Table 5 Classification of hepatic encephalopathy depending on the grading of hepatic come in adult patients proposed by the inuyama symposium in 1972

The grade of coma	Psychiatric disorders	Reference items
I	• Inversion of sleep pattern • Euphoria and/or occasional depression • Sloppy attitude with Shortened attention span	Recognized retrospectively in most cases
II	• Disorientation for time or place and confusion of objects • Inappropriate behaviors, such as to throwing away money or discarding cosmetics • Occasional somnolent tendency; able to open eyes and respond appropriately to questions • Makes impolite remarks, but follows doctors' instructions	Excitation state and incontinence of urine and service are absent, but lapping tremor is found on physical examination
III	• State of excitation and/or delirium showing defiant behavior • Somnolent tendency; sleeping most of the time • Opens eyes in response to stimulation, but cannot follow the instructions of doctors, except for simple orders	Flapping tremor is observed, and the extent of disorientation is severe
IV	• Coma; complete loss of consciousness • Response to painful stimuli	Brushes off hands and/or frowns in response to stimuli
V	• Deep coma • No response to painful stimuli	

however, differed from those of the patients with fulminant hepatitis, since liver damage in alcoholic liver disease patients develops in an acute-on-chronic liver disease pattern. Also, the survey revealed that the most frequent cause of death was complications of liver disease, but not the liver failure itself, in most patients with etiologies not listed among the etiologies of fulminant hepatitis or LOHF.

Based on these observations, "acute liver failure" in Japan is defined as an acute liver disease associated with prolongation of the prothrombin time, with an INR of 1.5 or more. To confirm the correspondence between the present and previous criteria, "prothrombin time values of 40% or less of the standardized values" was also used as a cutoff to define the patients with acute liver failure. Consequently, patients without hepatic encephalopathy may also be included in the disease entity of acute liver failure, if they show an INR of 1.5 or more. Thus, acute liver failure patients are classified into those with and without hepatic coma, and acute liver failure with hepatic coma is further subdivided into two disease types, namely, the "acute type" and the "subacute type," according to the interval from the onset of symptoms to the development of hepatic encephalopathy, similarly to the case for fulminant hepatitis.[1,4]

Regarding the etiology of acute liver failure, patients without histological evidence of inflammation in the

Table 6 Classification of hepatic encephalopathy depending on the grading of hepatic coma in pediatric and infantile patients as proposed in the 5th workshop on pediatric liver diseases in 1988

Grade of coma	Pediatric	Infantile
I	Low-spirited from before (poor activity)	Does not laugh aloud
II	Obedient attitude with somnolent tendency Disorientation for time or place	Does not laugh even when dandled Cannot maintain eye contact with the mother (later than 3 months after birth)
III	Open eyes in response to loud voice	
IV	Does not wake up in response to painful stimuli, but frowns and/or brushes off by his/her hands	
V	No response to painful stimuli	

Table 7 Classification of etiologies of acute liver failure modified from the criteria proposed by the Intractable Liver Diseases Study Group of Japan in 2002

I. Viral infection
  1 Hepatitis A virus (HAV)
  2 Hepatitis B virus (HBV)
    (1) Transient infection
    (2) Acute exacerbation in HBV carrier*
      i. Inactive carrier, without drug exposure
      ii. Reactivation in inactive carrier by immunosuppressant and/or anticancer drugs
      iii. Reactivation in transiently infected patients by immunosuppressant and/or anticancer drugs (de novo hepatitis)
    (3) Indeterminate infection patterns
  3 Hepatitis C virus (HBV)
  4 Hepatitis E virus (HEV)
  5 Other viruses
II. Autoimmune hepatitis
III. Drug-induced liver injuries
  1. Drug allergy-induced liver injury
  2. Drug toxicity-induced liver injury
IV. Circulatory disturbance
V. Infiltration of the liver by malignant cells
VI. Metabolic diseases
VII. Liver injuries after liver resection and transplantation
VIII. Miscellaneous etiologies
IX. Indeterminate etiology despite sufficient examinations
X. Unclassified due to insufficient examinations

Patients with etiologies I, II and III-1 are diagnosed as having "fulminant hepatitis" as well as "acute liver failure", whereas those with etiologies III-2 and IV to VIII are diagnosed as having "acute liver failure", but excluded from disease entity of "fulminant hepatitis". Diagnostic criteria for classification of etiology based on laboratory data should be established in the future.
Serum HBs antigen-negative patients following transient infection with HBV are classified as HBV carriers, in which HBV reactivation can be induced by immunosuppressant and/or anticancer drugs, but the significance of this causative etiology needs to be evaluated further.

liver, such as those with the disease caused by drug toxicity, circulatory disturbance or metabolic disease, were included under the disease entity of acute liver failure. In contrast, patients showing impaired liver function due to underlying chronic liver diseases before the worsening of the liver damage were excluded from the disease entity of acute liver failure. Thus, alcoholic liver disease patients were excluded from the disease entity, since they showed clinical features consistent with acute-on-chronic liver disease. However, patients with underlying chronic liver diseases such as fatty liver due to alcohol intake or metabolic diseases, including obesity, were included in the disease entity of acute liver failure, when the liver function impairment was retrospectively estimated to be minimal or absent prior to the current exacerbation of liver damage, since the incidence of metabolic syndrome has been increasing in the Japanese population. The patients with autoimmune hepatitis were defined similarly to those with underling fatty liver, because the presence of chronic liver disease preceding the hepatitis exacerbation is uncertain in most of these patients. On the other hand, patients with liver injury caused by viral infection, autoimmune hepatitis and drug allergy-induced hepatitis are included under the disease entities of "fulminant hepatitis" as well as "acute liver failure". The significance of diagnostic criteria for fulminant hepatitis should be further evaluated in the future.

In addition, the diagnostic criteria for LOHF as a disease related to "acute liver failure". LOHF was defined as grade II or more severe hepatic encephalopathy developing between 8 and 24 weeks of the onset of symptoms.[1,4] However, in the present criteria, patients with LOHF were defined as those showing INRs of 1.5 or more as well as prothrombin time values of 40% or less of the standardized values. Thus, patients without histological evidence of hepatitis are also included in the disease entity of LOHF, similar to the case of acute liver failure. On the other hand, the disease entity of "acute hepatitis severe type", in which

patients show no or grade I hepatic encephalopathy despite having prothrombin time values of 40% or less as compared to the standardized values, was excluded from the footnote of the present criteria, since patients classified under such a disease entity can also be diagnosed as having "acute liver failure without hepatic encephalopathy".

In conclusion, the diagnostic criteria for "acute liver failure" in Japanese patients were established. A nationwide survey of "acute liver failure" will be conducted by the Intractable Hepato-Biliary Diseases Study Group of Japan after 2011, based on these novel criteria. Also, the significance of the diagnostic criteria in Japan should be further evaluated in relation to the criteria in Europe and the United States.

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REFERENCES

1 Fujiwara K, Mochida S, Matsui A *et al.* Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol Res* 2008; **38**: 646–57.
2 Polson J, Lee WM. AASLD position paper: the management of acute liver failure. *Hepatology* 2005; **41**: 1179–97.
3 The Proceedings of the 12th Inuyama Symposium. *Hepatitis Type A and Fulminant Hepatitis*. Tokyo: Chugai Igaku-sha, 1982 (in Japanese).
4 Mochida S, Nakayama N, Matsui A *et al.* Re-evaluation of the Guideline published by the Acute Liver Failure Study Group of Japan in 1996 to determine the indications of liver transplantation in patients with fulminant hepatitis. *Hepatol Res* 2008; **38**: 970–9.
5 Gimson AES, O'Grady JG, Ede RJ, Portmann B, Williams R. Late onset hepatic failure: clinical, serological and histological features. *Hepatology* 1986; **6**: 288–94.
6 Tsubouchi H, Oketani M, Fulminant Hepatitis and Late Onset Hepatic Failure (LOHF) in Japan. *Annual Report of the Study Group of Intractable Hepato-Biliary Study Group in Japan Supported by the Ministry of Health, Labor, and Welfare. 2006*. 2004; 61–9. (in Japanese).
7 Tsubouchi H, Oketani M, Ido A *et al.* Fulminant Hepatitis and Late Onset Hepatic Failure (LOHF) in Japan. *Annual Report of the Study Group of Intractable Hepato-Biliary Study Group in Japan Supported by the Ministry of Health, Labor, and Welfare. 2007*. 2005; 90–100. (in Japanese).
8 Tsubouchi H, Oketani M, Fulminant Hepatitis and Late Onset Hepatic Failure (LOHF) in Japan. *Annual Report of the Study Group of Intractable Hepato-Biliary Study Group in Japan Supported by the Ministry of Health, Labor, and Welfare. 2008*. 2006; 83–94. (in Japanese).
9 Tsubouchi H, Oketani M, Ido A *et al.* Fulminant Hepatitis and Late Onset Hepatic Failure (LOHF) in Japan. *Annual Report of the Study Group of Intractable Hepato-Biliary Study Group in Japan Supported by the Ministry of Health, Labor, and Welfare. 2009*. 2007; 83–93. (in Japanese).
10 Tsubouchi H, Oketani M, Ido A *et al.* Fulminant Hepatitis and Late Onset Hepatic Failure (LOHF) in Japan. *Annual Report of the Study Group of Intractable Hepato-Biliary Study Group in Japan Supported by the Ministry of Health, Labor, and Welfare. 2010*. 2008; 95–106. (in Japanese).

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Original Article

Novel scoring system as a useful model to predict the outcome of patients with acute liver failure: Application to indication criteria for liver transplantation

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Aim: In Japan, the indication for liver transplantation in patients with acute liver failure (ALF) is currently determined according to the guideline published in 1996. However, its predictive accuracy has fallen in recent patients. Thus, we attempted to establish a new guideline.

Methods: The subjects were 1096 ALF patients enrolled in a nationwide survey. All patients showed a prothrombin time <40% of the standardized value and grade II or more severe hepatic encephalopathy. A multiple logistic regression analysis and receiver operating characteristic analysis were performed in 698 patients seen between 1998 and 2003 to identify significant parameters determining the outcome of patients. The extracted parameters were graded as numerical scores. An established scoring system was validated in patients seen between 2004 and 2008.

