with HCV, and also affected the clinical outcome, including spontaneous clearance of HCV.[13–15]

We previously reported that the expression of suppressor of cytokine signaling 3 (SOCS3), which is related to insulin resistance, impairs the response to interferon treatment and might be a useful predictor of HCV clearance by interferon therapy.[16]

In this study, we examined the relationship among the expression of SOCS 3 in the liver, single nucleotide polymorphisms (SNPs) in the IL28B locus, and the outcome of interferon therapy.

METHODS

NEEDLE BIOPSIES OF the liver were obtained from 67 patients with positive HCV antibodies prior to interferon treatment at Nagasaki University Hospital and National Hospital Organization (NHO) Nagasaki Medical Center. Twenty of 67 cases were also examined in a previous study.[16] All patients with genotype 1b received weekly injections of PEG-IFN. The clinical data of the patients are summarized in Table 1. Liver biopsy was performed by needle puncture for diagnostic purposes. The diagnosis of each case was independently confirmed histologically by liver pathologists according to the Japanese chronic hepatitis classification criteria (New Inuyama classification). According to these criteria, mild activity was defined as A0 or A1, severe activity as A2 or A3, mild fibrosis as F0 or F1, and severe fibrosis as F2, F3, or F4. Fatty changes in >5% of all areas were defined as steatosis.

Table 1 Clinical backgrounds of the patients

Age	56.8 ± 9.3
Gender	Male : Female = 37:30
BMI (kg/m^2)	23.5 ± 2.9
Viral load (KIU/mL)	2320 ± 1519
White blood cell (/uL)	5074 ± 1713
Hemoglobin (mg/dL)	14.1 ± 1.3
Platelet (×10^3/uL)	167.3 ± 75.6
AST (IU/L)	77.1 ± 45.2
ALT (IU/L)	101.2 ± 56.3
γGTP (IU/L)	70.6 ± 65.5
HCV core 70 wild	40 cases
HCV core 91 wild	50 cases
Steatosis (>5%)	37 cases
A (0–1:2–3)	36:31
F (0–1:2–4)	22:45

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

All patients received PEG-IFN (Schering-Plough, Tokyo, Japan) + ribavirin (Schering-Plough, Tokyo, Japan) therapy for 48 weeks. The patients who were treated with a dose of PEG-IFN or ribavirin reduced by more than 20% were excluded from the study. PEG-IFN (1.5 μg/kg) was administered once per week, and the ribavirin dose was titrated according to body weight. A sustained virological response (SVR) was defined as undetectable HCV RNA at 6 months after the end of interferon treatment.

Of 38 patients who could not achieve an end-of-treatment response, 28 patients required a re-elevation of their viral loads regardless of the fact that the HCV-RNA levels were temporarily negative, and 10 patients did not achieve an HCV negative result during the entire treatment period.

SOCS3 immunohistochemistry

All tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin, and 4 μm thick serial sections were cut from each paraffin block. In the immunohistochemical study, an anti-SOCS3 antibody (dilution 1:100, Affinity BioRegents, Golden, CO, USA) was used for SOCS3. Immunohistochemistry was performed with the labeled streptavidin biotinylate antibody (LSAB) method and a commercially available kit (Histofine, SAB-PO(R); Nichirei Corporation, Tokyo, Japan). The area immunostained for SOCS 3 was divided according to the number of immunoreactive cells per unit area. Immunoreactive cases were classified as those with less than 30% of the hepatocellular cells stained (low SOCS3 group) and those with 30% or more of the cells stained (high SOCS3 group), because our previous study showed that staining of more than 30% of the area was a significant predictor of viral clearance.[16]

Genetic variation near the IL28B gene

Genotyping for replication was performed by use of the Invader assay or direct sequencing. In this study, genetic variation near the IL28B gene (rs8099917), which was previously reported to be a predictor of the virological response was investigated.[13]

Statistical analysis

The SPSS 9.0 for Windows statistical software program was used to assess correlations among multiple variables. When appropriate, clinical and laboratory data

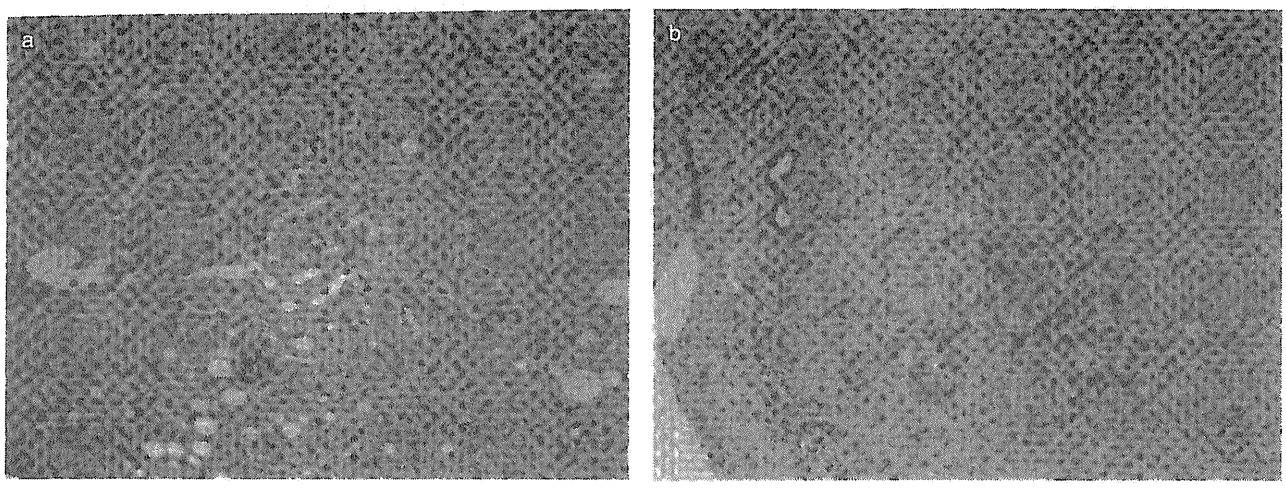

Figure 1 (a) This case showed less than 5% suppressor of cytokine signaling 3 (SOCS3) immunostained areas (low immunostaining). (b) This cases showed about 50% SOCS3 immunostaining areas (high immunostaining).

were compared with the Student's *t*-test or the Mann–Whitney test. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Immunostaining of SOCS3 in the liver (Figs 1,2)

IMMUNOSTAINING FOR SOCS3 was mainly seen in the periportal area. Less than 30% SOCS3 immunostained areas were found in 36 cases (54%) and areas with 30% or more immunostaining for SOCS3 were found in 31 cases (46%).

