Table 3 Factors associated with sustained virological response in patients with chronic hepatitis C who underwent 48 weeks of pegylated inferferon-α plus ribavirin therapy

	UVA			MVA	
	SVR	Non-SVR	*p*	OR (95 % CI)	*p*
Number	74 (38 male, 36 female)	45 (31 male, 14 female)	0.06		
Age (years)	55.4 ± 10.1	58.2 ± 10.0	0.122		
WBC (/mm^3)	5,043 ± 1,695	5,248 ± 1,363	0.247		
Hb (g/dL)	14.3 ± 1.5	14.4 ± 1.6	0.504		
Plt (×10^4/mm^3)	18.2 ± 4.6	16.9 ± 6.0	0.186		
TP (g/dL)	7.5 ± 0.6	7.6 ± 0.5	0.292		
Alb (g/dL)	4.2 ± 0.4	4.1 ± 0.4	0.575		
AST (U/L)	47.5 ± 27.9	66.5 ± 50.0	0.049	1.012 (0.997–1.027)	0.108
ALT (U/L)	66.4 ± 47.9	80.0 ± 62.9	0.286		
T-bil (mg/dL)	0.7 ± 0.3	0.9 ± 0.4	0.101		
T-chol (mg/dL)	178.1 ± 36.8	174.3 ± 37.7	0.717		
AFP (ng/mL)	7.1 ± 7.8	14.1 ± 18.8	0.062		
HCV RNA (log IU/mL)	6.3 ± 0.7	6.3 ± 0.5	0.753		
IFNL3 rs8099917 (TT/non-TT)	70:4	30:15	<0.0001	17.25 (3.34–89.13)	0.001
Histological activity score (A0-A1/A2-A3)	45:20	24:15	0.454		
Fibrosis score (F1–F2/F3-F4)	57:8	27:12	0.023	0.239 (0.072–0.798)	0.02
IFN-λ_3 (pg/mL)	17.3 ± 31.7	11.8 ± 14.9	0.262		
IP-10 (pg/mL)	458.0 ± 404.9	504.7 ± 364.0	0.208		
MIP-1α (pg/mL)	13.1 ± 36.1	4.2 ± 5.6	0.026	0.66 (0.457–0.956)	0.028
MIP-1β (pg/mL)	195.7 ± 204.3	154.9 ± 81.5	0.865		
RANTES (pg/mL)	18,125 ± 8,076	16,597 ± 7,946	0.187		
PDGF-BB (pg/mL)	3,931 ± 1,846	3,312 ± 1,803	0.079		

Alb albumin, *AFP* α-fetoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CI* confidence interval, *Hb* hemoglobin, *HCV* hepatitis C virus, *IFN-λ$_3$* interferon-λ$_3$, *IP-10* interferon-γ-inducible protein 10, *MIP-1α* macrophage inflammatory protein 1α, *MIP-1β* macrophage inflammatory protein 1β, *MVA* multivariate analysis, *OR* odds ratio, *PDGF-BB* platelet-derived growth factor BB, *Plt* platelets, *RANTES* regulated on activation, normally T cell expressed, and secreted, *T-bil* total bilirubin, *T-chol* total cholesterol, *TP* total protein, *UVA* univariate analysis, *WBC* white blood cells

function was reported for PDGF-BB, the level of which is reported to be increased in patients with advanced/fibrosis stages of HBV infection [32, 33]. These reports support the notion that IFN-λ_3 is related to liver inflammation and fibrosis. As well as in B-CH patients, a positive correlation was observed between serum IFN-λ_3 levels and inflammation (AST levels) and fibrosis markers (FIB-4 score and APRI). Secondly, we examined whether serum IFN-λ_3 and chemokines are involved or not involved in the SVR to PEG-IFN-α plus RBV therapy for C-CH patients. We confirmed that *IFNL3* genotypes, fibrosis score, and MIP-1α are associated with SVR in this cohort, but failed to do so with IP-10 and serum IFN-λ_3. Several studies showed that pretreatment IP-10 levels could be a predictor of SVR in PEG-IFN-α plus RBV therapy for C-CH [34], the significance of which became stronger in combination with *IFNL3* genotypes [35, 36]. One of the reasons why the IP-10 levels failed to be significant in this study may be a bias for the enrollment of patients from multiple hospitals and medical centers.

In summary, serum IFN-λ_3 levels are increased in patients with chronic HCV infection regardless of the *IFNL3* genotype, the level of which is associated with liver inflammation and fibrosis. The biological role and clinical impact of IFN-λ_3 in patients with chronic HCV infection need to be investigated further.

Acknowledgment This study was supported by grants (23-105) from the National Center for Global Health and Medicine in Japan.

Conflict of interest The authors declare that they have no conflict of interest.

