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Efficacy and Anticarcinogenic Activity of Ribavirin Combination Therapy for Hepatitis C Virus-Related Compensated Cirrhosis

Norio Akuta[a] Fumitaka Suzuki[a] Yuya Seko[a] Yusuke Kawamura[a] Hitomi Sezaki[a] Yoshiyuki Suzuki[a] Tetsuya Hosaka[a] Masahiro Kobayashi[a] Mariko Kobayashi[b] Satoshi Saitoh[a] Yasuji Arase[a] Kenji Ikeda[a] Hiromitsu Kumada[a]

[a]Department of Hepatology, Toranomon Hospital, and Okinaka Memorial Institute for Medical Research, and [b]Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan

Key Words
Hepatitis C virus · Interferon · Ribavirin · Hepatocellular carcinoma · Cirrhosis · Biochemical response

Abstract
Objective: Anticarcinogenic activity of ribavirin combination therapy for hepatitis C virus (HCV)-related compensated cirrhosis is still unclear. ***Methods:*** In study 1, in 157 consecutive patients with HCV-related compensated cirrhosis, treatment efficacy with interferon plus ribavirin therapy was evaluated for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b. In study 2, in 185 consecutive patients with HCV-related compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy, hepatocarcinogenesis rates were evaluated according to the additional treatment, and they were classified into three groups: no treatment, interferon monotherapy, and ribavirin combination therapy. ***Results:*** In study 1, in HCV-1b, rates of sustained virological response and sustained biochemical response were 21 and 56%, respectively. In HCV-2a/2b, rates of sustained virological response and sustained biochemical response were 70 and 78%, respectively. In HCV-1b, sustained biochemical response rates were significantly higher than those of sustained virological response. In study 2, the hepatocarcinogenesis rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy and no treatment, respectively. ***Conclusion:*** Ribavirin combination therapy for HCV-related compensated cirrhosis reduces the risk of hepatocarcinogenesis in comparison with interferon monotherapy, and higher rates of sustained biochemical response might be associated with lower hepatocarcinogenesis rates.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–5]. The life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of hepatocellular carcinoma during the clinical course [3]. Because an effective and curative therapy for hepatocellular carcinoma remains

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Norio Akuta, MD
Department of Hepatology, Toranomon Hospital
2-2-2 Toranomon, Minato-ku
Tokyo 105-0001 (Japan)
E-Mail akuta-gi@umin.ac.jp

Table 1. Profile and laboratory data at the start of ribavirin combination therapy in 157 patients with HCV-related compensated cirrhosis (study 1)

Demographic data	
Patients, n	157[1]
Sex (male/female), n	105/52
Age, years	58 (34–74)
Laboratory data	
Serum aspartate aminotransferase, IU/l	69 (7–235)
Serum alanine aminotransferase, IU/l	70 (14–585)
Leukocytes, /mm^3	4,100 (1,600–8,800)
Hemoglobin, g/dl	14.0 (9.4–17.6)
Platelet count, $\times 10^4$/mm^3	11.3 (6.1–32.2)
HCV genotype (1b/2a/2b), n	120/27/10
Levels of viremia, log IU/ml	6.1 (3.9–7.5)
Treatment	
Past history of interferon-based therapy, n	95 (60.5%)
PEG-IFNα-2b/IFNα-2b, n	110/47
Ribavirin dose, mg/kg	10.7 (2.7–15.1)
Duration of treatment, weeks	
Genotype 1b	48 (1–48)
Genotype 2a or 2b	24 (5–24)

Unless otherwise indicated, values represent median (range).

[1] 24 of the 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2. They showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks) and were treated additionally with ribavirin combination therapy (≥24 weeks).

limited at best, primary prevention of hepatocellular carcinoma in patients with chronic liver disease is of great importance at present.

Treatment of HCV-chronic hepatitis with interferon can induce viral clearance and marked biochemical and histological improvement [6, 7]. Furthermore, previous studies showed that interferon monotherapy reduced the risk of hepatocellular carcinoma [8–10]. However, an extended analysis of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) cohort recently showed that long-term peginterferon (PEG-IFN) monotherapy could not reduce the incidence of hepatocellular carcinoma among patients with advanced hepatitis C who did not achieve sustained virological response, and patients with cirrhosis who received PEG-IFN monotherapy had a lower risk of hepatocellular carcinoma than controls [11]. Thus, it is controversial whether interferon monotherapy for patients with liver cirrhosis might reduce hepatocarcinogensis. Furthermore, it is still unclear whether ribavirin combination therapy for patients with liver cirrhosis might reduce the risk of hepatocellular carcinoma, and there are also no reports on whether ribavirin combination therapy could reduce the risk in comparison with interferon monotherapy.

The present study investigated the efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis, especially in comparison with interferon monotherapy.

Materials and Methods

Study Population

Two retrospective cohort studies were performed to investigate treatment efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis.

In the study 1 cohort, 157 consecutive patients of HCV-related compensated cirrhosis were recruited into the study protocol of interferon (PEG-IFNα-2b or IFNα-2b) plus ribavirin combination therapy for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b, from 2001 to 2010 at Toranomon Hospital. In this retrospective study the rates of sustained virological response [HCV-RNA negativity at 24 weeks after the completion of therapy based on the COBAS TaqMan HCV test (Roche Diagnostics)] were evaluated as well as sustained biochemical response [normal level of serum alanine aminotransferase at 24 weeks after the completion of therapy (6–50 IU/l)]. Treatment efficacy was evaluated by intention-to-treat (ITT) analysis classified as treatment failure in patients who could not complete the treatment regimen and per protocol (PP) analysis. Table 1 summarizes the profiles and data of the 157 patients at the commencement of combination therapy with interferon plus ribavirin in study 1. They included 105 men and 52 women aged 34–74 years (median 58 years). 110 (70.1%) patients received PEG-IFNα-2b plus ribavirin, and the remaining 47 (29.9%) patients received IFNα-2b plus ribavirin. They received PEG-IFNα-2b at a median dose of 1.3 μg/kg (range 0.5–1.9 μg/kg) subcutaneously each week or IFNα-2b at a median dose of 6 million units (range 3–6 million units) intramuscularly each day (7 times per week for the initial 2 weeks followed by 3 times per week). They also received oral ribavirin at a median dose of 10.7 mg/kg (range 2.7–15.1 mg/kg) daily. In 56 of the 157 (35.7%) patients, the dose of ribavirin was reduced during treatment due to a fall in hemoglobin concentration. The median total duration of treatment in 120 patients of HCV-1b was 48 weeks (range 1–48 weeks), and that in 37 patients of genotype 2a or 2b was 24 weeks (range 5–24 weeks).

In the study 2 cohort (fig. 1), 185 consecutive patients of HCV-related compensated cirrhosis, who showed no sustained virological response following at the first course of interferon monotherapy (≥24 weeks) from 1987 to 2010 at Toranomon Hospital, were recruited. Hepatocarcinogenesis rates were evaluated according to the additional treatment (second course of treatment), and were classified into three groups: no treatment (106 patients), interferon monotherapy (≥24 weeks; 55 patients), and ribavirin combination therapy (≥24 weeks; 24 patients). 106 patients without treatment did not receive the additional treatment because of concerns about adverse effects, lack of time for treatment, physician recommendation based on the appearance of depression and car-

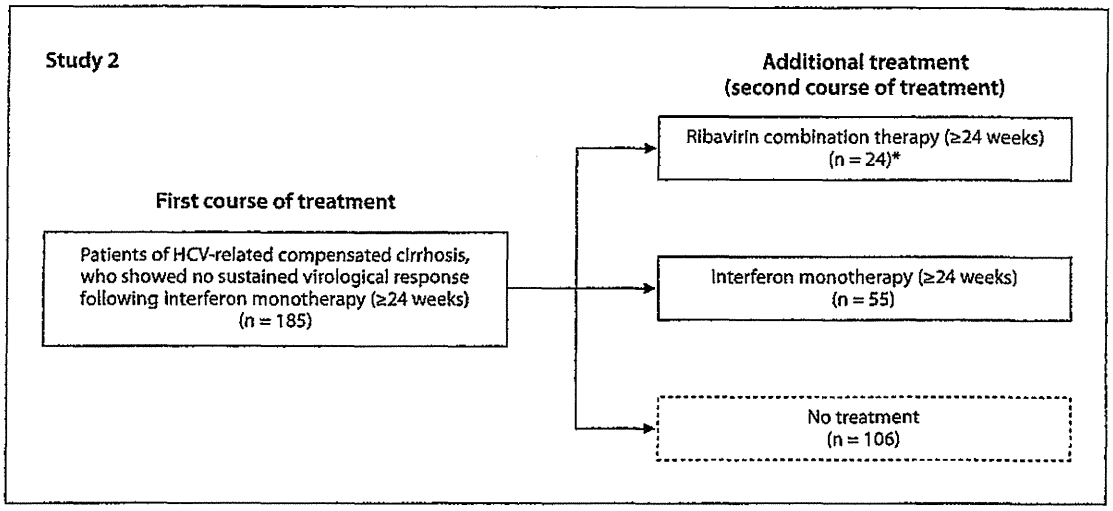

Fig. 1. For study 2, 185 patients with HCV-related compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks), were recruited. Hepatocarcinogenesis rates were evaluated according to the additional treatment (second course of treatment), and patients were classified into three groups: no treatment, interferon monotherapy (≥24 weeks), and ribavirin combination therapy (≥24 weeks). * 24 of 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2.

diopulmonary disease during and after the first course of interferon monotherapy or the lower levels of serum alanine aminotransferase. The median follow-up time, from the end of the first course of interferon monotherapy until the last visit, was 6.4 years (range 0.0–21.0 years). 24 of the 157 patients in study 1 were also included in study 2; they showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks) and were treated additionally with ribavirin combination therapy (≥24 weeks).