Results: Six parameters were identified and graded as 0, 1 and/or 2; the interval between disease onset and development of hepatic encephalopathy, prothrombin time, serum total bilirubin concentration, the ratio of direct to total bilirubin concentration, peripheral platelet count and the presence of liver atrophy. When the prognosis of the patients with total score of 5 or more was judged as "death", the predictive accuracy was 0.80 with sensitivity, specificity, positive predictive value and negative predictive value greater than 0.70. The values were similarly high in patients for validation.

Conclusion: Novel scoring system for predicting the outcome of ALF patients may be useful to determine the indication of liver transplantation, since the system showed high predictive accuracy even after validation.

Key words: acute liver failure, fulminant hepatitis, guideline, indication criteria, liver transplantation, outcome prediction

INTRODUCTION

ACUTE LIVER FAILURE is a disease entity characteristic with extensive destruction of liver parenchyma by hepatitis virus infection and other causes, and is typically represented by fulminant hepatitis. Although the outcome may differ depending on the etiology of acute liver failure, survival rates of patients receiving conventional medical care are generally low in cases with impaired liver regeneration. Hepatitis patients are diagnosed as fulminant hepatitis in Japan if grade II or deeper hepatic encephalopathy develops within 8 weeks of the onset of hepatitis symptoms due to severe abnormality of the liver function with prothrombin time lower than 40% of the standardized value. Fulminant hepatitis is further classified into two subtypes according to clinical

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course; acute type with hepatic encephalopathy developing within 10 days of disease onset and subacute type with hepatic encephalopathy at 11 days or later.[1,2] In addition, late onset hepatic failure (LOHF) is defined as a related disease of fulminant hepatitis, in which hepatic encephalopathy develops between 8 and 24 weeks after the onset of hepatitis symptoms.[2] Fulminant hepatitis in Japan is defined as acute liver failure with histological appearance of hepatic inflammation, such as lymphocyte infiltration in the liver. Thus, the etiology of fulminant hepatitis comprises viral hepatitis including persistent hepatitis B virus (HBV) carriers, autoimmune hepatitis, drug-induced/allergic hepatitis, and hepatitis with indeterminate etiologies, but excludes drug-induced/toxic liver damage, acute fatty liver in pregnancy, postoperative liver damage and ischemic liver damage. However, in Europe and the United States, the latter causes are included in the disease entity of acute liver failure, formerly called fulminant hepatic failure. Thus, for example, acetaminophen-induced liver damage is included in acute liver failure in Europe and the United States, while it is excluded from the disease entity of fulminant hepatitis in Japan.[3] Considering such differences of the definition and diagnostic criteria between fulminant hepatitis in Japan and acute liver failure in the United States and Europe, the guidelines in the latter countries to determine the indication of liver transplantation for acute liver failure are not directly applicable to fulminant hepatitis in Japan.

It is accepted worldwide that liver transplantation is the most effective therapeutic modality for patients with acute liver failure. In Japan, indication criteria for liver transplantation in patients with fulminant hepatitis were defined in 1996, as a two-step outcome prediction scoring system, by the Acute Liver Failure Study Group of Japan (Table 1).[4,5] According to this guideline, the outcome of fulminant hepatitis patients is predicted at the onset of grade II or more severe hepatic encephalopathy based on five parameters: age of patient, the interval between occurrence of hepatitis symptoms and development of hepatic encephalopathy, prothrombin time, serum total bilirubin concentration and the ratio of the serum direct to total bilirubin concentration. Then, in patients undergoing intensive medical care including artificial liver support, their prognosis is reassessed 5 days later, according to the extent of improvement of hepatic encephalopathy and prothrombin time. This guideline was prepared based on the clinical findings in fulminant hepatitis patients seen between 1988 and 1992, and was considered to be useful, since the predictive accuracy was found to be 83% in a prospective analysis conducted in patients seen between 1993 and 1995.[4] However, the predictive accuracy of the guideline fell in patients with fulminant hepatitis seen between 1998 and 2003; 68% and 78% in the acute and subacute types, respectively, and the values did not improve following reassessment at 5 days later.[6]

Table 1 Guideline to determine the indications for liver transplantation in patients with fulminant hepatitis by the Acute Liver Failure Study Group of Japan in 1996

Patients may be registered as recipients of liver transplantation when at least two of the five criteria are satisfied at the time of onset of Grade II or more severe hepatic encephalopathy.

1 Age ≥45 years.
2 Interval from the appearance of the initial symptoms to the development of hepatic encephalopathy ≥11 days.
3 Prothrombin time <10% of the standardized value.
4 Serum bilirubin concentration ≥18.0 mg/dL.
5 Ratio of the direct to total bilirubin concentration <0.67.

If liver transplantation cannot be performed within 5 days and intensive medical therapy, including artificial liver support, is undertaken, the prognosis of the patients is evaluated again. If both of the following two criteria are positive at 5 days after the onset of hepatic encephalopathy, the patients are re-predicted as "alive" and excluded from the candidate list for liver transplantation.

1 The hepatic encephalopathy shows improvement to Grade I or less or attenuation by two more grades.
2 Prothrombin time improves to over 50% of the standardized value.

English version of this guideline was published in Mochida *et al.*[5]

To improve the predictive accuracy of indication criteria for liver transplantation in patients with fulminant hepatitis, the Study Group of Intractable Hepatobiliary Diseases supported by the Ministry of Health, Labor, and Welfare of Japan organized a task force in 2006. The task force first analyzed the database obtained from patients with fulminant hepatitis and LOHF seen between 1998 and 2003 in Japan to establish the novel scoring system to predict the outcome of the patients. Then, the established system was evaluated in the patients seen between 2004 and 2008. In the present paper, we report on the usefulness of this novel scoring system. We state here that the new system is intended for use in a general cohort of acute liver failure, but is actually organized on the database of registered patients with fulminant hepatitis and LOHF. Thus, validation of

the system for acute liver failure due to other etiologies as described earlier awaits future study.

METHODS

Patients

THE STUDY SUBJECTS are 1096 patients with acute liver failure who were enrolled in the nationwide survey by the Intractable Hepato-Biliary Disease Study Group of Japan between 1999 and 2008 (formerly the Intractable Liver Diseases Study Group of Japan before 2003). All of the patients showed grade II or more severe hepatic encephalopathy and prothrombin time of less than 40% of the standardized value and were admitted to 610 hospitals of Japan specializing in hepatology between 1998 and 2008. The patients consisted of three disease types; 505 and 449 patients, respectively, with acute and subacute types of fulminant hepatitis and 88 patients with LOHF. They were divided into two cohorts; 698 patients (316, 318 and 64 patients, respectively, with acute and subacute types of fulminant hepatitis and LOHF) seen between 1998 and 2003 (the estimation cohort) and 394 patients (189, 191 and 24 patients, respectively, of each disease type) seen between 2004 and 2008 (the validation cohort). From both cohorts, the patients with incomplete records and those treated with liver transplantation were excluded. Thus, the estimation cohort included 421 patients (201 and 178 patients, respectively, with acute and subacute types of fulminant hepatitis and 41 patients with LOHF) and the validation cohort recruited 231 patients (125, 95 and 11 patients, respectively, in each disease type).

Etiologies of hepatitis in the estimation and validation cohorts are given in Table 2. Demographic and clinical features of patients in each cohort are shown in Tables 3 and 4, respectively. These features did not differ between the two cohorts, except that the ages of the patients were greater in the validation cohort than in the estimation cohort. The survival rates of patients were equivalent between two cohorts; 37.4% in the estimation cohort and 37.7% in the validation cohort.

Table 2 Etiologies of fulminant hepatitis and late onset hepatic failure (LOHF) in the estimation cohort and validation cohort

	Estimation cohort 1998–2003	Validation cohort 2004–2008
HAV	33	15
HBV (acute onset)	104	51
HBV (career)	65	33
HBV (unclassified)	9	16
HCV	8	3
Other Virus	3	5
AIH	26	23
Drug	35	33
Undetermined	132	49
No record	6	3
Total	421	231

Data are expressed as the number of patients.
AIH, autoimmune hepatitis; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus.

Identification of prognostic factors responsible for the outcome of patients with fulminant hepatitis and LOHF in the estimation cohort

First, univariate logistic analysis was performed in patients of the estimation cohort to identify possible prognostic factors among demographic and clinical features at the onset of grade II or more severe hepatic encephalopathy as follows. (i) *Demographic features*; sex and age of patients, the types of disease (acute and subacute types of fulminant hepatitis and LOHF), the interval (days) between the onset of hepatitis symptoms and the development of hepatic encephalopathy. (ii) *Symptoms*; fever of 37.5°C or more, convulsion, tachycardia, disappearance of liver dullness on physical examination, flapping tremor, hepatic odor and edema. (iii) *Laboratory parameters*; prothrombin time (%), hepaplastin test (%), antithrombin III activity (%), serum concentrations of albumin (g/dL) and total and direct bilirubin (mg/dL), the ratio of direct to total bilirubin concentration, serum levels of aspartate aminotransferase (AST: IU/L) and alanine aminotransferase (AST: IU/L), serum α-fetoprotein concentration (ng/mL), blood ammonia concentration (μg/dL), plasma concentration of hepatocyte growth factor (HGF: ng/mL), peripheral platelet and white blood cell counts (/mm^3). (iv) *Imaging*; liver atrophy diagnosed by ultrasound sonography and/or computed tomography/magnetic resonance imaging (CT/MRI).

Extracted factors were subjected to multivariate logistic analyses through a stepwise elimination manner. Then a receiver operating characteristic (ROC) curve was constructed for each significant variable.

Scoring of prognostic factors and predicted mortality of patients with fulminant hepatitis and LOHF in the estimation and validation cohorts

The grading of variables was determined as numerical scores based on the inflection points of each ROC curve.

