The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the approval of the Ethics Committee of the Kurume University School of Medicine.

Diagnosis and staging of HCC

The diagnosis of HCC was based on the clinical practice manual proposed by the Japan Society of Hepatology,[25] by using serum AFP and DCP levels and imaging techniques including ultrasonography, computerized tomography, magnetic resonance imaging, hepatic angiography and/or tumor biopsy. The Milan criteria (single nodule ≤5 cm or three nodules <3 cm) were used for the staging of HCC.[26]

Data mining

An association between the Milan criteria and each risk factor was examined by Student's *t*-test and χ^2-test. Because of the insufficient scientific evidence for testing specific clinical hypotheses, graphical modeling and decision trees were employed to explore complex associations between the Milan criteria and a set of risk factors.

MIM software (http://www.hypergraph.dk/) was used for graphical modeling. R package rpart (recursive partitioning and regression trees by Terry Therneau and Beth Atkinson; http://www.mayo.edu/biostatistics) was used to construct a decision tree algorithm. In order to evaluate the prediction error, the original data ($n = 663$) were randomly divided into a training dataset ($n = 442$) and a test dataset ($n = 221$). Ten-hold cross-validation was conducted to construct the initial tree on the basis of the training dataset; then, the optimal-size tree was constructed by examining a set of cost–complexity parameters. The overall prediction error rate as well as the sensitivity and specificity were calculated by applying the results of the decision tree algorithm to the test dataset.

RESULTS

Characteristics of patients with NBNC-HCC

THE PATIENTS' CHARACTERISTICS are summarized in Table 1. Family history of liver disease and history of blood transfusion were not noted in more than 80% of the patients. Approximately 40% of the patients did not have any etiology of chronic liver disease.

Univariate analysis of variables associated with the Milan criteria

Univariate analysis showed that diagnosis of liver cirrhosis, serum AST level, serum ALT level, serum AFP level and serum DCP level were significantly associated with the Milan criteria (Table 2).

Table 1 Characteristics of all patients

Variable	
n	663
Diagnostic year of HCC (years)	2002 ± 3
Age (years)	68.1 ± 9.9
Male/female	480/183
Family history of liver disease (yes/no/unclear)	79/547/37
History of blood transfusion (no/before 1989/after 1989/unclear)	584/29/22/28
Daily alcohol intake (none/<60 g/60–100 g/>100 g)	254/183/141/85
Etiology of chronic liver disease (none/alcohol/others)	296/188/179
Diagnosis of liver cirrhosis (yes/no)	260/403
Diagnosis of diabetes mellitus (no/yes without medication/yes with medication)	396/109/158
Serum AST level (U/L)	53.3 ± 51.3
Serum ALT level (U/L)	51.8 ± 49.9
Serum AFP level (ng/mL)	9397 ± 71066
Serum DCP level (mAU/mL)	8003 ± 37377
Size of HCC (cm)	5.0 ± 3.4
Number of HCC	2.8 ± 2.9

Data are expressed as the mean ± standard deviation or the number of patients.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

Graphical modeling

Complex interactions of the risk factors associated with the Milan criteria were visualized graphically (Fig. 1). Graphical modeling identified six independent factors directly associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum AST level; serum ALT level; serum AFP level; and serum DCP level (Fig. 1). Although alcohol intake, diagnosis of liver disease and diagnosis of diabetes mellitus were not directly associated with the Milan criteria, they were associated with the Milan criteria through diagnosis of liver cirrhosis (Fig. 1).

Decision tree algorithm

With the training dataset ($n = 442$), a decision tree algorithm was created by using five variables to classify six groups of patients (Fig. 2). A serum AFP level of 200 ng/mL or less was the cut-off value for the initial

Table 2 Univariate analysis of the variables associated with the Milan criteria

Variable	Statistical method	Test statistics	Degree of freedom (df)	*P*
Diagnostic year of HCC (years)	χ^2	13.4013	11	0.2679
Age (years)	Pooled	−1.07	661	0.2843
Sex	χ^2	0.2975	1	0.5854
Family history of liver disease	χ^2	1.7412	1	0.187
History of blood transfusion	χ^2	4.9527	2	0.084
Daily alcohol intake	χ^2	2.4158	3	0.4907
Liver cirrhosis	χ^2	28.9521	1	<0.0001
Diabetes mellitus	χ^2	0.926	2	0.6294
AST level (U/L)	Satterthwaite	3.06	387.51	0.0023
ALT level (U/L)	Satterthwaite	4.79	546.95	<0.0001
AFP level (ng/mL)	χ^2	63.1357	2	<0.0001
DCP level (mAU/mL)	χ^2	47.7161	2	<0.0001

Associations between the variables and the Milan criteria were analyzed by the indicated statistical methods. $P < 0.05$ was considered significant.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

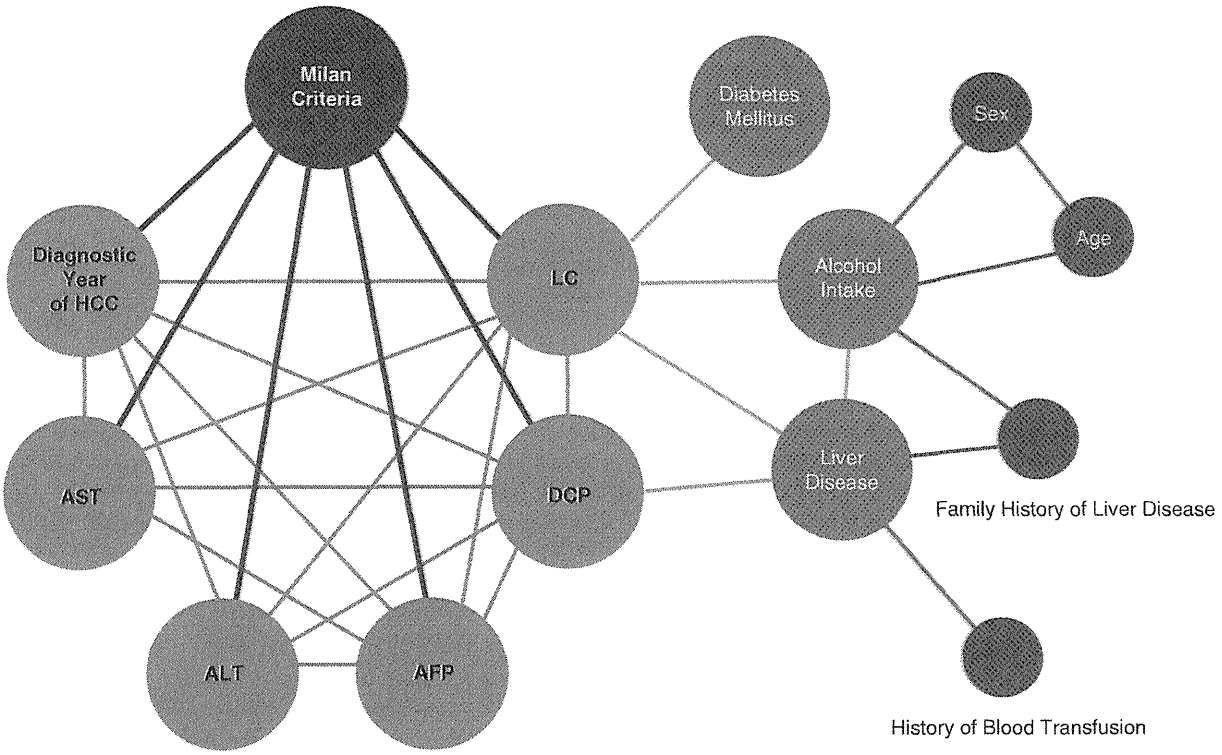

Figure 1 Graphical modeling of the interactions of the risk factors associated with the Milan criteria. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

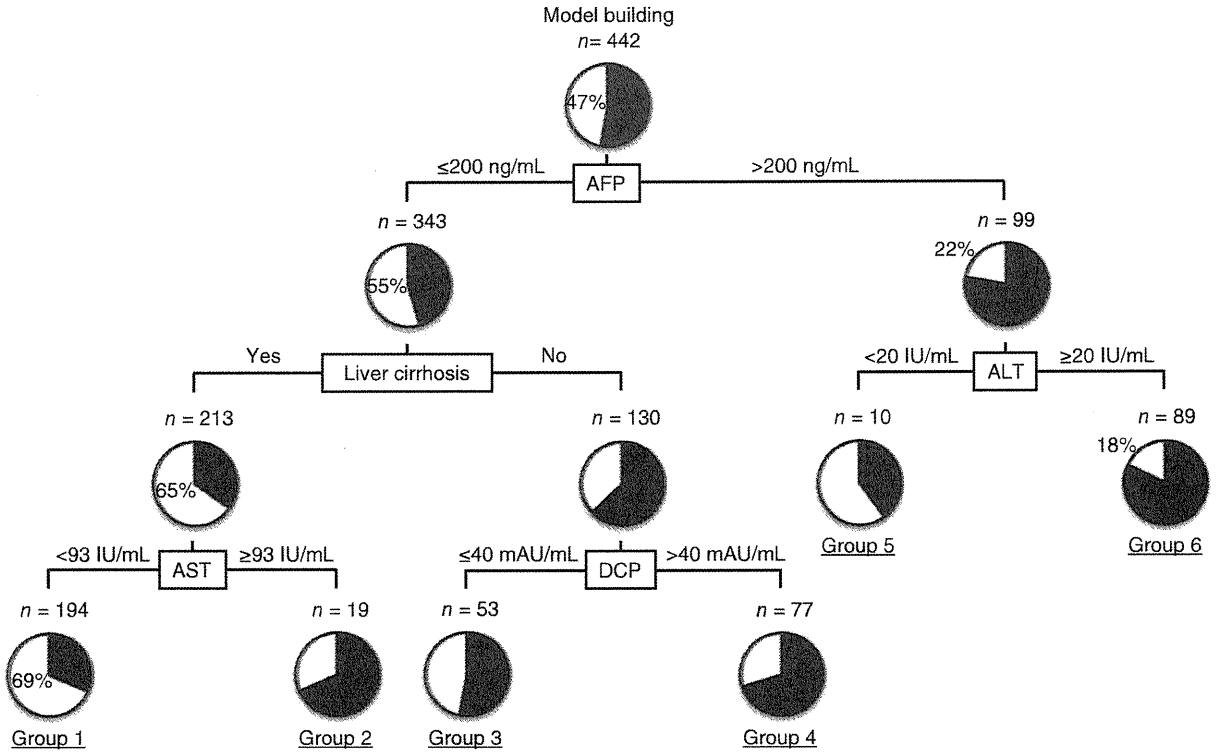

Figure 2 Decision tree algorithm of the variables associated with the Milan criteria. The patients were classified according to the indicated cut-off values of the variables. The pie graphs indicate the percentage of patients with HCC within (white)/beyond the Milan criteria in each group. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

classification. Among the patients with an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis was used as the variable for the second division. Among the patients with liver cirrhosis, a serum AST level of less than 93 IU/mL was the cut-off value for the third division. Thus, 69% of the patients were within the Milan criteria, when the patients met all of the following conditions: AFP of 200 ng/mL or less; diagnosis of liver cirrhosis; and AST of less than 93 IU/mL (group 1; Fig. 2). On the other hand, only 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and an ALT level of 20 IU/mL or more (group 6; Fig. 2).