At the additional treatment of interferon monotherapy, 43 patients (78.2%) received IFNα alone, and the remaining 12 patients (21.8%) received IFNβ alone. They received interferon monotherapy including initial aggressive induction therapy (every day for 8 weeks followed by 3 times per week), with a median treatment duration of 44 weeks (range 24–382 weeks) at a median dose of 3 million units (range 3–10 million units) intramuscularly each day.

At the additional treatment of ribavirin combination therapy, 11 patients (45.8%) received PEG-IFNα-2b plus ribavirin, and the remaining 13 patients (54.2%) received IFNα-2b plus ribavirin. They received PEG-IFNα-2b at a median dose of 1.5 μg/kg (range 0.8–1.7 μg/kg) subcutaneously each week or IFNα-2b at a median dose of 6 million units (range 3–6 million units) intramuscularly each day (7 times per week for the initial 2 weeks followed by 3 times per week), with a median treatment duration of 26 weeks (range 24–48 weeks). They also received oral ribavirin at a median dose of 11.0 mg/kg (range 3.0–12.5 mg/kg) daily.

In the present studies, the patients were selected based on the following criteria. (1) Patients had compensated cirrhosis, but no decompensated cirrhosis or hepatocellular carcinoma. The diagnosis of compensated cirrhosis was based on clinical features (absence of signs for decompensation of ascites, encephalopathy, or gastrointestinal bleeding), laboratory tests, and peritoneoscopy or liver biopsy. (2) Patients were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, Calif., USA), and positive for HCV-RNA by qualitative or quantitative analysis. (3) Patients were free of coinfection with human immunodeficiency virus. (4) Lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (5) Patients were free of other types of hepatitis, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (6) Each patient signed a consent form of the study protocol that had been approved by the human ethics review committee.

Laboratory Investigations

Blood samples were frozen at –80° within 4 h of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [12]. HCV-RNA quantitative analysis was measured by branched DNA assay version 2.0 (Chiron Corp., Emeryville, Calif., USA), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems Inc., Pleasanton, Calif., USA), or COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). High viral load of viremia levels was defined as branched DNA assay ≥1.0 MEq/ml, AMPLICOR GT HCV Monitor ≥100 × 10^3 IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay <1.0 MEq/ml, AMPLICOR GT HCV Monitor <100 × 10^3 IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV-RNA qualitative analysis (Amplicor, Roche Diagnostics, Mannheim, Germany) was 100 copies/ml, and that of

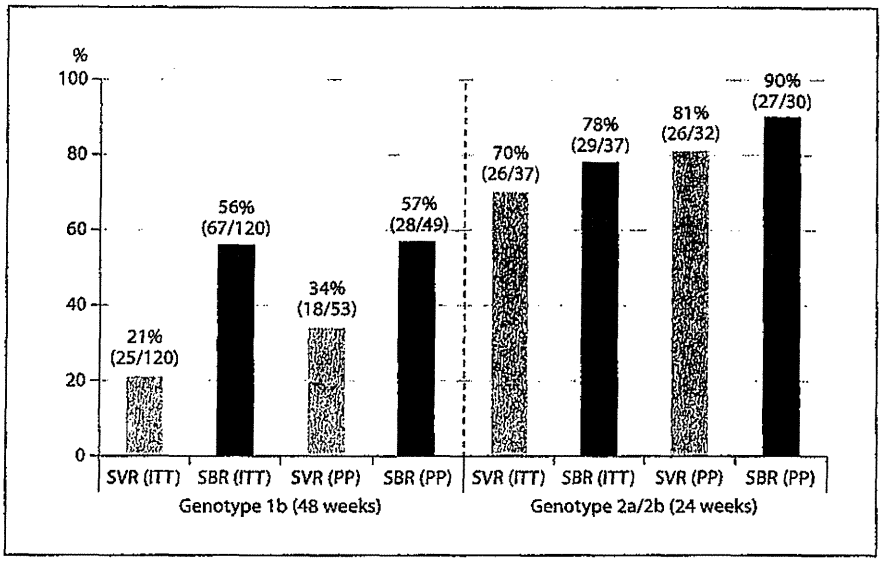

Fig. 2. In 157 patients with HCV-related compensated cirrhosis treatment efficacy with interferon plus ribavirin therapy was evaluated for 48 weeks of HCV genotype 1b or 24 weeks of genotype 2a/2b. In HCV genotype 1b, rates of sustained biochemical response (SBR) were significantly higher than those of sustained virological response (SVR; ITT analysis, $p < 0.001$, and PP analysis, $p = 0.028$).

COBAS TaqMan HCV test was 1.2 log IU/ml. The undetectable samples by HCV-RNA qualitative analysis or COBAS TaqMan HCV test were defined as negative HCV-RNA.

Follow-Up and Diagnosis of Hepatocellular Carcinoma

Clinical and laboratory assessments were performed at least once every month before, during, and after treatment. Adverse effects were monitored clinically by careful interviews and medical examination at least once every month. Patient compliance with treatment was evaluated with a questionnaire. Blood samples were also obtained at least once every month before, during, and after treatment, and were also analyzed for levels of serum alanine aminotransferase and HCV-RNA at various time points.

Patients were examined for hepatocellular carcinoma by abdominal ultrasonography every 3–6 months. If hepatocellular carcinoma was suspected based on ultrasonographic results, additional procedures, such as computed tomography, magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy if necessary, were used to confirm the diagnosis.

Statistical Analysis

χ^2 test, Fisher's exact probability test, and Mann-Whitney's U test were used to compare the background characteristics between groups. Multiple comparisons were examined by the Bonferroni test. The cumulative hepatocarcinogenesis rates were calculated using the Kaplan-Meier technique, and differences between the curves were tested using the log-rank test. Statistical analysis of the hepatocarcinogenesis rates according to groups was calculated using the period from the end of the first course of interferon monotherapy until the appearance of hepatocellular carcinoma or until the last visit or until the start of the third course of interferon-based treatment. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with hepatocarcinogenesis. The hazard ratio (HR) and 95% confidence interval were also calculated. Potential predictive factors associated with hepatocarcinogenesis included the following 13 variables: age, sex, serum aspartate aminotransferase, serum alanine aminotransferase, platelet count, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, HCV genotype, levels of viremia, total duration of additional treatment, and group of additional treatment. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. All p values <0.05 and <0.1 by the two-tailed test were considered significant ($p < 0.05$) and marginally significant ($p < 0.1$), respectively. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were tested by multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Efficacy of Ribavirin Combination Therapy (Study 1)

Treatment efficacy of a 48-week regimen of interferon plus ribavirin combination therapy in 120 patients infected with HCV-1b was evaluated. In ITT analysis, rates of sustained virological response and sustained biochemical response were 21% (25 of 120 patients) and 56% (67 of 120 patients), respectively. In the PP analysis, rates of sustained virological response and sustained biochemical response were 34% (18 of 53 patients) and 57% (28 of 49 patients), respectively (fig. 2). In both analyses, rates of sustained biochemical response were significantly higher than those of sustained virological response (ITT analysis, $p < 0.001$, and PP analysis, $p = 0.028$).

Table 2. Profile and laboratory data of 185 patients with HCV-related compensated cirrhosis according to additional treatment groups (study 2)

	No treatment	Interferon monotherapy (≥24 weeks)	Ribavirin combination therapy[1] (≥24 weeks)
Demographic data			
Patients, n	106	55	24
Sex (male/female), n	64/42	37/18	20/4
Age, years	56 (30–75)[a]	56 (35–76)[b]	51 (34–68)
Laboratory data			
Serum aspartate aminotransferase, IU/l	75 (26–285)	83 (35–213)	62 (30–160)
Serum alanine aminotransferase, IU/l	92 (17–400)	104 (30–316)	93 (36–250)
Platelet count, $\times 10^4/mm^3$	10.7 (2.5–18.2)[c]	10.8 (5.7–19.8)[d]	13.0 (5.2–23.5)
Total cholesterol, mg/dl	165 (103–273)[h]	152 (101–220)	160 (111–211)
High-density lipoprotein cholesterol, mg/dl	46 (25–93)	43 (21–65)	47 (28–56)
Low-density lipoprotein cholesterol, mg/dl	93 (38–168)	87 (45–139)	100 (34–135)
Triglycerides, mg/dl	96 (36–437)	80 (51–215)	108 (52–206)
HCV genotype (1b/2a or 2b), n	70/36	39/16	17/7
Levels of viremia (high viral load/low viral load), n	84/16	37/15[e]	24/0
Additional treatment			
Duration of additional treatment, weeks	–	44 (24–382)[f]	26 (24–48)
Sustained virological response (ITT), n	–	11 (20%)	7 (29%)
Sustained biochemical response (ITT), n	–	25 (45%)[g]	16 (67%)

Unless otherwise indicated, values represent median (range).

