studies also suggest that a shorter duration of treatment is effective in only some rapid viral responders with genotype 2 or 3.[12,13] The benefits of a short duration of combination therapy in patients with genotype 2 thus remain controversial.

In patients with genotype 2 who do not have viral clearance at week 4, the SVR rate decreases to about 50%,[8,12] indicating that the response to 24 weeks of combination therapy is unsatisfactory in slow viral responders. In genotype 1, extending therapy improves the SVR rate in patients with delayed viral response (i.e., HCV-RNA becomes undetectable between 13 and 24 weeks after starting treatment).[14–16] These findings indicate that response-guided therapy is one of the most efficient methods for treatment. A recent meta-analysis showed that 72-week extended treatment improved the SVR rate in slow responders with HCV genotype 1.[17] To our knowledge, however, no study has assessed the value of extending therapy for slow viral responders with genotype 2.

In the present study, we evaluated the antiviral effectiveness of response-guided therapy in patients with HCV genotype 2 who received both shortened and extended durations of SOC treatment. The duration of combination therapy was decided according to HCV RNA clearance on Taqman polymerase chain reaction (PCR) analysis at week 4. Patients who had a rapid viral response could select either 16 weeks or 24 weeks of therapy. Patients with a slow viral response could select 24 weeks or 48 weeks of therapy. We examined the SVR rate and evaluated correlations between IL28B genotype and viral response in different subgroups of patients.

METHODS

Patients

A TOTAL OF 154 patients infected with HCV genotype 2 received PEG-IFN plus ribavirin between March 2006 and December 2010 at Osaka City University Hospital, Osaka, Japan. We studied 105 patients who received more than 75% of the dosage specified in the protocol (Fig. 1).

Inclusion criteria were as follows: viral load (HCV RNA) ≥ 5 Log IU/mL of HCV genotype 2; white cell count ≥3000/mm^3; neutrophil count ≥1500/mm^3; platelet count ≥90 000/mm^3; and hemoglobin level ≥12 g/dL. Patients were excluded if they had hepatitis B surface antigen, human immunodeficiency virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, cirrhosis, current alcohol abuse or a history of alcohol abuse (>20 g/day), psychiatric conditions, previous liver transplantation, or evidence of hepatocellular carcinoma. The following demographic and virologic characteristics were recorded at baseline: age, sex, body mass index (BMI), HCV genotype, HCV RNA viral load, white cell count, hemoglobin level, platelet count, alanine aminotransferase (ALT) level, and γ-glutamyltransferase (γ-GTP) level.

Study design

All patients received PEG-IFN-α-2b (PegIntoron, MSD K.K., Tokyo, Japan) at a dose of 1.5 μg/kg subcutaneously once weekly and ribavirin (Rebetol, MSD K.K.) at a dose of 600 to 1000 mg/day according to body weight (<60 kg, 600 mg/day; 60–80 kg, 800 mg/day; >80 kg, 1000 mg/day).

Rapid viral response (RVR) was defined as no detectable HCV RNA in serum 4 weeks after the start of treatment, and SVR was defined as no detectable HCV RNA in serum at 24 weeks after the end of treatment.

The patients were classified into four groups according to the viral response to combination therapy. We proposed 16 weeks of therapy to patients with RVR. Patients who accepted our proposal were treated for a short duration (group A), and those who refused a short duration of treatment were treated for 24 weeks (group B). For patients without RVR, we proposed 48 weeks of therapy. Patients who accepted our proposal were treated for an extended duration (group D), and those who refused were treated for 24 weeks (group C).

Virology

HCV RNA analysis was performed with COBAS TaqMan HCV test (Taqman, Roche Diagnostics, K.K., Tokyo, Japan). The linear dynamic range of the assay was 1.2–7.8 Log IU/mL. Samples with undetectable HCV-RNA were defined as negative. Quantitative HCV RNA analyses were performed 4, 8, and 12 weeks after the start of treatment, at the end of treatment (weeks 16, 24, or 48), and 24 weeks after the end of treatment (weeks 40, 48, or 72). All quantifications were performed at a clinical testing laboratory (SRL, Inc., Tokyo, Japan) with the use of ethylene diamine tetraacetic acid plasma, which had been stored at −70 °C until use. The HCV genotype was determined by Okamoto's method.[18]

IL28B genotyping

We examined single nucleotide polymorphisms (SNP) of IL28B in patients who consented to genome analysis. Genomic DNA was extracted from whole blood samples

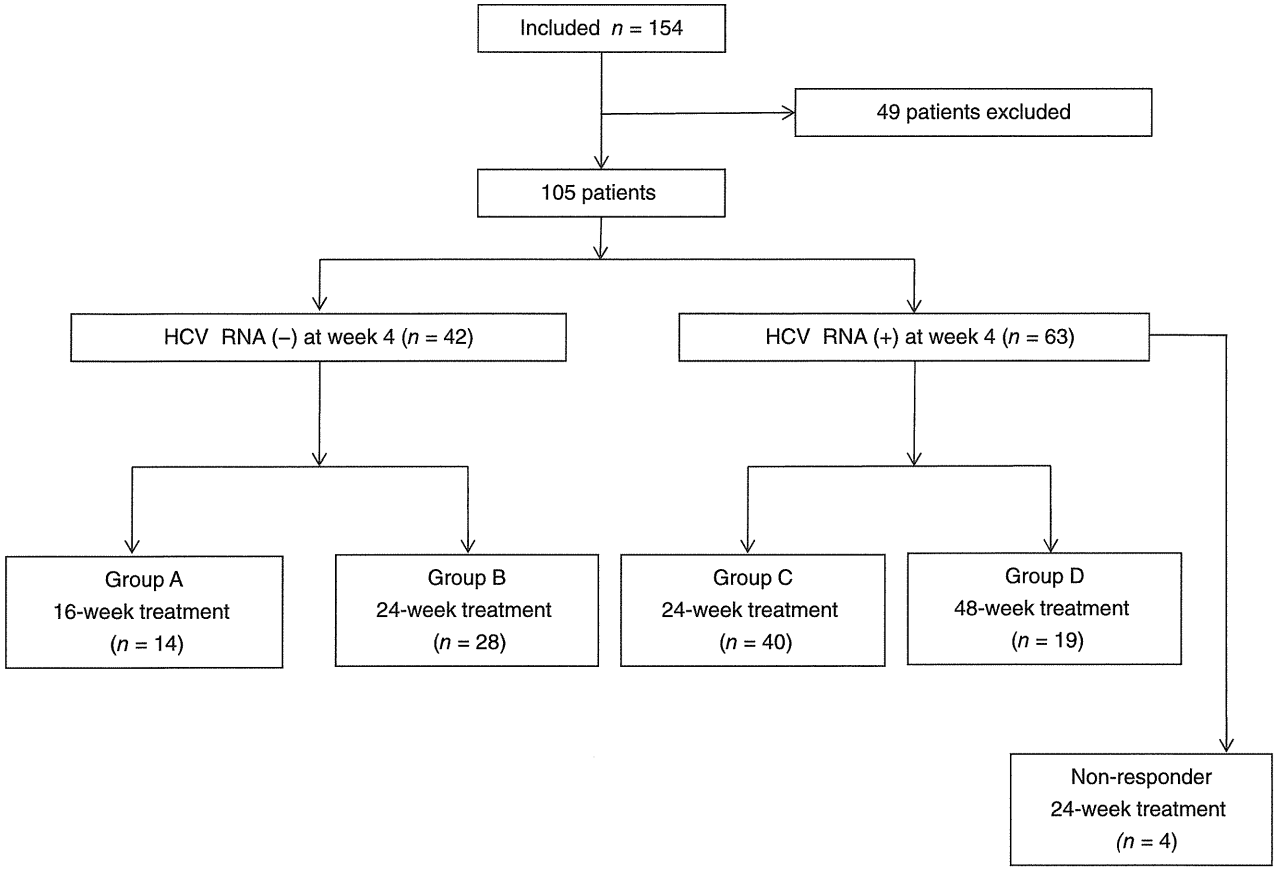

Figure 1 Enrollment and follow-up of patients receiving response-guided therapy. Forty-nine patients were excluded for the following reasons: 30 patients received less than 75% of the protocol dose of pegylated interferon, ribavirin, or both as specified in the protocol; seven patients were excluded because they discontinued treatment by week 12; and 12 patients did not comply with the protocol requirements for response-guided therapy.

obtained from each patient. A genetic polymorphism located upstream of the IL28B gene, rs8099917, was determined by a TaqMan PCR assay.[19] Heterozygosity (T/G) or homozygosity (G/G) for the minor allele (G) was defined as IL28B minor type, whereas homozygosity for the major allele (T/T) was defined as IL28B major type.

Statistical analysis

Statistical analysis was performed using SAS version 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). Distributions of continuous variables were analyzed with the Mann–Whitney *U*-test. Differences in proportions were tested by Fisher's exact test. The Cochran-Armitage test was used to check for trends in binomial proportions across levels of a single factor. The Cochran–Mantel–Haenszel test was used to analyze stratified categorical data. Two-tailed *P*-values of <0.05 were considered to indicate statistical significance.

Ethical considerations

Informed consent was obtained from all subjects. The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the Ethics Committee of Osaka City University Graduate School of Medicine.

RESULTS

Clinical differences between patients with RVR and those with non-RVR

THE CLINICAL CHARACTERISTICS and treatment response rates of the 105 patients are summarized in Tables 1, 2. Forty-two (40%) of the 105 patients

Table 1 Comparison of characteristics between patients with rapid viral response (RVR) and patients without RVR

	RVR ($n = 42$)	Non-RVR ($n = 63$)	*P*-value
Age, years	47 (20–71)	57 (27–69)	0.007
Gender (M/F)	21/21	20/43	0.061
BMI (kg/m^2)	21.9 (17.1–39.1)	22.6 (16.1–40.1)	0.857
HCV genotype (2a/2b)	30/12	42/21	0.606
HCV RNA load (LogIU/mL)	5.9 (5.0–7.1)	6.5 (5.0–7.4)	<0.001
WBC (/μL)	5100 (3200–8200)	4300 (3000–9700)	0.006
Neutrophils (/μL)	2491 (1504–4578)	2173 (1282–4788)	0.311
Hb (g/dl)	13.8 (12.0–17.0)	13.7 (12.0–17.9)	0.672
Platelets (10^4/μL)	20.4 (9.3–32.3)	16.3 (9–34.1)	0.087
ALT (IU/L)	65 (13–449)	39 (15–303)	0.143
γ-GTP (IU/L)	33 (9–269)	24 (9–266)	0.272
IL28B genotype (major/minor)	38/4	49/14	0.091

All quantitative variables are expressed as median values.
ALT, alanine aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyltransferase; Hb, hemoglobin; HCV, hepatitis C virus; WBC, white cell count.

achieved an RVR as determined by real-time PCR. HCV RNA was detected in 63 (60%) of 105 patients at week 4, 18 patients (17%) at week 8, and four patients (4%) at week 24. Analysis of IL28B genotype showed that 87 patients were homozygotes for the major allele (T/T), 18 patients were heterozygotes (T/G), and none were homozygotes for the minor allele (G/G). RVR was significantly associated with a lower viral load at enrollment, a higher white cell count, and younger age. Hemoglobin level, platelet count, HCV genotype, host IL28B genotype, and BMI were not related to RVR (Table 1).