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J Gastroenterol (2014) 49:737–747
DOI 10.1007/s00535-013-0824-z

The Japanese Society of Gastroenterology

Using early viral kinetics to predict antiviral outcome in response-guided pegylated interferon plus ribavirin therapy among patients with hepatitis C virus genotype 1

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Received: 5 March 2013 / Accepted: 18 April 2013 / Published online: 21 May 2013

Abstract
Background HCV kinetics during treatment demonstrated strong association with the antiviral outcome of patients treated with pegylated interferon (Peg-IFN) plus ribavirin. However, the relationship between HCV kinetics and pre-treatment factors remains unclear.
Methods Of 547 patients with HCV genotype 1 treated with Peg-IFN alfa-2b plus ribavirin, 401 completed the response-guided therapy and were assessed for per protocol analysis.
Results The sustained virologic response (SVR) rate was 53 % for all patients, 60 % for those with genotype TT, and 19 % for those with genotype TG/GG according to IL28B (rs8099917) single nucleotide polymorphisms. The SVR rates increased with HCV decrease at week 4; 4 % (2/56) with <1 $\log_{10}$ decrease, 13 % (7/56) with 1–2 $\log_{10}$ decrease, 51 % (44/87) with 2–3 $\log_{10}$ decrease, 64 % (56/87) with 3–4 $\log_{10}$ decrease, 88 % (72/82) with more than 4 $\log_{10}$ decrease but with detectable HCV RNA and 100 % (33/33) with undetectable HCV RNA ($p < 0.001$). Similarly, SVR rates increased step-by-step in proportion to HCV decrease in both IL28B TT and TG/GG groups, showing almost the same SVR rates for the same conditions. In multivariate analysis, age ($p = 0.005$) and the magnitude of HCV decrease at week 4 ($p < 0.001$) but not IL28B were associated with SVR. Advanced liver fibrosis ($p = 0.004$) and the magnitude of HCV decrease at week 4

Electronic supplementary material The online version of this article (doi:10.1007/s00535-013-0824-z) contains supplementary material, which is available to authorized users.

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($p < 0.001$) but not IL28B were associated with non-response.

Conclusions The magnitude of the HCV decrease at week 4 seems to be the most reliable marker for predicting antiviral outcome after starting Peg-IFN plus ribavirin therapy.

Keywords Chronic hepatitis C · Pegylated interferon plus ribavirin · HCV decrease · IL28B

Introduction

Triple therapy with pegylated interferon (Peg-IFN), ribavirin plus telaprevir (TVR) can improve the antiviral effect for patients infected with hepatitis C virus (HCV) genotype 1 [1–8]. However, this triple therapy is not indicated for some patients due to adverse effects such as severe anemia progression, severe eruption and deterioration of renal function. On the other hand, 48–55 % of genotype 1 chronic hepatitis C (CH-C) patients have been shown to attain sustained virologic response (SVR) by response-guided Peg-IFN plus ribavirin combination therapy [9, 10]. Accordingly, naïve patients for whom there is concern about the possibility of enhanced adverse effects due to the addition of TVR, especially elderly patients, should first be given dual therapy with Peg-IFN plus ribavirin [11]. However, if patients having a high risk for hepatocellular carcinoma show poor antiviral response to this dual therapy, starting them on the triple therapy with Peg-IFN, ribavirin plus TVR should be considered. What is important is the ability to predict the antiviral outcome early during treatment in response-guided Peg-IFN plus ribavirin combination therapy.

The magnitude of the HCV RNA decrease or the timing of HCV RNA negativiation during the treatment has been reported to be strongly associated with SVR rates among genotype 1 CH-C patients treated with Peg-IFN plus ribavirin combination therapy [12–16]. During a 48-week treatment, SVR was attained in 70–80 % of patients with complete EVR (c-EVR), defined as undetectable HCV RNA at 12 weeks of treatment, and in about 90 % of patients with rapid virological response (RVR), defined as undetectable HCV RNA at 4 weeks of treatment [14–16], while those with less than a 2 $\log_{10}$ decrease in the HCV RNA level at 12 weeks mostly were non-SVR [12, 13, 16]. Currently, the magnitude of the HCV RNA decrease at 4 weeks of treatment has been attracting attention as a predictive factor for the antiviral outcome to this combination therapy [16, 17]. However, the aforementioned study was based on an existing method (lower limit 50 IU/ml) [17]. It is now possible to more precisely predict antiviral outcome thanks to progress in measurement of HCV RNA levels, which can now be quantitated with a higher degree of accuracy (lower limit 15 IU/ml) and for a broader range with 2 $\log_{10}$ differences.

A genetic polymorphism near the interleukin 28B (IL28B) gene has been reported to be a strong factor associated with antiviral outcome to the Peg-IFN plus ribavirin combination therapy [18–24]. Patients with the TT genotype of IL28B single nucleotide polymorphism (SNP) (rs8099917) can attain a higher virologic response during treatment (RVR, c-EVR) and a higher SVR rate in comparison with those with non-TT genotype [19, 23].