Demographic data and laboratory data, at the start of the first course of interferon monotherapy, are shown.

[a] $p = 0.013$, [b] $p = 0.030$, [c] $p = 0.002$, [d] $p = 0.015$, [e] $p = 0.006$, [f] $p = 0.044$, [g] $p = 0.083$ compared with ribavirin combination therapy by Bonferroni test, Mann-Whitney U test, or χ^2 test. [h] $p = 0.039$ compared with interferon monotherapy by Bonferroni test.

[1] 24 of 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2. They showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks), and were additionally treated with ribavirin combination therapy (≥24 weeks).

Treatment efficacy of a 24-week regimen of interferon plus ribavirin combination therapy in 37 patients infected with HCV-2a or 2b was evaluated. In the ITT analysis, rates of sustained virological response and sustained biochemical response were 70% (26 of 37 patients) and 78% (29 of 37 patients), respectively. In the PP analysis, rates of sustained virological response and sustained biochemical response were 81% (26 of 32 patients) and 90% (27 of 30 patients), respectively (fig. 2). In both analyses, rates of the sustained biochemical response were not significantly higher than those of the sustained virological response.

Profile, Laboratory Data, and Efficacy according to Additional Treatment Groups (Study 2)

Profile and laboratory data, at the start of the first course of interferon monotherapy of 185 patients with HCV-related compensated cirrhosis, are summarized in table 2. The age of patients with ribavirin combination therapy was significantly lower than that of patients with no treatment ($p = 0.013$; Bonferroni test) and interferon monotherapy ($p = 0.030$; Bonferroni test). The platelet count of patients of ribavirin combination therapy was significantly higher than that of patients without treatment ($p = 0.002$; Bonferroni test) and interferon monotherapy ($p = 0.015$; Bonferroni test). The total cholesterol level of patients with interferon monotherapy was significantly lower than that of patients without treatment ($p = 0.039$; Bonferroni test). Low viral load rates of patients with interferon monotherapy were significantly higher than those of patients with ribavirin combination therapy ($p = 0.006$; Bonferroni test). There were no other significant differences in clinical features at the start of the first course of interferon monotherapy among the three groups.

Additional treatment duration of only 1 patient, who was diagnosed with hepatocellular carcinoma during additional treatment, was evaluated using the period from the start of the second course of interferon monotherapy

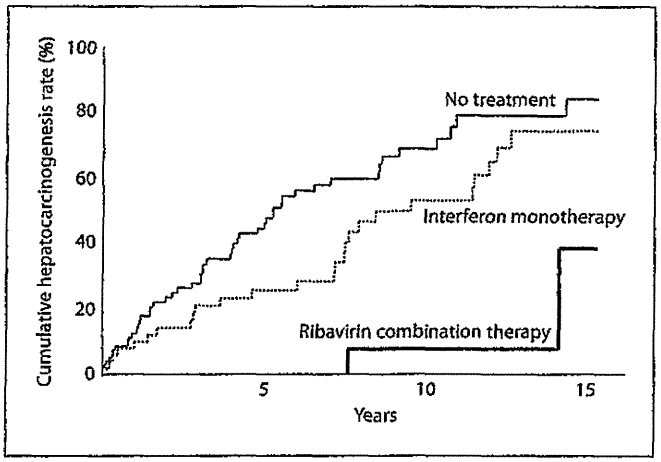

Fig. 3. Cumulative hepatocarcinogenesis rates in the three groups of additional treatment. The rates in no treatment were significantly higher than those in interferon monotherapy ($p = 0.047$; log-rank test) and ribavirin combination therapy ($p < 0.001$; log-rank test), and the rates in interferon monotherapy were significantly higher than those in ribavirin combination therapy ($p < 0.001$; log-rank test).

Table 3. Factors associated with hepatocarcinogenesis in 185 patients of HCV-related compensated cirrhosis identified by multivariate analysis (study 2): Cox proportional hazard model

Factors/category	Hazard ratio (95% confidence interval)	p
Additional treatment		
Ribavirin combination therapy	1	
Interferon monotherapy	4.47 (1.04–19.3)	0.045
No treatment	9.14 (2.19–38.2)	0.002
Age		
<55 years	1	
≥55 years	2.87 (1.76–4.67)	<0.001
Aspartate aminotransferase		
<58 IU/l	1	
≥58 IU/l	2.11 (1.20–3.74)	0.010

until the appearance of hepatocellular carcinoma. During additional treatment, the total duration of interferon monotherapy was significantly longer than that of ribavirin combination therapy ($p = 0.044$; Mann-Whitney U test). In ITT analysis, sustained virological response rates of ribavirin combination therapy (29%) were not different from those of interferon monotherapy (20%), but sustained biochemical response rates of ribavirin combination therapy (67%) tended to be higher than those of interferon monotherapy (45%; $p = 0.083$; χ^2 test) (table 2).

Predictive Factors Associated with Hepatocarcinogenesis by Multivariate Analysis

The data for the whole population sample were analyzed to determine those factors that could predict hepatocarcinogenesis. Hepatocarcinogenesis rates in older patients (≥55 years), in patients with higher levels of aspartate aminotransferase (≥58 IU/l), and lower levels of platelet count (<15.0 × 10^4/mm^3) were significantly higher than those in younger patients (<55 years), in patients with lower levels of aspartate aminotransferase (<58 IU/l), and higher levels of platelet count (≥15.0 × 10^4/mm^3), respectively ($p < 0.001$, $p = 0.006$, and $p = 0.017$; log-rank test). Furthermore, the rates in no treatment were significantly higher than those in interferon monotherapy ($p = 0.047$; log-rank test) and ribavirin combination therapy ($p < 0.001$; log-rank test), and the rates in interferon monotherapy were significantly higher than those in ribavirin combination therapy ($p < 0.001$; log-rank test) (fig. 3). Thus, univariate analysis identified four parameters that significantly correlated with hepatocarcinogenesis. These factors were entered into multivariate analysis, which then identified three parameters that significantly influenced hepatocarcinogenesis independently: additional treatment (no treatment; HR 9.14, $p = 0.002$), age (≥55 years; HR 2.87, $p < 0.001$), and levels of aspartate aminotransferase (≥58 IU/l; HR 2.11, $p = 0.010$) (table 3).

The data for 167 patients, except for 18 patients who showed a sustained virological response following additional treatment, were also analyzed to determine those factors that could predict hepatocarcinogenesis. Hepatocarcinogenesis rates in older age (≥55 years) and higher levels of aspartate aminotransferase (≥58 IU/l) were significantly higher than those in younger age (<55 years) and lower levels of aspartate aminotransferase (<58 IU/l), respectively ($p < 0.001$ and $p = 0.007$; log-rank test). Furthermore, the rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy ($p < 0.001$; log-rank test) and no treatment ($p < 0.001$; log-rank test) (fig. 4). Thus, univariate analysis identified three parameters that significantly correlated with hepatocarcinogenesis. These factors were entered into multivariate analysis, which then identified three parameters that significantly influenced hepatocarcinogenesis independently: additional treatment (no treatment; HR 7.87, $p = 0.005$), age (≥55 years; HR 2.52, $p < 0.001$), and levels of aspartate aminotransferase (≥58 IU/l; HR 2.13, $p = 0.010$) (table 4).

Discussion

One of our previous studies indicated that the cancer-suppressive activity of interferon monotherapy in patients with HCV-RNA eradication was similar to that in patients with alanine aminotransferase normalization without HCV-RNA elimination [9]. Other studies also indicated a higher incidence and more rapid development of hepatocellular carcinoma in HCV patients with high levels of alanine aminotransferase [13, 14]. Collectively, these results suggest that the carcinogenic process in patients with chronic HCV infection is enhanced by high levels and fluctuations of alanine aminotransferase, and indicate a close relationship between suppression of inflammatory necrosis of hepatocytes and a lower incidence of hepatocellular carcinoma in patients with HCV-associated chronic liver disease. Recent studies based on interferon plus ribavirin combination therapy also showed that the attainment of sustained virological response or lower levels of alanine aminotransferase after ribavirin combination therapy could reduce the rates of hepatocellular carcinoma [15, 16], but the small numbers of patients with compensated cirrhosis (5% or less of all patients) were recruited. The present study 1 based on the patients with compensated cirrhosis showed that rates of sustained virological response and sustained biochemical response in HCV-2a/2b were high rates of 70 and 78%, and that rates of sustained biochemical response (57%) were significantly higher than those of sustained virological response (34%) in HCV-1b. Furthermore, the present study 2 based on the patients with compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy, also showed that sustained biochemical response rates of ribavirin combination therapy (67%) tended to be higher than those of interferon monotherapy (45%). Thus, in ribavirin combination therapy for compensated cirrhosis, higher rates of sustained biochemical response might be associated with lower rates of hepatocarcinogenesis. One limitation is that the present study was performed based on the small numbers of patients who showed no sustained virological response with interferon monotherapy. In further prospective studies a larger number of patients need to be investigated to confirm this finding.