There were no significant differences in the patients' characteristics between the training dataset and the test dataset. Prediction error was obtained by applying the results of the decision tree algorithm to the test dataset. The sensitivity (proportion of patients with HCC correctly classified as beyond the Milan criteria) and specificity (proportion of patients with HCC correctly classified as within the Milan criteria) were 72.1% (75/104) and 68.4% (80/117), respectively; the overall prediction error rate was 29.8% (66/221).

DISCUSSION

IN THIS STUDY, we revealed the complex interactions of the risk factors associated with staging of NBNC-HCC using graphical modeling. In addition, we presented a decision tree algorithm to identify clinical feature profiling associated with the staging of NBNC-HCC.

Various factors seem to be intricately related to the progression of NBNC-HCC. In this study, by graphical modeling, we identified six variables directly associated with the Milan criteria: serum AST level; serum ALT level; serum AFP level; serum DCP level; diagnosis of liver cirrhosis; and diagnostic year of HCC. Chronic hepatic inflammation modulates many of the signaling cascades involved in cell proliferation, survival and invasion of

HCC.[27,28] Further, AFP and DCP are directly associated with HCC progression through the induction of cancer cell proliferation and angiogenesis, respectively.[29,30] Thus, our results are in good accordance with previous basic investigations and suggest that hepatic inflammation as well as elevated AFP and DCP levels independently accelerate the progression of NBNC-HCC.

Diagnostic year of HCC was also directly associated with the Milan criteria in this study. Although the reason for this association is unclear, a progress in serum tumor markers is a possible explanation. Because sensitivities of AFP and DCP were improved during this study period (1995–2006),[31–33] one would think that serum AFP and DCP levels are confounding factors for an association between diagnostic year of HCC and the Milan criteria.

Recently, lifestyle-related factors including alcohol intake and diabetes mellitus have been noted as risk factors for the development of NBNC-HCC.[2,10–12,34–38] Previous *in vitro* studies showed that ethanol and glucose stimulate the proliferation and migration of HCC,[39,40] indicating the direct association of alcohol intake and diabetes mellitus with NBNC-HCC progression. However, in this study, these factors were not directly associated with the Milan criteria. Although the reason for this discrepancy remains unclear, alcohol intake and diabetes mellitus were associated with the Milan criteria through diagnosis of liver cirrhosis in this study. Both ethanol consumption and diabetes mellitus can activate fibroblasts,[41,42] which are crucial components of the tumor microenvironment promoting the growth and invasion of cancer cells.[43,44] Thus, alcohol intake and diabetes mellitus may be associated with the clinical progression of NBNC-HCC through the tumor microenvironment.

Then, we created a decision tree algorithm to identify the clinical feature profiling associated with the staging of NBNC-HCC; the reproducibility of this model was confirmed by the independent validation datasets. Serum AFP level was selected for the initial classification, and serum DCP level was selected for the third division, creating groups 3 and 4. Although it is still unclear why the serum AFP level was associated with the Milan criteria to a greater extent than the serum DCP level, an association of the serum AFP level with the pathological features of HCC is a possible explanation. The AFP level is related to the number of HCC, whereas the DCP level is more specific to vascular invasion.[45–47] In this study, the staging of HCC was evaluated by using the Milan criteria, which include number and size of HCC but not vascular invasion,[26] explaining why serum AFP level was selected for the initial classification.

Diagnosis of liver cirrhosis was selected for the second division in the decision tree algorithm. Although liver cirrhosis is a well-known major risk factor for the development of HCC,[5,10,12,25,34,42] our result indicates that liver cirrhosis may suppress the progression of NBNC-HCC. We do not have any data accounting for the association between diagnosis of liver cirrhosis and suppression of the NBNC-HCC progression, the following is, however, a possible explanation for this contradiction. HCC surveillance may be performed more often in patients with liver cirrhosis than in those without liver cirrhosis,[12,25] so HCC could be identified at an early stage in patients with liver cirrhosis.

A limitation of this study is that a relationship between progression of NBNC-HCC and non-alcoholic steatohepatitis (NASH) was not evaluated. The reason is that NASH-related HCC is often diagnosed as cryptogenic cirrhosis-related HCC because of reduction of hepatic triglycerides according to the progression of NASH, so-called "burned-out NASH".[48] However, NASH is deeply involved in the development of HCC and a major reason for the increase in number of NBNC-HCC patients.[8,49,50] Recently, visceral fat accumulation is also reported to be an independent risk factor for HCC recurrence after curative treatment.[51] Thus, further study will be focused on a relationship between the progression of NBNC-HCC and NASH.

In conclusion, data mining disclosed complex associations of risk factors and clinical feature profiling associated with the staging of NBNC-HCC.

REFERENCES

1 El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1752–63.
2 Kawaguchi T, Taniguchi E, Itou M, Sumie S, Yamagishi SI, Sata M. The pathogenesis, complications and therapeutic strategy for hepatitis C virus-associated insulin resistance in the era of anti-viral treatment. *Rev Recent Clin Trials* 2010; **5**: 147–57.
3 Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820–32.
4 Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. *Intervirology* 2010; **53**: 39–43.
5 Nordenstedt H, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. *Dig Liver Dis* 2010; **42** (Suppl 3): S206–14.
6 Nouso K, Kobayashi Y, Nakamura S *et al.* Evolution of prognostic factors in hepatocellular carcinoma in Japan. *Aliment Pharmacol Ther* 2010; **31**: 407–14.

7 Tanaka H, Imai Y, Hiramatsu N *et al.* Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. *Ann Intern Med* 2008; **148**: 820–6.
8 Taura N, Fukushima N, Yatsuhashi H *et al.* The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased in Kyushu area. *Med Sci Monit* 2010; **17**: PH7–11.
9 Abe H, Yoshizawa K, Kitahara T, Aizawa R, Matsuoka M, Aizawa Y. Etiology of non-B non-C hepatocellular carcinoma in the eastern district of Tokyo. *J Gastroenterol* 2008; **43**: 967–74.
10 Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35–50.
11 Hassan MM, Hwang LY, Hatten CJ *et al.* Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206–13.
12 Kiyosawa K, Umemura T, Ichijo T *et al.* Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; **127**: S17–26.
13 Bellazzi R, Zupan B. Predictive data mining in clinical medicine: current issues and guidelines. *Int J Med Inform* 2008; **77**: 81–97.
14 Kalisch M, Fellinghauer BA, Grill E *et al.* Understanding human functioning using graphical models. *BMC Med Res Methodol* 2010; **10**: 14–23.
15 Edwards D. *Introduction to Graphical Modelling*. New York: Springer-Verlag, 2000.
16 Pielou EC. *The Interpretation of Ecological Data. A Primer on Classification and Ordination*, 1st edn. New York: John Wiley&Sons, Inc., 1984.
17 Legendre P, Legendre L. *Numerical Ecology*, 2nd edn. Amsterdam: Elsevier Science, 1998.
18 Tsai CL, Camargo CA Jr. Methodological considerations, such as directed acyclic graphs, for studying "acute on chronic" disease epidemiology: chronic obstructive pulmonary disease example. *J Clin Epidemiol* 2009; **62**: 982–90.
19 Kurosaki M, Matsunaga K, Hirayama I *et al.* A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. *Hepatol Res* 2010; **40**: 251–60.
20 Kurosaki M, Sakamoto N, Iwasaki M *et al.* Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis. *J Gastroenterol* 2010 (in press).
21 Pauker SG, Kassirer JP. The threshold approach to clinical decision making. *N Engl J Med* 1980; **302**: 1109–17.
22 Lee A, Joynt GM, Ho AM, Keitz S, McGinn T, Wyer PC. Tips for teachers of evidence-based medicine: making sense of decision analysis using a decision tree. *J Gen Intern Med* 2009; **24**: 642–8.
23 Guo J, Wang W, Liao P *et al.* Identification of serum biomarkers for pancreatic adenocarcinoma by proteomic analysis. *Cancer Sci* 2009; **100**: 2292–301.
24 Warwick J, Vardaki E, Fattizzi N *et al.* Defining the surgical management of suspected early-stage ovarian cancer by estimating patient numbers through alternative management strategies. *BJOG* 2009; **116**: 1225–41.
25 Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. *Oncology* 2007; **72** (Suppl 1): 2–15.
26 Mazzaferro V, Regalia E, Doci R *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693–9.
27 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57–70.
28 Sanz-Cameno P, Trapero-Marugan M, Chaparro M, Jones EA, Moreno-Otero R. Angiogenesis: from chronic liver inflammation to hepatocellular carcinoma. *J Oncol* 2010; article no.: 272170.
29 Inagaki Y, Tang W, Xu H *et al.* Des-gamma-carboxyprothrombin: clinical effectiveness and biochemical importance. *Biosci Trends* 2008; **2**: 53–60.
30 Wang XW, Xie H. Alpha-fetoprotein enhances the proliferation of human hepatoma cells in vitro. *Life Sci* 1999; **64**: 17–23.
31 Weitz IC, Liebman HA. Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. *Hepatology* 1993; **18**: 990–7.
32 Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R, Tomita K. Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. *Oncology* 2002; **62** (Suppl 1): 57–63.
33 Marrero JA, Lok AS. Newer markers for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S113–19.
34 El-Serag HB. Epidemiology of hepatocellular carcinoma in USA. *Hepatol Res* 2007; **37** (Suppl 2): S88–94.
35 El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460–8.
36 Kawaguchi T, Sata M. Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. *World J Gastroenterol* 2010; **16**: 1943–52.
37 Kawaguchi T, Taniguchi E, Morita Y *et al.* Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection. *Liver Int* 2010; **30**: 479–86.
38 Tazawa J, Maeda M, Nakagawa M *et al.* Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci* 2002; **47**: 710–15.
39 Brandon-Warner E, Sugg JA, Schrum LW, McKillop IH. Silibinin inhibits ethanol metabolism and ethanol-dependent cell proliferation in an in vitro model of hepatocellular carcinoma. *Cancer Lett* 2010; **291**: 120–9.
40 Chang YJ, Chiu CC, Wu CH *et al.* Glucose-regulated protein 78 (GRP78) silencing enhances cell migration but does not influence cell proliferation in hepatocellular carcinoma. *Ann Surg Oncol* 2010; **17**: 1703–9.