The frequency and distribution of the SOCS3 expression are shown in (Fig. 2)

Correlation between SOCS3 immunostaining and clinicopathlogical factors

A significant difference between low and high SOCS3 groups was found in age (low : high = 54.5 ± 9.8:59.5 ± 8.1, $P = 0.028$), the levels of platelets (low : high = 189.5 ± 90.0:141.6 ± 41.3, $P = 0.009$), aspartate aminotransferase (AST) (low : high = 94.5. ± 56.0:62.1 ± 33.5, $P = 0.003$), alanine aminotransferase; (ALT) (low : high = 85.8 ± 52.4:119.0. ± 56.3, $P = 0.015$), gamma-glutamyl transpeptidase (γGTP) (low : high = 48.8 ± 53.5:94.7 ± 70.6, $P = 0.004$). The incidence of steatosis (low : high = 33%: 81%, $P = 0.001$), severe activity (low : high = 27%: 67%, $P = 0.001$) and sever fibrosis (low : high = 52%: 84%, $P = 0.006$) was significantly higher in the SOCS3 high

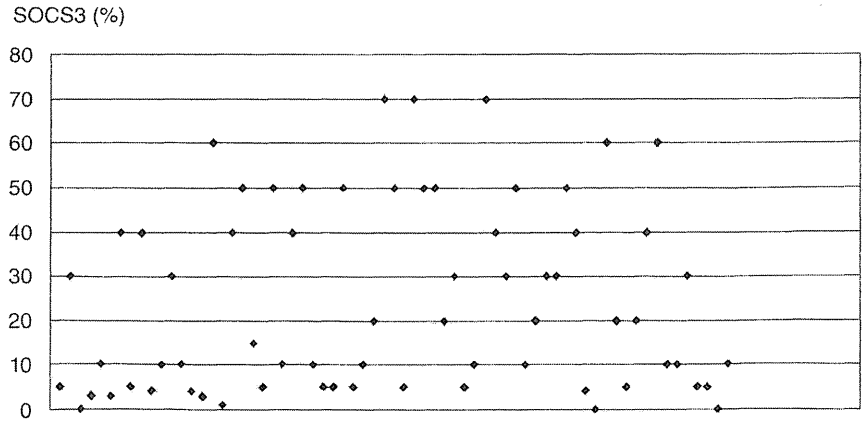

Figure 2 The distribution of the SOCS3 immunostaining area is shown.

Table 2 Comparison of the suppressor of cytokine signaling 3 (SOCS3) immunostaining groups

	SOCS3 high 31 cases	SOCS3 low 36cases	*P*-value
Age	59.5 ± 8.1	54.5 ± 9.8	0.028
Gender (male)	16 (53%)	21 (58%)	0.581
BMI (kg/m^2)	23.3 ± 2.2	23.6 ± 3.5	0.719
Viral load (KIU/mL)	2139 ± 1367	2475 ± 1950	0.427
White blood cell (/uL)	4935 ± 1386	5039 ± 1384	0.765
Hemoglobin (mg/dL)	14.1 ± 1.1	14.0 ± 1.3	0.570
Platelet (×10^3/uL)	141.6 ± 41.3	189.5 ± 90.0	0.009
AST (IU/L)	94.5. ± 56.0	62.1 ± 33.5	0.003
ALT (IU/L)	119.0 ± 56.3	85.8 ± 52.4	0.015
γGTP (IU/L)	94.7 ± 70.6	48.8 ± 53.5	0.004
Core 70 wild	17 (55%)	23 (63%)	0.451
Core 91 wild	23 (74%)	27 (75%)	0.939
Steatosis	25 (81%)	12 (33%)	0.001
Activity (severe)†	21 (67%)	10 (27%)	0.001
Fibrosis (severe)‡	26 (84%)	19 (52%)	0.006
IL28 TT rs8099917	22 (71%)	29 (80%)	0.358

†Severe activity was defined as A2 or A3.
‡Severe fibrosis was defined as F2, F3, or F4.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

immunostaining group than in the SOCS3 low immunostaining group. No significant difference was observed between the SOCS3 low and high groups in any of the other clinical factors (age, body mass index [BMI], viral load, white blood cell count, hemoglobin, substitution of the core 70, 91) (Table 2).

Comparison of SOCS3 expression and the genetic variation of IL28B gene

No significant difference in the genetic variation of the IL28 TT genotype was observed between the SOCS3 low and high immunostaining groups (low : high = 80%: 71%, $P = 0.250$).

Assessment of SOCS3 expression and genetic variation in IL28 as predictors of a sustained virological response

The age of patients in the non responder (NR) group was significantly higher than that in sustained virological response (SVR) group (SVR : NR = 52.3 ± 11.5: 59.6 ± 6.1, $P = 0.003$).

The incidence of the IL28 TT genotype was significantly lower, and that of SOCS3 high immunostaining group was significantly higher in the NR group than in the SVR group (Table 3).

As determined by a logistic regression analysis, the significant predictor of an SVR was high age (≥65 years old) (odds ratio 0.221 [0.120–0.966], $P = 0.045$), the IL28 TT genotype (odds ratio 5.422 [1.254–23.617], $P = 0.024$) and SOCS3 (high) (odds ratio 0.308 [0.104–0.948], $P = 0.040$) (Table 4).We found that two of nine (22%) patients with the IL28 TG genotype and SOCS3 high immunostaining showed a SVR, while one of seven (14%) patients with the IL28 TG genotype and SOCS3 low immunostaining, six of 22 (27%) patients with the IL28 TT genotype and SOCS3 high immunostaining, and 20 of 29 (69%) patients with the IL28 TG genotype and SOCS3 low immunostaining showed a SVR (Fig. 3).