Hepatitis C virus RNA was continuously detected during 24 weeks of combination therapy in four patients (non-responders) and treatment was stopped at week 24. As compared with the other patients, non-responders were older (mean age, 63 years; range, 58–69). Two of the four non-responders had IL28B minor type. SVR was achieved in 86 (85%) of the remaining 101 patients (Fig. 2).

Response-guided therapy for RVR patients

Fourteen patients with RVR selected 16 weeks of therapy (group A), and 28 selected 24 weeks of therapy (group

Table 2 Comparison of group A and group B among patients with rapid viral response (RVR)

	Group A (16 W) ($n = 14$)	Group B (24 W) ($n = 28$)	*P*-value
Age, years	45 (20–65)	58 (25–71)	0.256
Gender (M/F)	11/3	10/18	0.02
BMI (kg/m^2)	21.9 (18.1–28.1)	22.1 (17.1–39.2)	0.8
HCV genotype (2a/2b)	7/7	23/5	0.067
HCV RNA load (LogIU/mL)	6.4 (5.0–6.7)	5.7 (5.0–7.1)	0.109
WBC (/μL)	6100 (3700–8200)	4850 (3200–8100)	0.008
Neutrophils (/μL)	2551 (1642–4025)	2370 (1504–4571)	0.139
Hb (g/dl)	14.7 (12–17)	13.5 (12.1–16.9)	0.125
Platelets (10^4/μL)	18.0 (9.3–32.2)	21.2 (9.9–32.2)	0.566
ALT (IU/L)	60 (20–253)	74 (13–449)	0.989
γ-GTP (IU/L)	40 (13–174)	29 (9–269)	0.191
IL28B genotype (major/minor)	12/2	27/1	0.1

All quantitative variables are expressed as median values.
ALT, alanine aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyltransferase; Hb, hemoglobin; HCV, hepatitis C virus; WBC, white cell count.

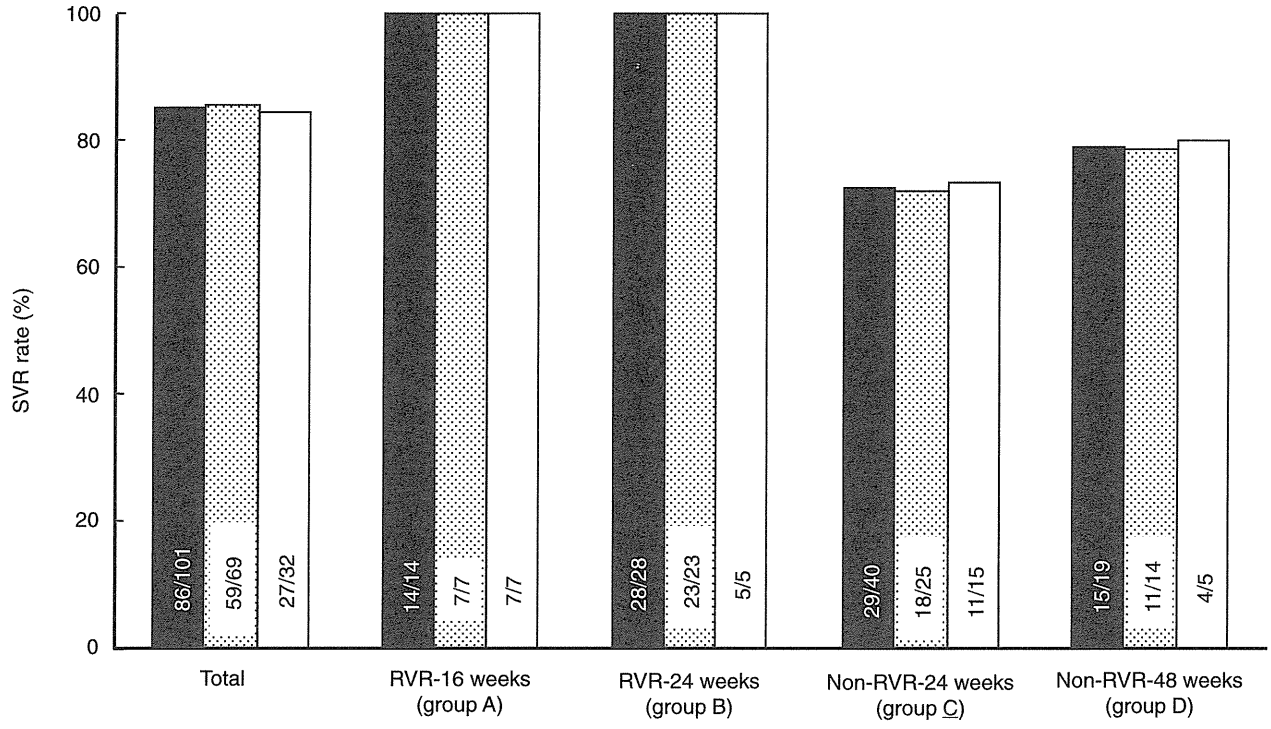

Figure 2 Rates of sustained viral response (SVR) in patients assigned to receive treatment for 16, 24, or 48 weeks in the per-protocol analysis. ■: All patients with genotype 2; ▨: Patients with genotype 2a; □: Patients with genotype 2b. All patients with rapid viral response (RVR) achieved SVR. In patients without RVR, the SVR rates in the 24- and 48-week treatment groups were almost equal.

B). There were no significant differences in age, BMI, viral load, hemoglobin level, platelet count, ALT, γ-GTP, HCV genotype, or IL28B genotype between the two groups. Only white cell count and gender differed significantly (Table 2). All patients in both groups achieved SVR (Fig. 3).

Clinical differences between SVR group and non-SVR group among patients without RVR

Among 59 patients with non-RVR, 44 achieved an SVR. Baseline HCV RNA load was significantly lower in the SVR group than in the non-SVR group ($P = 0.031$). The time of HCV RNA disappearance differed significantly between the SVR group and non-SVR group ($P = 0.006$; Table 3).

Response-guided therapy for non-RVR patients

Forty patients with non-RVR selected 24 weeks of therapy (group C), and 19 selected 48 weeks of therapy (group D). There were no significant differences in age, gender, viral load, white blood cell count, ALT, γ-GTP, HCV genotype, or IL28B genotype between the two groups (Table 4). Group C consisted of 34 patients in whom HCV RNA disappeared at 5–8 weeks, five at 9–12 weeks, and one at 13–16 weeks. Group D consisted of 11 patients in whom HCV RNA disappeared at 5–8 weeks, and 8 at 9–12 weeks. The proportion of patients in whom HCV RNA disappeared at a later week of treatment was slightly higher in group D ($P = 0.0578$). The SVR rate was similar in group C (73%) and group D (79%) (Fig. 2). As shown in detail in Figure 3, among patients in whom no HCV RNA was detected at week 8, SVR was achieved in 28 (82%) of 34 patients in group C and 10 (91%) of 11 patients in group D. Among patients in whom HCV RNA was positive at week 8 and negative at week 12, SVR was achieved in one (20%) of five patients in group C and five (63%) of eight patients in group D. Thus, in the slower viral responders, the SVR rate was improved by extending the duration of therapy.

In group C, 23 (74%) of 31 patients with IL28B major type achieved an SVR, and six (67%) of nine patients with IL28B minor type achieved an SVR ($P = 0.983$). In group D, 13 (81%) of 16 patients with IL28B major type

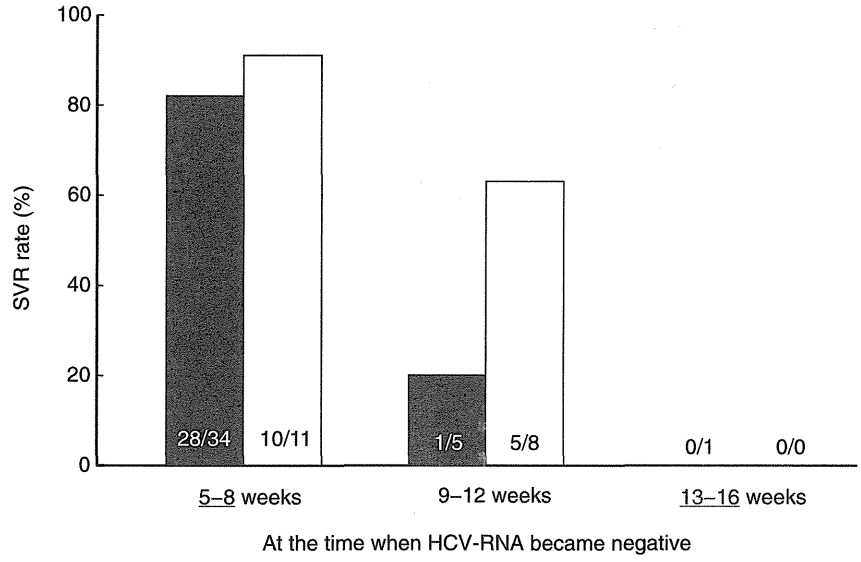

Figure 3 Rates of sustained viral response (SVR) among patients without rapid viral response (RVR). ▬: Patients given 24 weeks of treatment; □: Patients given 48 weeks of treatment. Patients in whom HCV RNA was positive at week 4 and negative at week 8 were classified into the 24-week treatment group or 48-week treatment group. The SVR rate was 82% in the 24-week treatment group and 91% in the 48-week treatment group. Patients in whom HCV RNA was positive at week 8 and negative at week 12 were classified into the 24-week treatment group or 48-week treatment group. The SVR rate was 20% in the former group and 63% in the latter. Patients in whom HCV RNA was positive at week 12 fail to achieve SVR in the 24-week treatment group. The SVR rate after extended treatment was higher than that of the standard duration of treatment.