41 Flanders KC. Smad3 as a mediator of the fibrotic response. *Int J Exp Pathol* 2004; **85**: 47–64.
42 Gyamfi MA, Wan YJ. Pathogenesis of alcoholic liver disease: the role of nuclear receptors. *Exp Biol Med (Maywood)* 2010; **235**: 547–60.
43 Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. *Exp Cell Res* 2010; **316**: 1324–31.
44 Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. *Front Biosci* 2010; **15**: 166–79.
45 Hamamura K, Shiratori Y, Shiina S *et al.* Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. *Cancer* 2000; **88**: 1557–64.
46 Miyaaki H, Nakashima O, Kurogi M, Eguchi K, Kojiro M. Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 962–8.
47 Toyoda H, Kumada T, Kiriyama S *et al.* Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2006; **4**: 111–17.
48 Ong J, Younossi ZM, Reddy V *et al.* Cryptogenic cirrhosis and posttransplantation nonalcoholic fatty liver disease. *Liver Transpl* 2001; **7**: 797–801.
49 Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972–8.
50 Hashimoto E, Yatsuji S, Kaneda H *et al.* The characteristics and natural history of Japanese patients with nonalcoholic fatty liver disease. *Hepatol Res* 2005; **33**: 72–6.
51 Ohki T, Tateishi R, Shiina S *et al.* Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. *Gut* 2009; **58**: 839–44.

INTERNAL MEDICINE

ORIGINAL ARTICLE

Predictive Value of the Fibrosis Scores in Patients with Chronic Hepatitis C Associated with Liver Fibrosis and Metabolic Syndrome

Hisamitsu Miyaaki, Tatsuki Ichikawa, Naota Taura, Satoshi Miuma, Hidetaka Shibata, Hajime Isomoto, Fuminao Takeshima and Kazuhiko Nakao

Abstract

Objective We evaluated patients with chronic hepatitis C (CHC) and compared the clinical and pathological features of steatosis and metabolic syndrome to identify the risk factors for CHC with severe fibrosis.

Methods One hundred seventy-one patients with biopsy-confirmed CHC were included in the study: 90 males and 81 females, age 56.2±12.8 years; 46 with obesity (BMI≥25 kg/m^2); 51 with hypertension; 36 with type 2 diabetes mellitus; and 20 with hypertriglyceridemia.

Results Steatosis was detected in 79 patients (46%); 92 patients (54%) showed no steatosis. Seventy-four patients (43%) showed mild fibrosis and 97 patients (56%) showed severe fibrosis. The variables that were significantly associated with steatosis were obesity [odds ratio 2.160 (1.010-4.727), p=0.046] and type 2 diabetes [odds ratio 3.667 (1.559-8.430), p=0.027]. The variables that were significantly associated with severe fibrosis were older age [odds ratio 2.675 (1.309-5.464), p=0.007], obesity [odds ratio 2.156 (1.006-4.619), p=0.048] and type 2 diabetes [odds ratio 8.739 (2.845-26.846), p=0.0002]. Nagasaki (N) score (the total number of specific risk factors, namely an older age, obesity, and type 2 diabetes) was higher in the severe fibrosis group than in the mild fibrosis group (mild fibrosis: severe fibrosis=1.48±1.14 vs.2.66±94, p<0.001).

Conclusion Metabolic syndrome factors, including obesity and diabetes, play a critical role in the pathogenesis of fibrosis in CHC. The N score was therefore found to be a significant predictor of severe fibrosis in CHC.

Key words: chronic hepatitis C, steatosis, liver fibrosis, type 2 diabetes mellitus, obesity

(Intern Med 50: 1137-1141, 2011)
(DOI: 10.2169/internalmedicine.50.4447)

Introduction

Hepatitis C virus (HCV) infection is one of the most common causes of chronic liver disease, affecting 2 million persons in Japan. It can lead to end-stage liver disease and hepatocellular carcinoma. Chronic HCV infection is associated with metabolic abnormalities, including insulin resistance (1-3). We recently reported that insulin resistance is associated with interferon signalling, which plays an important role in the clearance of chronic hepatitis C (CHC) during interferon therapy (4, 5). Previous studies have demonstrated the association between metabolic syndrome and hepatic fibrosis in patients with hepatitis C (6-11). Steatosis is a frequent histological finding in chronic hepatitis C virus infection, one that affects disease progression and occurrence of hepatocellular carcinoma (7, 12-15). Hepatic steatosis is associated with metabolic syndrome and non-alcoholic steatohepatitis (NASH). We previously reported that risk factors for severe fibrosis in patients with NASH were metabolic syndrome, hypertension, and, in particular, diabetes mellitus (16). In this study, we evaluated patients with chronic hepatitis C (CHC) and compared the clinical and pathological features of metabolic syndrome to identify the risk factors for CHC with severe fibrosis.

Department of Gastroenterology and Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Japan
Received for publication August 30, 2010; Accepted for publication February 20, 2011
Correspondence to Dr. Hisamitsu Miyaaki, miyaaki-hi@umin.ac.jp

Table 1. Clinical Data of the Patient Population

age	56.2 ± 12.8
Gender	male: female=90:81
HCV genotype (1:2)	123: 48
HCV viral load (Fmol/L)	5325 ± 5297
BMI (kg/m^2)	22.8 ± 2.9
BMI ≥ 25 (kg/m^2)	49 cases (29%)
Platelet (×10^4/mm^3)	15.9 ± 5.8
AST (IU/L)	72 ± 100
ALT (IU/L)	93 ± 110
Type 2 diabetes mellitus	36 cases (21%)
Hypertension	51 cases (30%)
Hyperlipidemia	20 cases (11%)

Table 2. Comparison between the Non-steatosis Group and Steatosis Group

	Steatosis n=79	Non-steatosis n=92	p value
Age (≥60 years old)	38 (48%)	45 (48%)	0.916*
Gender (male)	48 (60%)	42 (45%)	0.049*
HCV genotype 1	55 (69%)	68 (73%)	0.533*
HCV viral load (Fmol/L)	4611 ± 4872	5968 ± 5514	0.109
Platelet (×10^4/ mm^3)	15.4 ± 5.7	16.2 ± 5.7	0.324
ALT (IU/L)	106 ± 139	82 ± 77	0.173
Obesity	30(38%)	19 (20%)	0.013*
Diabetes mellitus	24 (36%)	11(12%)	0.001*
Hypertension	33 (42%)	18 (20%)	0.002*
Hypertriglycerides	13 (17%)	7 (7%)	0.070*

* χ^2 test

Methods

This retrospective study included 171 consecutive patients with biopsy-confirmed CHC who were assessed between 1996 and 2008 in Nagasaki Universities and associated hospitals. Inclusion criteria were an increased serum aminotransferase level for at least 6 months; serum anti-HCV (ELIZA; third generation); positive HCV RNA (PCR); negative serum HBs; and no other cause of liver disease, such as alcohol intake >30d/d or autoimmune or metabolic disorders (genetic hemochromatosis). Obesity was defined as a body mass index (BMI) >25 according to the World Health Organization criteria (17). Type 2 diabetes was diagnosed according to International Diabetes Federation criteria (18) (fasting glucose >110 mg/dL, or previously diagnosed type 2 diabetes). Hypertension was also diagnosed according to International Diabetes Federation criteria (18) (systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg). Hypertriglyceridemia was diagnosed if there was documented use of anti-hypertriglyceride medications, or if fasting triglyceride levels were >150 mg/dL. All liver biopsy tissue specimens were examined using Hematoxylin-Eosin, Azan-Mallory, and silver reticulum staining. The specimens were assessed by one reviewer blinded to patient clinical and biochemical data. The diagnosis of each case was independently and histologically confirmed by liver pathologists according to the Japanese chronic hepatitis classification (New Inuyama classification) (19). Fatty change in >5% of all areas was defined as steatosis. The patients were divided into two groups based on their degree of inflammation defined as mild activity and severe activity. Fibrosis staging was scored using a 5-grade scale: F0 indicated no fibrosis, F1 was defined as fibrous portal expansion, F2 was bridging fibrosis, F3 was bridging fibrosis with lobular distortion, and F4 indicated the presence of cirrhosis. The mild fibrosis group was defined as having a score of F0-2 and severe fibrosis was defined as a score of F3 or F4.

We defined the Nagasaki (N) score as the total number of risk factors for severe fibrosis. The risk factors were age (≥ 60 years old), obesity, and type 2 diabetes. In the N score, we doubled the presence of diabetes as a factor because the estimation value of DM was 2 times higher than that of the other factors (older age and obesity) in our logistic regression formula. Therefore, we defined N score as follows (20):

N score= age (≥60 years old) +Obesity (BMI≥25) +2× diabetes mellitus

The N score was compared with other non-invasive predictors of fibrosis stage, such as the AST to platelet ratio index (APRI), and the platelet APRI was defined as follows:

APRI=AST level (/ULN)/ platelet count (10^9/L) ×100.

Statistical significance was determined by SPSS analytical software (IBM, Armonk, NY). We used Student's t-test and the chi-square test to perform analyses. A multivariate analysis was performed using binary logistic regression analysis.

Results

Among the 171 patients, 90 males and 81 females were examined. The median body mass index (BMI) was 22.8± 2.9 kg/m^2. Type 2 diabetes was diagnosed in 21% of patients, hypertension in 30%, and hyperlipidemia in 11% (Table 1).

Histological findings

Steatosis was detected in 79 patients (46%). We found mild activity in 149 patients and severe activity in 22 patients. We found no fibrosis in 18 patients (11%) (F0); mild fibrosis in 56 patients (33%) (F1); moderate fibrosis in 33 patients (19%) (F2); bridging fibrosis in 30 patients (17%) (F3); and cirrhosis in 34 patients (20%) (F4).

Steatosis

The two steatosis groups are shown in Table 2. Males were significantly more common in the non-steatosis group than in the steatosis group (60% vs. 45%, p=0.049). The patients with obesity (38% vs. 20%, p=0.013), type 2 diabetes

Table 3. Multivariate Logistic Regression Analysis of the Association of Steatosis with the Risk of Fibrosis

	odds ratio	p value
Gender (male)	1.462 (0.765~2.888)	0.263
Obesity (BMI≥ 25 kg/m^2)	2.160 (1.010~4.227)	0.046
Type 2 diabetes	3.667 (1.559~8.430)	0.027
Hypertension	2.318 (1.151~4.864)	0.002

Table 4. Comparison between Mild Fibrosis and Severe Fibrosis

	Severe fibrosis n=97	Mild fibrosis n=74	p value*
Age (≥60 years old)	56 (59%)	23(32%)	0.001*
gender (male)	51(52%)	39 (52%)	0.987*
HCV genotype 1	73 (75%)	50 (67%)	0.348 *
HCV viral load	5924 ± 5490	4864 ± 5035	0.214
Platelet (×10^4/ mm^3)	13.5 ± 4.7	19.0 ± 5.4	<0.001
ALT	104.2 ± 129.9	79.5 ± 77.7	0.126
obesity	34 (31%)	15 (20%)	0.034*
Type 2 diabetes	32 (33%)	4 (5%)	<0.001*
Hypertension	35 (38%)	16 (18%)	0.041 *
Hypertriglyceride	11 (11%)	9 (12%)	0.868 *

χ^2 test

(36% vs. 12%, p=0.001), and hypertension (42% vs. 20%, p=0.002) were significantly more likely to be in the non-steatosis group than the steatosis group. There were no significant differences between the other clinical features (age, incidence of the HCV genotype, HCV viral load, ALT, platelet count, hypertriglycemia). In a multivariate logistic regression analysis, obesity, type 2 diabetes, and hypertension were independent predictors for steatosis [odds ratio 2.160 (1.010-4.227), p=0.046, odds ratio 3.667 (1.559-8.430), p=0.027, odds ratio 2.318 (1.151-4.864), p=0.002)] (Table 3).