In the present study, we investigated the magnitude of impact of the early HCV RNA kinetics on predicting the antiviral outcome of response-guided Peg-IFN plus ribavirin combination therapy among genotype 1 CH-C patients. We also examined the relationship between the early viral kinetics and pre-treatment factors.

Patients and methods

Patients

The current study was a retrospective, multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 547 patients with CH-C treated with a combination of Peg-IFN alfa-2b plus ribavirin between January 2008 and August 2010 were enrolled in this study. Of the 547 patients, 401 completed the response-guided therapy and were assessed for per protocol analysis.

Eligible patients for this study were those who were infected with HCV genotype 1 and had a viral load of more than 10^5 IU/ml, but were negative for hepatitis B surface antigen and anti-human immunodeficiency virus. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis). Informed consent was obtained from each patient included in this study. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki amended in 2002.

Treatment

All patients received Peg-IFN alfa-2b (PEGINTRON; Merck & Co. Inc., Whitehouse Station, NJ, USA) plus ribavirin (REBETOL; Merck & Co. Inc.). Peg-IFN alfa-2b was given subcutaneously once weekly at a dosage of 60–150 μg/kg based on body weight (body weight 35–45 kg, 60 μg; 46–60 kg, 80 μg; 61–75 kg, 100 μg; 76–90 kg, 120 μg; 91–120 kg, 150 μg) and ribavirin was given orally twice a day at a total dose of 600–1000 mg/day based on body weight (body weight <60 kg, 600 mg;

60–80 kg, 800 mg; >80 kg, 1000 mg), according to a standard treatment protocol for Japanese patients.

Dose reduction

Dose modification followed, as a rule, the manufacturer's drug information according to the intensity of the hematologic adverse effects. The dose of Peg-IFN alfa-2b was reduced to 50 % of the assigned dose if the white blood cell (WBC) count declined to $<1500/mm^3$, the neutrophil count to $<750/mm^3$, or the platelet count to $<8 \times 10^4/mm^3$, and was discontinued if the WBC count declined to $<1000/mm^3$, the neutrophil count to $<500/mm^3$, or the platelet count to $<5 \times 10^4/mm^3$. Ribavirin was also reduced from 1000 to 600, or 800 to 600, or 600 to 400 mg if the hemoglobin (Hb) level decreased to <10 g/dl, and was discontinued if the Hb level decreased to <8.5 g/dl.

Histological evaluation

Pre-treatment liver biopsies were conducted within 6 months before the start of the combination therapy. Histopathological interpretation of the specimens was done by experienced liver pathologists who had no clinical, biochemical and virological information. The histological appearances, activity and fibrosis, were evaluated according to the METAVIR histological score [25].

IL28B genotyping

Human genomic DNA was extracted from a whole blood sample for each patient. Genetic polymorphism in SNPs located near the IL28B gene (rs8099917) was determined by a real-time PCR system. Each extracted DNA was used for PCR with primers and probes from a commercial kit (Taqman SNP Genotyping Assays, Applied Biosystems). The SNP of IL28B rs8099917 was amplified, and the results were analyzed by real-time PCR in a thermal cycler (7900 Real-time PCR System, Applied Biosystems). Homozygosity for TT genotype was defined as having the IL28B TT genotype, whereas homozygosity for GG or heterozygosity for TT (TG) genotype was defined as having the IL28B non-TT genotype.

Virologic assessment and definition of viral response

Serum HCV RNA level was quantified with the COBAS Taqman HCV test, version 2.0 (detection range 1.2–7.8 log IU/ml; Roche Diagnotics, Branchburg, NJ, USA). Serum HCV RNA level was assessed before treatment, every 4 weeks during treatment and 24 weeks after the therapy. The patients were divided into six groups according to the magnitude of the decrease in HCV RNA from baseline at treatment week 4, 8 and 12: <1 $\log_{10}$ decrease, 1 to <2 $\log_{10}$ decrease, 2 to <3 $\log_{10}$ decrease, 3 to <4 $\log_{10}$ decrease, ≥ 4 $\log_{10}$ (detectable), and undetectable. The case of serum HCV RNA level lower than 1.2 log IU/ml but positive for the amplification signal was defined as ≥ 4 $\log_{10}$ (detectable). A RVR was defined as undetectable serum HCV RNA at week 4, a c-EVR as undetectable serum HCV RNA at week 12, and a late virologic response (LVR) as detectable HCV RNA at week 12 but undetectable at week 36. SVR was defined as undetectable serum HCV RNA at 24 weeks after discontinuation of treatment. The treatment duration followed the response-guided therapy, i.e., patients with c-EVR were treated for 48 weeks and those with LVR for 72 weeks. Treatment was stopped for patients with detectable HCV RNA at week 36; they were considered to have experienced treatment failure (non-response, NR).

Definition of PPV and NPV

The positive predictive value (PPV) was defined as the probability that a certain outcome would occur in subjects on implementing the prediction criterion of interest, and a negative predictive value (NPV) was defined as the probability that a certain outcome would not occur in subjects if the prediction criterion of interest were not implemented.