Table 3 Demographic and clinical features of patients with fulminant hepatitis and late onset hepatic failure (LOHF) seen between 1998 and 2003 (estimation cohort)

	Total ($n = 421$)	Dead patients ($n = 260$)	Surviving patients ($n = 161$)
Sex (Male : Female)	218:202:(1)†	148:111:(1)†	70:91
Age	48.6 ± 16.3‡	53.2 ± 14.8**	41.3 ± 16.0
HBV Carrier	15.2% (64/421)	18.8%** (49/260)	9.3% (15/161)
Disease Type (FHA : FHS : LOHF)	201:178:41	86:138:36**	115:40:5
HGF (ng/mL)	6.0 ± 11.4	7.6 ± 13.9*	3.9 ± 6.0
TB (mg/dL)	14.0 ± 9.1	16.6 ± 9.6**	9.7 ± 6.2
D/T ratio	0.63 ± 0.13	0.62 ± 0.14**	0.66 ± 0.12
PT (%)	22.7 ± 12.6	22.5 ± 13.8*	23.5 ± 11.3
AT (%)	37.3 ± 20.3	35.9 ± 20.3*	41.5 ± 19.9
NH3 (μg/dL)	138.7 ± 82.8	151.6 ± 87.8**	118.1 ± 69.6
PLT (10^4/μL)	12.7 ± 7.7	12.1 ± 8.1*	13.6 ± 6.9
Liver Atrophy (present : absent)	265:156	210:50**	55:106
O-C (days)	21.2 ± 26.7	26.4 ± 29.3**	12.8 ± 19.2

*$P < 0.05$, **$P < 0.01$ versus alive.
†A value in parenthesis means the number of patients with no record regarding the sex.
‡Values are expressed as mean ± standard deviation (SD).
AT, antithrombin III; D/T ratio, ratio of direct to total bilirubin concentrations; FHA, acute type of fulminant hepatitis; FHS, subacute type of fulminant hepatitis; HBV, hepatitis B virus; HGF, hepatocyte growth factor; O-C, intervals between hepatitis onset and hepatic encephalopathy development; PLT, platelet; PT, prothrombin time; TB, total bilirubin.

The total scores were calculated in each patient belonging to the estimation cohort, and the mortality rates were evaluated depending on total scores. Then, ROC analysis was performed again to identify the cut-off value of the total score that can discriminate sharply between survived and dead patients. Finally, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and predictive accuracy of the established scoring system were calculated when the predicted outcome of patients with the total score greater than the cut-off value was judged as "death".

Predictive accuracies of the established system were confirmed similarly in the validation cohort.

Statistical analysis

All statistical analyses were performed with JMP v7.0 for Macintosh (SAS Institute Inc., Cary, NC, USA). Univariate analyses were performed with analysis of variance (ANOVA) and χ^2 test. Multivariate analyses were performed by multiple logistic regression analysis with stepwise selection.

RESULTS

Prognostic factors responsible for the outcome of patients with fulminant hepatitis and LOHF

UNIVARIATE LOGISTIC ANALYSIS revealed 18 variables including demographic features and clinical characteristics at the appearance of grade II or more severe hepatic encephalopathy may affect the mortality of the patients; age of patients, the interval between disease onset and the development of hepatic encephalopathy, presence of tachycardia and edema, disappearance of liver dullness on physical examination and presence of liver atrophy on imaging examination, serum concentrations of albumin and total bilirubin, the ratio of direct to total bilirubin concentration, serum levels of AST and ALT, blood ammonia level, plasma HGF concentration, prothrombin time, hepaplastin test, antithrombin III activity and peripheral platelet count. These factors were subjected to multivariate logistic analysis with stepwise elimination manner, and 10 variables were identified as significant as shown in Table 5. At this step,

Table 4 Demographic and clinical features of patients with fulminant hepatitis and late onset hepatic failure (LOHF) seen between 2004 and 2008 (validation cohort)

	Total ($n = 231$)	Dead patients ($n = 144$)	Surviving patients ($n = 87$)
Sex (Male : Female)	120:111	74:70	46:41
Age	54.7 ± 15.0†	59.8 ± 12.2**	46.3 ± 15.6
HBV Carrier	14.2% (33/231)	19.4% (28/144)	5.7% (5/87)
Disease Type (FHA : FHS : LOHF)	125:95:11	61:74:9**	64:21:2
HGF (ng/mL)	6.0 ± 9.2	7.1 ± 10.4	3.2 ± 3.6
TB (mg/dL)	13.7 ± 8.9	16.6 ± 9.3**	8.8 ± 5.3
D/T ratio	0.64 ± 0.14	0.64 ± 0.13	0.63 ± 0.16
PT (%)	24.5 ± 12.7	22.0 ± 12.4**	28.4 ± 12.4
AT (%)	38.2 ± 20.4	34.4 ± 21.1*	44.3 ± 17.7
NH3 (μg/dL)	162.0 ± 141.8	191.1 ± 168.8**	116.4 ± 60.7
PLT (10^4/μL)	12.6 ± 7.2	11.5 ± 6.9**	14.3 ± 7.2
Liver Atrophy (Present/absent)	146:85	114:30**	32:50
O-C (days)	15.9 ± 17.0	19.0 ± 18.5**	10.6 ± 12.2

*$P < 0.05$, **$P < 0.01$ versus alive.
†Values are expressed as mean ± standard deviation (SD).
AT, antithrombin III; D/T ratio, ratio of direct to total bilirubin concentrations; FHA, acute type of fulminant hepatitis; FHS, subacute type of fulminant hepatitis; HBV, hepatitis B virus; HGF, hepatocyte growth factor; O-C, intervals between hepatitis onset and hepatic encephalopathy development; PLT, platelet; PT, prothrombin time; TB, total bilirubin.

"age of patients" was excluded from the list of candidate variables in order to facilitate the system to be available in pediatric patients. Then ROC curve was constructed for each variable, and six variables with the greatest area under the curve (AUC) were identified; the interval between disease onset and the development of hepatic encephalopathy, prothrombin time, serum concentration of total bilirubin, the ratio of direct to total bilirubin concentration, peripheral platelet count, and liver atrophy.

Table 5 Prognostic factors to affect the outcome of patients with fulminant hepatitis and late onset hepatic failure (LOHF): multivariate logistic analysis in those seen between 1998 and 2003 (estimation cohort)

	Odds ratio†	(95% confidence interval)	*P*-value
Liver atrophy	9.777		<0.0001
TB	1.0993	(1.043–1.168)	0.0009
D/T ratio	0.000446	(0.0001146–0.081412)	0.0062
NH3	1.007	(1.002–1.014)	0.0098
Age	1.0654	(1.010–1.136)	0.0113
PT%	0.9773	(0.959–0.995)	0.0115
HGF	1.1837	(1.049–1.374)	0.0139
O-C	1.0687	(1.014–1.141)	0.0270
ALB	0.0409	(0.129–0.906)	0.0312
PLT	0.9648	(0.931–0.999)	0.0489

†Odds ratio of dead patients to survived patients in relation to the presence or absence of liver atrophy and a unit increase of each continuous parameter.
ALB, albumin; D/T ratio, ratio of direct to total bilirubin concentration; HGF, hepatocyte growth factor; O-C, the interval between hepatitis onset and the development of hepatic encephalopathy; PLT, platelet; PT, prothrombin time; TB, total bilirubin.

Table 6 Scores for Predictive Variables Affecting the Mortality of Patients with Fulminant Hepatitis and late onset hepatic failure (LOHF)

Score	0	1	2
O-C (days)	≤5	6–10	11≤
PT (%)	20<	5< ≤20	≤5
TB (mg/dL)	<10	10≤ <15	15≤
D/T ratio	0.7≤	0.5≤ <0.7	<0.5
PLT (104/μL)	10<	5< ≤10	≤5
Liver atrophy	Absent	Present	

D/T ratio, ratio of direct to total bilirubin concentrations; O-C, the interval between hepatitis onset and the development of hepatic encephalopathy; PLT, platelet; PT, prothrombin time; TB, total bilirubin.

Scoring system to predict the possible outcome of patients with fulminant hepatitis and LOHF

Variables extracted through ROC curve analysis were graded as shown in Table 6, according to the inflection points of each curve. The interval between hepatitis onset and the development of hepatic encephalopathy, prothrombin time, serum concentration of total bilirubin, the ratio of direct to total bilirubin concentration and peripheral platelet count were classified into three grades (0, 1 and 2), and liver atrophy into two grades (0 and 1).

As shown in Table 7, the mortality rates rose in relation to total scores calculated in patients seen between 1998 and 2003 (estimation cohort). When the predictive outcome of patients showing total scores of 5 or more was judged as "death", PPV and NPV of the system were 0.84 and 0.70, respectively (Table 8), suggesting that total scores of 5 is sufficient enough as a cut-off value that can discriminate between dead and survived patients. The scoring system with such cut-off value showed sensitivity and specificity of 0.80 and 0.76, respectively, and resulted in predictive accuracy of 0.78 in patients in the estimation cohort. Predictive accuracies did not differ depending on the disease types; 0.75 in patients with acute type of fulminant hepatitis and 0.87 in those with subacute type of fulminant hepatitis.

Table 7 The outcome of patients with fulminant hepatitis and late onset hepatic failure (LOHF) seen between 1998 and 2003 (estimation cohort) depending on total scores calculated through established scoring system

Total scores	Mortality (%)	FHA/FHS/LOHF
9≤	**9/10 (90.0%)**	2/4/4
8	**26/27 (96.3%)**	2/20/5
7	**42/46 (91.3%)**	10/30/6
6	**71/83 (85.5%)**	20/52/11
5	**59/80 (73.8%)**	26/42/12
4	31/55 (56.3%)	32/22/1
3	12/50 (24.0%)	44/4/2
2	8/40 (20.0%)	35/5/0
1	2/25 (8.0%)	25/0/0
0	0/5 (0.0%)	5/0/0

FHA, acute type of fulminant hepatitis; FHS, subacute type of fulminant hepatitis.

Table 8 Accuracies of established scoring system in patients with fulminant hepatitis and late onset hepatic failure (LOHF) seen between 1998 and 2003 (estimation cohort) when predictive outcome of patients showing total scores of 5 or more are diagnosed as "death"

	Total scores		
	≥5	<5	Total
Number of patients			
Dead patients	207	53	260
Surviving patients	39	122	161
Total	246	175	421
Mortality	84.1%	30.3%	61.8%
The accuracies			
Positive predictive value (PPV)	207/246		0.84
Negative predictive value (NPV)	122/175		0.70
Sensitivity	207/260		0.80
Specificity	122/161		0.76
Predictive accuracy (PA)	(207+122)/421		0.78

The accuracies of the established scoring system were validated in patients with fulminant hepatitis and LOHF seen between 2004 and 2008 (validation cohort). As shown in Table 9, the mortality rate of patients in each total score was almost equivalent to that obtained in analysis with patients in the estimation cohort. Thus, predictive accuracy through analysis in the validation cohort was 0.75 with sensitivity, specificity, PPV and NPV of 0.75, 0.80, 0.86 and 0.65, respectively (Table 10).