The incidence of severe activity was not significantly different between the two groups (non-steatosis group: steatosis group= 9.7%: 16.4%, p=0.2849).

Severe fibrosis risk factors

The risk factors of the two fibrosis groups are shown in Table 4. The prevalence of older patients (≥60 years old) in the severe fibrosis group was significantly greater than that of younger patients (32% vs. 59%, p=0.001). The prevalence of obesity, type 2 diabetes, and hypertension was significantly higher in the severe fibrosis group than in the mild fibrosis group (31% vs. 20%, p=0.034, 33% vs. 5%, p <0.001, 38% vs. 18%, p=0.041, respectively). The platelet count was significantly lower in the severe fibrosis group than in the mild fibrosis group (13.5±4.7 vs. 19.0±5.4, < 0.001). There were no significant differences between the other clinical features (age, incidence of the HCV genotype, HCV viral load, ALT, hypertriglycemia). In a multivariate logistic regression analysis, older age, type 2 diabetes, and obesity were independent predictors for severe fibrosis [coefficient 0.984 odds ratio 2.675 (1.309-5.464), p=0.007, coefficient 2.168 odds ratio 8.739 (2.845-26.846), p=0.0002, coefficient 0.768 odds ratio 2.156 (1.006-4.619), p=0.048)] (Table 5).

Table 5. A Multivariate Logistic Regression Analysis of the Association of Severe Fibrosis with Various Risk Factors

	Odds ratio	p value
Age (60 years old)	2.675 (1.309~5.464)	0.007
Obesity	2.156 (1.006~4.619)	0.048
Type 2 DM	8.739 (2.845~26.846)	0.0002
Hypertension	1.087 (0.487~2.426)	0.8394

The fibrosis stage was significantly worse in the steatosis group than in the non-steatosis group (1.78±1.28 vs. 2.34± 1.29, p=0.005).

Predictive score of severe fibrosis

The N score is the total number of risk factors, including: older age (≥60 years old), obesity, and type 2 diabetes. N score were significantly higher in the severe fibrosis group than in the mild fibrosis group (1.48±1.14 vs. 2.66±94, p< 0.001).

We found that 17 of 53 patients (32%) with an N score of 0 had severe fibrosis, 31 of 63 (49%) with an N score of 1, 25 of 29 (86%) with an N score of 2, 21 of 23 (91%) patients with an N score of 3, and 3 of 3 (100%) patients with an N score of 4 had severe fibrosis.

The ROC curve (Fig. 1) shows the respective sensitivities and specificities for any combination of 1 to 4 of the N score. An N score of 2 provides the best combination of sensitivity (0.50) and specificity (0.91) for predicting severe fibrosis.

Compared with other non-invasive predictors of significant fibrosis, the sensitivity and specificity of an APRI of 1.5 were 46% and 93%, and those for a platelet count of 12×10^4/mm^3 were 44% and 91%.

Discussion

In this study, we analyzed the correlation between metabolic syndrome and pathological findings in CHC patients, and identified the clinical risk factors for severe fibrosis.

Obesity, type 2 diabetes, and hypertension were significant risk factors for severe steatsosis and fibrosis. These comorbidities of metabolic syndrome affected steatosis and the progression of severe fibrosis in the liver. Previous data suggest a strong association between the presence of steatosis and severe fibrosis in CHC (7, 12, 13). In this study, patients in the steatosis group had more severe fibrosis than

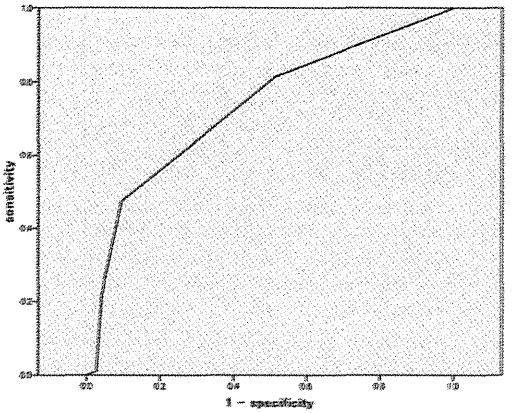

Figure 1. ROC curve for the Nagasaki (N) score. A cut-off N score of 2 gives the sensitivity (0.50) and specificity (0.91). The area under the ROC curve is 0.732.

Table 6. The Incidence of Severe Fibrosis in Patients with Different N Scores

	Severe fibrosis
N Score 0	17/53 (32%)
N Score 1	31/63 (49%)
N Score 2	25/29 (86%)
N Score 3	21/23 (91%)
N Score 4	3/3 (100%)

those in the non-steatosis group. It remains controversial whether or not hepatic steatosis may accelerate fibrosis by stimulating the activity of CHC. In this study, the ALT levels and the incidence of severe inflammation in the steatosis group were not significantly higher than in the non-steatosis group. Therefore, hepatic steatosis may not promote liver fibrosis by liver cell injury.

Evidence indicates that hepatic steatosis, which is affected by metabolic syndrome, may accelerate the progression of fibrosis in patients with CHC. The findings are consistent with those from other reports that demonstrate an association between effective weight loss and reductions in steatosis, ALT levels, and fibrosis stage in patients with CHC (21).

HCV core protein increases reactive oxygen species (ROS) and lipid peroxidation, leading to liver damage and fibrosis (22, 23). Core protein also reduces microsomal triglyceride transport protein function, leading to hepatic steatosis. Infection with the HCV virus affects liver steatosis as well as fibrosis (24). Thus, both host and viral factors induce steatosis and play a role in severe fibrosis in chronic hepatitis C.

We found a significant correlation between the severity of hepatic fibrosis and the comorbidites of metabolic syndrome, including obesity, diabetes mellitus, and hypertension. Our previous study showed that metabolic syndrome, including diabetes mellitus and hypertension, was a risk factor for severe fibrosis in patients with NASH (16). These risk factors in patients with NASH were similar to those in CHC patients, suggesting that the mechanism underlying the liver fibrosis in CHC patients resembles that of NASH.

Type 2 diabetes and obesity are correlated with insulin resistance. We previously reported that the development of liver fibrosis is associated with insulin resistance in CHC patients (25). Outcomes from the present study show the adverse effects of insulin resistance on liver fibrosis in CHC patients.

Previous data also have shown that obesity and diabetes mellitus are associated with progression of fibrosis in CHC (6, 8, 9, 26). Few prior studies have been conducted in Asian patients. The present study shows that metabolic syndrome, including obesity and diabetes, also predict severe fibrosis in Asian patients.

Taken together, although steatosis, fibrosis and metabolic syndrome seem to be associated with each other, our cross-sectional study did not identify any associations between these factors. Further studies will be necessary to confirm whether these conditions are associated, or whether they act as independent risk factors.

We defined the N score as the total number of risk factors for severe fibrosis. The risk factors were age ($\geq$60 years old), obesity, and type 2 diabetes. In the N score, we doubled the presence of diabetes as a factor because the estimation value of DM was 2 times that of other factors (older age and obesity) in our logistic regression formula (risk factor). The N score was significantly higher in the severe fibrosis group than in the mild fibrosis group. About 90% of the patients in the severe fibrosis group had an N score $\geq$2. An N score $\geq$2 indicates a high risk for severe fibrosis. Our results suggest that half of the patients in the severe fibrosis group also had metabolic disorders, including diabetes and obesity. Conversely, there was no association between metabolic syndrome and other factors.

While the specificity of the N score (0.91) is very good, the sensitivity (0.5) is not sufficient. This suggests that there are two or more mechanisms underlying the progression of fibrosis, and metabolic syndrome represents one of them.

Compared to other non-invasive markers, the sensitivity and specificity of the N score was equal to the platelet count and APRI. The N score is a simple score to calculate, and it adds together the three risk factors. Therefore, determining the N score is considered to be an easy way to predict the presence of severe liver fibrosis in CHC patients.

The present study has limitations. We cannot perform a validation due to the fact that our sample size was so small. Now, we are planning to perform a validation set to confirm the value of N score.

In conclusion, older age, obesity and type 2 diabetes are significant predictors of severe fibrosis in Japanese CHC patients. The total number of these risk factors in patients could be a useful marker for predicting severe fibrosis in pa-

tients with CHC.

The authors state that they have no Conflict of Interest (COI).