Previous studies have shown that gender, age, fibrosis stage, alanine aminotransferase, and interferon regimen are important pretreatment predictors of hepatocarcinogenesis [9, 10, 17]. In the present study 2 based on the patients with compensated cirrhosis, higher age and aspartate aminotransferase were associated with higher hepatocarcinogenesis rates in the whole population sample and in the sample which excluded patients who showed sustained virological response following additional treatment. Furthermore, as treatment-related factors, the hepatocarcinogenesis rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy. Thus, in patients with compensated cirrhosis representing a high-risk group of hepatocarcino-

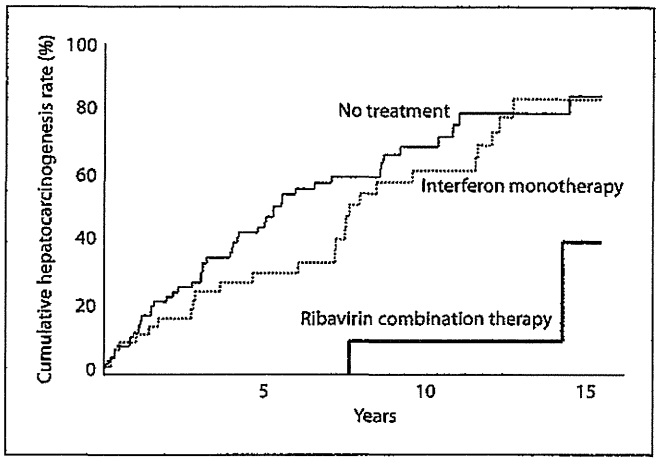

Fig. 4. Cumulative hepatocarcinogenesis rates in the three groups of additional treatment, except for patients who showed sustained virological response following additional treatment. The rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy ($p < 0.001$; log-rank test) and no treatment ($p < 0.001$; log-rank test).

Table 4. Factors associated with hepatocarcinogenesis in 167 patients of HCV-related compensated cirrhosis, except for 18 patients who showed sustained virological response following additional treatment identified by multivariate analysis (study 2): Cox proportional hazard model

Factors/category	Hazard ratio (95% confidence interval)	p
Additional treatment		
Ribavirin combination therapy	1	
Interferon monotherapy	4.68 (1.08–20.3)	0.039
No treatment	7.87 (1.89–32.9)	0.005
Age		
<55 years	1	
≥55 years	2.52 (1.54–4.11)	<0.001
Aspartate aminotransferase		
<58 IU/l	1	
≥58 IU/l	2.13 (1.20–3.79)	0.010

genesis, ribavirin combination therapy might reduce the risk of hepatocellular carcinoma in comparison with interferon monotherapy. One reason for the higher anticarcinogenic activity by ribavirin combination therapy might be due to higher rates of sustained biochemical response. The other reason might be due to the difference in the background (lower age and higher levels of platelet count as an indicator of fibrosis stage) of patients with ribavirin combination therapy. Further studies of a larger number of patients matched for background, including age, sex, genotype, and platelet count, are required to investigate the rates of hepatocarcinogenesis and the mechanism of anticarcinogenic activity by ribavirin combination therapy for HCV-related compensated cirrhosis.

Two previous studies (PROVE1 and PROVE2) showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin could achieve sustained virological response rates of 35–60 and 61–69% in patients infected with HCV-1, respectively [18, 19]. However, a recent study (PROVE3) also showed that the sustained virological response rates were the lower rates of 39 and 38% with the 24- and 48-week regimen of triple therapy in previously nonresponding patients infected with HCV-1, who do not become HCV-RNA negative during or at the end of the initial PEG-IFN/ribavirin treatment, respectively [20]. Furthermore, the telaprevir-based regimen induces resistant variants [21–23] and has side effects including anemia and rash [18–20, 24]. Hence, patients, who do not achieve sustained virological response by triple therapy, need to be identified, in order to avoid unnecessary side effects and telaprevir-resistant variants. Recent studies identified amino acid substitutions at position 70 and/or 91 in the HCV-1b core region, advanced fibrosis stage, and higher levels of α-fetoprotein as pretreatment predictors of poor virological response to PEG-IFN/ribavirin combination therapy or triple therapy of telaprevir/PEG-IFN/ribavirin [23, 25–28], and these factors are also risk factors and surrogate markers of hepatocarcinogenesis [29–34]. Hence, ribavirin combination therapy for these patients might be an efficacious therapeutic regimen for sustained biochemical response and thus a reduction of the risk of hepatocarcinogenesis. Large-scale prospective studies should be conducted in the future to confirm this finding.

In conclusion, the present retrospective study indicated that ribavirin combination therapy for HCV-related compensated cirrhosis could reduce the risk of hepatocarcinogenesis in comparison with interferon monotherapy. Large-scale prospective studies need to be conducted in the future to confirm these findings.

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Original Article

Discrimination of fibrotic staging of chronic hepatitis C using multiple fibrotic markers

Kenji Ikeda,[1,2] Namiki Izumi,[3] Eiji Tanaka,[8] Hiroshi Yotsuyanagi,[4] Yoshihisa Takahashi,[6] Junichi Fukushima,[7] Fukuo Kondo,[6] Toshio Fukusato,[6] Kazuhiko Koike,[5] Norio Hayashi,[9] Hirohito Tsubouchi[10] and Hiromitsu Kumada[1,2]

[1]Department of Hepatology, Toranomon Hospital, [2]Okinaka Memorial Institute for Medical Research, [3]Department of Gastroenterology, Musashino Red Cross Hospital, [4]Department of Infectious Disease, [5]Department of Gastroenterology, Graduate School of Medicine, Tokyo University, [6]Department of Pathology, Teikyo University School of Medicine, [7]Department of Pathology, NTT Medical Center Tokyo, Tokyo, [8]Department of Gastroenterology, Shinshu University of Medicine, Matsumoto, [9]Department of Gastroenterology, Kansai-Rosai Hospital, Hyogo, and [10]Department of Gastroenterology, Kagoshima University of Medicine, Kagoshima, Japan

Aim: In order to evaluate and judge a fibrotic stage of patients with chronic hepatitis C, multivariate regression analysis was performed using multiple fibrotic markers.

Methods: A total of 581 patients from eight hepatology units and institutes were diagnosed by needle biopsy as having chronic liver disease caused by hepatitis C virus. Twenty-three variables and their natural logarithmic transformation were employed in the multivariate analysis.

Results: Multivariate regression analysis finally obtained the following function: $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times 10^3/mm^3$) $+ 0.79 \times ln$ (total bilirubin) (mg/dL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$. Median values of the fibrotic score of F1 ($n = 172$), F2 ($n = 80$), F3 ($n = 37$) and F4 ($n = 16$) were calculated as 1.00, 1.45, 2.82 and 3.83, respectively. Multiple regression coefficient and coefficient of determination were 0.56 and 0.320, respectively. Validation with patient data from other institutions demonstrated good reproducibility of the fibrotic score for hepatitis C (FSC), showing 1.10 in F1 ($n = 156$), 2.35 in F2 ($n = 73$), 3.16 in F3 ($n = 36$) and 3.58 in F4 ($n = 11$).

Conclusion: A concise multiple regression function using four laboratory parameters successfully predicted pathological fibrotic stage of patients with hepatitis C virus infection.

Key words: chronic hepatitis, hepatitis C virus, liver cirrhosis, liver fibrosis, multiple regression analysis, stage

INTRODUCTION

WHEN HEPATITIS C virus (HCV)-related chronic liver disease was found by biochemical and virological examination, peritoneoscopy and/or liver biopsy can establish the definitive diagnosis of chronic hepatitis and liver cirrhosis. Although these pathological procedures are reliable and informative both in diagnosis and treatment, they sometimes require medical invasion and financial costs, including the risk of bleeding from needle puncture, some pain experienced during the examination, medical expenses and hospitalization for a few days. The pathological examination is, therefore, rarely performed repeatedly in a short period of time, even when disease activity is severe and progression of liver disease is highly suspected. Recently, many authors described the usefulness of ultrasonographic elastography and magnetic resonance imaging technology in the estimation of staging of chronic hepatitis and cirrhosis.[1–4] These ways of estimation using the imaging apparatuses seem truly useful for current patients, but it cannot evaluate and compare with past fibrotic states of patients retrospectively. Moreover, the same apparatus for elastometry will not be available for repeated measurement for a follow-up examination, several years later for example.

In spite of the accuracy of biopsy and of convenience of elastography in chronic liver disease, clinical diagnosis based on biochemistry and hematology is still indispensable for the daily practice of many patients with

Correspondence: Dr Kenji Ikeda, *Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: ikedakenji@tora.email.ne.jp*

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HCV-related liver disease. Recently, several studies were published about estimation of hepatitis stages, using one or more serum biomarkers. Discriminant functions or multivariate analyses demonstrated that approximately 60–90% of patients with chronic hepatitis C were correctly classified as mild hepatitis and severe hepatitis with advanced fibrosis.[5–16] The usefulness of the discriminant functions was, however, less valuable up to the present time for a few reasons. First, these functions were made for the purpose of discrimination of severe hepatic fibrosis from mild fibrosis, and four histological classifications (F1, F2, F3 and F4) were selected in almost of the studies. Second, some studies analyzed both hepatitis B virus and HCV infection, although the significance and actual values of each liver function test in the evaluation of the severity of liver disease were not similar among each viral hepatitis and alcoholic liver disease. Third, biochemical markers for liver fibrosis (e.g. hyaluronic acid, type IV collagen, procollagen III peptide)[17–19] were not always included in those previous studies.