DISCUSSION

LIVER TRANSPLANTATION IS regarded worldwide as the most effective therapeutic procedure for patients with end-stage liver diseases including acute liver

Table 9 The outcome of patients with fulminant hepatitis and late onset hepatic failure (LOHF) seen between 2004 and 2008 (validation cohort) depending on total scores calculated through established scoring system

Total scores	Mortality (%)	FHA/FHS/LOHF
9≤	4/4 (100.0%)	0/3/1
8	9/9 (100.0%)	1/6/2
7	26/30 (86.7%)	9/20/1
6	35/39 (89.7%)	9/29/1
5	33/42 (78.6%)	18/19/5
4	21/39 (53.8%)	28/10/1
3	10/30 (33.3%)	22/8/0
2	4/26 (15.4%)	26/0/0
1	2/8 (25.0%)	8/0/0
0	0/4 (0.0%)	4/0/0

FHA, acute type of fulminant hepatitis; FHS, subacute type of fulminant hepatitis.

failure. Japanese Society for the Study of Liver Transplantation revealed that survival rate at 1 year after liver transplantation was 72.7% in patients with acute liver failure,[7] while conventional medical care yielded insufficient prognosis in such patients; survival rates were 54.0% and 24.0%, respectively, in patients with fulminant hepatitis of acute and subacute types and 15.0% in LOHF patients according to the nationwide survey by the Study Group of Intractable Hepatobiliary Diseases.[8] In general, in Japan, patients with acute liver failure visit clinics or hospitals at the onset of hepatitis symptoms and derangement of liver function was diagnosed by physicians specialized in general medicine. Next, the patients were transferred to hospitals with specialists in the fields of hepatology and emergency medicine around the periods of the development of hepatic encephalopathy. Conventional medical care including artificial liver support with plasma exchange and hemodiafiltration was performed, and then the patients were introduced to transplant surgeons regarding the indication of liver transplantation. Thus, the simple criteria to predict the outcome of patients with fulminant hepatitis and LOHF with sufficient accuracies are required to facilitate communication among general physicians, hepatologists and transplant surgeons.

Table 10 Accuracies of established scoring system in patients with fulminant hepatitis and late onset hepatic failure (LOHF) seen between 2004 and 2008 (validation cohort) when predictive outcome of patients showing total scores of 5 or more are diagnosed as "death"

	Total scores		
	≥5	<5	Total
Number of patients			
Dead patients	107	17	124
Surviving patients	37	70	107
Total	144	87	231
Mortality	74.3%	19.5%	53.7%
The accuracies			
Positive predictive value (PPV)	107/144		0.75
Negative predictive value (NPV)	70/87		0.80
Sensitivity	107/124		0.86
Specificity	70/107		0.65
Predictive accuracy (PA)	(107+70)/231		0.77

In Europe and the United States, the indication of liver transplantation in patients with acute liver failure has been determined according to the guideline proposed by King's Collage Hospital[9] and Beaujon Hospital.[10] In addition, a scoring system of model for end-stage liver disease (MELD), initially designed for patients with chronic liver failure, has recently been applied also to those with acute liver failure.[11] However, these guidelines are not directly applicable to patients with fulminant hepatitis and LOHF in Japan, since social environment as well as demographic and clinical features of the patients differ among Japan, Europe and the United States; for example liver transplantation with brain death-related donor is hardly available and artificial liver support is routinely performed in Japan. Thus, novel guidelines should be established for Japanese patients with fulminant hepatitis and LOHF instead of the previous guideline proposed by the Acute Liver Failure Study Group in Japan at 1996,[4] which shows decline of predictive accuracy when applied to recent patients.[5]

In the present paper, a novel scoring system to predict the outcome of patients with fulminant hepatitis and LOHF was established based on demographic and clinical features of patients seen between 1998 and 2008. The predictive mortality rates were estimated through six variables at the occurrence of grade 2 or more severe hepatic encephalopathy; the interval between the onset of hepatitis symptoms and the development of hepatic encephalopathy, prothrombin time, serum concentration of total bilirubin, the ratio of direct to total bilirubin concentration, peripheral platelet count and presence of liver atrophy on imaging. When total scores were calculated through six variables in patients belonging to the estimation cohort, the mortality rate was 84.1% in those with scores of 5 or more, while it was 30.3% in those with scores of 4 or less. Thus, the cut-off value of total scores to discriminate possible dead

patients from surviving patients was set between 4 and 5. Consequently, excellent accuracies were obtained through analysis in patients belonging to the estimation cohort; predictive accuracy was 0.78 with either of PPV, NPV, sensitivity and specificity greater than 0.7. Such high predictive accuracy was also found through analysis in patients belonging to the validation cohort. It is noteworthy that peripheral platelet count and the presence of liver atrophy were added to the list of predictive variables in the present scoring system. Also, cut-off values to grade other variables, such as the interval between the onset of hepatitis symptoms and the development of hepatic encephalopathy, differ between the present system (Table 6) and previous guidelines (Table 1). These modifications may contribute to improve predictive accuracy of the novel scoring system when applied to recent patients.

In the present study, the age of patients was excluded from the list of predictive variables to facilitate the use of the system in pediatric patients. In our database, a patient showing total score of 6 died, while three cases with total scores of 4 or less survived, when the system was applied for patients aged less than 15 years old. Furthermore, the most recent report by Fujisawa showed 100% specificity and PPV by the scoring system in 40 pediatric patients.[12] Thus, the system seems to be useful even in such patients. Also, plasma HGF concentration was deleted from the list of predictive variables, because it is difficult to obtain the results within a day in most of the hospitals in Japan. In contrast, the presence of liver atrophy was included in the predictive variable list, but the quantitative criteria for liver atrophy were not specified in the present scoring system. The estimated liver volume is measured on CT examination, and the ratio of the value to the standardized liver volume was reported to correlate with mortality in patients with acute liver failure in Japan.[13] These problems, regarding age of patients, significance of plasma HGF concentration and diagnostic criteria to determine liver atrophy should be further investigated.

In conclusion, a novel scoring system for predicting outcome of patients with fulminant hepatitis and LOHF was established. This system may be useful to determine the indication of liver transplantation in patients with acute liver failure, since the system showed high predictive accuracies even after the validation.

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REFERENCES

1 Inuyama Symposium Kiroku Kanko-Kai. *Hepatitis Type A and Fulminant Hepatitis. The Proceedings of the 12th Inuyama Symposium*. Tokyo: Chugai Igaku-Sha, 1982 (in Japanese).
2 Mochida S, Fujiwara K. Symposium on clinical aspects in hepatitis virus infection. 2. Recent advances in acute and fulminant hepatitis in Japan. *Intern Med* 2001; **40**: 175–7.
3 Polson J, Lee WM. AASLD position paper: the management of acute liver failure. *Hepatology* 2005; **41**: 1179–97.
4 Sugihara J, Naito T, Ishiki Y *et al.* A multicenter study on the prognosis and indication of liver transplantation for fulminant hepatitis in Japan: details of decision of the guideline for liver transplantation in Japanese Acute Hepatic Failure Study Group (1996). *Acta Hepatol Jpn* 2001; **42**: 543–57 (in Japanese).
5 Mochida S, Nakayama N, Matsui A *et al.* Re-evaluation of the Guideline published by the Acute Liver Failure Study Group of Japan in 1996 to determine the indications of liver transplantation in patients with fulminant hepatitis. *Hepatol Res* 2008; **38**: 970–9.
6 Fujiwara K, Mochida S, Matsui A *et al.* Fulminant hepatitis and late onset hepatic failure in Japan: summary of 698 patients between 1998 and 2003 analyzed in annual nationwide survey. *Hepatol Res* 2008; **38**: 646–57.
7 Japanese Society for the Study of Liver Transplantation. Reports on the registered cases for liver transplantation in Japan (The Second Report). *Ishoku (Transplantation)* 2008; **43**: 45–55 (in Japanese).
8 Oketani M, Ido A, Tsubouchi H. Changing etiologies and outcomes of acute liver failure: a perspective from Japan. *J Gastroenterol Hepatol* 2011; **26** (Suppl 1): 65–71.
9 O'Grady JG, Alexander GJ, Thick M. Outcome of orthotopic liver transplantation in the aetiological and clinical variants of acute liver failure. *Q J Med* 1988; **68**: 817–24.
10 Bernuau J. Selection for emergency liver transplantation. *J Hepatol* 1993; **19**: 486–7.
11 Yantorno SE, Kremers WK, Ruf AE. MELD is superior to King's college and Clichy's criteria to assess prognosis in fulminant hepatic failure. *Liver Transpl* 2007; **13**: 822–8.
12 Fujisawa T. Pediatric patients with fulminant hepatic failure. Annual Report of the Intractable Hepato-Biliary Disease Study Group supported by the Ministry of Health, Labor, and Welfare of Japan, 2010. 116–17.
13 Yamagishi Y, Saito H, Tada S. Value of computed tomography-derived estimated liver volume/standard liver volume ratio for predicting the prognosis of adult fulminant hepatic failure in Japan. *J Gastroenterol Hepatol* 2005; **20**: 1843–9.

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REVIEW

Changing etiologies and outcomes of acute liver failure: A perspective from Japan

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Key words
acute liver failure, fulminant hepatitis, Japan, liver transplantation, viral hepatitis.

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Abstract

Acute liver failure in Japan usually consists of fulminant hepatitis (FH) due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury. The annual incidence of FH was estimated at 429 cases in 2004. FH is classified into acute or subacute type, and the prognosis of the latter is poor. Hepatitis B virus (HBV) is the most frequently identifiable agent that causes FH in Japan. Transient HBV infection is more prevalent in the acute than subacute type, whereas the frequency of HBV carriers is greater in the subacute type. FH due to HBV reactivation from resolved hepatitis B has been increasingly observed in patients with malignant lymphoma treated with rituximab and corticosteroid combination therapy. The prognosis is poor in HBV carriers with acute exacerbation, especially in patients with HBV reactivation from resolved hepatitis B. Despite careful investigation, the etiology is still unknown in 16% and 39% of the acute and subacute type of FH, respectively. Autoimmune hepatitis and drug-allergy-induced liver injury are found in 7% and 10%, respectively, and are more frequently observed in the subacute type of FH. Living donor liver transplantation is now the standard care for individuals with poor prognosis. Artificial liver support with plasmapheresis and hemodiafiltration plays a central role while waiting for a donor liver or for the native liver to regenerate. Further research is necessary to identify the causes of unknown origin. In addition, to improve the prognosis of FH, it is necessary to establish treatment modalities that are effective for liver regeneration.