References

1. Koike K. Hepatitis C as a metabolic disease: Implication for the pathogenesis of NASH. Hepatol Res **33**: 145-150, 2005.
2. Moucari R, Asselah T, Cazals-Hatem D, et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology **134**: 416-423, 2008.
3. Sheikh MY, Choi J, Qadri I, Friedman JE, Sanyal AJ. Hepatitis C virus infection: molecular pathways to metabolic syndrome. Hepatology **47**: 2127-2133, 2008.
4. Miyaaki H, Ichikawa T, Nakao K, et al. Predictive value of the phosphorylation of signal transducers and activators of transcription in the outcome of interferon therapy for chronic hepatitis C. Intervirology **51**: 394-399, 2008.
5. Miyaaki H, Ichikawa T, Nakao K, et al. Predictive value of suppressor of cytokine signal 3 (SOCS3) in the outcome of interferon therapy in chronic hepatitis C. Hepatol Res **39**: 850-855, 2009.
6. Hu KQ, Kyulo NL, Esrailian E, et al. Overweight and obesity, hepatic steatosis, and progression of chronic hepatitis C: a retrospective study on a large cohort of patients in the United States. J Hepatol **40**: 147-154, 2004.
7. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology **33**: 1358-1364, 2001.
8. Hwang SJ, Luo JC, Chu CW, et al. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. J Gastroenterol Hepatol **16**: 190-195, 2001.
9. Clouston AD, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. Hepatology **41**: 809-818, 2005.
10. Tsochatzis EA, Manolakopoulos S, Papatheodoridis GV, Archimandritis AJ. Insulin resistance and metabolic syndrome in chronic liver diseases: old entities with new implications. Scand J Gastroenterol **44**: 6-14, 2009.
11. Svegliati-Baroni G, Bugianesi E, Bouserhal T, et al. Post-load insulin resistance is an independent predictor of hepatic fibrosis in virus C chronic hepatitis and in non-alcoholic fatty liver disease. Gut **56**: 1296-1301, 2007.
12. Giannini E, Ceppa P, Botta F, et al. Steatosis and bile duct damage in chronic hepatitis C: distribution and relationships in a group of Northern Italian patients. Liver **19**: 432-437, 1999.
13. Hourigan LF, Macdonald GA, Purdie D, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology **29**: 1215-1219, 1999.
14. Ohata K, Hamasaki K, Toriyama K, et al. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Cancer **97**: 3036-3043, 2003.
15. Tanaka A, Uegaki S, Kurihara H, et al. Hepatic steatosis as a possible risk factor for the development of hepatocellular carcinoma after eradication of hepatitis C virus with antiviral therapy in patients with chronic hepatitis C. World J Gastroenterol **13**: 5180-5187, 2007.
16. Miyaaki H, Ichikawa T, Nakao K, et al. Clinicopathological study of nonalcoholic fatty liver disease in Japan: the risk factors for fibrosis. Liver Int **28**: 519-524, 2008.
17. World Health Organization. Difinition and Classification of Diabetes Mellitus and its Complications—Part 1: Diagnosis and Classification of Diabetes Mellitus. World Health Organization, 1999: 20-21.
18. Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group. The metabolic syndrome--a new worldwide definition. Lancet **366**: 1059-1062, 2005.
19. Ichida F, Tsuji T, Omata M, et al. New Inuyama classification; New criteria for histological assesment of chronic hpatitis. Int Hepatol Commun **6**: 112-119, 1996.
20. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology **38**: 518-526, 2003.
21. Hickman IJ, Clouston AD, Macdonald GA, et al. Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. Gut **51**: 89-94, 2002.
22. Korenaga M, Wang T, Li Y, et al. Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. J Biol Chem **280**: 37481-37488, 2005.
23. Dionisio N, Garcia-Mediavilla MV, Sanchez-Campos S, et al. Hepatitis C virus NS5A and core proteins induce oxidative stress-mediated calcium signalling alterations in hepatocytes. J Hepatol **50**: 872-882, 2009.
24. Perlemuter G, Sabile A, Letteron P, et al. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. Faseb J **16**: 185-194, 2002.
25. Taura N, Ichikawa T, Hamasaki K, et al. Association between liver fibrosis and insulin sensitivity in chronic hepatitis C patients. Am J Gastroenterol **101**: 2752-2759, 2006.
26. Mason AL, Lau JY, Hoang N, et al. Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology **29**: 328-333, 1999.

Hidaka et al. Journal of Medical Case Reports 2011, 5:276
http://www.jmedicalcasereports.com/content/5/1/276

JOURNAL OF MEDICAL CASE REPORTS

CASE REPORT Open Access

Living donor liver transplantation from a donor previously treated with interferon for hepatitis C virus: a case report

Masaaki Hidaka[1], Mitsuhisa Takatsuki[1], Akihiko Soyama[1], Hisamitsu Miyaaki[2], Tatsuki Ichikawa[2], Kazuhiko Nakao[2], Takashi Kanematsu[1] and Susumu Eguchi[1*]

Abstract

Introduction: Selecting a marginal donor in liver transplantation (LT) remains controversial but is necessary because of the small number of available donors.

Case presentation: A 46-year-old Japanese woman was a candidate to donate her liver to her brother, who had decompensated liver cirrhosis of unknown origin. Eight years before the donation, she had a mild liver dysfunction that was diagnosed as a hepatitis C virus (HCV) infection (serotype 2). She had received anti-viral therapy with interferon α-2b three times weekly for 24 weeks and had a sustained viral response (SVR). A biopsy of her liver before the donation showed normal findings without any active hepatitis, and her serum was negative for HCV-RNA. Only 67 patients have undergone LT from a cadaveric donor in Japan. The family in this case decided to have living donor LT. A careful selection for the liver graft donation was made; however, since she was the only candidate, we approved her as a living donor. She was discharged nine days after the liver donation. Her liver function recovered immediately. A computed tomography scan showed sufficient liver regeneration one year later. Her brother also had good liver function after LT and had no HCV infection 48 months after surgery and no *de novo* malignancy. Neither of the siblings has developed an HCV infection.

Conclusions: A patient with SVR status after interferon therapy might be considered a candidate for living donor LT but only if there are no other possibilities of LT for the recipient. A careful follow-up of the donor after donation is needed. The recipient also must have a very close follow-up because it is difficult to predict what might happen to the graft with post-transplant immunosuppression.

Introduction

The number of deceased donor liver transplantations (DDLTs) in Japan is extremely small. There were 67 cases between February 1999 and January 2010, according to the Japan Organ Transplant Network [1]. Therefore, living donor liver transplantation (LDLT) is the most frequent treatment option for patients with end-stage liver disease in Japan. The main advantage of LDLT over DDLT is that the donor can be completely evaluated, before the operation, to exclude many medical problems. However, the indications for a living donor should be strict and the risk to the donor must be avoided with the greatest care. Donors with possibly morbid liver conditions, including fatty infiltration or a history of viral hepatitis, and older donors offer "marginal grafts", which should be used only after very careful evaluation. A hepatitis B virus (HBV) core antibody seropositive donor can be accepted as long as HBV surface antigen is seronegative and anti-viral treatment is administered to the recipient after transplantation [2,3]. In this way, donor safety also is established, according to several reports of this type of case [4-6]. Some investigators reported that the patients obtained a sustained viral response (SVR) after interferon therapy showed that there was no tendency to develop fibrotic liver in the future [7,8]. HCV-RNA was not detected in 88% of the serum and liver biopsies of patients with an SVR [9]. The infection rate of the recipient from an HCV-

* Correspondence: sueguchi@nagasaki-u.ac.jp
[1]Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
Full list of author information is available at the end of the article

positive graft should be low after LT. The rate of carcinogenesis has increased at an annual rate of 0.11% after an SVR was maintained with anti-viral therapy [10]. Using a graft with a positive hepatitis C virus (HCV) antibody although HCV-RNA was not detected in the blood remains controversial in Western countries [11-13]. To the best of our knowledge, there is no actual data that shows the outcome of liver transplantation with a graft from patients who acquire an SVR after successful anti-viral therapy. Here, we report the case of a living donor who had an SVR before LDLT. The case is described and discussed in detail.

Case presentation

A 46-year-old Japanese woman donated the right lobe of her liver to her 36-year-old brother, who had decompensated cirrhosis of unknown origin. She experienced mild liver dysfunction (117IU/L in alanine aminotransferase, normal range of 5 to 30IU/L) eight years before the donation. Her condition was diagnosed as chronic active HCV infection (serotype 2) on the basis of a liver biopsy and viral study that showed that her level of HCV-RNA was 13 kcopy/mL by real-time polymerase chain reaction analysis. The histological diagnosis showed chronic hepatitis A1/F1 (Figure 1). She received anti-viral therapy with intra-muscular interferon α-2b three times weekly for 24 weeks. Her serum HCV assay results were negative after two weeks of effective anti-viral therapy.

She was doing well and no HCV-RNA had been detected. She maintained an SVR without any complications until she was evaluated as a living donor. The donor evaluation revealed anti-HCV antibody, but her liver function test results were normal and HCV-RNA was negative by polymerase chain reaction analysis. She underwent an ultrasound-guided needle biopsy of her liver, and the pathological findings were normal and there were no findings of active hepatitis (Figure 2). She was approved as a living donor after a thorough evaluation by the ethics committee of the Nagasaki University Graduate School of Biomedical Sciences. She was discharged nine days after the liver donation. Her liver function recovered immediately. A computed tomography scan one year later showed that she had sufficient liver regeneration. Her brother was also doing well after the LT and had no HCV infection 40 months after surgery and no *de novo* malignancy.

Discussion

Selecting a living donor in this case might be controversial, although a marginal donor can also sometimes be a candidate. The risks in this case included HCV transmission to the recipient, HCV reactivation in the recipient after LDLT, and donor risk during surgery. A number of studies have reported that the results with recipients of an HCV-infected graft were comparable to those of recipients of an HCV-negative graft [11-13]. The Scientific Registry of the United Network for Organ

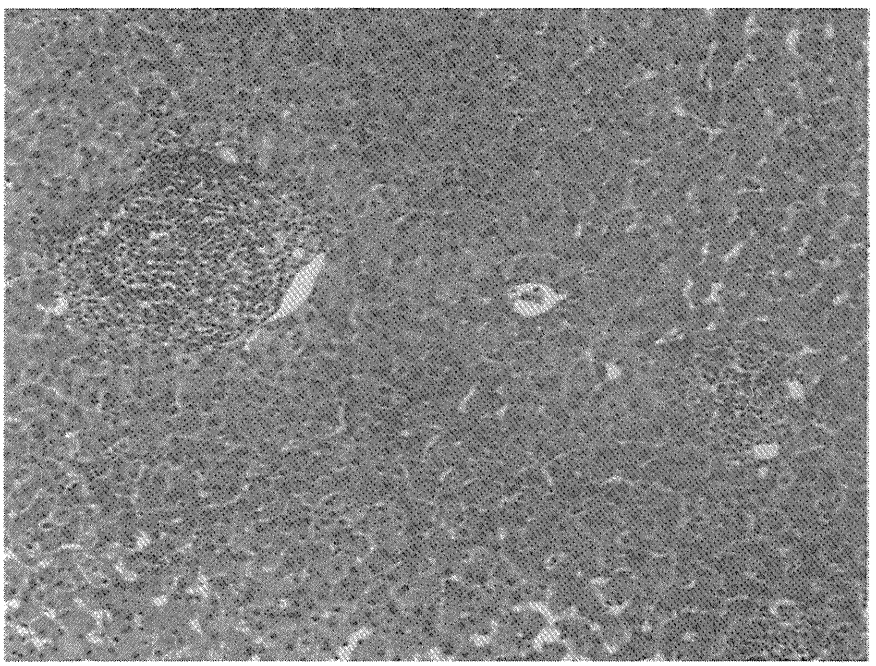

Figure 1 A liver biopsy showed chronic active hepatitis A1/F1 before interferon therapy.