Factors associated with SVR or NR on multivariate analysis

Factors associated with SVR or NR were assessed by multivariate analysis using two models; model 1 used the pre-treatment factors while model 2 also included the virologic response, i.e., the magnitude of the decrease in HCV RNA from baseline at treatment week 4, in addition to the factors used for model 1.

Statistical analysis

Baseline data for various demographic, biochemical, and virologic characteristics of the patients are expressed as mean ± SD or median. Viral response was evaluated using per protocol .analysis. The difference between the two groups was assessed by Chi square test or t test and the significance trend was determined with the Mantel–Haenszel Chi square test. The factors associated with the virologic response were assessed by univariate and multivariate analyses using logistic regression analysis. A p value <0.05 was considered significant. Statistical analysis was conducted with SPSS version 19.0J (IBM, Armonk, NY, USA).

Results

The baseline characteristics of the 401 patients who completed the response-guided regimen are summarized in Table 1. All were assessed for the HCV RNA level at baseline and treatment weeks 4, 8 and 12. The IL28B genotype was assessed for 174 patients. The RVR rate was 8 % (33/401), the c-EVR rate was 41 % (164/401) and the LVR rate was 33 % (133/401). The NR rate was 26 % (104/401) and the SVR rate was 53 % (214/401).

Patient prevalence and SVR rates according to the magnitude of the decrease in HCV RNA from baseline at treatment week 4, 8 and 12

The patient prevalence for the different magnitudes of decrease in HCV RNA from baseline at treatment week 4, 8 and 12 are shown (Fig. 1a). The SVR rate on response-guided therapy was assessed for the six groups classified according to the magnitude of decrease in HCV RNA from the baseline (Fig. 1b). At week 4, the SVR rate significantly increased with the magnitude of the decrease in HCV RNA from the baseline; while all patients achieved SVR among those with undetectable HCV RNA (33/33), the SVR rates were very low among patients with <1 $\log_{10}$ decrease (4 %, 2/56). At week 8, none of the patients achieved SVR among those with <1 $\log_{10}$ decrease (0/32) and the SVR rates were very low among patients with 1 to <2 $\log_{10}$ decrease (6 %, 2/36). At week 12, none of the patients achieved SVR among those with <1 $\log_{10}$ decrease (0/32) or 1 to <2 $\log_{10}$ decrease (0/23) and the SVR rates were very low if HCV RNA did not decrease more than 4 $\log_{10}$ compared to the baseline (among patients with 2 to <3 $\log_{10}$ decrease, 8 %, 2/25, 3 to <4 $\log_{10}$ decrease, 7 %, 2/27).

Next, the relationship between the virologic response during the treatment (c-EVR, LVR and NR) and the magnitude of the decrease in HCV RNA from baseline were assessed (Fig. 2). The timing of HCV RNA negativiation tended to shift to an earlier time during the treatment with an increase in the magnitude of the decrease in HCV RNA from the baseline at any week (week 4, $p < 0.0001$, week 8, $p < 0.0001$, week 12, $p < 0.0001$, respectively). At week 4, 89 % of the patients with a <1 $\log_{10}$ decrease resulted in NR; none of those with a <2 $\log_{10}$ decrease achieved c-EVR. At week 8, all patients resulted in NR among those with a <1 $\log_{10}$ decrease (32/32, PPV for NR = 100 %); none of those with a < 3 $\log_{10}$ decrease achieved c-EVR. At week 12, all the patients with a <2 $\log_{10}$ decrease resulted in NR (55/55, PPV for NR = 100 %).

Table 1 Baseline characteristics of patients

Factor	Number or mean ± SD
Number	401
Age (y.o)	56.0 ± 10.6
Sex: male/female	190/211
Past history of IFN therapy[a]: naïve/experienced	316/66
Liver histology (METAVIR)[b]	
Activity, A0–1/2–3	188/120
Fibrosis, F0–2/3–4	265/42
IL28B SNP[c]: TT/TG/GG	131/40/3
HCV RNA (log IU/ml)	6.5 ± 0.6
White blood cells (/mm^3)	5152 ± 1486
Hemoglobin (g/dl)	13.8 ± 1.4
Platelets ($\times 10^4$/mm^3)	16.9 ± 5.5
ALT (IU/l)	65 ± 47
γGTP (IU/l)	62 ± 68