Table 3 Comparison of characteristics between sustained viral response (SVR) group and non-SVR group among patients without rapid viral response (RVR)

	SVR ($n = 44$)	Non-SVR ($n = 15$)	*P*-value
Age, years	56 (27–67)	60 (28–69)	0.589
Gender (M/F)	14/30	5/10	0.914
BMI (kg/m^2)	22.7 (16.1–40.1)	22.7 (19.8–34.1)	0.338
HCV genotype (2a/2b)	29/15	10/5	0.957
HCV RNA load (LogIU/mL)	5.88 (5.0–7.1)	6.48 (5.0–7.4)	0.031
WBC (/μL)	4350 (3000–9700)	4800 (3500–8500)	0.219
Neutrophils (/μL)	2186 (1344–4788)	2294 (1536–4581)	0.414
Hb (g/dl)	13.7 (12.0–17.5)	14.1 (12.0–17.9)	0.276
Platelets (10^4/μL)	17.1 (9–28.7)	16.3 (10.1–34.1)	0.903
ALT (IU/L)	47 (17–303)	30 (15–114)	0.077
γ-GTP (IU/L)	24 (9–266)	21 (14–99)	0.944
IL28B genotype (major/minor)	36/8	11/4	0.481
Treatment duration (24 w/48 w)	29/15	11/4	0.832
No. patients at week with no detectable HCV RNA	38 at week 8, 7 at week-12,	7 at week 8, 6 at week-12 1 at week-16	0.006

All quantitative variables are expressed as median values.
ALT, alanine aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyltransferase; Hb, hemoglobin; HCV, hepatitis C virus; WBC, white cell count.

Table 4 Comparison of group C and group D among patients without rapid viral response (RVR)

	Group C (24 W) ($n = 40$)	Group D (48 W) ($n = 19$)	*P*-value
Age, years	56 (27–69)	57 (38–69)	0.111
Gender (M/F)	13/27	6/13	1
BMI (kg/m^2)	22.5 (17.5–40.1)	23.2 (16.1–29.4)	0.82
HCV genotype (2a/2b)	25/15	5/14	0.558
HCV RNA load (LogIU/mL)	6.5 (5.0–7.4)	6.7 (5.7–7.2)	0.124
WBC (/μL)	4350 (3000–8500)	4400 (3100–9700)	0.465
Neutrophils (/μL)	2001 (1510–4788)	2415 (1502–4548)	0.094
Hb (g/dl)	13.8 (12–17.9)	13.4 (12.2–17.1)	0.532
Platelets (10^4/μL)	16.2 (9–34.1)	17.0 (10.1–23.7)	0.548
ALT (IU/L)	49 (15–259)	32 (15–303)	0.202
γ-GTP (IU/L)	25 (9–266)	20 (12–155)	0.758
IL28B genotyping (major/minor)	31/9	16/3	0.734

All quantitative variables are expressed as median values.
ALT, alanine aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyltransferase; Hb, hemoglobin; HCV, hepatitis C virus; WBC, white cell count.

achieved an SVR, and two of three patients with IL28B minor type achieved an SVR ($P = 0.839$; Fig. 4).

DISCUSSION

IN THE PRESENT study, we performed response-guided therapy according to whether HCV RNA disappeared at week 4 in patients with HCV genotype 2 who received SOC treatment. The initial 16 weeks of combination therapy completely eradicated HCV in all RVR patients with genotype 2, in contrast to the results of previous studies.[9,10] Disparate results may have been caused by critical differences in study design. First, we monitored HCV RNA by real-time PCR (lower detection

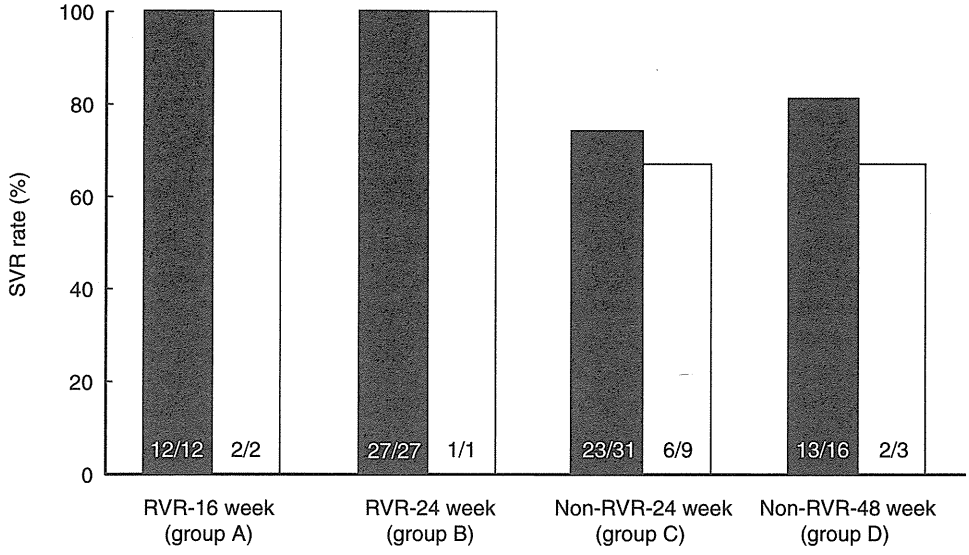

Figure 4 Rates of sustained viral response (SVR) classified according to IL28B (rs8099917) genotype and treatment group. ■, Patients with IL28B major type; □, Patients with IL28B minor type. In patients with rapid viral response (RVR), patients with both major and minor types of IL-28B single nucleotide polymorphism (SNP) achieved an SVR. In patients without RVR, SVR rates in the 24-week treatment group were 74% in IL28B major type (TT) and 67% in IL28B minor type (TG) ($P = 0.983$). SVR rates in the 48 weeks treatment group were 81% in IL28B major type and 67% in minor type ($P = 0.839$).

limit, 15 IU/mL), whereas previous studies used the COBAS AMPLICORE HCV test (lower detection limit, 50 IU/mL).[9,11,12] We thus speculate that RVR was more accurately determined in the present study than in previous reports. Second, we studied patients who received more than 75% of the dosage specified in the protocol to avoid potential effects of inadequate treatment. Third, we limited our study group to patients with an HCV RNA load exceeding 5 Log copies/mL. Japanese guidelines recommend combination therapy only for patients with a high viral load, because a high proportion of patients with an HCV-RNA load of less than 5 Log copies/mL achieve a higher SVR after PEG-IFN monotherapy (81%).[20] However, our study also had several limitations. For example, it was not a randomized controlled study, and the number of subjects was lower than that in previous studies. The antiviral effectiveness of short-term therapy in adequate numbers of patients with RVR should therefore be confirmed by randomized controlled studies using sensitive methods to detection HCV RNA, such as real-time PCR.

After excluding patients with no response, we compared antiviral effectiveness between standard therapy and extended therapy. In non-RVR patients, the SVR rate did not differ significantly between 24 weeks of therapy and 48 weeks of therapy. However, among patients in whom HCV RNA was positive at week 4 and negative at week 8, as well as those in whom HCV RNA was positive at week 8 and negative at week 12, 48-week extended therapy achieved a higher SVR rate than 24 weeks of therapy. These results indicate that extended therapy is an effective treatment option for patients with genotype 2 who have a slow viral response. In patients with genotype 1 who have a late viral response, prolonging therapy prevents viral relapse and has been recommended by treatment guidelines.[21,22] To our knowledge, similar clinical trials have not been performed previously in patients with genotype 2. Randomized control studies assessing the benefits of extending therapy in patients with genotype 2 are not easy to perform because only a small proportion of such patients have a slow viral response to combination therapy. Our findings will hopefully encourage further studies designed to determine the optimal duration of combination therapy for patients with HCV genotype 2.

We examined the antiviral effectiveness of extending therapy according to IL28B genotype. In patients with IL28B major type, extending therapy prevented viral relapse slightly, but not significantly as compared with 24 weeks of therapy. Previous studies using a standard duration of combination therapy showed that IL28B genotype was associated with RVR and SVR in Asian patients with genotype 2.[23–25] An Italian group found that IL28B genotype was associated with SVR in genotype 2 or 3 patients with non-RVR.[26] A recent study suggested that IL28B genotyping was of limited clinical value for the management of response-guided therapy in patients with genotype 1.[27] It is thus necessary to further evaluate the usefulness of IL28B genotyping as a predictor of the need for extending therapy in patients with genotype 2.

Only a few patients with genotype 2 had a slow viral response or no response to PEG-IFN plus ribavirin. Our results suggest that extending therapy can achieve an SVR in approximately 50% of patients with slow viral response. However, PEG-IFN plus ribavirin is unlikely to eradicate HCV in non-responders, even if therapy is extended. A recent study reported that adding telaprevir to combination therapy achieved an SVR in all enrolled patients with genotype 2 or 3.[28] Triple therapy may thus be a good option for genotype 2 patients with a slow or no response to SOC treatment. However, it is important to recognize that not all patients with genotype 2 require triple therapy to achieve an SVR.

In conclusion, 16 weeks of response-guided therapy with PEG-IFN plus ribavirin produced an adequate antiviral effect in RVR patients with HCV genotype 2 who received more than 75% of the dosage specified in the protocol. Extending therapy could not significantly improve SVR rate in non-RVR patients of the present study. In patients with a slower viral response, extending therapy achieved a higher rate of SVR.

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REFERENCES

1 Lauer G, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41–52.
2 Alter MJ. Global epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 2436–41.
3 Butt AA. Hepatitis C virus infection: the new global epidemic. *Expert Rev Anti Infect Ther* 2005; **3**: 241–9.
4 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.