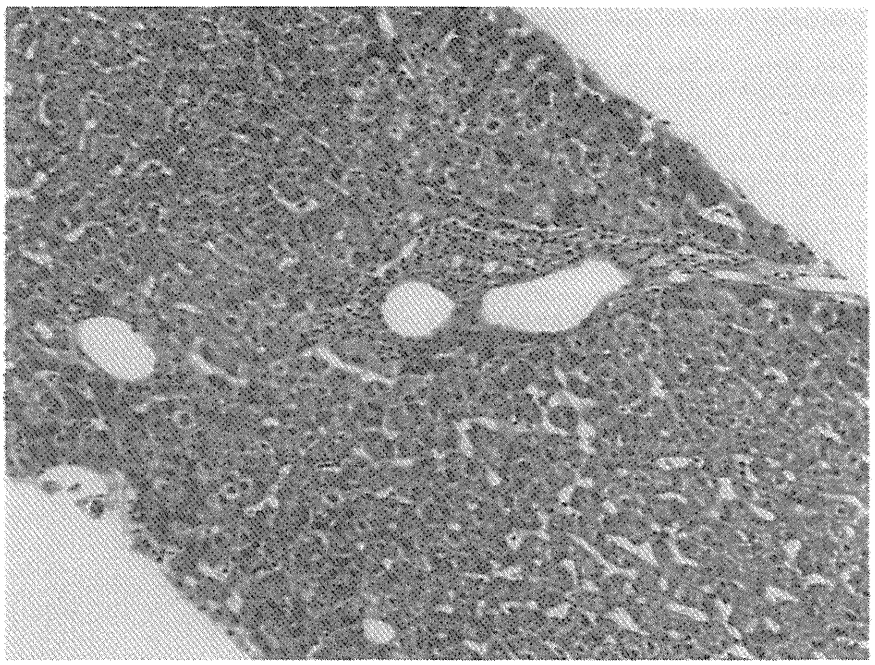

Figure 2 A liver biopsy shows normal liver tissue without hepatitis before the liver donation.

Sharing reported that the survival rate of 96 patients was significantly higher in the recipients of HCV-positive grafts than in recipients of HCV-negative grafts [13]. These results demonstrated that the use of an HCV-positive graft may also be acceptable in cadaveric LT. In contrast, an HCV-infected graft was not acceptable in LDLT. Patients who have HCV and who acquire an SVR after interferon therapy can be considered living donor candidates.

In this case, it was difficult to determine the indications for the donor selection before transplantation. Patients with serotype 2 HCV are more likely to achieve an SVR after interferon therapy than those with serotype 1. Our donor achieved an SVR of HCV. She and her brother were fully informed of the risk of peri-operative complications and the possibility that he would receive an HCV infection from the graft although she had obtained an SVR after anti-viral therapy. In theory, it is unlikely that a recipient would develop a viral infection from a graft that achieved an SVR.

Conclusions

We report a case of LDLT from a donor previously treated with interferon for HCV. A patient with SVR status after interferon therapy might be considered a candidate for LDLT only if there are no other possibilities of LT for the recipient. A careful follow-up of the donor after donation is needed. The recipient also must have a very close follow-up because it is difficult to predict what might happen to the graft with post-transplant immunosuppression.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Abbreviations

DDLT: deceased donor liver transplantation; HBV: hepatitis B virus; HCV: hepatitis C virus; LDLT: living donor liver transplantation; LT: liver transplantation; SVR: sustained viral response.

Author details

[1]Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. [2]Department of Gastroenterology and Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Authors' contributions

MH and SE shared responsibility for the management of this patient and were involved in drafting the manuscript or revising it critically for important intellectual content. MT shared responsibility for the management of this patient. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 25 October 2010 Accepted: 3 July 2011 Published: 3 July 2011

References

1. Japan Organ Transplant Network homepage. , http://www.jotnw.or.jp (in Japanese), http://www.jotnw.or.jp/english/index.html (in English).

2. Cholongitas E, Papatheodoridis GV, Burroughs AK: **Liver grafts from anti-hepatitis B core positive donors: a systematic review.** *J Hepatol* 2010, **52**:272-279.
3. Yu L, Koepsell T, Manhart L, Ioannou G: **Survival after orthotopic liver transplantation: the impact of antibody against hepatitis B core antigen in the donor.** *Liver Transplant* 2009, **15**:1343-1350.
4. Hwang S, Moon DB, Lee SG, Park KM, Kim KH, Ahn CS, Lee YJ, Chu CW, Yang HS, Cho SH, Oh KB, Ha TY, Min PC: **Safety of anti-hepatitis B core antibody-positive donors for living-donor liver transplantation.** *Transplantation* 2003, **75**:S45-48.
5. Celebi Kobak A, Karasu Z, Kilic M, Ozacar T, Tekin F, Gunsar F, Ersoz G, Yuzer Y, Tokat Y: **Living donor liver transplantation from hepatitis B core antibody positive donors.** *Transplant Proc* 2007, **39**:1488-1490.
6. Van Thiel DH, De Maria N, Colantoni A, Friedlander L: **Can hepatitis B core antibody positive livers be used safely for transplantation: hepatitis B virus detection in the liver of individuals who are hepatitis B core antibody positive.** *Transplantation* 1999, **68**:519-522.
7. Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M: **Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy.** *Ann Intern Med* 2000, **132**:517-524.
8. Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, Wilkinson J, Adair D, Rakela J, Laskus T: **Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C.** *Hepatology* 2005, **41**:106-114.
9. Larghi A, Tagger A, Crosignani A, Ribero ML, Bruno S, Portera G, Battezzati PM, Maggioni M, Fasola M, Zuin M, Podda M: **Clinical significance of hepatic HCV RNA in patients with chronic hepatitis C demonstrating long-term sustained response to interferon-alpha therapy.** *J Med Virol* 1998, **55**:7-11.
10. Ikeda M, Fujiyama S, Tanaka M, Sata M, Ide T, Yatsuhashi H, Watanabe H: **Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon.** *J Gastroenterol* 2005, **40**:148-156.
11. Saab S, Ghobrial RM, Ibrahim AB, Kunder G, Durazo F, Han S, Farmer DG, Yersiz H, Goldstein LI, Busuttil RW: **Hepatitis C positive grafts may be used in orthotopic liver transplantation: a matched analysis.** *Am J Transplant* 2003, **3**:1167-1172.
12. Fan X, Lang DM, Xu Y, Lyra AC, Yusim K, Everhart JE, Korber BT, Perelson AS, Di Bisceglie AM: **Liver transplantation with hepatitis C virus-infected graft: interaction between donor and recipient viral strains.** *Hepatology* 2003, **38**:25-33.
13. Marroquin CE, Marino G, Kuo PC, Plotkin JS, Rustgi VK, Lu AD, Edwards E, Taranto S, Johnson LB: **Transplantation of hepatitis C-positive livers in hepatitis C-positive patients is equivalent to transplanting hepatitis C-positive livers.** *Liver Transplant* 2001, **7**:762-768.

doi:10.1186/1752-1947-5-276
Cite this article as: Hidaka *et al.*: Living donor liver transplantation from a donor previously treated with interferon for hepatitis C virus: a case report. *Journal of Medical Case Reports* 2011 **5**:276.

Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/j.1440-1746.2011.06743.x

HEPATOLOGY

Ferritin/alanine aminotransferase ratio as a possible marker for predicting the prognosis of acute liver injury

Eisuke Ozawa,*,† Seigo Abiru,* Shinya Nagaoka,* Koji Yano,* Atsumasa Komori,* Kiyoshi Migita,* Hiroshi Yatsuhashi,* Naota Taura,† Tatsuki Ichikawa,† Hiromi Ishibashi* and Kazuhiko Nakao†

*Clinical Research Center, National Hospital Organization Nagasaki Medical Center, Omura, and †Department Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Key words
acute liver failure, ferritin, heme oxygenase-1 (HO-1), prognostic predictor.

Accepted for publication 24 March 2011.

Correspondence
Dr Seigo Abiru, Clinical Research Center, National Hospital Organization Nagasaki Medical Center, Kubara 2-1001-1, Omura, Nagasaki 856-8562, Japan. Email: s.abiru@nmc.hosp.go.jp

Financial Support and Conflict of Interests: No potential conflicts of interest relevant to this article were reported from any of the authors.

Abstract

Background and Aims: Serum levels of ferritin and heme oxygenase (HO)-1 are both markers of macrophage activation. We evaluated simple markers for predicting the prognosis of severe acute liver injury in which macrophage activation plays an important role.
Methods: Subjects comprised 114 patients with acute liver injury, admitted to the liver unit of Nagasaki Medical Center between January 2001 and September 2010. Subjects included 11 patients with fulminant hepatic failure (FHF), 82 patients with ordinary acute hepatitis (AH), and 21 patients with severe-form AH (AHS). We determined serum levels of ferritin, HO-1 and other biochemical makers, and analyzed relationships between clinical outcomes of patients and each of these parameters alone and in combination.
Results: Median serum ferritin levels were significantly higher in FHF (25 900 ng/mL) and AHS (3060 ng/mL) than in AH (700 ng/mL; $P < 0.01$ each). Median HO-1 levels were also significantly higher in FHF (123 ng/mL) and AHS (51 ng/mL) than in AH (19 ng/mL; $P < 0.01$ each). Similarly, median ferritin/alanine aminotransferase (F/A) ratio was significantly higher in FHF (6.7) than in AHS (1.6, $P < 0.05$) or AH (0.5, $P < 0.01$). Among the 11 FHF patients, three recovered, seven died and one underwent liver transplantation. The ability of F/A ratio to distinguish non-survivors from survivors was analyzed using receiver operating characteristics curves. A cut-off level of 3.12 provided high sensitivity (87.5%) and specificity (81.2%).
Conclusion: These results suggest that F/A ratio offer a quick and simple marker for predicting the prognosis of acute liver injury.

Introduction

Fulminant hepatic failure (FHF) is associated with high mortality rates, despite recent advances in medical management. In contrast, outcomes for acute hepatitis (AH), and even severe-form AH (AHS), are not fatal. The pathogenesis of FHF has not been elucidated in detail, but antigen-specific cytotoxic T lymphocytes, polyclonal cytokines, immune modulators, and products of oxidative stress have been shown to induce damage and destruction of hepatocytes in these patients.[1]

Activated macrophages have been suggested to play important roles in the pathogenesis of FHF, as reflected by the activation of both pro- and anti-inflammatory cascades in the innate immune system.[2] Corticosteroids have been used to suppress macrophage activation in the treatment of severe acute hepatic failure.[3] Recent reports have found that serum concentrations of interleukin (IL)-10 and soluble-form CD163 (sCD163), a macrophage-activating factor, are both highly elevated in FHF.[4,5] Macrophages and their expression of Fas ligands may also play important roles in the pathogenesis of FHF.[6]

Ferritin, on the other hand, is a ubiquitous and highly conserved iron-binding protein. The serum ferritin level is an indicator of iron stores, and is also used as a marker of macrophage activation. Very high serum ferritin levels are observed in macrophage activation syndromes (MASs), such as hemophagocytic syndrome (HPS) and adult-onset Still's disease (AOSD), although the mechanisms underlying this increase are unclear.[7,8] Iron metabolism is known to be regulated by iron-responsive proteins (IRPs) affecting ferritin at the mRNA level. Inflammation dramatically affects iron metabolism and a variety of inflammatory mediators act via IRPs.[7]

Heme oxygenase (HO) is an enzyme that catalyzes the conversion of heme into carbon monoxide (CO), Fe2+ and biliverdin. HO-1, an inducible form of HO, is a 32-kD heat-shock protein that is expressed in response to various noxious stimuli, including heavy metals, hyperoxia, hypoxia, endotoxin, hydrogen peroxide, and inflammatory cytokines.[8–12] Recent studies have identified serum HO-1 as a novel marker for diagnosing macrophage activation state under conditions such as sepsis, HPS, and AOSD.[13,14]

Hepatocyte growth factor (HGF) was first discovered as a potent mitogen for adult hepatocytes.[15] The significance of evaluating serum HGF levels in liver diseases has been addressed in patients with FHF, who show markedly increased levels of serum HGF.[16,17] Measurement of serum HGF levels, which is commonly performed in Japan, may thus be useful for predicting fulminant progression and prognosis of acute liver disease.[18]

The difficult decision of whether to perform liver transplantation for patients with FHF should be made in the early stage of the disease.[19] The King's College criteria have been widely applied,[20] but offers unacceptably low predictive accuracy.[21] The aim of the present study was to identify a simple marker such as ferritin, F/A ratio and HO-1 for predicting the prognosis of acute liver injury in relation to serum HGF and to clarify the involvement of macrophage activation in FHF.