We tried to generate a function estimating fibrotic stages of HCV-related chronic hepatitis, which were objectively diagnosed by liver biopsy. The purpose of this study is, therefore, to make a reliable multiple regression function and to obtain practical coefficients for significant variables also using fibrotic markers.

METHODS

Patients

A TOTAL OF 605 Japanese patients with chronic hepatitis C were recruited for the study from eight hospitals in Japan: Toranomon Hospital, Hiroshima University Hospital (K. Chayama, M.D.), Ehime University Hospital (M. Onji, M.D.), Musashino Red Cross Hospital (N. Izumi, M.D.), Shishu University Hospital (E. Tanaka, M.D.), Showa University Hospital (M. Imawari, M.D.), Osaka University Hospital (T. Takehara, M.D.) and Kagoshima University Hospital (H. Tsubouchi, M.D.). Inclusion criteria for this study were: (i) positive HCV antibody for more than 6 months; (ii) persistent or intermittent elevation in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels; and (iii) liver biopsy showing chronic hepatitis (F1, F2, F3 or F4). We excluded those patients with overt alcoholic liver disease or fatty liver, association of other types of liver disease (e.g. hepatitis B, primary biliary cirrhosis, autoimmune hepatitis), or those associated with hepatocellular carcinoma or other malignancy. Among the patients, 603 fulfilled the conditions for the study: complete demographic data, basic laboratory data of hematology and biochemistry, required liver biopsy specimens, and sufficient amount of frozen sera. We also excluded an additional 22 patients with eventual histological diagnosis of F0 stage.

Finally, a total of 581 patients who were diagnosed as having chronic hepatitis or cirrhosis (F1, F2, F3 or F4) were analyzed for the following hematological, biochemical and histopathological examination. There were 305 males and 276 females aged 15–78 with a median of 55 years.

All the patients presented written informed consent in individual hospitals and medical centers, and the study was approved by each ethical committee.

Hematological and biochemical examination

Hematological and standard biochemical evaluation had been performed in each medical institution: white blood cell, red blood cell count, hemoglobin, platelet count, total bilirubin, AST, ALT, AST/ALT ratio (AAR), γ-glutamyltransferase (GGT), total protein, albumin and γ-globulin.

Special biochemical examinations including fibrotic markers were carried out using stored frozen sera at −20°C or lower: α2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, tissue inhibitor of matrix metalloproteinase (TIMP)-1, TIMP-2, procollagen III peptide and type IV collagen 7S.

Histological diagnosis of chronic hepatitis and cirrhosis

All of the 581 cases fulfilled required standards of histological evaluation: sufficient length of specimen, hematoxylin–eosin staining and at least one specimen with fiber staining. Four independent pathologists (Y. T., J. F., F. K. and T. F.), who were not informed of patients' background and laboratory features except for age and sex, evaluated the 581 specimens regarding the stages of fibrosis and activity. Pathological classification of chronic hepatitis staging was based on Desmet *et al.*[20]

Before judgment of histological staging of individual specimens, the pathologists discussed objective and reproducible judgment of pathological diagnosis of hepatitis. They made a panel for obvious criteria using typical microscopic pictures for each stage, and it was always referred to during the procedure of pathological judgment. When inconsistent results were found in the diagnosis of stage of hepatitis among the pathologists, the final judgment was accepted as the majority rule among them.

Statistical analysis

Non-parametric procedures were employed for the analysis of background characteristics and laboratory data among patients in each stage, including Mann–Whitney *U*-test, Kruskal–Wallis test and χ^2-test.

The normality of the distribution of the data was evaluated by Kolmogorov–Smirnov one-sample test. Because certain variables partly did not conform to a normal distribution, natural logarithmic transformation of bilirubin, AST, ALT, GGT, α2-macroglobulin, hyaluronic acid, type IV collagen 7S and TIMP-2 were also analyzed in the following calculation. The natural logarithmic transformation of the results yielded a normal distribution or symmetrical distribution for all the analyzed factors. After the procedures, the following multiple regression analysis became rationally robust against deviations from normal distribution. In order to avoid introducing into the model any variables that were mutually correlated, we checked the interaction between all pairs of the variables by calculating variance of inflation factors. Of the highly correlated variables, less significant factors were removed from the viewpoint of multicollinearity.

Multivariate regression analysis was performed using 305 patient data from Toranomon Hospital (training dataset), to generate training data of predicting function. We used a stepwise method for selection of informative subsets of explanatory variables in the model. Multiple regression coefficient and coefficient of determination are also taken into account in the selection of variables. Next, we validated the obtained predictive function using the remaining 276 patient data from the other seven liver institutions (validation dataset).

A *P*-value of less than 0.05 with two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS version 19.[21]

For evaluation of the efficiency and usefulness of obtained function for estimation of fibrosis, we compared various fibrotic scores for hepatitis C, including AAR,[8] AST-to-platelet ratio index (APRI),[12] FIB-4[13] and FibroTest.[9]

RESULTS

Pathological diagnosis

FOUR PATHOLOGISTS INDEPENDENTLY judged the fibrotic stages and inflammatory activity for 581 specimens of chronic hepatitis/cirrhosis caused by HCV. A total of 328 patients (56.5%) had a fibrotic stage of F1, 153 (26.3%) F2, 73 (12.6%) F3 and 27 (4.6%) F4. In the training subgroup ($n = 305$), judgment of F1 was made in 172, F2 in 80, F3 in 37 and F4 in 16. In the validation group ($n = 276$), judgment as F1 was made in 156, F2 in 73, F3 in 36 and F4 in 11.

According to hepatitis activity classification, A0 was found in nine patients (1.52%), A1 in 350 (60.2%), A2 in 198 (34.1%) and A3 in 24 (4.1%).

Laboratory data of each hepatitis stage in training group

There were 161 males and 144 females with a median age of 54 years (range, 22–69). Laboratory data of the 305 patients in the training group are shown in Table 1. Although several individual items were well correlated with the severity of hepatic fibrosis, significant overlap values were noted among F1 to F4 stages: platelet count, GGT, γ-globulin, hyaluronic acid and type IV collagen 7S.

Regression function generated from training patient group

After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times 10^3$/mm^3) $+ 0.79 \times ln$ (total bilirubin) (ng/mL) $+ 0.39 \times ln$ (hyaluronic acid) (μm/L) $-$ 1.87. Median values of the fibrotic score of F1 ($n = 172$), F2 ($n = 80$), F3 ($n = 37$) and F4 stages ($n = 16$) were calculated as 1.00, 1.45, 2.82 and 3.83, respectively (Fig. 1). The multiple regression coefficient and coefficient of determination were 0.56 and 0.32, respectively.

A 55-year-old man with F1 fibrotic stage (Fig. 2a) showed serum type IV collagen concentration as 3.8 ng/mL, platelet as 152×10^3 count/mm^3, total bilirubin as 0.8 mg/dL and hyaluronic acid as 16 μg/L. The regression function provided his fibrotic score as 1.16. Another man aged 43 years had F3 fibrosis with severe hepatitis activity of A3 on histological examination (Fig. 2b). His type IV collagen was 11.0 ng/mL, platelet 162×10^3 count/mm^3, total bilirubin 0.7 mg/dL and hyaluronic acid 189 μg/L, and regression function calculated his fibrotic score as 4.98.

Validation of discriminant function

Validation data of 276 patients (Table 2) were collected from the other seven institutions in Japan. When applying the regression function for the validation set, the fibrotic score for hepatitis C (FSC) demonstrated good reproducibility, showing 1.10 in patients with chronic hepatitis of F1 ($n = 156$), 2.35 in F2 ($n = 73$), 3.16 in F3 ($n = 36$) and 3.58 in F4 ($n = 11$) (Fig. 3). Although F4