5 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958–65.
6 Jacobson IM, McHutchison JG, Dusheiko G *et al.* Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405–16.
7 Poordad F, McCone J Jr, Bacon BR *et al.* Boceprevir for untreated chronic HCV genotype1 infection. *N Engl J Med* 2011; **364**: 1195–206.
8 von Wagner M, Huber M, Berg T *et al.* Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; **129**: 522–7.
9 Mangia A, Santoro R, Minerva N *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; **352**: 2609–17.
10 Yu ML, Dai CY, Huang JF *et al.* A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; **56**: 553–9.
11 Shiffman ML, Suter F, Bacon BR *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; **357**: 124–34.
12 Dalgard O, Bjøro K, Ring-Larsen H *et al.* Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. *Hepatology* 2008; **47**: 35–42.
13 Lagging M, Langeland N, Pedersen C *et al.* Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. *Hepatology* 2008; **47**: 1837–45.
14 Watanabe S, Enomoto N, Koike K *et al.* Prolonged treatment with pegylated interferon alpha 2b plus ribavirin improves sustained virological response in chronic hepatitis C genotype 1 patients with late response in a clinical real-life setting in Japan. *Hepatol Res* 2010; **40**: 135–44.
15 Chayama K, Hayes CN, Yoshioka K *et al.* Factors predictive of sustained virological response following 72 weeks of combination therapy for genotype 1b hepatitis C. *J Gastroenterol* 2011; **46**: 545–55.
16 Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; **46**: 1688–94.
17 Di Martino V, Richou C, Cervoni JP *et al.* Response-guided Peg-interferon plus ribavirin treatment duration in chronic hepatitis C: meta-analyses of randomized controlled trials and implications for the future. *Hepatology* 2011; **54**: 789–800.
18 Okamoto H, Tokita H, Sakamoto M *et al.* Characterization of the genomic sequence of type V (or 3a) hepatitis C virus isolates and PCR primers for specific detection. *J Gen Virol* 1993; **74**: 2385–90.
19 Nishida N, Tanabe T, Takasu M *et al.* Further development of multiplex single nucleotide polymorphism typing method, the DigiTag2 assay. *Anal Biochem* 2007; **364**: 78–85.
20 Iwasaki Y, Shiratori Y, Hige S *et al.* A randomized trial of 24 versus 48 weeks of peginterferon α-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan. *Hepatol Int* 2009; **3**: 468–79.
21 Ghany MG, Strader DB, Thomas DL *et al.* American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335–74.
22 Kumada H, Okanoue T, Onji M *et al.* The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, Ministry of Health, Labour and Welfare of Japan. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 8–13.
23 Kawaoka T, Hayes CN, Ohishi W *et al.* Predictive value of the IL28B polymorphism on the effect of interferon therapy in chronic hepatitis C patients with genotypes 2a and 2b. *J Hepatol* 2011; **54**: 408–14.
24 Yu ML, Huang CF, Huang JF *et al.* Role of Interleukin-28B Polymorphisms in the Treatment of Hepatitis C Virus Genotype 2 Infection in Asian Patients. *Hepatology* 2011; **53**: 7–13.
25 Sakamoto N, Nakagawa M, Tanaka Y *et al.* Association of IL28B variants with response to pegylated-interferon alpha plus ribavirin combination therapy reveals intersubgenotypic differences between genotypes 2a and 2b. *J Med Virol* 2011; **83**: 871–8.
26 Mangia A, Thompson AJ, Santoro R, Piazzolla V *et al.* An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010; **139**: 821–7.
27 Mangia A, Thompson AJ, Santoro R *et al.* Limited utility of IL28B in the setting of response-guided treatment with detailed on-treatment virological monitoring. *Hepatology* 2011; **54**: 722–80.
28 Foster GR, Hézode C, Bronowicki JP *et al.* Telaprevir alone or with peginterferon and ribavirin reduces HCV RNA in patients with chronic genotype 2 but Not 3 infections. *Gastroenterology* 2011; **141**: 881–9.

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Letter to the Editor

Anti-hepatitis B virus therapy: To stop, or not to stop: Has the question been solved?

Nucleos(t)ide analogs (NA) induce on-treatment response in most patients with chronic hepatitis B, but post-treatment relapse is common. Long-term use of NA triggers drug resistance. Therefore, the end-point of NA treatment remains unclear. Hepatitis B core-related antigen (HBcrAg) reflects intrahepatic covalently closed circular DNA[1–3] and can be a marker for the safe discontinuation of NA,[4,5] similar to hepatitis B surface antigen (HBsAg).[6,7]

We read with interest the report by Matsumoto *et al.*,[8] who proposed a model using a combination of HBcrAg and HBsAg to predict the risk of relapse after discontinuation of NA. The utility of this model remains to be validated by an independent group. We studied the reliability of the model for predicting post-treatment relapse.

First, we retrospectively studied 14 patients who discontinued NA therapy. The median duration of treatment was 1.8 years (1.1–8.3). All patients were hepatitis B e antigen (HBeAg) negative at discontinuation of NA. Nine patients (64%) had relapse after a median post-treatment period of 1.0 years (0.1–7.6). A univariate comparison of relapsers versus non-relapsers showed that the HBsAg level at discontinuation was higher in relapsers than in non-relapsers (median, 3.4 vs 2.5 $\log_{10}$ IU/mL; $P = 0.039$); HBcrAg (median, 4.2 vs 3.4 $\log_{10}$ U/mL; $P = 0.11$) and duration of treatment (median, 1.6 vs 2.2 years; $P = 0.74$) did not differ between the groups. When classified into three groups according to the sum scores of HBcrAg and HBsAg levels at discontinuation, the proportion of patients without relapse in each group was consistent with their findings (Fig. 1).

Second, we measured HBcrAg and HBsAg at the most recent visit in 100 patients who were receiving entecavir (73 NA-naïve and 27 switched from lamivudine). Inclusion criteria were as follows: entecavir administrated for more than 6 months; HBeAg negative; and hepatitis B virus DNA of less than 3.0 $\log_{10}$ copies/mL. The median duration of treatment was 3.5 years (0.5–10.8). Eight patients (8.0%) fulfilled the criteria for group 1 of Matsumoto's classification, associated with the lowest risk of relapse (i.e. HBcrAg <3.0 $\log_{10}$ U/mL and HBsAg <1.9 $\log_{10}$ IU/mL).

Lastly, we studied 37 patients with HBeAg positive chronic hepatitis B who had received sequential therapy starting with lamivudine for 6 months ($n = 28$) or entecavir for 12 months ($n = 9$), followed by interferon for 6 months (after a short period of concomitant administration). The changes in serum levels of HBcrAg and HBsAg during and after sequential therapy are shown in Supplementary Figure S1. HBcrAg did not decrease to 4.0 $\log_{10}$ U/mL in any patient, and HBsAg fell to between 1.9 and 2.8 $\log_{10}$ IU/mL in only three patients (11%) at NA discontinuation. However, sustained

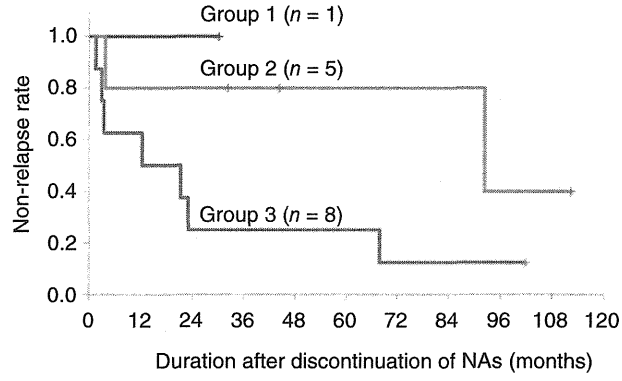

Figure 1 Comparison of non-relapse rates as estimated by the Kaplan–Meier method among three groups classified according to the sum scores of hepatitis B core-related antigen (HBcrAg) and hepatitis B surface antigen (HBsAg) levels at the time of nucleos(t)ide analog (NA) discontinuation. Group 1 consists of patients with a total score of 0, group 2 of patients with a total score of 1–2 and group 3 of patients with a total score of 3–4. The scores were calculated by assigning a score of 0 for an HBcrAg level of <3.0 $\log_{10}$ U/mL, 1 for a level from 3.0–3.9 $\log_{10}$ U/mL, and 2 for a level of ≥4.0 $\log_{10}$ U/mL; and a score of 0 for an HBsAg level of <1.9 $\log_{10}$ IU/mL, 1 for a level from 1.9–2.8 $\log_{10}$ IU/mL and 2 for a level of ≥2.9 $\log_{10}$ IU/mL. The log-rank test was used to test hypotheses concerning differences in the occurrence of non-relapse between the groups ($P = 0.16$).

Conflict of interest: none to declare.

response was achieved in 11 patients (30%) 24 weeks after completion of therapy.

In conclusion, our results suggest that the combination of HBcrAg and HBsAg as reported by Matsumoto *et al.*[8] can be used to guide the safe discontinuation of NA in patients with chronic hepatitis B infection, albeit only a small proportion fulfilled the proposed criteria. Sequential switching of therapy to interferon potentially prevents relapse after discontinuation of NA, even in patients not meeting the criteria. Prospective studies are required to further validate the utility of the proposed model.

Ritsuzo Kozuka, Masaru Enomoto,
Hiroyasu Morikawa, Akihiro Tamori
and Norifumi Kawada
Department of Hepatology, Osaka City University Graduate School of Medicine, Osaka, Japan

REFERENCES

1 Rokuhara A, Tanaka E, Matsumoto A *et al.* Clinical evaluation of a new enzyme immunoassay for hepatitis B virus core-related antigen; a marker distinct from viral DNA for monitoring lamivudine treatment. *J Viral Hepat* 2003; **10:** 324–30.
2 Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. *J Clin Microbiol* 2007; **45:** 3942–7.
3 Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. *J Med Virol* 2009; **81:** 27–33.
4 Shinkai N, Tanaka Y, Orito E *et al.* Measurement of hepatitis B virus core-related antigen as predicting factor for relapse after cessation of lamivudine therapy for chronic hepatitis B virus infection. *Hepatol Res* 2006; **36:** 272–6.
5 Matsumoto A, Tanaka E, Minami M *et al.* Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. *Hepatol Res* 2007; **37:** 661–6.
6 Chan HL, Wong GL, Chim AM, Chan HY, Chu SH, Wong VW. Prediction of off-treatment response to lamivudine by serum hepatitis B surface antigen quantification in hepatitis B e antigen-negative patients. *Antivir Ther* 2011; **16:** 1249–57.
7 Liu F, Wang L, Li XY *et al.* Poor durability of lamivudine effectiveness despite stringent cessation criteria: a prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients. *J Gastroenterol Hepatol* 2011; **26:** 456–60.
8 Matsumoto A, Tanaka E, Suzuki Y *et al.* Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B. *Hepatol Res* 2012; **42:** 139–49.

SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article:

Figure S1 Changes in serum levels of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) during and after sequential therapy with NA and interferon. When sustained responders were compared with non-responders, there was no significant difference in the serum HBsAg level. In contrast, the serum HBcrAg level was significantly lower in sustained responders than in non-responders at 24 weeks after completion of sequential therapy ($P = 0.0006$).

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RESEARCH ARTICLE Open Access

Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population

Yoshio Sumida[1], Masato Yoneda[2], Hideyuki Hyogo[3], Yoshito Itoh[4], Masafumi Ono[5*], Hideki Fujii[6], Yuichiro Eguchi[7], Yasuaki Suzuki[8], Noriaki Aoki[9], Kazuyuki Kanemasa[1], Koji Fujita[2], Kazuaki Chayama[3], Toshiji Saibara[5], Norifumi Kawada[6], Kazuma Fujimoto[7], Yutaka Kohgo[8], Toshikazu Yoshikawa[4] and Takeshi Okanoue[10], for Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD)

Abstract

Background: A reliable and inexpensive noninvasive marker of hepatic fibrosis is required in patients with nonalcoholic fatty liver disease (NAFLD). FIB4 index (based on age, aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels, and platelet counts) is expected to be useful for evaluating hepatic fibrosis. We validated the performance of FIB4 index in a Japanese cohort with NAFLD.