ALT alanine aminotransferase, *γGTP* γ-glutamyl transferase

[a] Past history of IFN was unknown in 19 patients

[b] 94 missing

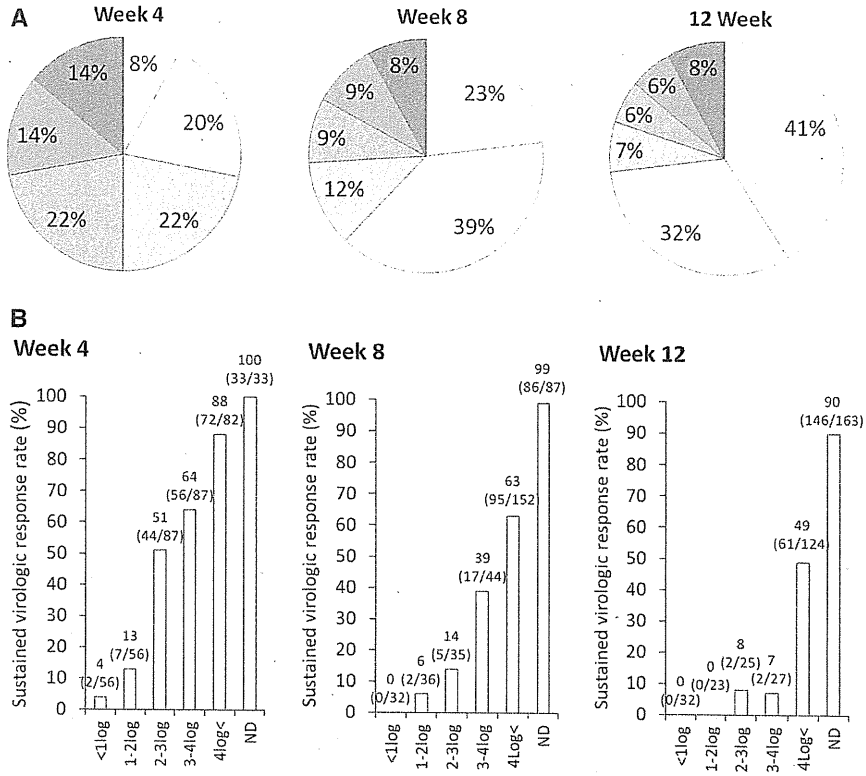
[c] Single nucleotide polymorphism of IL28B gene determined for rs8099917, 227 missing

Patient prevalence and virologic responses according to IL28B genotype

Among the patients who were assessed for their IL28B genotype, 131 had the IL28B TT genotype and 25 had the IL28B non-TT genotype (Fig. 3A). The SVR rate for response-guided therapy was significantly higher in patients with the IL28B TT genotype than those with the non-TT genotype ($p < 0.001$) (Fig. 3B). The treatment responses distributing c-EVR, LVR, and NR between the two groups were assessed. The c-EVR rate was significantly higher in patients with the IL28B TT genotype and the NR rate was significantly higher in those with the IL28B non-TT genotype ($p < 0.001$) (Fig. 3C).

Patient prevalence and SVR rates according to the magnitude of the decrease in HCV RNA from baseline at treatment week 4, 8 and 12 among patients with IL28B genotype TT or non-TT genotype

The patient prevalence and SVR rates according to the magnitude of the decrease in HCV RNA from baseline at treatment week 4, 8 and 12 are shown by IL28B genotype (Figs. 4a, 5a). The magnitudes of the decrease in HCV RNA from baseline at treatment week 4, 8 and 12 were significantly higher in patients with the IL28B TT genotype than in those with the IL28B non-TT genotype. The SVR rate for response-guided therapy was assessed according to