DISCUSSION

RECENT IMPROVEMENTS IN the efficiency of antiviral therapy have led to approximately 50% of patients with HCV genotype 1 achieving sustained viral clearance.[1–5] However, some patients are refractory to interferon therapy. A recent study reported that the presence of genetic variation near the IL28B gene (rs8099917, rs1297860) can be used as a pretreatment predictor of virlogical response to a 48-week PEG-IFN plus combination therapy in patients with HCV geno-

Table 3 Factors associated with the response to peginterferon-α (PEG-IFN) and ribavirin

	SVR 29 cases	NR 38 cases	*P*-value
Age	52.8 ± 11.0	59.8 ± 6.4	0.002
Gender (male)	17 (58%)	20 (52%)	0.625
BMI (kg/m^2)	23.9 ± 3.1	22.9 ± 3.1	0.190
Viral load (KIU/mL)	2188 ± 1764	2420 ± 1689	0.587
White blood cell (/uL)	4816 ± 1427	5225 ± 1287	0.242
Hemoglobin (mg/dL)	14.1 ± 1.1	14.0 ± 1.3	0.626
Platelet (×10^3/uL)	176.5 ± 52.8	160.3 ± 89.2	0.350
AST (IU/L)	75.5 ± 36.1	78.3 ± 51.5	0.795
ALT (IU/L)	108.9 ± 56.8	95.3 ± 56.0	0.333
γGTP (IU/L)	63.9 ± 61.9	75.7 ± 68.6	0.464
Core 70 wild	20 (69%)	20 (53%)	0.176
Core 91 wild	21 (72%)	29 (71%)	0.173
IL28 TT rs8099917	26 (90%)	25 (65%)	0.022
steatosis	14 (47%)	23 (61%)	0.452
Activity (severe)†	10 (34%)	21 (64%)	0.091
Fibrosis (severe)‡	18 (62%)	27 (71%)	0.437
SOCS3 (Positive)	8 (27%)	23 (61%)	0.015

†Severe activity was defined as A2 or A3.
‡Severe fibrosis was defined as F2, F3, or F4.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus; NR, non responder; SOCS3, suppressor of cytokine signal 3; SVR, sustained virological response.

type 1.[13–15] We previously reported that SOCS3 was a factor associated with the response to PEG-IFN treatment.[16] We compared these factors and clarified their usefulness as predictors of PEG-IFN plus combination therapy.

In the laboratory data from our patients, a significant difference between the groups with weak and strong SOCS3 staining was found in the level of AST, ALT, and platelets. These laboratory data suggested that the SOCS3 immunostained area was significantly associated with the presence of inflammation and the fibrosis stage. Indeed, in a pathological study, the inflammation and fibrosis stage were significantly different between the low and high SOCS3 immunostaining groups. This finding was consistent with our previous study that showed that the SOCS3 immunostained area was influenced by inflammation and the fibrosis stage.[16]

Table 4 Results of a multilogistic regression analysis

	Odds ratio	*P*-value
Age (>65 years)	0.221 (0.120~0.966)	0.045
IL28 TT	5.422 (1.254~23.617)	0.024
SOCS3 (low)	0.308 (0.104~0.948)	0.040

SOCS3, suppressor of cytokine signal 3.

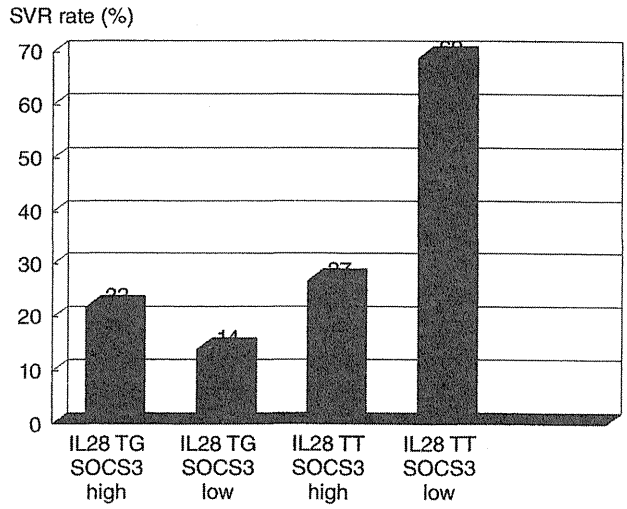

Figure 3 A total of 12.5% of patients with IL28 TG and suppressor of cytokine signaling 3 (SOCS3) high immunostaining showed a sustained virological response (SVR), 20% of patients with IL28 TG and SOCS3 low immunostaining, 31% of patients with IL28 TT and SOCS3 high immunostaining, and 68% of patients with IL28 TG and SOCS3 low immunostaining showed a SVR.

Moreover, a significant difference between the low and high SOCS3 groups was also found in the level of γGTP. Several previous reports showed that the level of γGTP was correlated with steatosis in the liver.[7,17] In this study, the presence of steatosis also was significantly different in the low and high SOCS3 immunostaining groups. Together with our results, these results demonstrated that the SOCS3 immunostained area in the liver was associated with obesity, insulin resistance, and hepatic steatosis.[18,19]

Although recent reports showed that genetic variation of IL28B was also associated with liver inflammation and fibrosis,[20] this was not associated with the SOCS3 immunostained area in the present study. The SOCS3 proteins are known for their role as negative regulators and inhibitors of Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling, where they mediate a classical negative feedback loop in the IFN-α/β receptor signaling pathway.[21,22] The mechanism that leads to the association between genetic variation of IL28B and the effect of interferon therapy is clear, because it has been demonstrated that IL28B inhibits hepatitis C virus replication through the JAK-STAT pathway.[23] Taken together, both the SOCS3 immunostained area and IL28B polymorphisms were associated with the JAK-STAT pathway, but the different factors might interfere with JAK-STAT signaling in different ways.