Table 1 Demography and laboratory data of 305 patients in training group

	F1 ($n=172$)	F2 ($n=80$)	F3 ($n=37$)	F4 ($n=16$)
Demography				
Males : females	97:75	38:42	20:17	6:10
Age (median, range)	51 (22–69)	55 (29–68)	55 (27–69)	56.5 (29–65)
Laboratory data (median, range)				
WBC ($\times10^3$/mm^3)	4.7 (2.0–10.1)	4.3 (2.3–8.5)	4.5 (2.9–6.8)	4.7 (3.3–6.9)
Hemoglobin(g/dL)	14.6 (11.0–18.2)	14.4 (9.3–17.4)	14.6 (11.5–17.7)	14.55 (12.1–16.5)
Platelet ($\times10^3$/mm^3)	183 (52–364)	161 (82–387)	131 (74–237)	124 (7.7–191)
Albumin (g/dL)	4.1 (2.3–4.9)	4.0 (3.5–4.6)	3.9 (3.1–4.6)	3.8 (3.3–4.3)
Bilirubin (mg/dL)	0.8 (0.2–1.9)	0.7 (0.3–1.7)	0.9 (0.4–7.5)	0.8 (0.5–7.4)
AST (IU/L)	42 (16–386)	61 (16–332)	63 (13–238)	71 (30–160)
ALT (IU/L)	60.5 (12–1664)	84.5 (10–647)	108 (27–415)	90.5 (36–264)
γ-GTP (IU/L)	40 (7–383)	48 (10–262)	54 (13–209)	58 (21–195)
γ-Globulin (g/dL)	1.47 (0.58–3.40)	1.61 (1.02–2.41)	1.69 (0.66–2.64)	1.79 (1.22–2.73)
γ-Globulin (%)	19.4 (10.0–40.5)	20.9 (14.0–28.3)	21.3 (8.1–30.4)	22.7 (16.5–36.9)
α2-Macroglobulin (mg/dL)	269 (123–505)	335 (154–551)	369 (183–627)	317 (207–511)
Haptoglobin (mg/dL)	94.5 (<5–265)	75.5 (<5–263)	56 (<5–2031)	75 (30–142)
Apolipoprotein A1 (mg/dL)	132 (71–209)	131 (73–207)	124 (98–166)	121 (83–153)
Hyaluronic acid (μg/L)	25 (<5–407)	41.5 (<5–263)	71 (<5–326)	89.5 (5–246)
TIMP-1 (ng/mL)	165 (73–291)	173 (97–302)	182 (126–308)	192.5 (128–260)
TIMP-2 (ng/mL)	77.5 (31–210)	80 (34–307)	76 (46–143)	78 (58–110)
Procollagen III peptide (U/mL)	0.75 (0.47–1.50)	0.805 (0.61–1.70)	0.86 (0.53–1.50)	1.05 (0.66–1.60)
Type IV collagen 7S (ng/mL)	4.0 (1.7–73)	4.3 (2.1–11.0)	5.2 (3.2–11.0)	5.8 (4.3–9.4)

γ-GTP, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cell.

fibrotic stage consisted of only 11 patients and the score 3.58 was regarded as a rather low value, the scores of other stages of fibrosis were concordant with histological fibrosis.

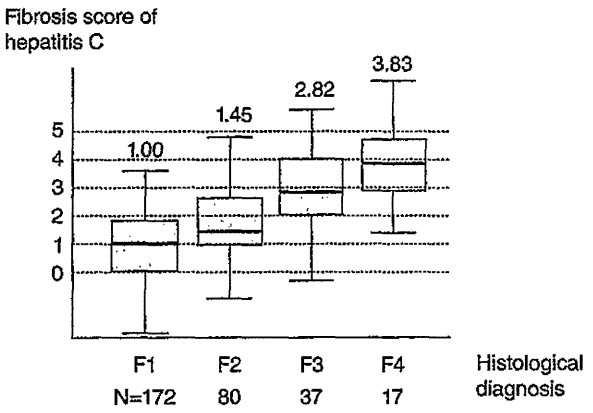

Figure 1 Box and whisker plots of fibrotic score of each group of histological fibrosis in the training dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times10^3$/mm^3) $+ 0.79 \times ln$ (total bilirubin) (mg/dL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$.

Comparisons of efficacy with various fibrotic scores (Fig. 4)

In order to evaluate the efficacy and usefulness of the obtained FSC, we compared with previously reported fibrotic scores using training data. AAR, APRI, FIB-4 and FibroTest showed only slight correlation with actual histological stage. APRI and FIB-4 demonstrated increasing trends of the score associated with histological fibrosis, but significant overlapping scores were found through F1 to F4. Spearman's correlation coefficients of AAR, APRI, FIB-4 and FibroTest were 0.021 ($P = 0.707$), 0.462 ($P < 0.001$), 0.440 ($P < 0.001$) and 0.415 ($P < 0.001$), respectively. Our FSC showed Spearman's correlation coefficient of 0.572 ($P < 0.001$), and was of much higher value than the others.

DISCUSSION

RECOGNITION OF SEVERITY of chronic hepatitis is essential in managing patients with chronic HCV infection: estimation of length of infection, existence of any previous hepatitis activity, presumption of current fibrotic stage, and prediction of future fibrotic progression and hepatocarcinogenesis. Differential diagnosis of cirrhosis from chronic hepatitis is especially important

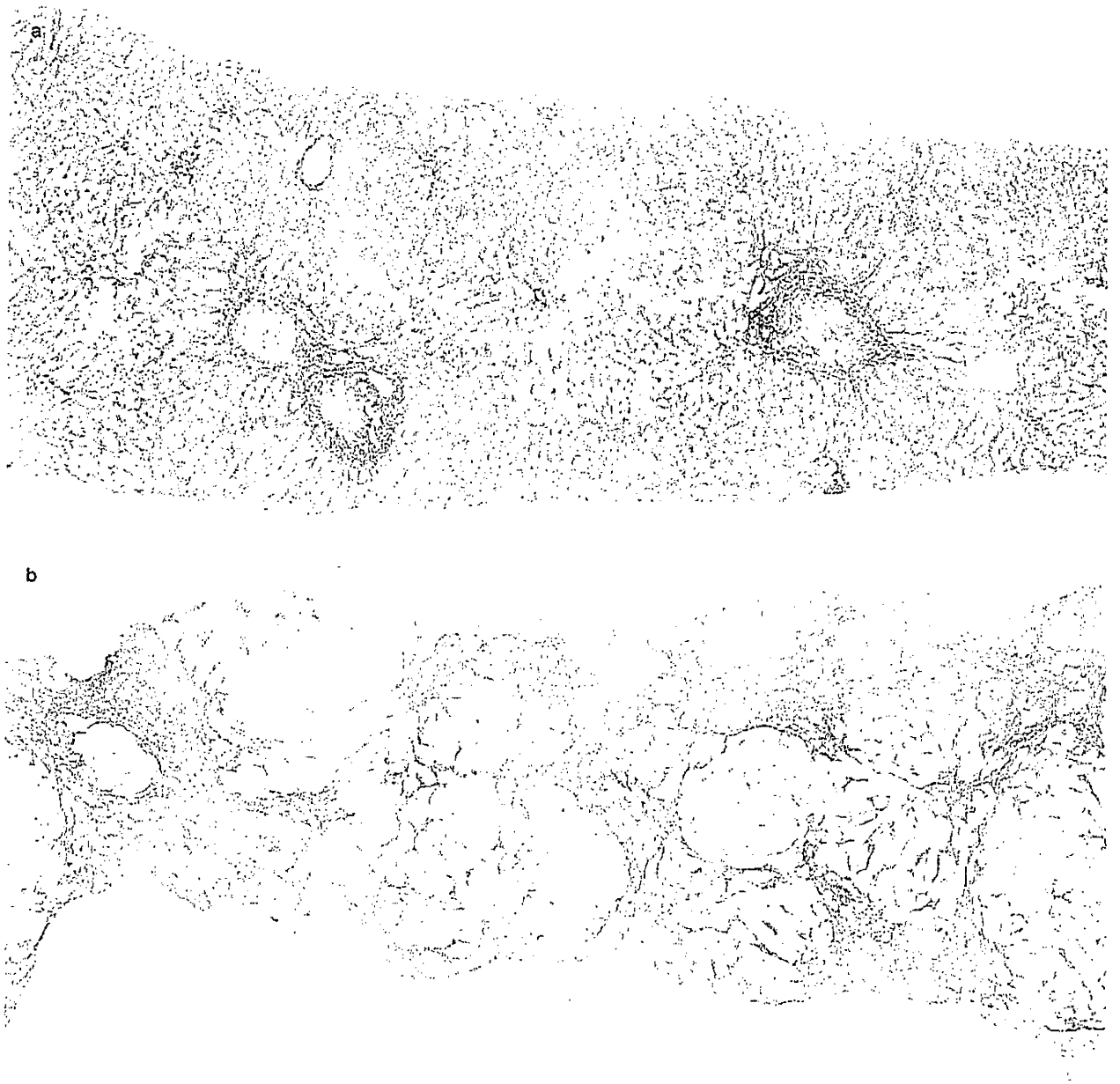

Figure 2 Case presentations of the training set. (a) A 55-year-old man with F1 fibrosis. Final regression function provided his fibrotic score as 1.16. (b) A 43-year-old man with F3 fibrosis with severe hepatitis activity. His regression coefficient was calculated as 4.98 (silver stain, ×40).

in the evaluation of chronic HCV infection. Identification of liver cirrhosis often leads to an important change in management of the patients: needs for fiberscopic examination for esophageal varices, ultrasonographic exploration for the association of liver cancer, and prediction of hepatic decompensation.