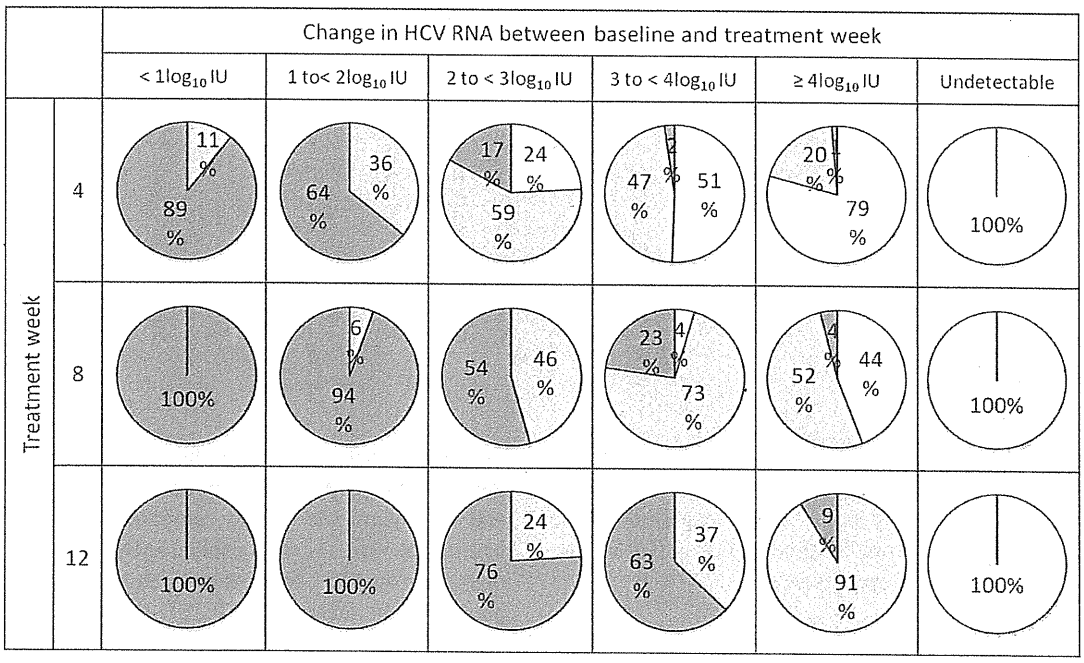


Fig. 1 **a** Patient prevalence according to the magnitude of HCV RNA decrease from baseline. ■ <1 log_{10} decrease. ▨ 1 to <2 log_{10} decrease. ▩ 2 to <3 log_{10} decrease. ▧ 3 to <4 log_{10} decrease. ≥4 log_{10} (detectable). □ undetectable. **b** SVR rate according to the magnitude of the decrease in HCV RNA from baseline

Fig. 2 Relationship between the virologic response during treatment and the magnitude of the decrease in HCV. RNA from baseline. □ early virologic response. ▨ late virologic response. ■ non-response

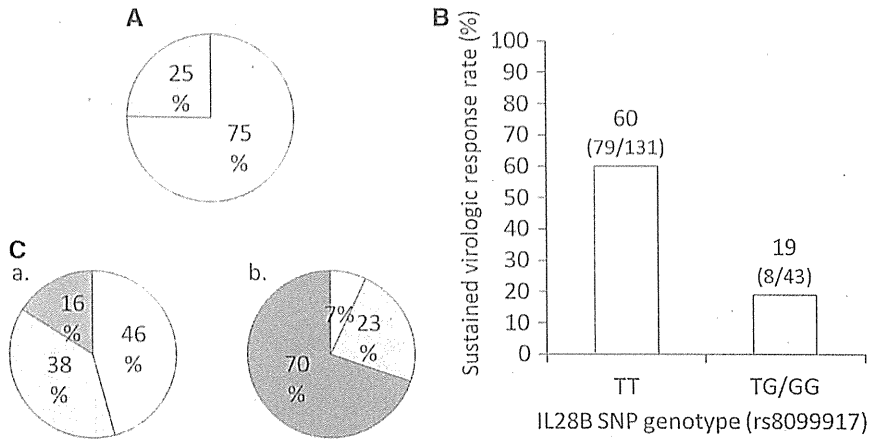


Fig. 3 **A** Patient prevalence according to IL28B genotype. □ TT genotype. ▒ non-TT genotype (TG of GG genotype). **B** SVR rate according to IL28B genotype. **C** Relationship between the virologic response during treatment and IL28B genotype. *a* Patients with IL28B TT genotype. *b* Patients with IL28B non-TT genotype. □ early virologic response. ▒ late virologic response. ■ non-response

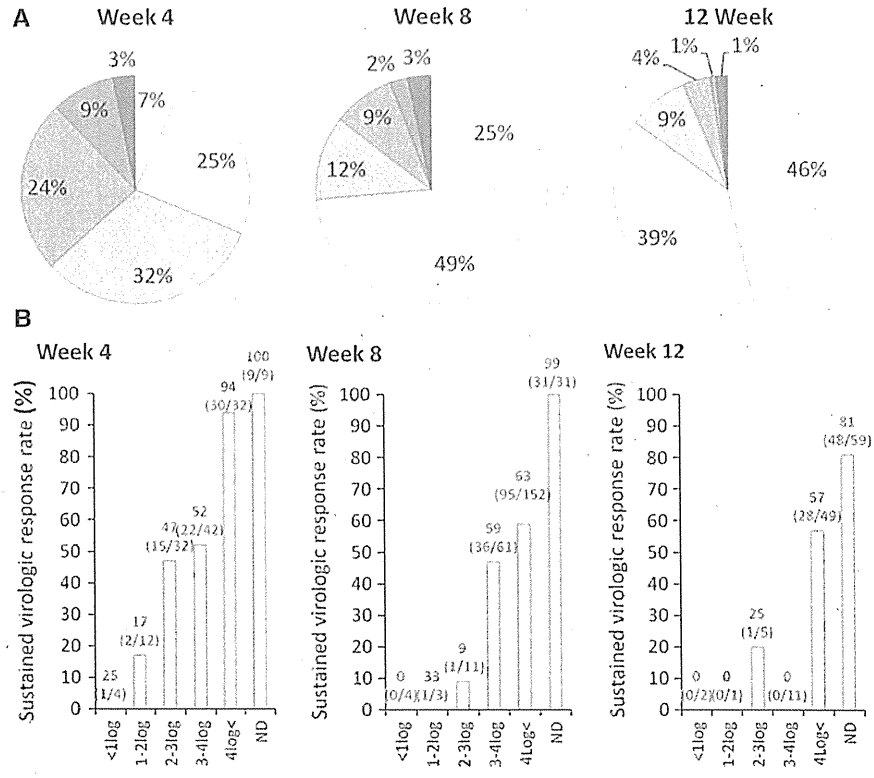


Fig. 4 **a** Patient prevalence according to the magnitude of HCV RNA decrease from baseline among patients with IL28B TT genotype. ■ <1 $\log_{10}$ decrease. ▒ 1 to <2 $\log_{10}$ decrease. ▒ 2 to <3 $\log_{10}$ decrease. ▒ 3 to <4 $\log_{10}$ decrease. ≥4 $\log_{10}$ (detectable). □ undetectable. **b** SVR rate according to the magnitude of the decrease in HCV RNA from baseline among patients with IL28B TT genotype