Introduction

Acute liver failure is a clinical syndrome that is marked by the sudden loss of hepatic function in a person without chronic liver disease. The causes of acute hepatic failure are varied and differ geographically. In Japan, fulminant hepatitis (FH) is defined as having hepatitis, when grade II or worse hepatic encephalopathy develops within 8 weeks of the onset of the disease symptoms, with a prothrombin time of ≤40%. FH due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury is the main cause of acute liver failure in Japan. In contrast, other causes, including paracetamol overdose, other drug toxicity, metabolic liver disease, and acute fatty liver of pregnancy, are infrequent.

The Intractable Hepato-biliary Diseases Study Group of Japan annually performs a nationwide survey of patients with FH and late-onset hepatic failure (LOHF). This paper summarizes the results of the survey and addresses the characteristics and trends of acute liver failure in Japan.

Definition and methods

In 1969, Trey and Davidson defined acute liver failure as the occurrence of encephalopathy within 8 weeks of the onset of acute hepatic illness, and in the absence of pre-existing liver disease.[1] Thereafter, patients with hepatic encephalopathy that develops between 8 and 24 weeks after disease onset are defined as having LOHF.[2] Other definitions based on the duration of illness have subsequently been used to classify patients:[2–4] hyperacute, <7 days; acute, 7–28 days; and subacute, 28 days to 6 months. In Japan, patients with FH are classified into acute or subacute type, in which the encephalopathy occurs within 10 days, or later than 11 days, respectively, of the onset of disease symptoms.[5,6] Based on the previous survey, patients with FH who present within 10 days of symptom onset have significantly higher survival rates than similar patients who present with encephalopathy at 10 days after symptom onset.[7,8]

The survey was performed in hospital with active members of the Japan Society of Hepatology and the Japanese Society of Gastroenterology. Patients who meet the diagnostic criteria for FH and LOHF were entered into the survey (Table 1). Besides the diagnostic criteria, patients under 1 year of age and those with alcoholic hepatitis were excluded from the analysis.

The etiology of acute liver failure is classified into five categories: viral infection, autoimmune hepatitis, drug-allergy-induced liver injury, unknown, and indeterminate (Table 2). Patients with viral infection consist of those with hepatitis A virus (HAV),

Table 1 Diagnostic criteria for fulminant hepatitis in Japan according to the Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labour (2003)

Fulminant hepatitis (FH) is defined as hepatitis in which hepatic encephalopathy of coma grade greater than II develops in the patients within 8 weeks after the onset of disease symptoms with highly deranged liver functions showing prothrombin time less than 40% of the standardized values.

FH is classified into two subtypes: the acute typeand subacute type in which the encephalopothy occurs within 10 days and later than 11 days, respecitvely.

Note 1: Patients with chronic liver diseases are excluded from FH, but asymptomatic HBV carriers who develop acute exacerbation are diagnosed with FH.

Note 2: Acute liver failure accompanying no liver inflammation, such as drug or chemical intoxication, microcirculatory disturbance, acute fatty liver of pregnancy, and Reye's syndrome are excluded from FH.

Note 3: The grading of hepatic encephalopathy is based on the criteria from the Inuyama Symposium in 1972.

Note 4: The etiology of FH is based on the criteria from the Intractable Liver Diseases Study Group of Japan in 2002 (Table 2).

Note 5: Patients with no hepatic encephalopathy or encephalopathy of coma grade I, even showing prothrombin time <40% of the standardized values, are diagnosed with severe acute hepatitis. Patients in whom encephalopathy develops between 8 and 24 weeks after disease onset, with prothrombin time <40% of the standardized values, are diagnosed with late onset hepatic failure (LOHF). Both are related to FH, but are regarded differently from FH.

hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV) and other viruses. Patients with HBV infection are further classified into transient infection and acute exacerbation of HBV carrier status. In 2002, the criteria were modified to define FH due to autoimmune hepatitis and HEV, and the etiology of patients between 1998 and 2001 was re-assessed according to these new criteria.

Demographic features

From 1998 to 2006, 934 patients were enrolled in the surveillance.[9] Among these patients, 856 (432, acute type and 424, subacute type) were classified as having FH and 78 as having LOHF (Table 3). Based on the nationwide epidemiology surveillance, the annual incidence of FH was estimated at 3700 cases in 1972, 1050 cases in 1995, and 429 cases in 2004.[10] About 30% of patients with severe acute hepatitis were presumed to develop hepatic encephalopathy of coma grade II or more.[11]

The male : female ratio was higher for the acute type than subacute type and LOHF. The age of the patients was significantly higher for the subacute type and LOHF than for the acute type. The frequency of HBV carriers was highest for the subacute type and lowest for LOHF. There were many patients with complications, such as metabolic syndrome, malignancy and psychiatric disorders, which preceded the onset of acute liver failure, and most of these patients had received daily medication. This tendency was more obvious in patients with the subacute type and LOHF.

The survival rates of non-liver-transplanted patients were 54% for acute and 24% for subacute type FH, and 15% for LOHF. The prognosis of patients with subacute type FH and LOHF was evidently poor. These annual rates have not improved between 1998 and 2006. When compared to a previous survey,[12] prognosis of FH in acute type patients improved until 1998, although the prognosis remained poor in the subacute type with no liver transplantation during that period (Fig. 1). This improvement was probably achieved by progress in artificial liver support.

Table 2 Criteria for etiology of fulminant hepatitis and late onset hepatic failure

I. Viral infection
  1. HAV: positive for serum IgM anti-HAV
  2. HBV: positive for either serum HBsAg, IgM anti-HBc or HBV DNA
    A. Transient infection: fulfilling either (a) or (b):
      (a) Negative for serum HBsAg before onset of acute liver injury.
      (b) Positive for serum IgM anti-HBc and negative for anti-HBc in serum diluted to 1:200.
    B. Acute exacerbation of carrier status: fulfilling either (a) or (b):
      (a) Positive for serum HBsAg before onset of acute liver injury
      (b) Negative for serum IgM anti-HBc and positive for anti-HBc in the serum diluted to 1:200.
    C. Undetermined: neither (a) nor (b)
  3. HCV: fulfilling either (a) or (b):
    (a) Negative for serum anti-HCV or HCV RNA before onset of acute liver injury.
    (b) Positive for serum HCV RNA and low titer positive for serum anti-HCV core protein.
  4. HEV: positive for serum HEV-RNA
  5. Other virus: e.g. EBV.

II. Autoimmune hepatitis: fulfilling either (a) (b) or (c):
  (a) Diagnosed as definite or probable according to the International Scoring System for autoimmune hepatitis.
  (b) Attenuation of liver injury after glucocorticosteroid administration and/or aggravation of liver injury following withdrawal of glucocorticoid.
  (c) Positive for serum antinuclear antigen and/or serum IgG levels >2 g/dL.

III. Drug-allergy-induced: drugs responsible for liver injury are determined by clinical course of liver injury and/or d-LST.

IV. Unknown: etiology is unknown despite sufficient examinations available.

V. Undetermined: etiology is undetermined because of insufficient examinations.

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; EBV, Epstein-Barr virus; d-LST, drug-induced lymphocyte stimulation test.

Causes of FH

Viral hepatitis

In Japan, the cause of FH has been identified as HAV, HBV or other viruses in about 50% of patients (Table 4). The causes of acute liver failure differed depending on the disease type. The frequencies of viral infection were 69% and 31% for patients with the acute and subacute types of FH, respectively, and 17% for LOHF patients.

Journal of Gastroenterology and Hepatology **26** (2011) Suppl. 1; 65–71

Table 3 Demographic features of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Men/women	431/423	228/203	197/226	33/45
Age (years; mean ± SD)	48 ± 17	46 ± 16	49 ± 17**	53 ± 15**
HBV carrier rate (%)	14	12	16*	7***
Complications (%)	39	35	44*	49*
History of medication (%)	46	41	51**	54*
Survival rate (no LT) (%)	40	54	24**	15**
Survival rate (LT) (%)	77	73	79	81

*$P < 0.05$; **$P < 0.01$ versus acute type; ***$P < 0.05$ versus subacute type.
HBV, hepatitis B virus; LT, liver transplantation.

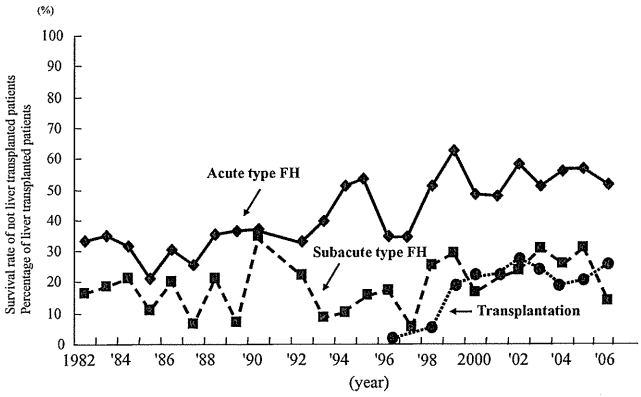

Figure 1 Survival rate of not liver transplanted patients with fulminant hepatitis(FH) and percentage of liver transplanted patients.

Table 4 Percentage etiology of fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Viral infection	51	69	31	17
HAV	6	11	1	1
HBV	42	56	27	13
(Transient infection)	(25)	(40)	(9)	(5)
(Carrier)	(13)	(11)	(16)	(4)
(Undetermined)	(4)	(6)	(2)	(4)
HCV	1	1	1	1
HEV	1	1	1	0
Other virus	1	1	1	1
Autoimmune hepatitis	7	2	12	18
Drug-allergy-induced	10	8	13	15
Unknown	30	18	42	47
Indeterminate	3	3	3	3

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Infection with HAV was found in 6% of patients with FH and frequently observed in the acute type. As annual incidence of acute hepatitis A has declined over the past decade,[13] so too has the incidence of FH. However, as the overall immunity of the Japanese population to hepatitis A is only 12%[14] and is decreasing gradually as in other non-endemic areas, the increasing risk of future outbreaks of acute hepatitis A is probable. With regard to the severity of hepatitis A, age, sex, and drug toxicity have been identified as potential contributing factors.[15] HAV susceptibility and the risk of severity have likely increased recently.

