使用した時は,E型肝炎をDILIと誤って診断する可能性を指摘している. さらに2011年10月1日よりIgA-HE抗体測定が本邦において保険収載され[16)],臨床現場でE型肝炎の検査が保険診療で可能となった. これらの状況を踏まえて,我々は,現行のDILI診断基準にE型肝炎マーカー測定によるE型肝炎の除外を加えることを再検討する必要性があると考えた. そこで2003年から2012年の間に鈴鹿中央総合病院消化器内科で経験し報告してきた症例[17)~19)]を含めたE型肝炎症例群,及び同時期に経験したDILI症例群を用いて現行のDILI診断基準で両群の鑑別が可能か否かを検討し,診断基準へのE型肝炎測定系の追加の必要性の検討を行った.

対象と方法

2003年10月から2012年9月までの108カ月間,鈴鹿中央総合病院消化器内科を受診し,入院または外来で精査加療を受けた199例の急性肝障害症例の内,急性E型肝炎と診断された13例とDILIと診断された61例を対象とした. 急性E型肝炎の診断は,IgM-HE抗体陽性,IgA-HE抗体陽性,HEV RNA陽性の各項目のいずれかを使用して陽性反応を認めた症例を急性E型肝炎と診断した. 急性肝障害例に対して行われたE型肝炎測定状況をTable 1に示す. 急性肝障害199例中,103例でE型肝炎診断が行われており,IgM-HE抗体測定のみで診断したものが4例,IgA-HE抗体測定のみで診断したものが39例,これらの抗体測定に加えHEV RNA測定も行い診断したものが60例であった. DILIと診断された61例中36例でE型肝炎マーカーの測定が行われ感染が否定されていたが,残り25例はE型肝炎マーカーの測定が行われていなかった. DILIの診断は,「DDW-J 2004薬物性肝障害ワークショップ」において提示された診断基準により除外診断とスコアリングを行い,必要と思われた症例ではエコー下肝生検を行った. またE型肝炎,DILI症例群共に,他のウイルス性肝疾患,自己免疫性肝疾患,胆道系疾患,ショック肝を血清学的検査,画像診断等にて除外した. 急性E型肝炎症例13例で,E型肝炎感染を考慮せずDILIを疑ったと仮定して,「DDW-J 2004薬物性肝障害ワークショップ」において提示された診断基準によりスコアリングを行い点数化した. 薬物服用歴のある症例では,「DDW-J 2004薬物性肝障害スコアリングシステム」の「項目1」から「項目8」までの全てを使用して計算を行った. 薬物服用歴のない症例では,薬物服用が認められ,DILIが強く疑われたと仮定し,「項目2」,「項

Table 2 Causes of acute liver injury in 199 cases

Cause		Number of cases (n = 199)
HAV		7
HBV		33
HCV		1
HEV		13
EBV + CMV + HSV		27
Others* + unknown origin		57
DILI		61
Type of disease	Hepatocellular type	42
	Cholestatic type	5
	Mixed type	14

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HSV, herpes simplex virus; DILI, drug-induced liver injury.
*: Others include liver injuries associated with alcohol, shock, disseminated intravascular coagulation (DIC), starvation, portal vein thrombosis or acuteonset autoimmune hepatitis.

Table 3 Comparison of number of cases positive for HEV RNA, IgM-HE Ab or IgA-HE Ab between hepatitis E cases and DILI cases

HEV markers*	No. of cases (positive case/tested case)	
	Hepatitis E**	DILI
HEV RNA	7/7	0/19
IgM-HE Ab	2/2	0/1
IgA-HE Ab	4/4	0/16
Total	13/13	0/36

*: The HEV markers were tested as the initial screening in a commercial laboratory (SRL, Tokyo, Japan and Mitsubishi Chemical Medience Corporation, Tokyo, Japan).
**: The presence of HEV RNA and IgM-HE/IgA-HEV Abs were confirmed in 12 cases, whose stored serum samples were available, in our research laboratory in Jichi Medical University.
All viremic cases had also IgM-HEV and IgA-HE Abs.

目3」,「項目4」,「項目6」を使用して計算を行った. 薬物服用歴のある症例はもちろん, 薬物服用歴のない症例でも仮に薬物服用歴があれば, DILIを強く疑った可能性が考えられたため,「項目4」については, カテゴリー1, 2がすべて除外されたと考え, +2を採用した. なお急性E型肝炎群は, E型肝炎と診断された後のスコアリングも計算した. DILI群と急性E型肝炎群間の比較には, Fisher's exact test, Student's t-test, 及びMann-Whitney U testを使用し, $p<0.05$を有意差ありと判断した.

結　果

Table 2に, 2003年10月から2012年9月までの108カ月間に当院で発生した急性肝障害199例の内容を示す. 上記の期間に急性E型肝炎は計13例発生し, DILIは計61例発生していた. 同期間に急性A型肝炎は7例, 急性B型肝炎は33例, 急性C型肝炎は1例発生していた. DILI 61例は, 肝細胞障害型が42例, 胆汁うっ滞型が5例, 混合型が14例で, 各型の頻度は肝細胞障害型, 胆汁うっ滞型, 混合型がそれぞれ69%, 8%, 23%であった. 急性E型肝炎例13例のうち, 6例は抗体検査(IgM-HE抗体陽性, あるいはIgA-HE抗体陽性)のみで診断されたが, 残りの7例は抗体検査に加え, HEV RNAも陽性であることが確認された. IgM-HE抗体とIgA-HE抗体の一方, あるいは両者が陰性でHEV RNA陽性という症例はなかった. すなわち, HEV RNA陽性の7例はIgM-HE抗体とIgA-HE抗体の両者が同時に陽性であった. DILIはE型肝炎マーカーの測定が行われた36例全例でHEV RNA, IgM-HE抗体, IgA-HE抗体のいずれもが陰性であった (Table 3).

当院で発生した急性E型肝炎13例は, 12例が男性で, 女性は1例のみであった. これら13例の急性E型肝炎症例は, 受診時点で10例が薬物服用の既往を認めた (Table 4). 急性E型肝炎発症3カ月前までの海外渡航歴は2例 (No. 2；中国, No. 3；米国) に認めた. 一方急性E型肝炎13例中イノシシ肉, 鹿肉, 豚レバーの摂取歴を認めた症例は1例のみであった. 他の生物の摂取では急性E型肝炎例では生の馬肉摂取が2例, 刺身あるいは寿司の摂取が11例を, またDILIでは生の牛肉摂取が3例, 刺身あるいは寿司の摂取が27例を認めた (Table 5). 以上の結果から, 急性E型肝炎症例13例の中で, 経過中に鑑別診断としてHEV感染を考慮せずにE型肝炎マーカー測定を施行しなかった場合, 結果として8例の急性E型肝炎例がDILIと誤って診断されてしまうことが疑われた (Table 4). これら8例の急性E型肝炎例が誤ってDILIと診断された場合, 当院でのDILIは計69例となり, 全DILI症例69例中に8例 (11.6%) のE型肝炎をDILIと誤診した症例を含む