the six-group classification of the magnitude of the decrease in HCV RNA from baseline (Figs. 4b, 5b). The SVR rate increased step-by-step with the magnitude of the decrease in HCV RNA from baseline at 4, 8 and 12 weeks, irrespective of the IL28B genotype.

Factors associated with SVR

The factors associated with SVR were assessed for the variables shown in Table 1 and the magnitude of the decrease in HCV RNA from baseline at week 4 (Table 2). The factors selected as significant by univariate analysis (age, stage of liver fibrosis, platelet count, IL28B genotype and the magnitude of the decrease in HCV RNA from baseline at week 4) were evaluated by multivariate logistic regression analysis. In model 1, which consisted of only the pre-treatment factors, the IL28B genotype was the most powerful independent factor for SVR (Odds ratio, OR; 6.92, $p < 0.001$), apart from age (OR; 0.96, $p = 0.048$) and the stage of liver fibrosis (OR; 0.23, $p = 0.021$). However, in model 2, which included the parameter of viral kinetics at week 4, the magnitude of the decrease in HCV RNA

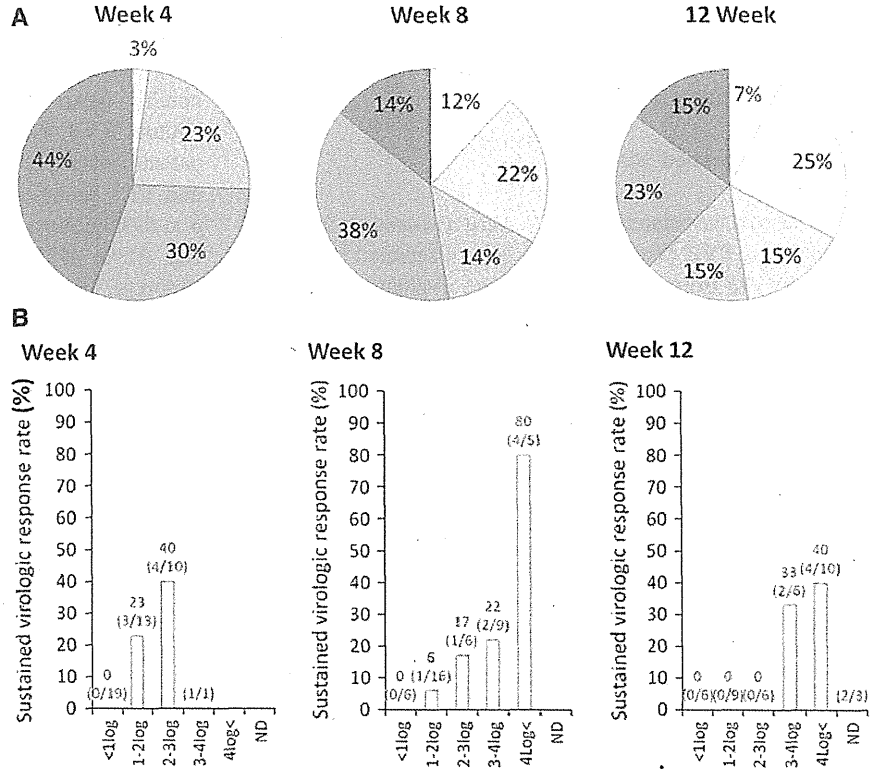


Fig. 5 **a** Patient prevalence according to the magnitude of HCV RNA decrease from baseline among patients with IL28B non-TT genotype. ■ <1 log_{10} decrease. ▩ 1 to <2 log_{10} decrease. ▨ 2 to <3 log_{10} decrease. ▧ 3 to <4 log_{10} decrease. ▩ ≥4 log_{10} (detectable). □ undetectable. **b** SVR rate according to the magnitude of the decrease in HCV RNA from baseline among patients with IL28B non-TT genotype