The NR rate to combination PEG-IFN plus ribavirin therapy in patients with the non-TT genotype was 10–20%. The value of NR for the prediction of the genetic variation of IL28B was therefore very high. On the other hand, the SVR rate in patients with the TG genotype was about 50%. The value of SVR prediction based only on the genetic variation of IL28B was therefore not as strong for this genotype.

The substitution of core amino acids was also reported to be a predictive factor for the response to interferon therapy and was significantly associated with the genetic variation of IL28B.[24] On the other hand, the SOCS3 immunostained area was independent of both of these factors. Thus, we suggested that using a combination of the SOCS3 immunostained area with the IL28B genotype can provide the best prediction of the response to PEG-IFN plus ribavirin therapy.

Indeed, in TT genotype patients, the SVR rate in the SOCS3 weak group was about 70%, and NVR rate in the SOCS3 low immunostained group was 27%. If a liver biopsy was performed, immunostaining for SOCS3 was easy, and provided a useful predictor of the response to interferon therapy.

Our study has some limitations. Our sample size was too small. Further large-scale studies are necessary to confirm the present results and to provide a better understanding of the interactions between the SOCS3 immunostained area and the genetic variation of IL28B.

In conclusion, a combination of the SOCS3 immunostained area in the liver and the assessment of the genetic variation of IL28B seem to be good predictors of the response to PEG-IFN plus ribavirin therapy.

REFERENCES

1 Mangia A, Ricci GL, Persico M *et al.* A randomized controlled trial of pegylated interferon alpha-2a (40 KD) or interferon alpha-2a plus ribavirin and amantadine vs interferon alpha-2a and ribavirin in treatment-naive patients with chronic hepatitis C. *J Viral Hepat* 2005; **12**: 292–9.

2 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; **46**: 403–10.

3 Wedemeyer H, Wiegand J, Cornberg M, Manns MP. Polyethylene glycol-interferon: current status in hepatitis C virus therapy. *J Gastroenterol Hepatol* 2002; **17** (Suppl 3): S344–50.

4 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339**: 1493–9.

5 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352**: 1426–32.

6 Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**: 639–44.

7 Yaginuma R, Ikejima K, Okumura K *et al.* Hepatic steatosis is a predictor of poor response to interferon alpha-2b and ribavirin combination therapy in Japanese patients with chronic hepatitis C. *Hepatol Res* 2006; **35**: 19–25.

8 Zografos TA, Liaskos C, Rigopoulou EI *et al.* Adiponectin: a new independent predictor of liver steatosis and response to IFN-alpha treatment in chronic hepatitis C. *Am J Gastroenterol* 2008; **103**: 605–14.

9 Yamada G, Iino S, Okuno T *et al.* Virological response in patients with hepatitis C virus genotype 1b and a high viral load: impact of peginterferon-alpha-2a plus ribavirin dose reductions and host-related factors. *Clin Drug Investig* 2008; **28**: 9–16.

10 Iwasaki Y, Ikeda H, Araki Y *et al.* Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; **43**: 54–63.
11 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; **334**: 77–81.
12 Akuta N, Suzuki F, Hirakawa M *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 2a high viral load and virological response to interferon-ribavirin combination therapy. *Intervirology* 2009; **52**: 301–9.
13 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105–9.
14 Thomas DL, Thio CL, Martin MP *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798–801.
15 Suppiah V, Moldovan M, Ahlenstiel G *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100–4.
16 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* 2009; **39**: 850–5.
17 Ikai E, Ishizaki M, Suzuki Y, Ishida M, Noborizaka Y, Yamada Y. Association between hepatic steatosis, insulin resistance and hyperinsulinaemia as related to hypertension in alcohol consumers and obese people. *J Hum Hypertens* 1995; **9**: 101–5.
18 Walsh MJ, Jonsson JR, Richardson MM *et al.* Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. *Gut* 2006; **55**: 529–35.
19 Ueki K, Kondo T, Tseng YH, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl Acad Sci U.S.A.* 2004; **101**: 10422–7.
20 Abe H, Ochi H, Maekawa T *et al.* Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol* 2010; **53**: 439–43.
21 Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* 2002; **2**: 410–6.
22 Yasukawa H, Sasaki A, Yoshimura A. Negative regulation of cytokine signaling pathways. *Annu Rev Immunol* 2000; **18**: 143–64.
23 Zhang L, Jilg N, Shao RX *et al.* IL28B inhibits Hepatitis C virus replication through the JAK-STAT pathway. *J Hepatol* 2011; **55**: 289–98.
24 Akuta N, Suzuki F, Hirakawa M *et al.* Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* 2010; **52**: 421–9.

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Original Article

Data mining reveals complex interactions of risk factors and clinical feature profiling associated with the staging of non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma

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Aim: Non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma (NBNC-HCC) is often detected at an advanced stage, and the pathology associated with the staging of NBNC-HCC remains unclear. Data mining is a set of statistical techniques which uncovers interactions and meaningful patterns of factors from a large data collection. The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

Methods: A database was created from 663 patients with NBNC-HCC at 20 institutions. The Milan criteria were used as staging of HCC. Complex associations of variables and clinical feature profiling with the Milan criteria were analyzed by graphical modeling and decision tree algorithm methods, respectively.

Results: Graphical modeling identified six factors independently associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum aspartate aminotransferase (AST); alanine aminotransferase (ALT); α-fetoprotein (AFP); and des-γ-carboxy prothrombin (DCP) levels. The decision trees were created with five variables to classify six groups of patients. Sixty-nine percent of the patients were

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within the Milan criteria, when patients showed an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis and an AST level of less than 93 IU/mL. On the other hand, 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and ALT level of 20 IU/mL or more.

Conclusion: Data mining disclosed complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC.