Methods: The areas under the receiver operating characteristic curves (AUROC) for FIB4 and six other markers were compared, based on data from 576 biopsy-proven NAFLD patients. Advanced fibrosis was defined as stage 3-4 fibrosis. FIB4 index was assessed as: age (yr) × AST (IU/L)/(platelet count (10^9/L) × √ALT (IU/L))

Results: Advanced fibrosis was found in 64 (11%) patients. The AUROC for FIB4 index was superior to those for the other scoring systems for differentiating between advanced and mild fibrosis. Only 6 of 308 patients with a FIB4 index below the proposed low cut-off point (< 1.45) were under-staged, giving a high negative predictive value of 98%. Twenty-eight of 59 patients with a FIB4 index above the high cut-off point (> 3.25) were over-staged, giving a low positive predictive value of 53%. Using these cutoffs, 91% of the 395 patients with FIB-4 values outside 1.45-3.25 would be correctly classified. Implementation of the FIB4 index in the Japanese population would avoid 58% of liver biopsies.

Conclusion: The FIB4 index was superior to other tested noninvasive markers of fibrosis in Japanese patients with NAFLD, with a high negative predictive value for excluding advanced fibrosis. The small number of cases of advanced fibrosis in this cohort meant that this study had limited power for validating the high cut-off point.

Background

Type 2 diabetes mellitus is associated with nonalcoholic fatty liver disease (NAFLD) in clinical practice. NAFLD includes a wide spectrum of liver diseases ranging from simple steatosis, which is usually a benign and non-progressive condition, to nonalcoholic steatohepatitis (NASH), which can progress to liver cirrhosis (LC) and hepatocellular carcinoma (HCC) in the absence of significant alcohol consumption [1-4]. Liver biopsy remains a reliable tool for the diagnosis of NASH [1,5,6], and the most sensitive and specific method for providing prognostic information. However, it may not be practical to perform liver biopsies in every patient with NAFLD to ascertain the presence of NASH [6]. Moreover, biopsies are associated with significant limitations such as pain, risk of severe complications, sampling errors [7,8], cost, and patient unwillingness to undergo invasive testing. Since it is not easy to distinguish simple steatosis from NASH in diabetes clinics, simple scoring systems to derive progressive NASH are required. Numerous noninvasive panels of tests have been developed to stage liver disease, including a combination of clinical and routine laboratory parameters, as well as specialized tests involving direct markers of fibrosis and elastography [9-20]. Of these, the BAAT (body mass index [BMI], age, alanine aminotransferase [ALT],

* Correspondence: onom@kochi-u.ac.jp
[5]Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi, Japan
Full list of author information is available at the end of the article

triglycerides) [14], European liver fibrosis (ELF) score [10], Fibrotest (BioPredictive, Paris, France) [9], Fibroscan (Echosens, Paris, France) [12], acoustic radiation force impulse elastography (Mochida Siemens Medical System Co. Ltd., Tokyo, Japan) [15], hyaluronic acid (HA) [16,17], type IV collagen 7S [18], BARD (BMI, aspartate aminotransferase [AST]/ALT ratio [AAR], diabetes mellitus [DM]) [19], N (Nippon) score [20] and the NAFLD fibrosis score (NFS) [21] have been tested in subjects with NAFLD.

The FIB4 index was developed as a noninvasive panel to stage liver disease in subjects with human immunodeficiency virus and hepatitis C virus (HCV) co-infection [22]. It relies on patient age, AST, ALT, and platelet count, which are routinely measured and are thus available for virtually all subjects with liver disease. This index has also been independently validated in subjects with HCV infection alone [23]. It has recently been demonstrated that its performance characteristics for the diagnosis of advanced fibrosis in NAFLD are better than those of other similar panels that do not require additional testing [24]. However, 74% of the subjects enrolled in the study were Caucasian, and validation of the FIB4 index in other ethnic groups is required before it can be applied globally. In this study, we therefore aimed to assess the accuracy of the FIB4 index for predicting advanced liver fibrosis in a cohort of Japanese patients with NAFLD.

Methods

Patients

A total of 576 patients with well-characterized and liver-biopsy-confirmed NAFLD between 2002 and 2008 were enrolled from the Japan Study Group of NAFLD (JSG-NAFLD), which includes nine hepatology centers in Japan: Center for Digestive and Liver Diseases, Nara City Hospital; Division of Gastroenterology, Yokohama City University Graduate School of Medicine; Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University; Department of Gastroenterology and Hepatology, Kochi Medical School; Department of Internal Medicine, Saga Medical School, Saga University; Department of Hepatology, Graduate School of Medicine, Osaka City University; Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine; Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College; and Hepatology Center, Saiseikai Suita Hospital. All patients were also involved in the previous JSG-NAFLD study [25].

The diagnosis of NAFLD was based on the following criteria: (1) liver biopsy showing steatosis in at least 5% of hepatocytes [26]; and (2) appropriate exclusion of liver diseases of other etiologies, including viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, biliary obstruction, hemochromatosis, Wilson's disease, or α-1-antitrypsin- deficiency-associated liver disease. Patients who consumed > 20 g alcohol per day and patients with evidence of decompensated LC or HCC were excluded. Written informed consent was obtained from all patients at the time of liver biopsy, and the study was conducted in accordance with the Helsinki Declaration [27]. The study protocol was approved by the ethical committee of Nara City Hospital in Nara, Japan.

Anthropometric and laboratory evaluation

Venous blood samples were taken in the morning after a 12-h overnight fast. Laboratory evaluations in all patients included a blood cell count and measurement of AST, ALT, γ-glutamyl transpeptidase (GGT), cholinesterase (ChE), total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, albumin, fasting plasma glucose (FPG), immunoreactive insulin (IRI), and ferritin. These parameters were measured using standard clinical chemistry techniques. BMI was also calculated; obesity was defined as BMI > 25, according to the criteria of the Japan Society for the Study of Obesity [28]. Patients were assigned a diagnosis of DM if they had documented use of oral hypoglycemic medication, a random glucose level > 200 mg/dL, or FPG > 126 mg/dL [29]. Hypertension was defined as a systolic blood pressure ≥ 130 mmHg or a diastolic blood pressure ≥ 85 mmHg or by the use of antihypertensive agents. Dyslipidemia was defined as serum concentrations of triglycerides ≥ 150 mg/dL or HDL cholesterol < 40 mg/dL and < 50 mg/dL for men and women, respectively, or by the use of specific medication [30]. Based on a review of the literature, the following scores were calculated for each patient: FIB4 [22], AAR, AST to platelet ratio index (APRI) [31], age-platelet index (AP index) [32], BARD score [19], N score [20], and NFS [13]. The values for the upper limit of normal were set according to the International Federation of Clinical Chemistry: AST 35 U/L for men, 30 U/L for women, and were comparable to the values used in other analyses. The specific formulae used to determine these scores are shown in Table 1.

Histologic evaluation

All patients enrolled in this study underwent percutaneous liver biopsy under ultrasonic guidance. The liver specimens were embedded in paraffin and stained with hematoxylin and eosin, and Masson's trichrome. The minimum biopsy size was 20 mm and the number of portal areas was 10. The liver biopsy specimens were reviewed by two hepatopathologists (T.O. and Y.S.) who were blinded to the clinical data. Fatty liver was defined

Table 1 Formulae for determining noninvasive marker panels for detection of liver fibrosis.

Formula	Equation	
FIB4 index	(Age [years] × AST [IU/L])/(platelet count [10^9/L] × $\sqrt{\text{ALT[IU/L]}}$)	
AST to ALT ratio (AAR)	AST/ALT	
AST to platelet ratio index (APRI) [a]	([AST/ULN]/platelet count [10^9/L]) × 100	
Age-platelet index (AP index)	Age (years)	platelet count (10^9/L)
	< 30 = 0	< 225 = 0
	30-39 = 1	200-224 = 1
	40-49 = 2	175-199 = 2
	50-59 = 3	150-174 = 3
	60-69 = 4	125-149 = 4
	≥ 70 = 5	< 125 = 5
	Score is the sum of two (0-10)	
NAFLD fibrosis score	-1.675 + 0.037 × age (years) + 0.094 ×BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT - 0.013 × platelet count (× 10^9/L) - 0.66 × albumin (g/dL).	
BARD score	Scale 0-4	
	BMI ≥ 28 kg/m^2 = 1 point	
	AST/ALT ≥ 0.8 = 2 points	
	Diabetes = 1 point	
N (Nippon) score	Scale 0-4	
	female sex = 1 point	
	older age (> 60 years) = 1 point	
	type 2 diabetes = 1 point	
	hypertension = 1 point	

BMI, body mass index; IFG, impaired fasting glucose; INR, international normalized ratio; ULN, upper limit of normal.[a]ULN for AST: 30 in women, 35 in men.

as the presence of steatosis in at least 5% hepatocytes, while steatohepatitis was diagnosed by steatosis, inflammation, and hepatocyte ballooning [2,3,26]. The individual parameters of NASH histology, including fibrosis, were scored independently using the NASH Clinical Research Network (CRN) scoring system developed by the NASH CRN [26]. Advanced fibrosis was classified as stage 3 or 4 disease (bridging fibrosis or cirrhosis).

Statistical analysis

Statistical analysis was conducted using SPSS 19.0 software (SPSS, Inc., Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD), or median (interquartile range). Qualitative data were presented as numbers with percentages in parentheses. Statistical differences in quantitative data were determined using the *t* test or Mann-Whitney U test. Fisher's exact probability test or χ^2 analysis was used for qualitative data (Table 2). The sensitivity and specificity for each value of each test were calculated to assess the accuracy of the clinical scoring system in differentiating between advanced and mild fibrosis, and receiver operating characteristic (ROC) curves were constructed by plotting the sensitivity against (1 - specificity) at each value (Figure 1). The diagnostic performances of the scoring systems were assessed by analysis of ROC curves. The most commonly used index of accuracy was the area under the ROC curve (AUROC), with values close to 1.0 indicating high diagnostic accuracy. (Table 3). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the two cut-off values (< 1.45 and > 3.25) proposed by Sterling [22] and those (< 1.30 and > 2.67) proposed by Shah [24]. Differences were considered statistically significant at $p < 0.05$.

Results

A total of 576 subjects were included in this analysis. Of these, 280 (49%) were women and 418 (73%) were obese (Table 2); 241 (42%) had type 2 DM and 184 (32%) were hypertensive. A total of 319 subjects had steatohepatitis, of whom 64 subjects had advanced fibrosis. As expected, subjects with more advanced fibrosis were significantly older, predominantly female, and more likely to be hypertensive, to have type 2 DM, to have higher AST, AAR, GGT, FPG, and IRI, and to have lower hemoglobin, platelet count, albumin, ChE, total cholesterol, and triglyceride. Regarding the individual components of the FIB4 score, the mean (± SD) or median [interquartile range] values were as follows: age (52.3 ± 15.4 years); AST (43 [30-67] IU/L); ALT (69 [43-112] IU/L), and platelets (227 ± 67 × 10^9/L) (Table 2). The distribution of fibrosis stages included stage 0 (n = 263), stage 1 (n = 169), stage 2 (n = 80), stage 3 (n = 45), and

Table 2 Characteristics of study population and values of noninvasive fibrosis marker panels[a].