In most of the patients, viral infections were due to HBV. HBV infection was found in 42% of patients with FH and 13% of those with LOHF. Among these, transient HBV infection was more frequent than acute exacerbation of HBV carrier status. Transient HBV infection was more frequent in the acute type (40%) than subacute type (9%) of FH, whereas the frequency of HBV carrier status was greater in the subacute type (16%) than in the acute type (11%). Annual incidence of FH due to HBV infection, both in transient HBV infection and acute exacerbation of HBV carrier status, has declined over the past decade. The routes of transmission of HBV indicate that, at present, sexual transmission from HBV carriers is a major route for FH. The preventive administration of HBV hyperimmune globulin and vaccination against HBV of neonates born to HBV-carrier mothers has been practiced nationwide since 1985 in Japan.[16] Therefore, the HBV carrier rate in the population has significantly decreased, and as a result, a marked decrease in the incidence of FH caused by HBV is expected.

Reactivation of HBV is a well-recognized complication in patients with chronic HBV infection who are undergoing cytotoxic chemotherapy or immunosuppressive therapy. HBV reactivation can be clinically severe and result in death from acute liver failure. Among acute exacerbation of HBV carrier status in the survey, HBV reactivation has been increasingly observed in patients with hematological malignancies. Furthermore, among the 12 patients with HBV reactivation, six with serological evidence of resolved hepatitis B [without hepatitis B surface antigen (HBsAg), but with antibody to hepatitis B core antigen (anti-HBc) and/or antibody to HBsAg (anti-HBs) in serum] developed reactivation with reappearance of HBsAg in serum. Most of these patients had received rituximab and coriticosteroid. Recently, combination therapy with rituximab and corticosteroid has been identified as a risk factor for HBV reactivation in HBsAg-negative patients with malignant lymphoma.[17,18] A study in Japan has revealed that 22% of *de novo* hepatitis B and that caused by HBV reactivation from resolved

hepatitis developed into fulminant hepatic failure, and mortality was 100%.[19] This problem deserves careful attention, because HBsAg-negative, anti-HBc-and/or anti-HBs-positive patients, which account for 20–25% of hospitalized patients in Japan, represent a high-risk group.[20]

HCV infection is rare in the etiology of patients with FH and LOHF. HCV infection was found in 1% of patients with FH, independent of the disease type. Reactivation of HCV as a cause of acute liver failure following chemotherapy has been reported.[21] However, none of these patients were found in the survey.

HEV infection was found in 1% of FH patients. HEV is a common cause of acute hepatitis in endemic areas, such as South Asia, Africa and South America.[22] The virus is now also known to exist indigenously in Japan, and can contribute to acute liver disease.[23,24] In Japan, the zoonotic transmission from pigs, wild boar and deer, either food-borne or otherwise, is the cause of HEV infection in non-endemic areas.[24,25] As for the geographical distribution of clinical HEV infection in Japan, it has been reported that there was wide variation with a higher prevalence in the northern part of Japan (Hokkaido Island and the northern part of mainland Honshu).[26] In the survey, two-thirds of the patients were from this area. Moreover, most of the patients were elderly men and there were no pregnant women, who have the highest attack rate of the virus in endemic areas.

In the survey, Epstein–Barr virus, cytomegalovirus, herpes simplex virus, human herpesvirus type-6 and parvovirus were infrequent causes of other forms of viral hepatitis.

Autoimmune hepatitis

Although autoimmune hepatitis is a chronic disease, an acute presentation occurs in approximately 22% of patients, and an even smaller number present with acute liver failure.[27] In the survey, autoimmune hepatitis was found in 7% of patients with FH and 18% of those with LOHF, respectively. In 2001, FH due to autoimmune hepatitis was recognized in Japan, because there were patients with non-HAV/HBV FH in which IgG levels were >2 g/dL, with positive antinuclear antigen in the serum. Although the diagnosis generally relies on the presence of serum autoantibodies, higher IgG levels (>2 g/dL), liver histology (if available), and response to corticosteroid therapy, the diagnosis of acute-onset autoimmune hepatitis is often difficult. The serum gammaglobulin or IgG concentrations are often lower than those in patients with chronic hepatitis.[28]

Drug-allergy-induced liver injury

Formation of toxic reactive metabolites has been suggested as a potential mechanism for causing idiosyncratic drug-induced liver injury.[29] Drug-allergy-induced liver injury was seen in 13% of patients with subacute type FH and in 15% of those with LOHF. The diagnosis relied mostly on the clinical course or drug-induced lymphocyte stimulation test (D-LST). Numerous types and classes of drugs have been implicated. Anti-tuberculosis agents (isoniazid, rifampicin, ethambutol and pyrazinamide), nonsteroidal anti-inflammatory drugs (loxoprofen, lornoxicam and acetaminophen), anti-cancer agents (tegafur, UFT and flutamide), drugs for metabolic syndrome (allopurinol and acarbose), and various herbal and natural remedies were the probable causative agents in the survey.

Table 5 Survival rates and etiology of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total (n = 678)	Acute type (n = 369)	Subacute type (n = 309)	(n = 62)
Viral infection	45	55	23*	36*
HAV	74	77	40	100
HBV	39	50	18*	38
(Transient infection)	(51)	(56)	(32*)	(33)
(Carrier)	(22)	(35)	(13*)	(67)
(Undetermined)	(23)	(33)	(0)	(0)
HCV	67	75	60	0
HEV	60	100	33	—
Other virus	60	50	67	0
Autoimmune hepatitis	21	25	21	18
Drug allergy-induced	42	58	29*	0*
Unknown	36	54	26*	10*
Indeterminate	28	36	14	0

*P < 0.05 versus acute type.
HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Unknown etiology

The etiology was unknown in 42% and 47% of patients with subacute type FH and LOHF, respectively. Although the roles of GB virus C (GBV-C)/hepatitis G virus (HGV) and transfusion transmitted virus (TTV) have been discussed, in this survey, neither GBV-C/HGV or TTV appeared to be a major cause of FH. It is possible that the patients with drug-allergy-induced liver injury were contaminated with those of unknown etiology, because the ratio of medication history was high in these patients. The relationship between daily dose of oral medication or medication with significant hepatic metabolism and idiosyncratic drug-induced liver injury has been reported.[30,31] The higher numbers of patients with complications and daily medication in the survey support this evidence. Furthermore, HEV infection needs further investigation, because serum HEV RNA and IgM antibody to HEV were measured less in the survey.

Prognosis

The prognosis of patients with FH and LOHF differed depending on the etiology (Table 5). It was excellent in patients with HAV infection: the survival rate was 77% and 40% in patients with acute and subacute types of FH, respectively, and 100% in those with LOHF. In contrast, the prognosis was especially poor in HBV carriers who showed acute exacerbation. The survival rates of acute and subacute types of FH were 35% and 13%, respectively. It is noteworthy that none of the patients with HBV reactivation from resolved hepatitis B after rituximab and corticosteroid combination therapy survived. In contrast, the survival rate was 56% in acute type FH and 32% in subacute type in patients with transient HBV infection. The prognosis was poor in autoimmune hepatitis independent of disease type. Prognosis was also poor in patients

Journal of Gastroenterology and Hepatology **26** (2011) Suppl. 1; 65–71

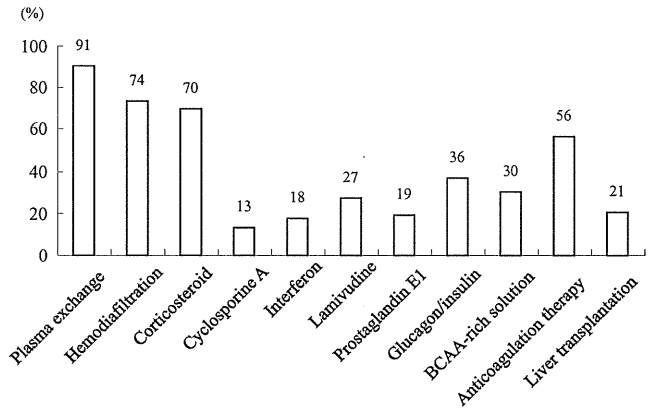

Figure 2 Percentage incidence of therapies performed for fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006). BCAA, branched-chain amino acid.

with subacute type FH and LOHF caused by drug-allergy-induced liver injury, and in those of the unknown etiology.

Complications

Complications that occurred during the course of acute liver failure also seemed to affect patient prognosis. Disseminated intravascular coagulation, renal failure and bacterial infection were found as complications in >30% of patients. Brain edema, gastrointestinal bleeding and congestive heart failure were seen in about 30%, 20% and 10%, respectively. Any of these complications significantly decreased survival rate. Furthermore, the number of these complications influenced prognosis.

Management

Specific therapies

The frequency of antiviral therapy with lamivudine has increased since 1998. As antiviral agents, lamivudine and interferon have been used in 27% and 18% of patients with FH and LOHF, respectively, between 1998 and 2006 (Fig. 2). Lamivudine has been used in 67% of patients with HBV-related FH or LOHF. Lamivudine has been reported to be efficacious for acute liver failure.[31,32] Recently, another guanosine nucleoside analog, entecavir, has been administered more frequently.[33] A preliminary study of entecavir for acute liver failure has revealed that the agent beneficially affects disease course. Lamivudine therapy is more efficacious when started early in acute liver failure. However, in the case of HBV reactivation from HBsAg-negative patients, it is difficult to prevent development of liver failure, even when lamivudine is administered after the onset of hepatitis. Two study groups in Japan have proposed guidelines for prevention of immunosuppressive-therapy- or chemotherapy-induced HBV reactivation. These guidelines recommend that patients with resolved infection should be routinely monitored for liver function and HBV DNA levels during and after chemotherapy, and antiviral therapy should be administered immediately when HBV DNA increases above the detection levels.