Key words: data mining, disease progression, hepatoma, non-viral hepatitis, tumor marker

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common cancer and the third most common cause of cancer-related deaths worldwide.[1–3] Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is a risk factor for HCC. Recent developments in the management of patients with viral hepatitis have resulted in early detection of HCC and improvement of prognosis.[4–8]

The number of patients with non-HBV/non-HCV-related HCC (NBNC-HCC) has been increasing, and NBNC-HCC now accounts for 12–16% of all the HCC cases in Japan.[8,9] A variety of factors are involved in the development and progression of this cancer including age, sex, alcoholic liver disease and diabetes mellitus.[10–12] Therefore, neither early detection nor improved prognosis has been achieved in NBNC-HCC.[6] Radical treatment is applicable to patients with NBNC-HCC who meet the Milan criteria;[13] however, this cancer is often detected at an advanced stage. For earlier detection, it is important to understand the complex interactions of the risk factors and clinical feature profiling associated with the Milan criteria, a staging system for NBNC-HCC.

Data mining, a set of statistical techniques, uncovers meaningful patterns and interactions of variables from a large data collection even when there is no a priori hypothesis imposed.[13] Graphical modeling is an exploratory multivariate analysis of data mining that reveals complex associations between variables.[14] This analysis assumes that the response variable is influenced by multiple factors.[15] Therefore, different from results of univariate analysis, an association between a risk factor and an outcome variable may disappear or appear because of the effects of another set of variables known as "confounding factors".[16,17] Furthermore, its findings are visualized as a graph, which provides an idea of how variables interact and denotes the conditional independence structure between random variables.[15] Therefore, graphical modeling is now identified as a new approach to model clinical data.[18]

Decision tree making is another exploratory technique of data mining that represents a series of rules for classification by identifying priorities.[19–21] It is an explicit, quantitative and systematic approach to decision-making under conditions of uncertainty and allows clinicians to choose an option that maximizes the net benefit to the patient.[22] Recently, decision trees were used to reveal the clinical feature profiling for staging of pancreatic cancer[23] and ovarian cancer.[24] However, decision trees have never been applied to identify the clinical feature profiling associated with the staging of NBNC-HCC.

The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

METHODS

Patient database

BETWEEN 1995 AND 2006, a total of 10 133 patients were diagnosed with HCC at 23 institutions located in Kyushu, a high morbidity area of HCC in Japan. Among them, 1363 patients were diagnosed with NBNC-HCC according to the negative results of both serum hepatitis B surface antigen and serum anti-HCV antibody or HCV RNA.

In order to examine the clinical variables associated with the staging of NBNC-HCC, a database of 663 patients with NBNC-HCC at 20 institutions was created on the basis of the following variables: diagnostic year of HCC; age; sex; family history of liver disease; past history of blood transfusion; alcohol intake; diagnosis of liver cirrhosis; diagnosis of liver disease; diagnosis of diabetes mellitus; serum aspartate aminotransferase (AST) level; serum alanine aminotransferase (ALT) level; serum α-fetoprotein (AFP) level; serum des-γ-carboxy prothrombin (DCP) level; size of HCC; and number of HCC.

For practical use, alcohol intake, serum AFP level and serum DCP level were categorized as follows. Alcohol intake: none; 60 g/day or less; 60–100 g/day; or more than 100 g/day. AFP level: 20 ng/mL or less; 20–200 ng/mL; or more than 200 ng/mL. DCP level: 40 mAU/mL or less; 40–100 mAU/mL; or more than 100 mAU/mL.

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.

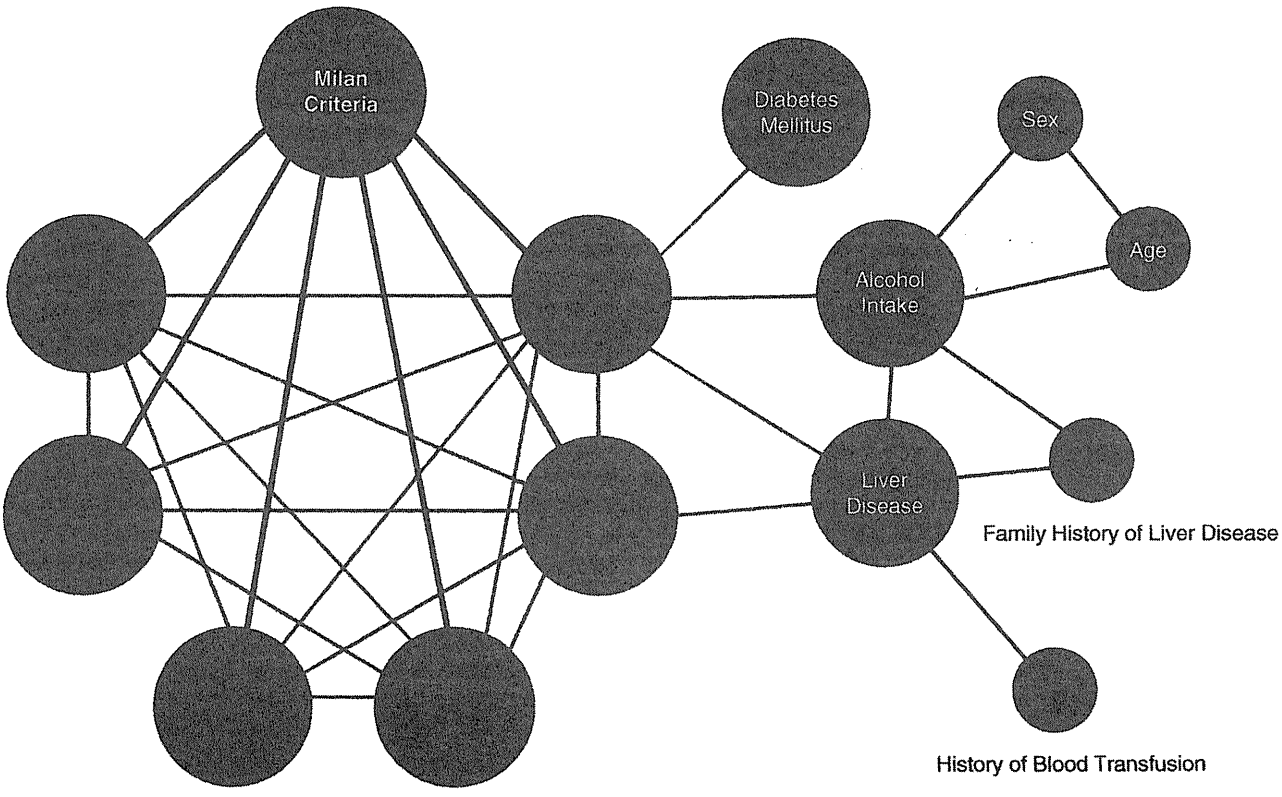

Figure 1 Graphical modeling of the interactions of the risk factors associated with the Milan criteria. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