Recently, non-invasive estimation of severity of liver fibrosis has been reported in patients with HCV-related chronic hepatitis.[6-14] However, these studies were principally aimed at differentiation of advanced fibrotic stages of F3 or F4 from mild fibrotic stages of F1 or F2. Those discriminative functions were insufficient to

Table 2 Demography and laboratory data of 276 patients in validation group

	F1 ($n=156$)	F2 ($n=73$)	F3 ($n=36$)	F4 ($n=11$)
Demography				
Males : females	83:73	42:31	13:23	6:5
Age (median, range)	55 (15–74)	58 (32–77)	62.5 (30–78)	51 (38–73)
Laboratory data (median, range)				
WBC ($\times10^3$/mm^3)	5.1 (2.1–10.5)	4.8 (2.6–9.0)	4.85 (2.3–14.2)	3.9 (3.2–6.0)
Hemoglobin (g/dL)	14.2 (8.9–17.7)	14.4 (11.8–17.4)	14.1 (10.1–16.4)	13.6 (8.9–16.3)
Platelet ($\times10^3$/mm^3)	183 (59–440)	153 (80–265)	136 (64–348)	135 (79–153)
Albumin (g/dL)	4.3 (3.1–5.3)	4.3 (3.3–5.2)	4.05 (3.0–5.5)	3.9 (3.0–4.7)
Bilirubin (mg/dL)	0.7 (0.2–8.7)	0.7 (0.2–1.7)	0.8 (0.2–2.5)	0.8 (0.4–11.0)
AST (IU/L)	35 (11–1390)	49 (19–183)	80 (20–190)	96 (29–257)
ALT (IU/L)	49 (11–1635)	62 (12–575)	84 (14–218)	115 (29–303)
γ-GTP (IU/L)	35 (11–600)	52 (10–497)	51 (14–236)	112 (17–312)
γ-Globulin (g/dL)	1.47 (0.70–2.14)	1.60 (0.80–2.37)	1.71 (0.63–2.62)	2.19 (1.70–2.82)
γ-Globulin (%)	19.5 (9.2–26.4)	20.8 (10.8–30.8)	22.4 (9.5–29.9)	27.4 (21.8–35.3)
α2-Macroglobulin (mg/dL)	271.5 (126–572)	381 (172–573)	405.5 (196–594)	468 (242–655)
Haptoglobin (mg/dL)	95 (<5–305)	80 (<5–223)	63.5 (<5–192)	65 (<5–130)
Apolipoprotein A1 (mg/dL)	126 (45–198)	127 (63–191)	116 (46–172)	108 (62–171)
Hyaluronic acid (μg/L)	37.5 (<5–1260)	68 (5–1000)	140.5 (23–2610)	159 (33–364)
TIMP-1 (ng/mL)	157.5 (77–301)	172 (89–355)	188.5 (99–430)	192 (112–320)
TIMP-2 (ng/mL)	70 (21–294)	73 (21–207)	89 (27–280)	76 (36–120)
Procollagen III peptide (U/mL)	0.73 (0.52–8.30)	0.81 (0.53–1.60)	1.00 (0.63–1.90)	1.00 (0.68–1.60)
Type IV collagen 7S (ng/mL)	3.9 (1.2–12.0)	4.5 (2.3–9.9)	5.8 (2.8–16.0)	6.1 (4.6–10.0)

γ-GTP, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cell.

recognize the stepwise progression of viral hepatitis from F1 through F4. This dichotomy (mild or severe) of chronic hepatitis C seemed less valuable in the study of disease progression, disease control abilities of antiviral drugs and estimation of histological improvement after anti-inflammatory drugs. A histology-oriented, practical and reliable formula is therefore required for the diagnosis and investigation of chronic hepatitis C.

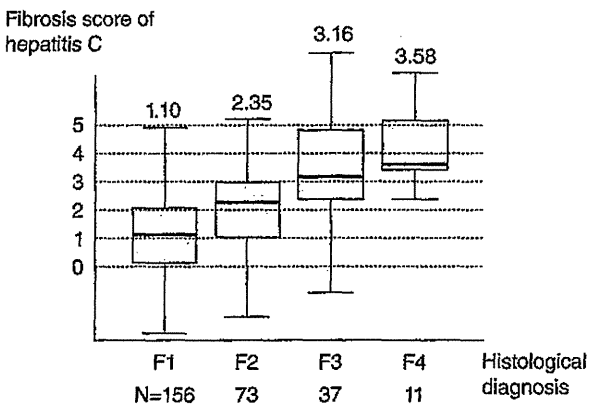

Figure 3 Box and whisker plots of fibrotic score of each group of histological fibrosis in the validation dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times10^3$/mm^3) $+ 0.79 \times ln$ (total bilirubin) (ng/mL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$.

This study was aimed to establish non-invasive evaluation and calculation of liver fibrosis for patients with chronic HCV infection. Although it was retrospectively performed as a multicenter study of eight institutions, judgment of histological diagnosis was independently performed by four pathologists in the other hospital, informed of nothing except for the patient's age, sex and positive HCV infection. Objective judgment of the histological staging and grading in sufficient biopsy specimens could be obtained.

As many as 581 patients with chronic hepatitis C were analyzed in this study, who had been diagnosed as having chronic hepatitis or cirrhosis by liver biopsy performed in experienced liver units in Japan. To obtain the most suitable equation approximating histological fibrotic stage, multivariate analysis was performed using two demographic parameters (age and sex) and 21 hematological and biochemical markers with or without logarithmic transformation. They included many kinds of fibrotic markers: α2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipo-

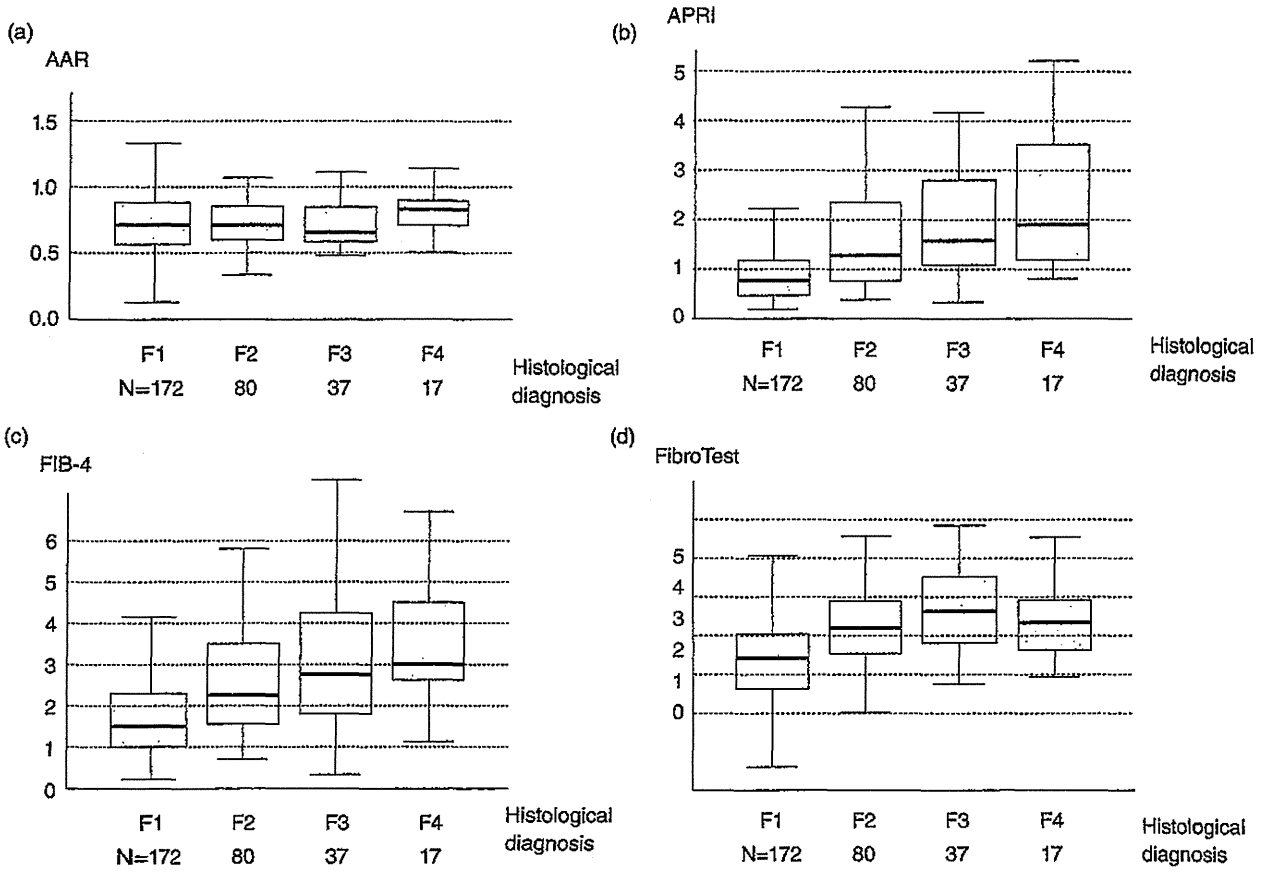

Figure 4 Previously published fibrotic scores: (a) aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR),[8] (b) AST-to-platelet ratio index (APRI), calculated by AST / (upper limit of normal of AST) / (platelet count [$\times 10^9$/L]) $\times$ 100.[12] (c) FIB-4 score, calculated by age $\times$ AST [IU/L] / (platelet count [$\times 10^9$/L] $\times$ ALT [IU/L]$^{0.5}$).[13] (d) FibroTest score regression coefficient was: $Z = 4.467 \times \log^{10}$ (α2-macroglobulin [g/L]) $- 1.357 \times \log^{10}$ (haptoglobin [g/L]) $+ 1.017 \times \log^{10}$ [γ-glutamyltransferase [GGT] [IU/L]) $+ 0.0281 \times$ (age [years]) $+ 1.737 \times \log^{10}$ (bilirubin [μm/L]) $- 1.184 \times \log^{10}$ (apolipoprotein A1 [g/L]) $+ 0.301 \times$ (sex [female = 0, male = 1]) $- 5.54$.[9]

protein A1, hyaluronic acid, TIMP-1, TIMP-2, procollagen III peptide and type IV collagen 7S. Multiple regression analysis finally generated a first-degree polynomial function consisting of four variables: type IV collagen 7S, platelet count, bilirubin and hyaluronic acid. A constant numeral (−1.87) was finally adjusted in the regression equation in order to obtain fitted figures for fibrotic stages of F1, F2, F3 and F4. From the magnitude of the standardized partial regression coefficient of individual variable in the function, *ln* (type IV collagen 7S) demonstrated the most potent contribution toward the prediction of liver fibrosis. Platelet count and *ln* (bilirubin) proved to be the second and third distinctive power in the model, respectively.