Corticosteroids were administered in 70% of patients with FH and LOHF. Steroid pulse therapy, methylprednisolone at a daily dose of 1 g injected intravenously, was administered to attenuate liver necrosis by suppressing excessive immune response. The efficacy of corticosteroids for improving the prognosis of acute liver failure is still obscure. Some randomized controlled trials have shown that corticosteroids provide no benefit overall in acute liver failure.[34] However, FH due to autoimmune hepatitis might be a candidate for therapy.[35] Anticoagulant therapy was performed in 56% of patients with FH and LOHF. Antithrombin III concentrate and protease inhibitor compounds such as gabexate mesylate and nafamostat mesylate were used as anticoagulants. They were effective for inhibition of disseminated intravascular coagulation and microcirculatory disturbance due to sinusoidal fibrin deposition. Glucagon/insulin, branched-chain amino acid-rich solution, cyclosporine A and prostaglandin E1 therapy was administered less frequently, and the frequency decreased compared to that in patients in the previous survey between 1995 to 1997.

Methods of liver support

In Japan, powerful artificial liver support with plasmapheresis and hemodiafiltration plays a central role in the treatment of acute liver failure. Plasmapheresis and hemodiafiltration were performed in 91% and 74% of patients with FH and LOHF, respectively (Fig. 2). In the late 1990s, hemodiafiltration therapy was developed and plasma exchange combined with hemodiafiltration therapy became popular. The increased frequency of this combination therapy in the 1990s could be implicated in the tendency for the survival rate to increase for acute type FH (Fig. 1). The effect of plasmapheresis on survival from acute liver failure has been difficult to determine. However, these support systems are efficacious for helping patients to remain in good condition until sufficient regeneration of the liver can be obtained, or liver transplantation can be performed. Recently, more powerful hemodiafiltration using large buffer volumes[36] or on-line hemodiafiltration[37] has been developed and has shown greater efficacy for improving hepatic coma.

Liver transplantation

Despite significant advances in critical care and an improved understanding of the pathophysiology of acute liver failure, the mortality rate remains high. Liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. In Japan, living donors have been used because of the insufficiency of organ donation since 1988. Living donor liver transplantation was performed in 17% of patients with FH and LOHF between 1998 and 2006, and the frequency in those patients was significantly greater in the subacute type (21%) than in the acute type (13%). Recently, these frequency ratios have been almost steady (Fig. 1). The survival rates were 77% and 81% in patients with FH and LOHF, respectively, and there was no difference in the rates among the disease types. Patient and graft survival rates were 94% and 87% at 1 year, and 91% and 81% at 5 years, respectively. There was no significant difference in patient and graft survival according to etiology.[38]

Appropriate judgment to move forward to liver transplantation is the most important step. The indications for liver transplantation

in cases of FH are determined according to the 1996 Guidelines of the Acute Liver Failure Study Group of Japan. Re-evaluation of the guidelines has revealed that the accuracy in patients not receiving liver transplantation was 68% and 78% in acute and subacute types of FH, respectively, and 84% among those with LOHF.[39] The sensitivity and specificity of the assessment in patients with acute and subacute types were very low. To improve this situation, new guidelines for using a scoring system have been proposed by the Intractable Hepato-biliary Disease Study Group of Japan.[40] By using these guidelines, the accuracy in patients not receiving liver transplantation was increased to 75% and 87% in acute and subacute types of FH, respectively.

Experimental methods of liver support

To improve the prognosis of acute liver failure, advances in the treatment for liver regeneration are urgently needed. Hepatocyte growth factor (HGF) acts as a stimulator of liver regeneration, as well as an anti-apoptotic factor. We have started a clinical trial to examine the effects of recombinant human HGF (rhHGF) in patients with FHor LOHF, and in the four patients with FH or LOHF enrolled in this study; repeated doses of rh-HGF did not produce any severe side effects. Although two patients were rescued in this study, evaluation of this therapeutic agent is still under investigation.[41]

Several clinical trials of bone marrow cell infusion in patients with liver cirrhosis have shown clinical improvement. A clinical trial of autologous bone marrow infusion for patients with advanced liver cirrhosis due to chronic HBV infection has shown clinical improvement with no serious adverse events.[42] The recent discovery of pluripotent stem cells has yielded a new cell type for potential application in regenerative medicine. Strategies to achieve high levels of hepatocyte survival and the development of methods to engineer a functional liver system *in vivo* are expected in the future.

Conclusion

In Japan, the incidence of FH has decreased gradually and the clinical characteristics of patients and the therapeutic approach have changed in the past decade. The prognosis differs in patients with FH and LOHF depending on the disease type and etiology. HBV is the major cause of FH in Japan. Recently, careful attention has been necessary because of an increase in HBV reactivation from resolved hepatitis B. Despite careful investigation, a significant group with FH of unknown origin remains and needs further investigation. Living donor liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. Artificial liver support systems are efficacious while waiting until the native liver regenerates or a donor is found. New therapeutic modalities are required to regenerate the liver, in particular, for the subacute type of FH.

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References

1 Trey C, Davidson CS. The management of fulminant hepatic failure. In: *Progress in Liver Failure*. New York: Grune and Stratton, 1970; 292–8.
2 Gimson AE, O'Grady J, Ede RJ, Portmann B, Williams R. Late onset hepatic failure: clinical, serological and histological features. *Hepatology* 1986; **6**: 288–94.
3 Bernuau J, Rueff B, Benhamou JP. Fulminant and subfulminant liver failure: definitions and causes. *Semin. Liver Dis.* 1986; **6**: 97–106.
4 O'Grady JG, Schalm SW, Williams R. Acute liver failure: redefining the syndromes. *Lancet* 1993; **342**: 273–5.
5 The Proceedings of the 12th Inuyama Symposium. *Hepatitis Type A and Fulminant Hepatitis*. Tokyo: Chugai Igaku-Sha, 1982; (In Japanese.).
6 Mochida S, Fujiwara K. Symposium on clinical aspects in hepatitis virus infection. Recent advances in acute and fulminant hepatitis in Japan. *Intern. Med.* 2001; **40**: 175–7.
7 Takahashi Y. Acute hepatic failure—in special relation to treatment. *Jpn. J. Med.* 1983; **22**: 140–5.
8 Fujiwara K, Mochida S, Matsui A. Fulminant hepatitis and LOHF in Japan. Annual Report of Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labor (2001). 2002; 62–77. (In Japanese).
9 Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G; Intractable Liver Diseases Study Group of Japan. Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol. Res.* 2008; **38**: 646–57.
10 Mori M, Itanai F, Washio S *et al.* Estimated number of patients with intractable liver diseases in Japan based on nationwide epidemiology surveillance. Annual Report of Epidemiology Research for Intractable Diseases in Japan, the Ministry of Health, Welfare and Labor (2005). 2006; 39–42. (In Japanese).
11 Suzuki K, Takikawa Y, Endo T. Prospective study to clarify the prognosis of patients with fulminant hepatitis and acute hepatitis severe type. Annual Report of Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labor (2001).2002; 87–96. (In Japanese).
12 Sato S, Suzuki K, Takikawa Y *et al.* Japanese National Study Group of Fulminant Hepatitis. Clinical epidemiology of fulminant hepatitis in Japan before the substantial introduction of liver transplantation: an analysis of 1309 cases in a 15-year national survey. *Hepatol. Res.* 2004; **30**: 155–61.
13 Yano K, Tamada Y, Yatsuhashi H *et al.* Japan National Hospital Acute Hepatitis Study Group. Dynamic epidemiology of acute viral hepatitis in Japan. *Intervirology* 2010; **53**: 70–5.
14 Kiyohara T, Sato T, Totsuka A, Miyamura T, Ito T, Yoneyama T. Shifting seroepidemiology of hepatitis A in Japan, 1973–2003. *Microbiol. Immunol.* 2007; **51**: 185–91.
15 Rezende G, Roque-Afonso AM, Samuel D *et al.* Viral and clinical factors associated with the fulminant course of hepatitis A infection. *Hepatology* 2003; **38**: 613–8.
16 Eto T, Shiraki K. National project on the prevention of mother-to-infant infection by hepatitis B virus in Japan. *Acta Paediatr. Jpn.* 1989; **31**: 681–4.
17 Hui CK, Cheung WW, Zhang HY *et al.* Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; **131**: 59–68.
18 Yeo W, Chan TC, Leung NW *et al.* Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J. Clin. Oncol.* 2009; **27**: 605–11.