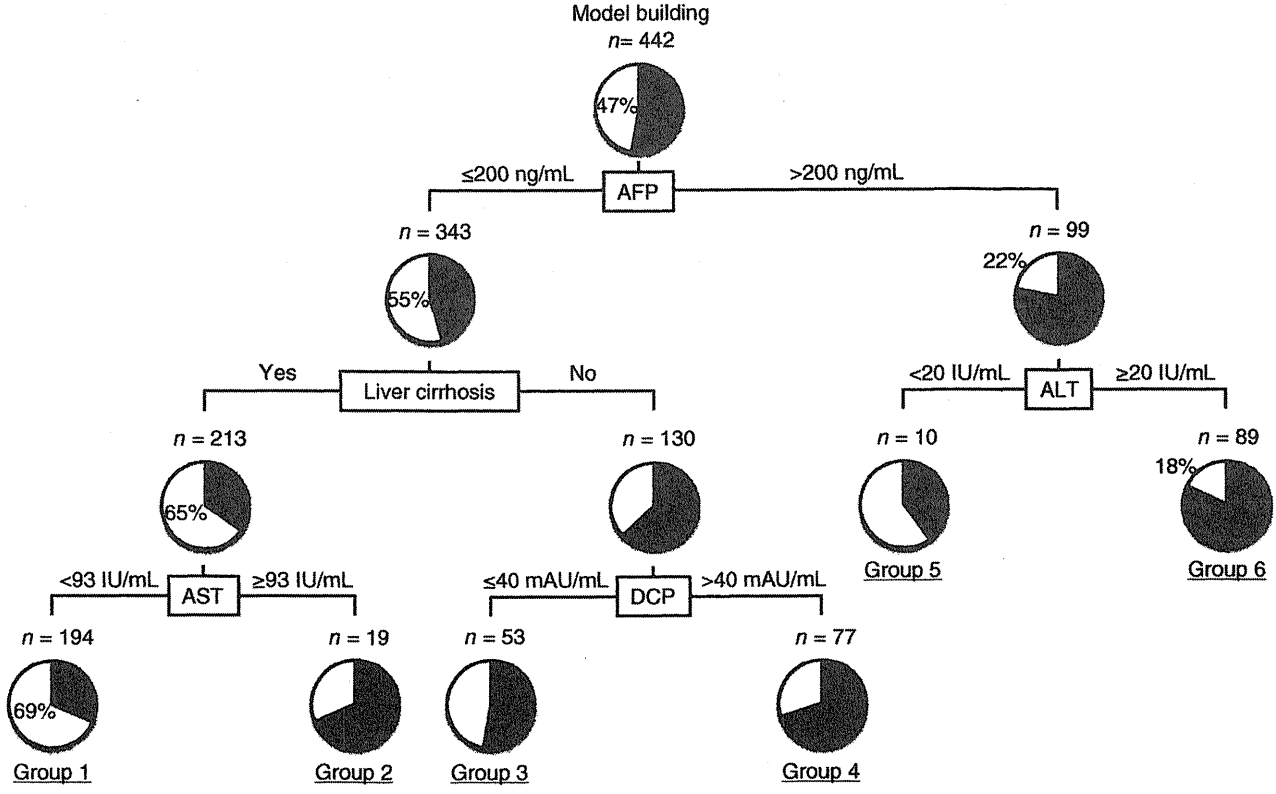

Figure 2 Decision tree algorithm of the variables associated with the Milan criteria. The patients were classified according to the indicated cut-off values of the variables. The pie graphs indicate the percentage of patients with HCC within (white)/beyond the Milan criteria in each group. AFP, α-fetoprotein; 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.

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HEPATOLOGY

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

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

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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.

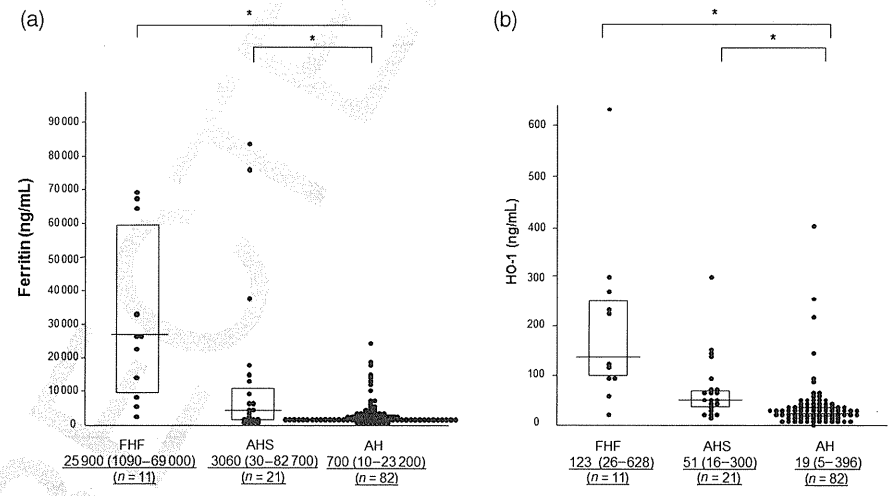

Figure 1 (a) Serum level of ferritin in subjects on admission. (b) Serum level of heme oxygenase (HO)-1 in subjects on admission. Box plots represent median and 25–75th percentiles. Upper and lower lines show minimum and maximum values, respectively. *$P < 0.01$, as determined by the Kruskal–Wallis test.

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).