The obtained figure of FSC was generated to imitate actual "F factor" of histological staging. FSC was sufficiently fitted to actual fibrotic stages with certain overlapping as was usually found in histological ambiguity judged by pathologists. Because judgment of fibrosis in chronic hepatitis often shows a transitional histological staging, pathological examination could not always achieve a clear-cut diagnosis discriminating F1, F2, F3 or F4. Considering the limitation of pathological difficulty in differentiation of the four continuous disease entities, the obtained regression function showed satisfactory high accuracy rates in the prediction of liver disease severity. FSC can provide one or two decimal places (e.g. 2.4 or 2.46) and the utility of the score is possibly higher

than mere histological staging of F1, F2, F3 or F4. The reproducibility was confirmed by the remaining 276 patients' data obtained from the other seven hospitals. Although the validation data were collected from different geographic area and different chronologic situation, FSC showed similar results in prediction of histological staging.

Fibrotic score for hepatitis C seemed a very useful quantitative marker in evaluating severity of fibrotic severity of hepatitis C patients without invasive procedures and without any specialized ultrasonography or magnetic resonance imaging. FSC also has an advantage of measurement, in which old blood samples are available for retrospective assessment of varied clinical settings: old sera from 20 years ago at the time of initial liver biopsy, or paired sera before and after a long-term anti-inflammatory therapy, for example. These kinds of retrospective assessments of fibrotic staging will be valuable in estimating a long-term progression of liver disease, in evaluating efficacy of a long-term medication or other medical intervention, or in making a political judgment from the viewpoint of socioeconomic efficacy.

The score can be calculated for any patients with chronic HCV infection. Although this multiple regression model dealt with appropriate logarithmic transformation for non-normal distribution parameters, the regression analysis was based on a linear regression model. Very slight fibrosis can be calculated as less than 1.00, which is commonly found with a slight degree of chronic hepatitis with a tiny fibrotic change as F0. Very severe fibrosis may be calculated as more than 4.00, which is an imaginable and nonsense number in the scoring system of fibrosis. FSC is, however, very useful and valuable in real clinical setting. Estimation of severity of liver fibrosis in outpatient clinics, evaluation of natural progression of patients' fibrosis over 10 years, and assessment of a long-term administration of interferon in patients with chronic hepatitis C from the viewpoint of fibrotic change. In this study, because certain patients actually had a history of interferon administration, regression of liver fibrosis during and after the treatment could be assessed when prior sera were available for serial evaluation of FSC. We can also expect the usefulness of evaluation of carcinogenic risk after sustained virological response, and stage progression with alcohol intake or obesity-induced steatosis. Recent development of new directly acting antiviral agents require evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HCV mutation, estimation of future carcinogenic risk, and even for the best way of management of patients with chronic hepatitis C. FSC seems one of the ideal methods of approximation for fibrotic stage of chronic hepatitis C. Repeated measurement is quite suitable for patients with an unestablished treatment or trial, every 1 or 2 years, for example. Because the current regression function was generated from the data of HCV-related chronic liver disease, this equation would not be suitable for the recognition of HBV-related chronic liver disease,[22] alcoholic liver disease and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies about individual diseases must be performed.

We compared the usefulness of the FSC with that of other fibrotic scores.[8,9,12,13] More simple and inexpensive AAR or APRI could not well estimate fibrotic stages with poor correlation coefficients of 0.021 and 0.462, which were much lower than the coefficient of FSC of 0.572. FibroTest, which contained three costly fibrotic markers (α2-macroglobulin, haptoglobin and apolipoprotein A1), also showed a low correlation coefficient of 0.415, suggesting that the usefulness was limited in HCV positive Asian patients. Although FIB-4 demonstrated the best coefficient of 0.440 among the fibrotic scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for rea17l histological classification. Because this study also measured those special markers included in FibroTest, the ability of discrimination of fibrotic stages could be compared among the five fibrotic scoring systems.

In conclusion, FSC was a useful and reliable biomarker for prediction of liver fibrosis in patients with chronic HCV infection. FSC is expected to be introduced and utilized in varied kinds of studies and trials. Its accuracy and reproducibility require further validation using more numbers of patients in several countries other than Japan.

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Original Article

Serum albumin level is a notable profiling factor for non-B, non-C hepatitis virus-related hepatocellular carcinoma: A data-mining analysis

Shingo Yamada,[1] Atsushi Kawaguchi,[4] Takumi Kawaguchi,[1,2] Nobuyoshi Fukushima,[1,6] Ryoko Kuromatsu,[1] Shuji Sumie,[1] Akio Takata,[1] Masahito Nakano,[1] Manabu Satani,[1] Tatsuyuki Tonan,[3] Kiminori Fujimoto,[3,7] Hiroji Shima,[8] Tatsuyuki Kakuma,[4] Takuji Torimura,[1,5] Michael R. Charlton[9] and Michio Sata[1,2]

[1]*Division of Gastroenterology, Department of Medicine, and Departments of* [2]*Digestive Disease Information and Research and* [3]*Radiology, Kurume University School of Medicine,* [4]*Biostatistics Center,* [5]*Liver Cancer Research Division, Research Center for Innovative Cancer Therapy, Kurume University,* [6]*Department of Gastroenterology, National Hospital Organization, Kyushu Medical Center,* [7]*Center for Diagnostic Imaging, Kurume University Hospital,* [8]*St Mary's Hospital, Kurume, Japan; and* [9]*Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA*

Aim: Various factors are underlying for the onset of non-B, non-C hepatitis virus-related hepatocellular carcinoma (NBNC-HCC). We aimed to investigate the independent risk factors and profiles associated with NBNC-HCC using a data-mining technique.

Methods: We conducted a case-control study and enrolled 223 NBNC-HCC patients and 669 controls from a health checkup database ($n = 176\,886$). Multivariate analysis, random forest analysis and a decision-tree algorithm were employed to examine the independent risk factors, factors distinguishing between the case and control groups, and to identify profiles for the incidence of NBNC-HCC, respectively.

Results: In multivariate analysis, besides γ-glutamyltransferase (GGT) levels and the Brinkman index, albumin level was an independent negative risk factor for the incidence of NBNC-HCC (odds ratio = 0.67; 95% confidence interval = 0.60–0.70; $P < 0.0001$). In random forest analysis, serum albumin level was the highest-ranked variable for distinguishing between the case and control groups (98 variable importance). A decision-tree algorithm was created for albumin and GGT levels, the aspartate aminotransferase-to-platelet ratio index (APRI) and the Brinkman index. The serum albumin level was selected as the initial split variable, and 82.5% of the subjects with albumin levels of less than 4.01 g/dL were found to have NBNC-HCC.

Conclusion: Data-mining analysis revealed that serum albumin level is an independent risk factor and the most distinguishable factor associated with the incidence of NBNC-HCC. Furthermore, we created an NBNC-HCC profile consisting of albumin and GGT levels, the APRI and the Brinkman index. This profile could be used in the screening strategy for NBNC-HCC.

Key words: lifestyle, metabolism, non-viral-related hepatoma, smoking

INTRODUCTION

LIVER CANCER IS the sixth most frequently diagnosed cancer worldwide and was the third most frequent cause of cancer-related death in 2008.[1] Although the incidence of liver cancer is increasing worldwide, the highest rate is found in East Asia. Hepatocellular carcinoma (HCC) accounts for 70–85% of the cases of primary liver cancer. The most significant risk factors for HCC are hepatitis C virus (HCV) and hepatitis B virus (HBV) infection. Although the incidence of HCV-related HCC has recently been decreasing,[2] the incidence of non-B, non-C hepatitis-related HCC (NBNC-HCC) in Japan has risen to 27.6% from 7.6% in the last 15 years.[2]

Correspondence: Dr Takumi Kawaguchi, Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine. 67 Asahi-machi, Kurume 830-0011, Japan. Email: takumi@med.kurume-u.ac.jp

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