19 Umemura T, Tanaka E, Kiyosawa K *et al.* Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. *Clin. Infect. Dis.* 2008; **47**: e52–6.
20 Kusumoto S, Tanaka Y, Mizokami M, Ueda R. Reactivation of hepatitis B virus following systemic chemotherapy for malignant lymphoma. *Int. J. Hematol.* 2009; **90**: 13–23.
21 Pitini V, Sturniolo G, Arrigo C, Leonardi S, Pino S, Altavilla G. HCV genotype 2 as a risk factor for reactivation in patients with B-cell lymphoma undergoing rituximab combination chemotherapy. *Br. J. Haematol.* 2010; **150**: 116–8.
22 Aggarwal R, Naik S. Epidemiology of hepatitis E: current status. *J. Gastroenterol. Hepatol.* 2009; **24**: 1484–93.
23 Suzuki K, Aikawa T, Okamoto H. Fulminant hepatitis E in Japan. *N. Engl. J. Med.* 2002; **347**: 1456.
24 Yazaki Y, Mizuo H, Takahashi M *et al.* Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J. Gen. Virol.* 2003; **84**: 2351–7.
25 Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* 2003; **362**: 371–3.
26 Inoue J, Ueno Y, Nagasaki F *et al.* Sporadic acute hepatitis E occurred constantly during the last decade in northeast Japan. *J. Gastroenterol.* 2009; **44**: 329–37.
27 Ferrari R, Pappas G, Agostinelli D *et al.* Type 1 autoimmune hepatitis: patterns of clinical presentation and differential diagnosis of the "acute" type. *QJM* 2004; **97**: 407–12.
28 Abe M, Hiasa Y, Masumoto T *et al.* Clinical characteristics of autoimmune hepatitis with histological features of acute hepatitis. *Hepatol. Res.* 2001; **21**: 213–9.
29 Uetrecht J. Idiosyncratic drug reactions: past, present, and future. *Chem. Res. Toxicol.* 2008; **21**: 84–92.
30 Lammert C, Bjornsson E, Niklasson A, Chalasani N. Oral medications with significant hepatic metabolism at higher risk for hepatic adverse events. *Hepatology* 2010; **51**: 615–20.
31 Miyake Y, Iwasaki Y, Takaki A *et al.* Lamivudine treatment improves the prognosis of fulminant hepatitis B. *Intern. Med.* 2008; **47**: 1293–9.
32 Degertekin B, Lok AS. Indications for therapy in hepatitis B. *Hepatology* 2009; **49** (Suppl. 5): S129–37.
33 Jochum C, Gieseler RK, Gawlista I *et al.* Hepatitis B-associated acute liver failure: immediate treatment with entecavir inhibits hepatitis B virus replication and potentially its sequelae. *Digestion* 2009; **80**: 235–40.
34 Harry R, Auzinger G, Wendon J. The effects of supraphysiological doses of corticosteroids in hypotensive liver failure. *Liver Int.* 2003; **23**: 71–7.
35 Ichai P, Duclos-Vallée JC, Guettier C *et al.* Usefulness of corticosteroids for the treatment of severe and fulminant forms of autoimmune hepatitis. *Liver Transpl.* 2007; **13**: 996–1003.
36 Yokoi T, Oda S, Shiga H *et al.* Efficacy of high-flow dialysate continuous hemodiafiltration in the treatment of fulminant hepatic failure. *Transfus. Apher. Sci.* 2009; **40**: 61–70.
37 Inoue K, Kourin A, Watanabe T *et al.* Artificial liver support system using large buffer volumes removes significant glutamine and is an ideal bridge to liver transplantation. *Transplant. Proc.* 2009; **41**: 259–61.
38 Matsui Y, Sugawara Y, Yamashiki N *et al.* Living donor liver transplantation for fulminant hepatic failure. *Hepatol. Res.* 2008; **38**: 987–96.
39 Mochida S, Nakayama N, Matsui A *et al.* Re-evaluation of the Guideline published by the Acute Liver Failure Study Group of Japan in 1996 to determine the indications of liver transplantation in patients with fulminant hepatitis. *Hepatol. Res.* 2008; **38**: 970–9.
40 Mochida S, Naiki T, Takikawa Y *et al.* Fulminant hepatitis and LOHF in Japan. Revision of the guideline for liver transplantation Annual Report of Intractable Hepato-biliary Diseases Study Group of Japan, the Ministry of Health, Welfare and Labor (2001). 2009; 16–8. (In Japanese).
41 Ido A, Tsubouchi H. Translational research to identify clinical applications of hepatocyte growth factor. *Hepatol. Res.* 2009; **39**: 739–47.
42 Terai S, Sakaida I. Current status of autologous bone marrow cell infusion therapy for liver cirrhosis patients. *Hepatol. Res.* 2008; **38** (suppl. 1): S72–5.

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Original article

De novo activation of HBV with escape mutations from hepatitis B surface antibody after living donor liver transplantation

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Background: *De novo* activation of HBV occurs after liver transplantation from hepatitis B surface antigen (HBsAg)-negative and hepatitis B core antibody (anti-HBc)-positive donors, even under hepatitis B immunoglobulin (HBIG) prophylaxis. One reason for the activation of HBV is the emergence of HBV with escape mutations from hepatitis B surface antibody (anti-HBs). The aim of this study is to clarify the clinical features for *de novo* activation of HBV with anti-HBs escape mutations after liver transplantation.

Methods: Clinical features of 75 patients who received HBIG prophylaxis >6 months after liver transplantation with liver grafts from anti-HBc-positive donors were retrospectively analysed.

Results: Among the 75 recipients, 19 (25%) developed *de novo* activation of HBV. Of the 19 recipients, the emergence of HBV with anti-HBs escape mutations was confirmed in 7 patients. The rate of *de novo* activation of HBV with anti-HBs escape mutations was 12% at 5 years. Sequence analysis revealed mutations in the common 'a' determinant region of the surface gene, including G145R, G145A and Q129P, in HBsAg. Administration of entecavir immediately after the occurrence of *de novo* HBV activation resolved hepatitis and induced clearance of serum HBsAg and HBV DNA in all four patients receiving entecavir.

Conclusions: Escape mutations from anti-HBs caused *de novo* activation of HBV under HBIG prophylaxis after liver transplantation. Early administration of entecavir was effective on *de novo* activation of HBV with anti-HBs escape mutations.

Introduction

Most individuals who are negative for hepatitis B surface antigen (HBsAg) but positive for hepatitis B core antibody (anti-HBc) – which is indicative of resolved hepatitis B – have persistent viral infection in the liver [1,2]. We previously demonstrated that latent HBV infection is accompanied by ongoing viral replication in the liver but not in the serum or lymphatic cells of healthy anti-HBc-positive liver transplant donors [3,4]. It is possible for latently infected HBV to be transmitted from anti-HBc-positive donors to recipients via liver grafts and reactivated under the immunosuppressive conditions imposed after liver transplantation. This reactivation is called *de novo* activation of HBV [5–7].

To prevent *de novo* activation of HBV after liver transplantation, hepatitis B immunoglobulin (HBIG) had been widely used as a prophylaxis post-surgery [7–9], although lamivudine with or without HBIG has recently become the standard prophylaxis [10,11]. Even under HBIG prophylaxis, occurrence of *de novo* activation of HBV has been reported [8,12,13]. Recently, we reported that *de novo* hepatitis B occurred in 24% of HBV-naive recipients who received liver grafts from anti-HBc-positive donors [13]. Among these cases, one of the most important factors associated with HBV activation was found to be the emergence of HBV with escape mutations from hepatitis B surface antibody (anti-HBs). Escape mutations from anti-HBs occur in the common 'a' determinant region of the surface gene, which is a highly conformational region of the HBsAg protein. Mutations in and around the 'a' determinant region have been shown to alter the antigenicity of the HBsAg protein; consequently, anti-HBs fails to neutralize HBV

[14–16]. Anti-HBs escape mutations have been found in patients vaccinated for HBV [17,18], in patients with chronic hepatitis B [19,20] and in liver transplant recipients after HBIG administration [21,22]. The clinical significance of the anti-HBs escape mutant HBV has been well-analysed in patients after HBV vaccination. The prevalence of anti-HBs escape mutants after HBV vaccination was reported to have increased from 7.8% in 1984 to 19.6% in 1989; after a 1994 survey, prevalence was reported to be 28.1% [18]. Commonly reported mutations in HBsAg with the potential to escape neutralization by vaccine-induced antibody in patients after HBV vaccination include G145R, D144A, P142S, K141E, Q129H, I/T126N/A and M133L [18,23]. By contrast, the clinical features of anti-HBs escape mutants after liver transplantation under HBIG prophylaxis have not been well-analysed.

Treatment strategies for HBV with anti-HBs escape mutations have not been clarified. At present, several nucleoside analogues such as lamivudine, adefovir and entecavir are available for the treatment of chronic hepatitis B [24]. Among them, entecavir, a carbocyclic analogue of 2′-deoxyguanosine, has been shown to have higher efficacy and lower rates of resistance than lamivudine for patients with chronic hepatitis B [24]; therefore, entecavir is now used as a first-line therapy in the treatment of chronic hepatitis B worldwide. However, the efficacy of nucleoside analogues for HBV with escape mutations from anti-HBs is unknown.

The aim of this study was to clarify the clinical features of *de novo* activation of HBV with escape mutations from anti-HBs under HBIG prophylaxis after liver transplantation.

Methods

Patients

We retrospectively analysed the medical records of 157 patients who underwent living donor liver transplantation (LDLT) using liver grafts from HBsAg-negative but anti-HBc-positive donors from July 1995 to August 2008 (Figure 1A). Of these, 57 recipients were excluded from our study because their sera were pre-operatively positive for HBsAg and/or HBV DNA. An additional 25 patients were also excluded from the study because of the short duration (<6 months) of their follow-up in our hospital. Accordingly, 75 patients with a follow-up period of >6 months were enrolled in this study. The study protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (Kyoto, Japan), and all patients provided informed consent.

Prophylaxis with HBIG and immunosuppressive protocol

HBIG monotherapy was given to all recipients with grafts from anti-HBc-positive donors, as reported previously [7]. The first application of HBIG at a dose of 200 IU/kg body mass was administered during the anhepatic phase of LDLT, and 100 IU/kg of HBIG was administered, if required, to maintain serum anti-HBs titres at >500 IU/l during the first post-operative month. Subsequently, HBV serological markers were examined at monthly intervals after the transplant operation, and 1,000 IU of HBIG was periodically administered to maintain serum anti-HBs titres at >200 IU/l throughout the follow-up period.

The standard immunosuppression protocol comprised tacrolimus and low-dose steroid therapy. The target whole blood lower level for tacrolimus was 10–15 ng/ml during the first 2 weeks, 10 ng/ml thereafter and 5–8 ng/ml starting from the second month. Steroid therapy was initiated at a dose of 10 mg/kg of prednisolone before graft reperfusion, then tapered down from 1 mg/kg per day on the first day to 0.3 mg/kg per day until the end of the first month, followed by 0.1 mg/kg per day until the end of the third month. After that, steroid administration was terminated.

Diagnosis of *de novo* activation of HBV

De novo activation of HBV was diagnosed when HBsAg and HBV DNA became positive in the serum of the liver transplant recipient. Serological HBV markers, including HBsAg, anti-HBs, anti-HBc, hepatitis B e antigen (HBeAg) and antibodies to HBeAg (anti-HBe), were measured by chemiluminescent enzyme immunoassay (CLEIA; Fuji Rebio, Tokyo, Japan). Serum HBV DNA titre was analysed using a commercial PCR assay (Amplicor HBV Monitor; Roche, Branchburg, NJ, USA).

PCR amplification of HBV DNA and sequencing of the surface gene

Serum samples were obtained at the diagnosis of *de novo* activation of HBV for the analysis of HBV DNA sequencing. Preparation of DNA samples and detection of HBV genomes by PCR have been described previously [3,13]. The nucleotide sequence spanning the S region was amplified by PCR using specific primers, 5′-TGCCCTTGGATAAAGGCATT-3′ and 5′-AAGTTAAGGGAGTAGCCCCA-3′, followed by direct sequencing analyses using primers 5′-CCTGCTGGTGGCTCCAGTTC-3′ and 5′-AAGTTAAGGGAGTAGCCCCA-3′.

Statistical analysis

Baseline characteristics were tabulated and compared between patients with activation of HBV with anti-HBs escape mutations and patients without HBV activation (Table 1). For continuous variables, medians and ranges are given, and the data were analysed by the Wilcoxon rank-sum test. For categorical variables, counts