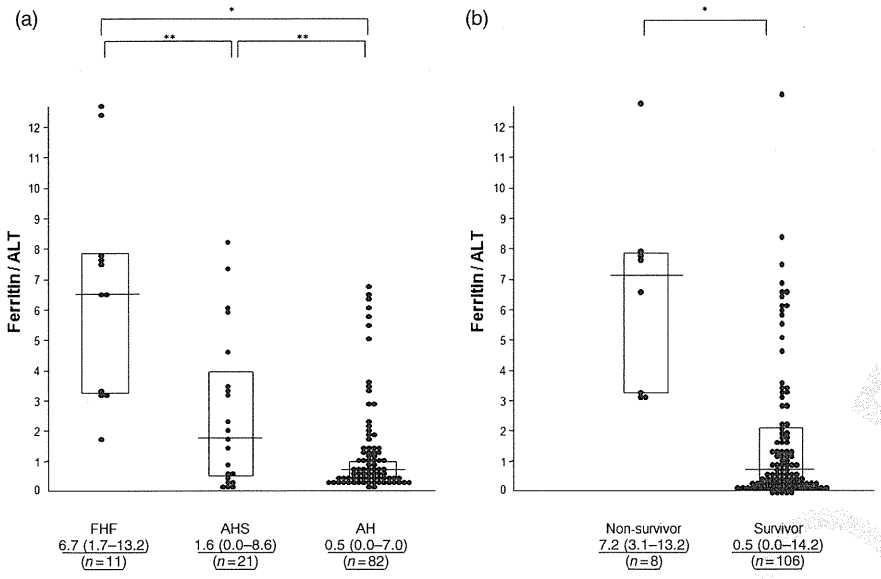

Figure 2 (a) Ferritin/alanine aminotransferase (ALT) ratio in subjects on admission. (b) Ferritin/ALT ratio in non-survivors and survivors on admission. $*P < 0.01$, $**P < 0.05$, as determined by the non-paired Mann–Whitney *U*-test.

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 (%)	33.1 (7.3–120.8)	76.9 (15.2–47.7)	< 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)	4.68 (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

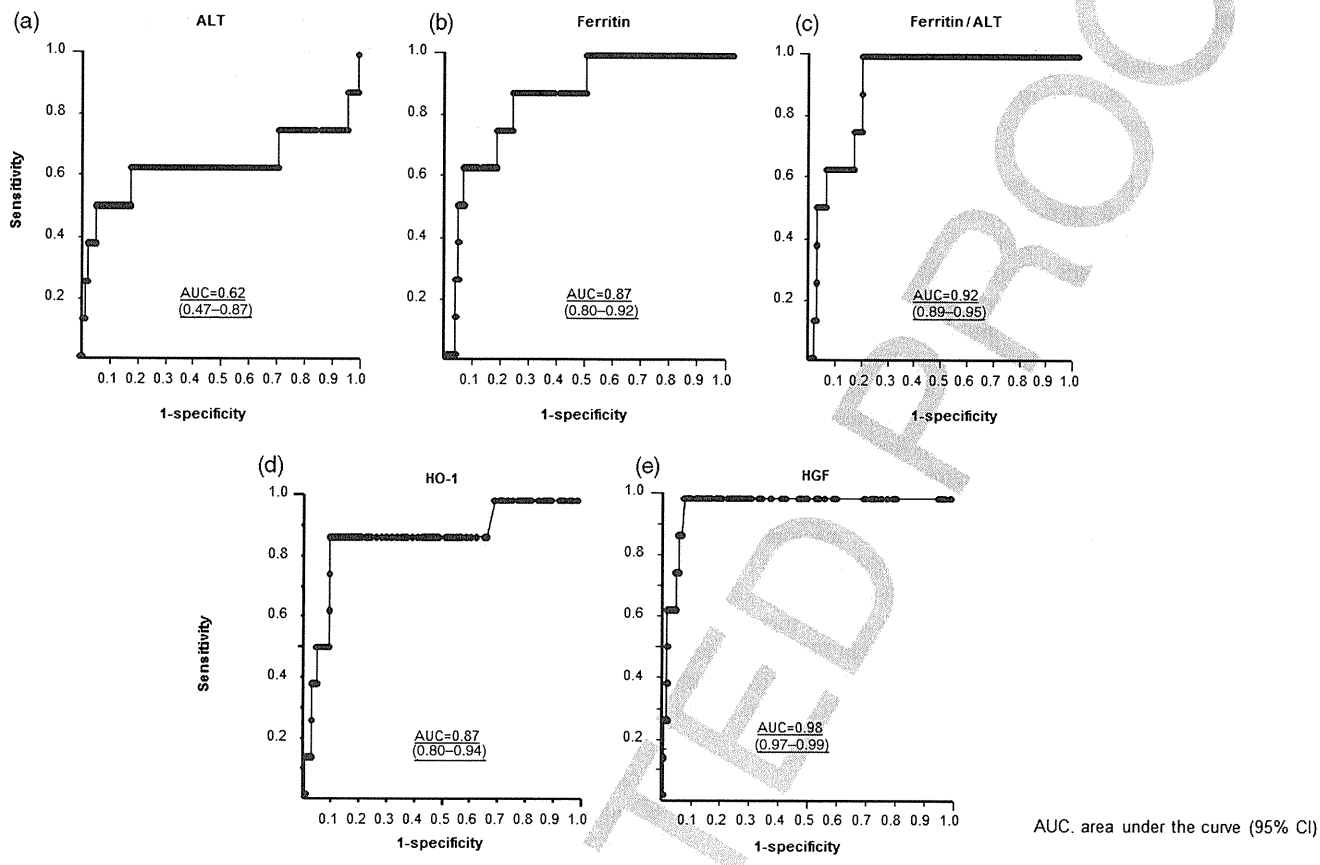

Figure 3 Receiver operating characteristics (ROC) curve for predicting fatal outcome in patients with acute liver failure. (a) Alanine aminotransferase (ALT) level on admission. (b) Serum ferritin level on admission. (c) Ferritin/ALT ratio on admission. (d) Serum HO-1 level on admission. (e) Serum hepatocyte 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 γ1.0 ng/mL and F/A ratio was γ3.0. For predicting mortality, the combined parameter of F/A ratio γ3.0 and HGF γ1 ng/mL showed superior specificity and likelihood ratio (sensitivity, 100%; specificity, 94.3%; likelihood ratio, 17.7) than the single parameter of HGF γ1.0 ng/mL (sensitivity, 100%; specificity, 81.1%; likelihood ratio, 5.3) (Table 4).