	Total (n = 576)	Fibrosis stage 0-2 (n = 512)	Fibrosis stage 3-4 (n = 64)	*p-value*[b]
Age (yr)	52.3 ± 15.4	51.2 ± 15.5	62.0 ± 10.1	< 0.0001
Gender (female)	280 (49%)	235 (46%)	45 (70%)	0.0003
BMI (kg/m^2)	27.9 ± 4.9	27.8 ± 4.9	28.6 ± 4.8	0.2138
Obesity (BMI > 25)	418 (73%)	369 (72%)	49 (77%)	0.5524
Hypertension (yes)	184 (32%)	150 (29%)	34 (53%)	0.0062
Type 2 diabetes (yes)	241 (42%)	199 (39%)	42 (66%)	0.0001
Hemoglobin (g/dL)	14.6 ± 2.0	14.7 ± 2.0	13.7 ± 2.0	0.0001
Platelet count (×10^9/L)	227 ± 67	235 ± 64	162 ± 52	< 0.0001
AST (IU/L)	43 (30-67)	41 (29-64)	61 (47-77)	< 0.0001
ALT (IU/L)	69 (43-112)	69 (43-69)	62 (46-94)	0.5074
AST/ALT ratio	0.65 (0.52-0.82)	0.63 (0.51-0.78)	0.98 (0.73-1.21)	< 0.0001
GGT (IU/L)	60 (39-99) (n = 572)	57 (36-92) (n = 508)	84 (59-128)	< 0.0001
Albumin (g/dL)	4.4 ± 0.4	4.4 ± 0.4	4.1 ± 0.4	< 0.0001
Cholinesterase (IU/L)	380 (330-433) (n = 527)	385 (337-439) (n = 466)	297 (244-367) (n = 61)	< 0.0001
Total cholesterol (mg/dL)	209 ± 40 (n = 467)	210 ± 39 (n = 409)	198 ± 42 (n = 58)	0.0484
Triglyceride (mg/dL)	147 (107-207) (n = 566)	150 (109-212) (n = 502)	131 (95-184) (n = 64)	0.0364
HDL-C (mg/dL)	50 ± 17 (n = 548)	50 ± 17 (n = 487)	51 ± 13 (n = 61)	0.7516
LDL-C (mg/dL)	128 ± 33 (n = 405)	129 ± 32 (n = 363)	120 ± 42 (n = 42)	0.1666
Ferritin (ng/mL)	173 (92-300)	169 (91-292)	216 (128-349)	0.0627
FPG (mg/dL)	103 (94-122) (n = 524)	103 (94-119) (n = 462)	111 (95-138) (n = 62)	0.0166
IRI (μU/mL)	11.6 (7.8-18.4)	11.3 (7.5-17.4)	17.3 (11.3-26.2)	< 0.0001
FIB4 index	1.23 (0.77-2.02)	1.13 (0.71-1.79)	3.17 (1.88-4.25)	< 0.0001
AST/ALT ratio (AAR)	0.65 (0.52-0.82)	0.63 (0.51-0.78)	0.98 (0.73-1.21)	< 0.0001
AST to platelet ratio index (APRI)	0.61 (0.40-0.98)	0.57 (0.38-0.92)	1.22 (0.86-1.79)	< 0.0001
Age-platelet index (AP index)	4 (2-6)	3 (2-5)	7 (5-8)	< 0.0001
NAFLD fibrosis score	-1.82 (-3.04 to -0.58)	-2.07 (-3.25 to -0.95)	0.25 (-0.60-1.06)	< 0.0001
BARD score				< 0.0001
0	144 (25%)	138 (27%)	6 (9%)	
1	201 (35%)	194 (38%)	7 (11%)	
2	112 (19%)	99 (19%)	13 (20%)	
3	88 (15%)	62 (12%)	26 (41%)	
4	31 (5%)	19 (4%)	12 (19%)	
N score				< 0.0001
0	135 (23%)	135 (26%)	0 (0%)	
1	170 (30%)	157 (31%)	13 (20%)	
2	118 (20%)	96 (19%)	22 (34%)	
3	99 (17%)	82 (16%)	17 (27%)	
4	54 (9%)	42 (8%)	12 (19%)	

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; and IRI, immuno-reactive insulin. *a* Values are mean ± SD, median (interquartile range), counts (%), as appropriate. *b*Values from univariate ordinal logistic regression, Mann-Whitney, or χ^2 analysis, as appropriate.

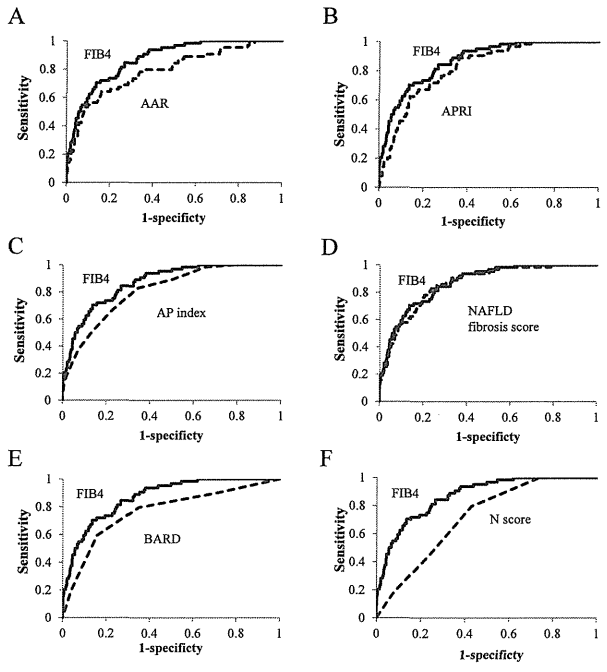

Figure 1 Comparison of ROCs of FIB4 and other panels for the diagnosis of advanced fibrosis.

stage 4 (n = 19). FIB4 values for the whole sample ranged from 0.17-10.74. The median FIB4 score was 1.23 (interquartile range, 0.77-2.02) (Table 3). The mean (interquartile range) FIB4 indices for stages 0, 1, 2, 3, and 4 were 1.09 (0.61-1.34), 1.40 (0.77-1.88), 2.36 (1.44-3.15), 3.23 (1.82-4.04), and 4.48 (3.19-5.17), respectively (p < 0.0001 by analysis of variance). The mean (interquartile range) FIB4 index was 1.13 (0.71-1.79) in patients with stage 0-2 fibrosis and 3.17 (1.88-4.25) in patients with stage 3-4 fibrosis (p <0.0001) (Table 2).

The sensitivity and specificity of FIB4 along the ROC were assessed first. At a sensitivity of 90% (FIB4 = 1.45) the specificity was 35%, while at a specificity of 90% (FIB4 = 2.67), the sensitivity was 52%. ROC curves were then developed for each of the noninvasive marker panels and superimposed, to determine which score would have the most clinical utility (Figure 1). ROC curves were created to determine the utility of the indices for predicting advanced fibrosis (stage 3 and 4 versus lower scores). The AUROC was greatest for FIB4 (0.871), followed by NFS (0.863), APRI (0.823), AP index (0.810), AAR (0.788), BARD score (0.765), and N score (0.715) (Table 3). As the NPVs for FIB4 index, AAR, APRI, AP index, NFS, BARD score, and N score were all greater than 95% using their lower cut-offs, these tests may have sufficient accuracy to be used clinically to exclude advanced fibrosis. Using this approach, a significant proportion of patients could avoid liver biopsy using each of these tests (Table 3). As the PPV were modest for all noninvasive tests, ranging from 19% to 53%, it was felt they were not accurate enough to be used as an alternative to liver biopsy. The PPV for FIB4 is highest among other noninvasive tests.

Using the low cut-off point proposed by Sterling and colleagues (< 1.45)[22], 330 of 336 (98.3%) patients without stage 3 or 4 fibrosis were correctly staged, while only 6 (1.7%) were under-staged (Table 4). All of the 6 patients with advanced fibrosis but FIB4 index below the low cut-off point had stage 3 fibrosis, none had stage 4 fibrosis. The NPV of this cut-off for stage 3 or 4 fibrosis was 98%. Using the high cut-off point proposed by Sterling and colleagues (> 3.25) [24], 31 of 59 (52.5%) patients with stage 3 or 4 fibrosis were correctly staged, while 28 (47.5%) were over-staged. Among the 28 patients without advanced fibrosis but FIB4 index above the high cut-off point, 18 had stage 2 fibrosis, 6 had stage 1, and 4 had no fibrosis. The PPV of this cut-off for stage 3 or 4 fibrosis was 53%. A total of 395 patients (69% of the cohort) had a FIB4 index < 1.45 or > 3.25; FIB4 identified the absence or presence of advanced fibrosis with 91% accuracy in these 361 subjects. A total of 181 subjects (31%) had FIB4 values in the indeterminate range (1.4-3.25).

Table 3 Accuracy of noninvasive fibrosis marker panels.

Fibrosis panel	AUROC	Cut-off values	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
FIB4 index	0.871	1.45	90	64	24	98
		3.25	48	95	53	94
AST/ALT ratio (AAR)	0.788	0.8	66	76	26	95
		1	48	92	44	94
AST to platelet ratio index (APRI)	0.823	1	67	81	31	95
Age-platelet index (AP index)	0.810	6	66	78	27	95
NAFLD fibrosis score	0.863	-1.455	92	63	24	98
		0.676	33	96	50	92
BARD score	0.765	2	80	65	22	97
N score	0.715	2	80	58	19	96

AUROC, area under the receiver operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value

Table 4 Proportion of patients who may potentially avoid liver biopsy using the simple non-invasive tests to exclude advanced fibrosis.

Fibrosis panel	Cut-off values	Patients avoiding liver biopsy[a]	False negative result
FIB4 index	< 1.45	336/576 (58%)	6 (2%)
	< 1.30	308/576 (53%)	4 (1%)
AST/ALT ratio (AAR)	< 0.8	413/576 (72%)	22 (5%)
AST to platelet ratio index (APRI)	< 1	435/576 (76%)	21 (5%)
Age-platelet index (AP index)	< 6	421/576 (73%)	22 (5%)
NAFLD fibrosis score	< -1.455	328/576 (57%)	5 (2%)
BARD score	< 2	355/576 (62%)	13 (4%)
N score	< 2	305/576 (53%)	13 (4%)

[a]Patients with a value below the cut-off.