Methods

Inclusion criteria

Subjects in the present study were patients with AH, AHS or FHF. AH was diagnosed as an acute increase in levels of serum alanine aminotransferase (ALT) to > 10 times the upper limit of normal, with or without an increase in total bilirubin level. AHS was defined as AH without hepatic encephalopathy in addition to prothrombin activity < 40% of normal control or international normalized ratio (INR) > 2.0. FHF is defined most widely as a potentially reversible condition resulting from severe liver injury, with onset of encephalopathy occurring within 8 weeks after the symptom onset and in the absence of pre-existing liver disease.[22] In this study, FHF was defined in accordance with Japanese criteria: development of hepatic encephalopathy of grade II or above within 8 weeks after the symptom onset in addition to prothrombin activity < 40% of normal control or > INR 2.0.

Patient management

Plasma exchange and blood filtration were performed for all FHF patients using a membrane plasma separator in addition to intensive total care management, including hemodynamic monitoring, mannitol therapy for cerebral edema, infusion of an H_2 antagonist, and nutritional support. AHS patients received similar intensive total care management, with the exception of extracorporeal circulation.

Patients

Subjects comprised 81 patients who were admitted with acute liver injury between January 2001 and September 2010 to the Liver Unit at Nagasaki Medical Center, Omura, Nagasaki, Japan. Of these, 11 patients were diagnosed with FHF, 21 patients with AHS, and 84 patients with AH. Of the 11 FH patients, three recovered, seven died and one underwent liver transplantation.

This study was performed after obtaining written informed consent from each patient or the appropriate guardian in accordance with the Ethics Guidelines for Clinical Study issued by the Ministry of Health, Labor and Welfare in Japan.

Biochemical assays

Blood samples were obtained on admission for analysis of biochemical data, including ALT, ferritin and HGF, and serum samples were stored at −80°C until use. HO-1 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (Human HO-1 ELISA kit; Stressgen, Ann Arbor, MI, USA). In brief, mouse monoclonal rabbit anti-HO-1 was coated onto microtiter wells. Each sample of 100 μL (diluted 1:20 in sample diluent) was added and incubated for 30 min. After washing, 100 μL of anti-human HO-1 antibody (diluted 1:500) was added and incubated for 1 h. After further washing, 100 μL of anti-rabbit immunoglobulin (Ig) G conjugated with horseradish peroxidase (diluted 1:4000) was added and incubated for 30 min. Wells were washed, and 100 μL of stabilized teramethylbenzine substrate solution was added. After 15 min, 100 μL of acid stop solution was added, and plates were read at 450 nm. Control samples and standards of purified HO-1 were co-analyzed in each run. Inter-assay coefficient of variation (CV) was < 10%.

Serum concentrations of HGF were determined using commercially available ELISA kits (Otsuka, Tokyo, Japan), with absorbance read at 490 nm on an ELISA plate reader (Molecular Devices E-max, Concord, ON, Canada).

Statistical analysis

Non-parametric tests were used for comparisons between groups (Mann–Whitney test for unpaired data, Kruskal–Wallis test for comparisons among three groups) and for correlation analysis (Spearman ρ). The 95% confidence intervals for the area under the curve (AUC) in receiver operating characteristics (ROC) curves were calculated non-parametrically. All tests were two-sided, and values of $P < 0.05$ were considered significant. All statistical analyses were performed using Stat-flex version 5.0 software (Artech, Osaka, Japan).

Results

Clinical features

Clinical features of subjects are shown in Table 1. No significant differences in age and sex were seen among FHF, AHS and AH groups. Median serum ALT levels did not differ significantly among patients with FHF (5168 IU/L; range, 349–9670 IU/L) AHS (2802 IU/L; range, 400–13 200 IU/L) and AH (1523 IU/L; range, 327–5050 IU/L) ($P < 0.01$ each). The total serum bilirubin level was significantly higher in FHF (11.9 mg/dL; range, 2.5–32.2 mg/dL) than in AHS (7.0 mg/dL; range, 1.2–32.9 mg/dL) or AH (3.9 mg/dL; range, 0.3–34.0 mg/dL) ($P < 0.01$ each). Prothrombin activity was lowest in FHF (18.2%; range, 7.3–47.7%). FHF was caused by viral hepatitis in three patients (hepatitis A, $n = 1$; hepatitis B, $n = 1$; Epstein–Barr virus, $n = 1$), severe alcoholic hepatitis in two patients, hematological malignancy in three patients, acute heart failure in one patient, drug-induced hepatitis in one patient and indeterminate cause in one patient. AHS was caused by viral hepatitis in 10 patients (hepatitis A, $n = 6$; hepatitis B, $n = 4$), severe alcoholic hepatitis in one patient, drug-induced hepatitis in seven patients, and indeterminate cause in three patients. AH was caused by viral hepatitis in 41 patients (hepatitis

Table 1 Clinical characteristics of patients

	FHF ($n = 11$)	AHS ($n = 21$)	AH ($n = 84$)
Age (years)	64 (16–83)	48 (21–75)	45 (15–87)
Sex (male : female)	7:4	13:8	47:37
Total bilirubin (mg/dL)	11.9 (2.5–32.2)*	7.0 (1.2–32.9)	3.9 (0.3–34.0)
PT (%)	18.2 (7.3–47.7)**	37.0 (20.2–91.3)**	84.6 (48.9–120.8)
ALT (IU/L)	5168 (349–9670)	2 802 (400–13 200)	1523 (327–5050)
Plt ($*10^3/\mu L$)	143 (22–224)	149 (105–373)	185 (20–397)
Etiology			
Viral	3 (27.3%)	10 (47.6%)	41 (48.8%)
Hepatitis A	1	6	9
Hepatitis B	1	4	21
Other virus	1	0	11
Non-viral	8 (72.7%)	11 (52.4%)	43 (51.2%)
Drug	1	7	33
Other	7	4	10

Results are provided as median (range) or number (%).

*$P < 0.05$ versus AHS and $P < 0.01$ versus AH; **$P < 0.01$ versus AH.

AH, acute hepatitis; AHS, severe-form acute hepatitis; ALT, alanine aminotransferase; FHF, fulminant hepatic failure; PT, prothrombin time.

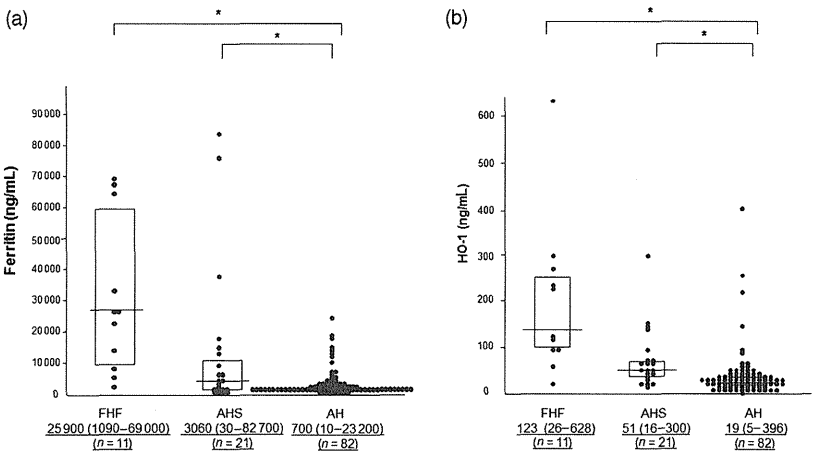

Figure 1 (a) Serum level of ferritin in subjects on admission. (b) Serum level of heme oxygenase (HO)-1 in subjects on admission. Box plots represent median and 25–75th percentiles. Upper and lower lines show minimum and maximum values, respectively. *$P < 0.01$, as determined by the Kruskal–Wallis test.

A, $n = 9$; hepatitis B, $n = 21$; hepatitis C, $n = 3$; hepatitis E, $n = 5$; Epstein–Barr virus, $n = 1$; and cytomegalovirus, $n = 1$), drug-induced hepatitis in 33 patients, and indeterminate causes in eight patients.

Highly increased serum levels of ferritin and HO-1 in FHF and AHS

Median levels of serum ferritin on admission were significantly higher in FHF (25 900 ng/mL; range, 1090–69 000 ng/mL) and AHS (3060 ng/mL; range, 30–82 700 ng/mL) than in AH (700 ng/mL; range, 10–23 200 ng/mL; $P < 0.01$). However, no significant difference was identified between FHF and AHS (Fig. 1a). Similarly, median serum HO-1 levels on admission were significantly higher in FHF (123 ng/mL; range, 26–628 ng/mL) and AHS (51 ng/mL; range, 16–300 ng/mL) than in AH (19 ng/mL; range, 5–396 ng/mL; $P < 0.01$), and no significant difference was apparent between FHF and AHS (Fig. 1b). HO-1 levels correlated significantly with ferritin levels ($r = 0.57$, $P < 0.01$; data not shown).

Highly increased ferritin/ALT (F/A) ratio in FHF and AH

Since ferritin is released into the circulation not only from activated macrophages but also from damaged hepatocytes,[23,24] distinguishing the origin of ferritin in serum is difficult in patients with acute liver injury. We therefore evaluated the F/A ratio, as a reflection of the fraction of ferritin released from activated macrophages rather than that released from hepatocytes. Median F/A ratio was significantly higher in FHF (6.7; range, 1.7–13.2) than in AHS (1.6; range, 0.0–8.6; $P < 0.05$) or AH (0.5; range, 0.0–7.0; $P \leq 0.01$ each) (Fig. 2a).