Discussion

The decision whether to perform liver transplantation for FHF patients is critical, but also often difficult.[19] Marker molecules in more robust prognostic models may thus have important clinical value.

Several reports have noted that macrophage-related factors may play a dominant role in determining the severity of disease in patients with FHF.[4–6,23] Expression of osteopontin is high in Kupffer cells and hepatic macrophages in rat liver after carbon tetrachloride intoxication.[25] Expression of CD163 in liver tissue is higher in patients with acute viral hepatitis than in those with chronic viral hepatitis.[26,27] Serum levels of IL-10 and tumor necrosis factor-α are high in patients with FHF and correlate with risk of fatal outcomes.[4] Serum levels of sCD163, a lineage-specific scavenger receptor regulated by IL-10 that is involved in several anti-inflammatory functions of the immune system, are significantly higher in patients with FHF compared to patients with AH[26] and again correlate with fatal outcomes.[28] *In vitro*, macrophages can take up different phenotypes dependent on the cytokine environment,[29] as reflected *in vivo* by pro- and anti-inflammatory activation states balancing the immune response. Taken together, the high levels of macrophage activation markers in FHF may represent an anti-inflammatory imbalance, particularly in patients with poor disease outcomes. The present results indicate that serum levels of ferritin and HO-1, both of which originate from activated macrophages, and are highly increased in acute liver injury.

As ferritin synthesis is stimulated by Fe2+, which is generated by HO-1-mediated heme degradation, hyperferritinemia might be caused by high HO-1 activity, irrespective of the underlying disease. Induction of HO-1 by extracellular heme has been shown to increase the free iron pool relevant for subsequent sequestration into ferritin.[14]

HO-1, an inducible heme-degrading enzyme converting heme into CO, Fe2+, and biliverdin, is a 32-kD heat-shock protein. HO-1 is expressed by macrophages and endothelial cells in response to various noxious stresses, and plays an important role against oxidative injuries.[10–12] Recent studies have shown that in MAS such as HPS and AOSD, serum HO-1 levels correlate closely with serum ferritin levels. Serum HO-1 levels could thus prove useful in differential diagnosis of hyperferritinemia and perhaps also in monitoring disease activity.[14] Although HO-1 expression was markedly increased at both transcriptional and protein levels in hepatocytes with a rat model of carbon tetrachloride-induced acute liver injury,[30] an increase in free heme concentration may upregulate HO-1 gene expression in patients with acute liver injury. In the present study, serum levels of ferritin and HO-1 were significantly higher in FHF and AHS than in AH, suggesting that activated macrophages may play a role in progression to FHF.

Serum ferritin levels are increased not only by release from hepatocytes as a result of liver damage, but also from activated macrophages.[23,24,31] Distinguishing the origin of ferritin between activated macrophages and liver cell cytolysis may be difficult. A recent report demonstrated a high ferritin level with a low percentage of glycosylated ferritin in patients with MAS, such as HPS and AOSD.[32,33] However, assaying glycosylated ferritin is not easy. We therefore determined the F/A ratio to reflect ferritin released from activated macrophages, revealing a significant difference in F/A ratio between FHF and AHS.

In clinical situations, assessing whether patients presenting with features of acute liver damage are likely to recover after an acute attack of hepatitis or will eventually develop FHF is very important. The present study found that serum levels of HO-1 and ferritin, both of which are macrophage-activation markers, were high in patients with FHF and AHS. These results suggest that activation of macrophages occurs in FHF and AHS, and that inflammatory cytokines can interact in the initiation and progression of liver cell damage. HO-1 and ferritin may be produced directly by activated macrophages in the liver of FHF patients, as macrophages play a dominant role in the pathogenesis of severe inflammation in FHF.

F/A ratio could reflect the amount of ferritin released from activated macrophages, and was significantly high in non-surviving patients with FHF in this study. In addition, the combi-

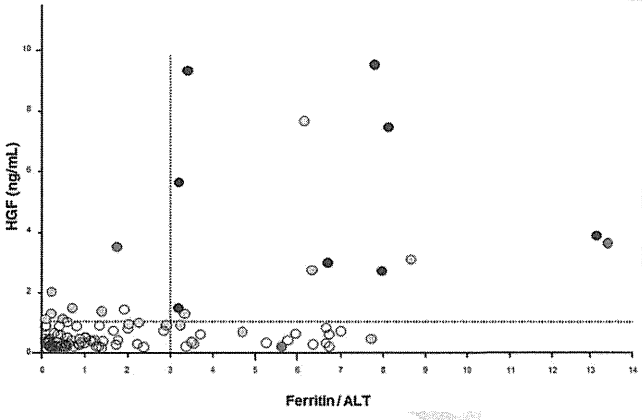

Figure 4 Ferritin/alanine aminotransferase (ALT) ratio with serum hepatocyte growth factor (HGF) level related to clinical outcomes. Horizontal dotted line indicates HGF at 1.60 ng/mL, as determined on the basis of receiver operating characteristics (ROC) curve. Vertical dotted line indicates an arbitrary cutoff value of 3.12 ng/mL for HO-1. ●, fulminant hepatic failure (FHF) non-survivor or transplantation; ●, FHF survivor; ◎, severe-form acute hepatitis (AHS); ○, acute hepatitis (AH).

Table 4 Prognostic prediction by combining HGF and F/A ratio

	Non-survivors	Survivors	Total	Sensitivity	Specificity	Likelihood ratio
HGF ≧ 1 ng/mL	8	20	28	100% (8/8)	81.1% (86/106)	5.3
HGF < 1 ng/mL	0	86	86			
HGF ≧ 1 ng/mL and F/A ratio ≧ 3	8	6	14	100% (8/8)	94.3% (100/106)	17.7
HGF < 1 ng/mL or F/A ratio < 3	0	100	100			

F/A ratio, ferritin/ALT ratio; HGF, hepatocyte growth factor.