On the other hand, using the low cut-off point proposed by Shah and colleagues (< 1.30) [24], 304 of 308 (99%) patients without stage 3 or 4 fibrosis were correctly staged, while only 4 (1%) were under-staged (Table 4). All of the 4 patients with advanced fibrosis but FIB4 index below the low cut-off point had stage 3 fibrosis and none had stage 4 fibrosis. The NPV of this cut-off for stage 3 or 4 fibrosis was 99%. Using the high cut-off point proposed by Shah and colleagues (> 2.67), 38 of 89 (43%) patients with stage 3 or 4 fibrosis were correctly staged, while 51 (57%) were over-staged. Among the 51 patients without advanced fibrosis but NAFLD fibrosis scores above the high cut-off point, 28 had stage 2 fibrosis, 14 had stage 1, and 9 had no fibrosis. The PPV of this cut-off for stage 3 or 4 fibrosis was 43%. A total of 397 patients (69% of the cohort) had a FIB4 index < 1.30 or > 2.67; FIB4 identified the absence or presence of advanced fibrosis with 86% accuracy in these 342 subjects. A total of 179 subjects (31%) had FIB4 values in the indeterminate range (1.30-2.67). Thus the prevalence of patients in the indeterminate range was similar using the two different cut-off values, but the number of patients with true positive or true negative predictions (accuracy) was higher using Sterling *et al.*'s cut-off values compared with Shah *et al.*'s (361 patients versus 342 patients). If liver biopsies were only performed in patients with an FIB4 index above the low cut-off point (> 1.45) proposed by Sterling, 336 (58%) of 576 biopsies could be avoided (Table 4).

The diagnostic accuracy of FIB4 index for detecting advanced fibrosis (stage 3-4) was also compared to that of NFS (Table 5). Three hundred and seventy patients (64% of the cohort) had an NFS <-1.455 or > 0.676; NFS identified the absence or presence of advanced fibrosis with 93% accuracy in these 344 subjects. A total of 206 subjects (36%) had NFS values in the indeterminate range (-1.455-0.676). Although the accuracy of NFS was higher (93%) than that of FIB4 (86%), more patients were correctly staged with FIB4 (n = 361) than with NFS (n = 344). Moreover, the percentage of patients in the undetermined range was lower for the FIB4 index (31%) than for NFS (36%). Using the cut-off values reported by Sterling and colleagues, discrepancies between FIB4 index and NFS were observed in 146 (39%) patients (Table 5). Patients were categorized into three groups, "low-risk" (< 10%), "intermediate-risk" (10-30%) and "high-risk" (> 30%), based on the combination of FIB4 index and NFS (Table 5). Only 1 patient (0.4%)

Table 5 Categorized risk groups for advanced fibrosis according to combined FIB4 index and NAFLD fibrosis score (NFS).

		FIB4 index (cut-off values proposed by Sterling et al.)			Total
		Low cut-off point (< 1.45)	Indeterminate (1.45-3.25)	High cut-off point (> 3.25)	
NFS	Low cut-off point (<-1.455)	283 [1 (0.4%)] [a]	42 [4 (9.5%)] [a]	3 [0 (0.0%)] [a]	328 (56.9%) [5 (1.5%)]
	Indeterminate (-1.455-0.676)	53 [5 (9.4%)] [a]	122 [19 (15.6%)] [b]	31 [14 (45.2%)] [c]	206 (35.8%) [38 (18.4%)]
	High cut-off point (> 0.676)	0	17 [4 (23.5%)] [b]	25 [17 (68.0%)] [c]	42 (7.3%) [21 (50.0%)]
Total		336 (58.3%) [6 (1.7%)]	181 (31.4%) [27 (14.9%)]	59 (10.2%) [31 (52.5%)}	576 (100%) [64 (11.1%)]

Total number of patients [stage 3-4 (%)]

Patients were categorized into three groups, "low-risk" (< 10%) [a], "intermediate-risk" (10-30%) [b] and "high-risk" (> 30%) [c], based on the combination of FIB4 index and NFS.

of 243 patients with the low cut-off points for both FIB4 index and NFS had advanced fibrosis.

Discussion

The AUROC of FIB4 was 0.871 for the diagnosis of advanced fibrosis, which was superior to those of the other noninvasive panels tested. For a value < 1.45, fibrosis could be excluded with 98% certainty (NPV 98%) whereas for a value > 3.25, the presence of significant fibrosis could be predicted with 53%. Despite the limited sensitivity of the FIB4 index in a population with a low prevalence of advanced fibrosis, the score was useful for ruling out advanced fibrosis. In our cohort, 58% of the liver biopsies could have been avoided if the procedure was not performed in patients with a FIB4 index below the low cut-off point (< 1.45). The score would therefore be particularly useful for reducing the number of unnecessary liver biopsies performed, and thus the costs of managing NAFLD patients in Asia, where advanced fibrosis is uncommon. A high cut-off FIB4 index of 2.67 which has been proposed by Shah and colleagues [24] had a low PPV (43%) in predicting stage 3 or 4 fibrosis. Our results contrast with those reported by Shah and colleagues [24], where a high cut-off FIB4 index of 2.67 had an 80% PPV in predicting stage 3 or 4 fibrosis; however the prevalence of advanced fibrosis in our study was only 11%, compared to 23% in Shah *et al.*'s study. Our study was therefore unable to reliably validate the high cut-off point, and larger Asian studies are warranted to investigate this. The FIB4 index was higher in our population than in Shah *et al.*'s study; stage 0-2: 1.13 (0.71-1.79) versus 0.97 (0.68-1.37), stage 3-4: 3.17 (1.88-4.25) versus 1.98 (1.28-3.08), probably because of older age, higher levels of ALT, and lower levels of platelets in our population.

The BARD score developed by Harrison *et al.* represents the weighted sum of three easily available variables (BMI ≥ 28 kg/m^2 [1 point], AAR ≥ 0.8 [2 points], and DM [1 point]), and the authors demonstrated that a score of 2-4 was associated with an odds ratio of 17 for predicting advanced fibrosis [19]. Although BARD score is simple to calculate, our validation study failed to detect any advantage of this score over FIB4; a BARD score of ≥ 2 was associated with a sensitivity, specificity, PPV and NPV for detecting advanced fibrosis of 80, 65, 22 and 97%, respectively. Consistent with the present study, Fujii and colleagues reported significantly poorer applicability of BARD in Japanese patients with NAFLD, compared with Caucasian subjects [33]. It has been suggested that BARD score is less predictive of advanced fibrosis in Japanese NAFLD patients because they are less obese than those in western countries. The N score (the total number of the following risk factors: female sex, age > 60 years, type 2 DM, and hypertension), which was established on the basis of data from 182 Japanese NAFLD patients in multiple centers in Nagasaki [20], requires no detailed laboratory measurements, but was not found to be superior to FIB4 index in our validation study. Angulo *et al.* found that the NFS, which consists of six variables (age, BMI, AAR, IFG/DM, platelet count, and albumin), reliably predicted advanced fibrosis in NAFLD patients [21]. In 428 (74%) of the subjects in the present study, FIB4 index was in accordance with NFS. The combination of two scoring systems could help to identify patients likely to have advanced fibrosis. Patients with FIB4 values above the high cut-off point (> 3.25) and NFS values above the low cut-off point (> -1.455) were at high risk (> 30%) for advanced fibrosis. If both FIB4 and NFS were applied to Japanese patients with NAFLD, patients with either FIB4 or NFS values below the low cut-off points (376/576, 65.3%) could avoid liver biopsies. In this way, when FIB4 was combined with NFS, its ability to predict or exclude advanced fibrosis improved further. In summary, the current study demonstrated that the FIB4 index, which can be established using a simple, relatively inexpensive method, correlated with the stage of fibrosis in adult subjects with NAFLD.

Type IV collagen is one of extracellular matrices that are produced by hepatic fibroblasts. The 7S domain in the N-terminus of type IV collagen is inserted in tissues and released into the blood by turnover in connective tissues. Therefore, the serum 7S domain level increases in parallel with the amount of fibrosis and in synthesis from stellate cells and myofibroblasts following increased liver fibrosis. In Japan, type IV collagen 7S is now widely used for assessing the extent of hepatic fibrosis in chronic liver diseases. Our data demonstrated that a cutoff point of 5.4 ng/ml provided a sensitivity and specificity of 86% and 87%, respectively, to detect advanced stage of NASH. The AUROC of type IV collagen 7s was: 0.926 for the diagnosis of advanced fibrosis, which was superior to FIB4 (data not shown). This data suggest that type IV collagen 7S is one of the best parameters among non-invasive parameters, but it costs too much to be determined routinely.

On the other hand, hepatic steatosis is frequently found in patients with HCV infection. Therefore, we also evaluated the value of FIB4 index in 185 HCV-infected patients with hepatic steatosis, including those with 72 advanced and 113 mild fibrosis. The AUROC of FIB4 was 0.808 for the diagnosis of advanced fibrosis. For a value < 1.45, fibrosis could be excluded with 89% certainty (NPV 89%) whereas for a value > 3.25, the presence of advanced fibrosis could be predicted with 82% (data not shown).

This study had several limitations. First, the proportion of subjects with advanced fibrosis was small, as

reported in other Asian studies [34], and further Asian studies with more patients with advanced fibrosis are warranted. Second, patients were recruited from hepatology centers in Japan with a particular interest in studying NAFLD, and the possibility of some referral bias could therefore not be ruled out. Patient selection bias could also have existed, because liver biopsy might have been considered for NAFLD patients who were likely to have NASH. The findings may thus not represent NAFLD patients in the wider community. However, this would introduce a negative bias, as NAFLD patients in the community would be likely to have milder liver disease, thus increasing the NPV of the FIB4 index. We also acknowledge that pathologic diagnosis was mainly determined using liver tissues derived from percutaneous liver biopsies, which are prone to sampling errors or interobserver variability [7,8]. As recent studies suggest that low normal ALT value does not guarantee freedom from underlying NASH with advanced fibrosis [35-37], it remains to be solved whether FIB4 index can be useful for predicting advanced fibrosis in NAFLD subjects with normal ALT. According to our preliminary data by JSG-NAFLD, the AUROC of FIB4 was 0.810 for the diagnosis of advanced fibrosis in 187 biopsy-proven NAFLD patients with normal ALT levels (data not shown). Our data support the hypothesis that FIB4 index could also be used in the Japanese NAFLD population with normal ALT.

Conclusion

The FIB4 index demonstrated a good NPV for excluding advanced fibrosis in Japanese NAFLD patients, and could thus be used to reduce the burden of liver biopsies. Larger Asian studies are required to validate the high cut-off point of the FIB4 index. However, the FIB4 test also has several serious limitations, in common with other noninvasive tests for fibrosis, and further research is needed before simple noninvasive tests, including the FIB4 test, can replace liver biopsies in the vast majority of patients.

Abbreviations

AAR: AST/ALT ratio; AST: aspartate aminotransferase; ALT: alanine aminotransferase; AP index: age to platelet index; APRI- aspartate aminotransferase to platelet ratio index; AUROC: area under the receiver operating characteristic; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; NFS: NAFLD fibrosis score; NPV: negative predictive value; PPV: positive predictive value.