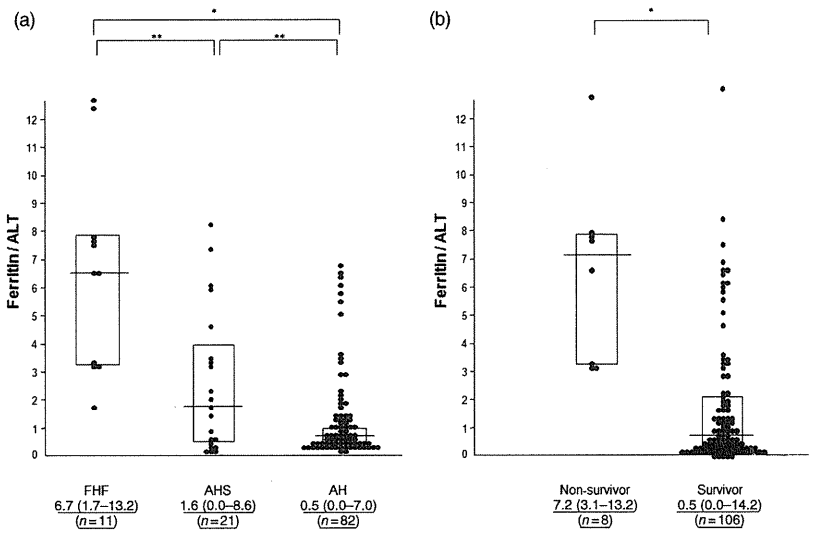

Figure 2 (a) Ferritin/alanine aminotransferase (ALT) ratio in subjects on admission. (b) Ferritin/ALT ratio in non-survivors and survivors on admission. $*P < 0.01$, $**P < 0.05$, as determined by the non-paired Mann–Whitney *U*-test.

Table 2 Comparisons of various parameters between survivors and non-survivors as assessed by univariate analysis

	Survivors ($n = 108$)	Non-survivors ($n = 8$)	*P*-value
Age (years)	46.0 (15–87)	64.0 (16–83)	n.s.
Sex (male : female)	61:47	6:2	n.s.
Type (AH : AHS : FHF)	84:21:3	0:0:8	< 0.01
Total bilirubin (mg/dL)	4.20 (3.00–32.2)	15.4 (0.30–34.0)	< 0.01
PT (%)	76.9 (15.2–47.7)	33.1 (7.3–120.8)	< 0.01
ALT (IU/L)	1 787 (327–13 200)	4 227 (349–9 670)	n.s.
Plt ($*10^3/\mu L$)	137 (20–397)	46.8 (22–191)	< 0.01
Viral/non-viral (*n*)	52/56	2/6	n.s.
Ferritin (ng/mL)	1 025 (14–82 700)	23 800 (1 090–66 900)	< 0.01
HO-1 (ng/mL)	34.0 (5.0–1 048.5)	106.0 (26.0–628.0)	< 0.01
F/A ratio	0.7 (0.01–13.2)	7.2 (3.12–12.95)	< 0.01
HGF (ng/mL)	0.5 (0.22–8.0)	5.0 (1.60–9.94)	< 0.01

Results are provided as median (range) or number.
ALT, alanine aminotransferase; FA ratio, ferritin/alanine aminotransferase ratio; HGF, hepatocyte growth factor; HO-1, heme oxygenase-1; n.s., not significant; Plt, platelet count; PT, prothrombin time.

Predicting survival by ferritin, HO-1, and F/A ratio

When we compared non-survivors ($n = 8$) and survivors ($n = 106$), median F/A ratio was significantly higher in non-survivors (7.2; range, 3.1–13.2) than in survivors (0.5; range, 0.0–14.2; $P < 0.01$). F/A ratio was > 3.0 in all non-survivors and liver-transplanted patients (Fig. 2b).

Comparisons of various parameters between survivors and non-survivors are shown in Table 2. Median serum ferritin level on admission was significantly higher in non-survivors (23 800 ng/mL; range, 1090–66 900 ng/mL) than in survivors (1025 ng/mL; range, 14–82 700 ng/mL; $P < 0.01$). Median serum HO-1 level was also significantly higher in non-survivors (106.0 ng/mL; range, 26.0–628.0 ng/mL) than in survivors (34.0 ng/mL; range, 5.0–1048.5 ng/mL; $P < 0.01$). No significant differences in age, sex, etiology or ALT level were seen among these three groups. Serum total bilirubin levels were significantly higher in non-survivors than in survivors, whereas prothrombin activities were significantly lower in non-survivors than in survivors. Median serum HGF levels were significantly higher in non-survivors (5.0 ng/mL; range, 1.60–9.94 ng/mL) than in survivors (0.5 ng/mL; range, 0.22–8.0 ng/mL; $P < 0.01$).

The ability to distinguish non-survivors from survivors was analyzed by generating ROC curves for sensitivity and specificity using different cut-off levels (Fig. 3, Table 3). For F/A ratio, a cut-off of 3.12 generated 87.5% sensitivity and 81.2% specificity. The odds ratio for the F/A ratio was 30.1. For serum HGF levels, a cut-off of 1.60 ng/mL generated 87.5% sensitivity and 94.1% specificity. The odds ratio for the serum HGF level was 110.0. The odds ratios for the F/A ratio and serum HGF level were higher than or approximately equivalent to those for bilirubin, prothrombin

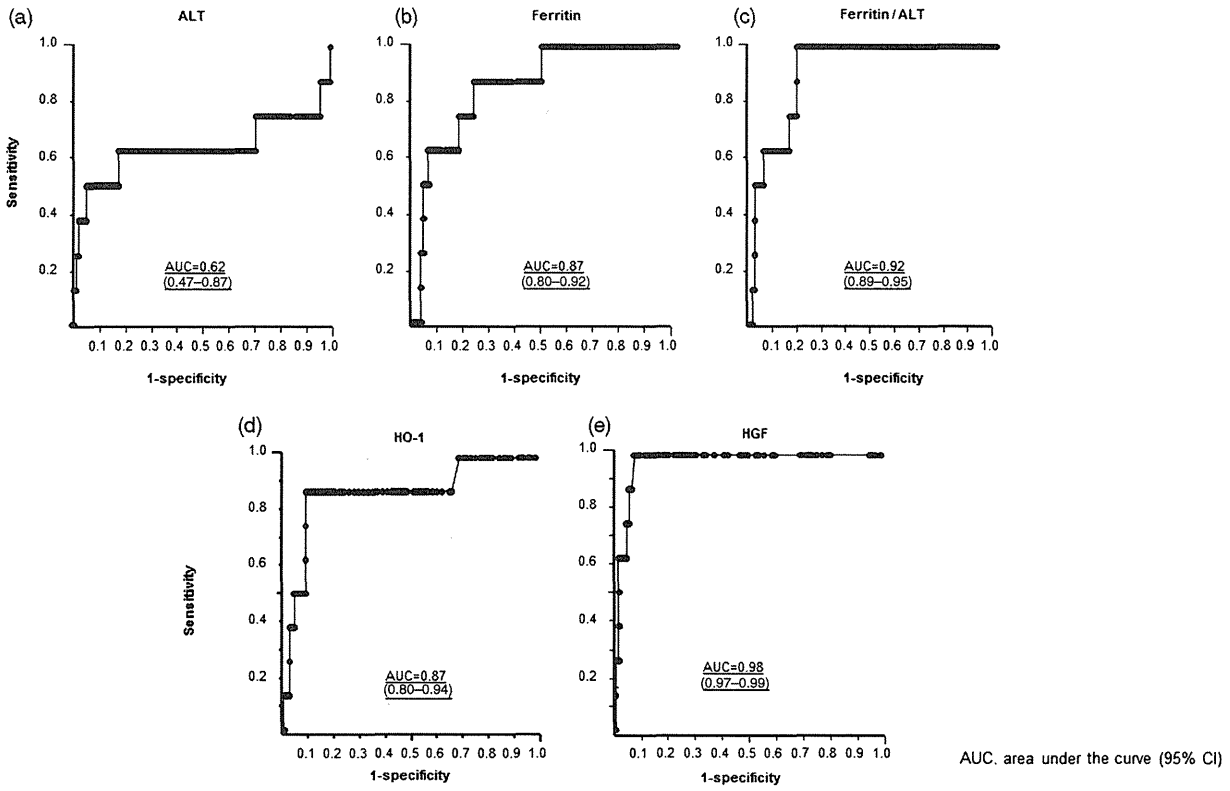

Figure 3 Receiver operating characteristics (ROC) curve for predicting fatal outcome in patients with acute liver failure. (a) Alanine aminotransferase (ALT) level on admission. (b) Serum ferritin level on admission. (c) Ferritin/ALT ratio on admission. (d) Serum HO-1 level on admission. (e) Serum hepatocyte growth factor (HGF) level on admission. AUC, area under the curve (95% confidence interval).

Table 3 Ability of ferritin/ALT ratio to distinguish non-survivors from survivors as described by ROC curves

	Cut off	Sensitivity	Specificity	PPV	NPV	Likelihood ratio	Odds ratio
ALT (IU/L)	2888	62.5%	74.4%	15.1%	96.2%	2.36	4.64
T. bil (mg/dL)	11.8	62.5%	80.6%	20.0%	96.5%	3.21	6.91
Cr (mg/dL)	0.9	37.5%	87.7%	17.6%	94.7%	2.81	3.90
PT (%)	33.8	57.1%	95.2%	28.5%	96.9%	6.0	12.6
Plt (*10^3/μL)	122	60.0%	85.9%	16.6%	97.8%	4.24	9.1
Ferritin (ng/mL)	4040	87.5%	77.4%	22.5%	98.7%	3.86	23.9
F/A ratio	3.12	87.5%	81.2%	25.9%	98.9%	4.63	30.1
HO-1 (ng/mL)	97.0	87.5%	92.5%	43.7%	99.0%	10.3	75.4
HGF (ng/mL)	1.60	87.5%	94.1%	53.3%	98.9%	14.7	110.0

ALT, alanine aminotransferase; F/A ratio, ferritin/alanine aminotransferase ratio; HGF, hepatocyte growth factor; HO-1, heme oxygenase 1; NPV, negative predictive value; PPV, positive predictive value.

time, platelet count and creatinine. AUCs for both F/A ratio and serum HGF levels were > 0.9.

Predicting prognosis using a combination of F/A ratio and serum HGF level

As both F/A ratio and serum HGF level were correlated with clinical outcomes for patients with FHF, we analyzed F/A ratio together with the serum HGF level. In the liver-transplanted patient and patients who died (black dots in Fig. 4), HGF level was ≥1.0 ng/mL and F/A ratio was ≥3.0. For predicting mortality, the combined parameter of F/A ratio ≥3.0 and HGF ≥1 ng/mL showed superior specificity and likelihood ratio (sensitivity, 100%; specificity, 94.3%; likelihood ratio, 17.7) than the single parameter of HGF ≥1.0 ng/mL (sensitivity, 100%; specificity, 81.1%; likelihood ratio, 5.3) (Table 4).