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Author details

[1]Center for Digestive and Liver Diseases, Nara City Hospital, Nara, Japan. [2]Division of Gastroenterology, Yokohama City University Graduate School of Medicine, Yokohama, Japan. [3]Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan. [4]Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan. [5]Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi, Japan. [6]Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, Japan. [7]Department of Internal Medicine, Saga Medical School, Saga University, Saga, Japan. [8]Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College, Asahikawa, Japan. [9]School of Biomedical Informatics, University of Texas Health Science Center at Houston, Houston, TX, USA. [10]Hepatology Center, Saiseikai Suita Hospital, Suita, Japan.

Authors' contributions

YS: study concept and design, drafting of the manuscript, MY: acquisition of data, HH: acquisition of data, YI: critical revision of the manuscript for important intellectual content. MO: study concept and design, HF: acquisition of data, YE: acquisition of data, YS: acquisition of data, NA: statistical analysis, KK: critical revision of the manuscript for important intellectual content, KF: acquisition of data, KC: critical revision of the manuscript for important intellectual content TS: critical revision of the manuscript for important intellectual content NK: critical revision of the manuscript for important intellectual content KF: critical revision of the manuscript for important intellectual content YK: critical revision of the manuscript for important intellectual content, TY: critical revision of the manuscript for important intellectual content, TO: study supervision, JSG-NAFLD: acquisition of data, study supervision. All authors read and approved the final manuscript.

Competing interests

The authors declare that there is no duality of interest associated with this manuscript.

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References

1. Ludwig J, Viggiano TR, McGill DB, Ott BJ: **Non-alcoholic steatohepatitis. Mayo Clinic experiences with a hitherto unnamed disease.** *Mayo Clin Proc* 1980, **55**:434-438.
2. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ: **Nonalcoholic fatty liver diseases: a spectrum of clinical and pathological severity.** *Gastroenterology* 1999, **116**:1413-1419.
3. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM: **Long-term follow-up of patients with nonalcoholic fatty liver.** *Clin Gastroenterol Hepatol* 2009, **7**:234-238.
4. Ono M, Saibara T: **Clinical features of nonalcoholic steatohepatitis in Japan: evidence from literature.** *J Gastroenterol* 2006, **41**:725-732.
5. Vuppalanchi R, Chalasani N: **Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management.** *Hepatology* 2009, **49**:306-317.
6. Wieckowska A, McCullough AJ, Feldstein AE: **Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future.** *Hepatology* 2007, **46**:582-589.
7. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T, LIDO Study Group: **Sampling variability of liver biopsy in nonalcoholic fatty liver disease.** *Gastroenterology* 2005, **128**:1898-1906.
8. Merriman RB, Ferrell LD, Patti MG, Weston SR, Pabst MS, Aouizerat BE, Bass NM: **Correlation of paired liver biopsies in morbidly obese patients**

with suspected nonalcoholic fatty liver disease. *Hepatology* 2006, **44**:874-880.

9. Ratziu V, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, Tahiri M, Munteanu M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V, Poynard T, LIDO Study Group; CYTOL study group: **Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease.** *BMC Gastroenterol* 2006, **6**:6.
10. Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM: **Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers.** *Hepatology* 2008, **47**:455-460.
11. Huwart L, Sempoux C, Salameh N, Jamart J, Annet L, Sinkus R, Peeters F, ter Beek LC, Horsmans Y, Van Beers BE: **Liver fibrosis: noninvasive assessment with MR elastography versus aspartate aminotransferase- to-platelet ratio index.** *Radiology* 2007, **245**:458-466.
12. Yoneda M, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo S, Nakajima A: **Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD).** *Dig Liver Dis* 2008, **40**:371-378.
13. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP: **The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD.** *Hepatology* 2007, **45**:846-854.
14. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T: **Liver fibrosis in overweight patients.** *Gastroenterology* 2000, **118**:1117-1123.
15. Yoneda M, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A: **Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography.** *Radiology* 2010, **256**:640-647.
16. Suzuki A, Angulo P, Lymp J, Li D, Satomura S, Lindor K: **Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with nonalcoholic fatty liver disease.** *Liver Int* 2005, **25**:779-786.
17. Kaneda H, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K: **Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease.** *J Gastroenterol Hepatol* 2006, **21**:1459-1465.
18. Yoneda M, Mawatari H, Fujita K, Yonemitsu K, Kato S, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Abe Y, Kubota K, Saito S, Iwasaki T, Terauchi Y, Togo S, Maeyama S, Nakajima A: **Type IV collagen 7s domain is an independent clinical marker of the severity of fibrosis in patients with nonalcoholic steatohepatitis before the cirrhotic stage.** *J Gastroenterol* 2007, **42**:375-381.
19. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA: **Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease.** *Gut* 2008, **57**:1441-1447.
20. Miyaaki H, Ichikawa T, Nakao K, Yatsuhashi H, Furukawa R, Ohba K, Omagari K, Kusumoto Y, Yanagi K, Inoue O, Kinoshita N, Ishibashi H, Yano M, Eguchi K: **Clinicopathological study of nonalcoholic fatty liver disease in Japan: the risk factors for fibrosis.** *Liver Int* 2008, **28**:519-524.
21. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP: **The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD.** *Hepatology* 2007, **45**:846-854.
22. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M, APRICOT Clinical Investigators: **Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection.** *Hepatology* 2006, **43**:1317-1325.
23. Vallet-Pichard A, Mallet V, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S: **FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest.** *Hepatology* 2007, **46**:32-36.
24. Shah A, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, NASH Clinical Research Network: **Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease.** *Clin Gastroenterol Hepatol* 2009, **7**:1104-1112.
25. Sumida Y, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, Eguchi Y, Suzuki Y, Imai S, Kanemasa K, Fujita K, Chayama K, Yasui K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Okanoue T, Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD): **A simple clinical scoring system using ferritin, fasting insulin and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease.** *J Gastroenterol* 2011, **46**:257-268.
26. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ, Nonalcoholic Steatohepatitis Clinical Research Network: **Design and validation of a histological scoring system for nonalcoholic fatty liver disease.** *Hepatology* 2005, **41**:1313-1321.
27. Rickham PP: **Human Experimentation. Code of ethics of the world medical association. Declaration of Helsinki.** *Br Med J* 1964, **2**:177.
28. Japanese Society for the Study of Obesity: **New criteria of obesity (in Japanese).** *J Jpn Soc Study Obes* 2000, **6**:18-28.
29. American Diabetes Association: **Report of the expert committee on the diagnosis and classification of diabetes mellitus.** *Diabetes Care* 1997, **20**:1183-1197.
30. Expert Panel on Detection, Treatment of High Blood Cholesterol in Adults: **Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III).** *JAMA* 2001, **285**:2486-2497.
31. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS: **A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C.** *Hepatology* 2003, **38**:518-526.
32. Poynard T, Bedossa P: **Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups.** *J Viral Hepat* 1997, **4**:199-208.
33. Fujii H, Enomoto M, Fukushima W, Tamori A, Sakaguchi H, Kawada N: **Applicability of BARD score to Japanese patients with NAFLD.** *Gut* 2009, **58**:1566-1567.
34. Wong VW, Wong GL, Chim AM, Tse AM, Tsang SW, Hui AY, Choi PC, Chan AW, So WY, Chan FK, Sung JJ, Chan HL: **Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis.** *Am J Gastroenterol* 2008, **103**:1682-1688.
35. Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S: **Risk of Severe Liver Disease in Nonalcoholic Fatty Liver Disease with Normal Aminotransferase Levels: A Role for Insulin Resistance and Diabetes.** *Hepatology* 2008, **48**:792-798.
36. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ: **Clinical and Histologic Spectrum of Nonalcoholic Fatty Liver Disease Associated With Normal ALT Values.** *Hepatology* 2003, **37**:1286-1292.
37. Uslusoy HS, Nak SG, Gülten M, Biyikli Z: **Non-alcoholic steatohepatitis with normal aminotransferase values.** *World J Gastroenterol* 2009, **21**:1863-1868.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinicopathological features of liver injury in patients with type 2 diabetes mellitus and comparative study of histologically proven nonalcoholic fatty liver diseases with or without type 2 diabetes mellitus

Toshihide Shima · Hirofumi Uto · Kohjiro Ueki · Toshinari Takamura · Yutaka Kohgo · Sumio Kawata · Kohichiroh Yasui · Hyohun Park · Naoto Nakamura · Tatsuaki Nakatou · Nobuyoshi Tanaka · Atsushi Umemura · Masayuki Mizuno · Junko Tanaka · Takeshi Okanoue

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Abstract
Background The Japan Society of Diabetes Mellitus reported that the leading cause of death in patients with diabetes mellitus (DM) was chronic liver disease; however, there are limited studies investigating the cause of liver injury in these patients. Our study aimed to clarify the clinicopathological features of liver injury and the characteristics of nonalcoholic fatty liver disease (NAFLD) in DM patients.
Methods In total, 5,642 DM patients and 365 histologically proven NAFLD patients were enrolled. Clinical and laboratory parameters and liver biopsy results were, respectively, recorded and analyzed for the two sets of patients.
Results Positivity rates for Hepatitis B surface antigens (HBsAg) and anti-hepatitis C virus antibodies (anti-HCV Ab) were 1.7 and 5.1 %, respectively. The proportion of drinkers consuming 20–59 g and ≥60 g alcohol daily was 14.9 and 4.3 %, respectively. The percentage of DM patients with elevated serum alanine aminotransferase (ALT) levels (≥31 IU/L) was 28.6 %. Alcohol consumption had no significant effect on serum ALT levels. Seventy-two percent of HBsAg-positive patients were serum hepatitis B virus (HBV)-DNA negative, whereas 10 % exhibited high levels of the same (>4.0 log copies/ml).

T. Shima · A. Umemura · M. Mizuno · T. Okanoue (✉)
Department of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Suita, Japan
e-mail: okanoue@suita.saiseikai.or.jp

H. Uto
Digestive and Life-style Related Disease, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

K. Ueki
Diabetes and Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

T. Takamura
Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

Y. Kohgo
Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College, Asahikawa, Japan

S. Kawata
Gastroenterology, Yamagata University Faculty of Medicine, Yamagata, Japan

K. Yasui
Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan

H. Park
Diabetes and Metabolic Diseases, Saiseikai Suita Hospital, Suita, Japan

N. Nakamura
Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Kyoto, Japan

T. Nakatou
Diabetes Center, Okayama Saiseikai General Hospital, Okayama, Japan

N. Tanaka
Gastroenterology, Fukui-ken Saiseikai Hospital, Fukui, Japan

J. Tanaka
Department of Epidemiology, Infectious Disease Control and Prevention, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

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