Table 2 Factors associated with SVR

Factor	Category	Univariate analysis			Multivariate analysis					
					Model 1			Model 2		
		OR	95 % CI	*p* value	OR	95 % CI	*p* value	OR	95 % CI	*p* value
Age		0.967	0.95–0.99	0.001	0.963	0.93–1.00	0.048	0.936	0.89–0.98	0.005
Sex	M/F	0.679	0.46–1.01	0.055						
Activity	A0–1/A2–3	0.671	0.42–1.06	0.090						
Fibrosis	F0–2/F3–4	0.301	0.15– 0.62	0.001	0.231	0.07–0.80	0.021	–	–	NS
HCV-RNA	By 1 log	0.657	0.53–1.03	0.075						
WBC		1.000	1.00–1.00	0.167						
Hb		1.080	0.94–1.24	0.272						
PLT		1.069	1.03–1.11	0.001	–	–	NS	–	–	NS
ALT		0.999	0.96–1.00	0.756						
γGTP		0.998	0.99–1.00	0.160						
IL28	TG or GG/TT	6.647	2.86–15.5	<0.001	6.924	2.60–18.4	<0.001	–	–	NS
Change of HCV RNA at 4 weeks	By 1 log increase	3.472	2.74–4.40	<0.001				3.338	2.20–5.06	<0.001

Model 1 consisted of pre-treatment factors. Model 2 consisted of pre-treatment factors and virologic response, that is the magnitude of the decrease in HCV RNA from baseline at treatment week 4

OR Odds ratio, *SVR* sustained virologic response, *WBC* white blood cells, *Hb* hemoglobin, *PLT* platelets, *ALT* alanine aminotransferase, *γGTP* γ-glutamyltransferase

from baseline at week 4 was the most powerful independent factor for SVR (OR; 3.4, $p < 0.001$) apart from age (OR; 0.936, $p = 0.005$), and the factor of IL28B was not a significant independent factor.

Factors associated with NR

The factors associated with NR were assessed in the same manner as SVR (Table 3). The factors selected as significant by the univariate analysis were evaluated by multivariate logistic regression analysis: grade of liver activity, stage of liver fibrosis, WBC count, platelet count, serum γ-glutamyltransferase level, IL28B genotype and the magnitude of the decrease in HCV RNA from baseline at week 4. In model 1, IL28B genotype was the most powerful independent factor for NR (OR; 39.75, $p < 0.001$), apart from the degree of liver fibrosis (OR; 10.31, $p = 0.021$) and platelet count (OR; 0.84, $p = 0.01$). However, in model 2, the magnitude of the decrease in HCV RNA from baseline at week 4 was the most powerful independent factor for NR (OR; 9.29, $p < 0.001$) apart from the degree of liver fibrosis (OR; 14.48, $p = 0.004$). IL28B was not selected as a significant independent factor.

SVR rate according to IL28B genotype and the timing of HCV RNA negativiation during treatment

Lastly, the SVR rate according to the IL28B genotype and the timing of HCV RNA negativiation during the treatment were assessed (Supplementary Figure 1). RVR was attained in nine patients with the IL28B TT genotype, and SVR was attained by all, but none of those with the IL28B non-TT genotype attained RVR. Among the patients without RVR, the SVR rate was significantly higher in the patients with the IL28B TT genotype than those with the non-TT genotype (57 %, 70/122 vs. 19 %, 8/43, $p < 0.001$). Among the patients with c-EVR, the SVR rate was 81 % (48/59) in the patients with the IL28B TT genotype and 67 % (2/3) in those with the non-TT genotype ($p = 0.48$). On the other hand, among the patients without c-EVR, the SVR rate was significantly higher in the patients with the IL28B TT genotype than those with the non-TT genotype (43 %, 30/70 vs. 16 %, 6/37, $p < 0.01$). Among the patients with LVR, the SVR rate was 60 % in both patients with the IL28B TT genotype (30/50) and the non-TT genotype (6/10) ($p = 1.00$). Next, the HCV RNA decrease among the patients without RVR or c-EVR was examined. The results revealed a significantly greater HCV RNA decrease in the IL28 TT group compared with the non-TT group among the patients without RVR (3.3 ± 1.2 $\log_{10}$ IU/ml vs. 1.3 ± 0.9 $\log_{10}$ IU/ml, $p < 0.001$) and those without c-EVR (4.7 ± 1.5 $\log_{10}$ IU/ml vs. 2.6 ± 1.5 $\log_{10}$ IU/ml, $p < 0.001$). The mean level of HCV RNA was significantly lower in the IL28 TT group compared with the non-TT group among the patients without RVR (3.3 ± 1.3 vs. 5.1 ± 1.2 $\log_{10}$ IU/ml, $p < 0.001$) and those without c-EVR (2.0 ± 1.4 vs. 3.9 ± 1.8 $\log_{10}$ IU/ml, $p < 0.001$).

Table 3 Factors associated with NR

Factor	Category	Univariate analysis			Multivariate analysis					
					Model 1			Model 2		
		OR	95 % CI	*p* value	OR	95 % CI	*p* value	OR	95 % CI	*p* value
Age		1.015	0.99–1.04	0.185						
Sex	M/F	1.187	0.76–1.86	0.455						
Activity	A0–1/A2–3	2.186	1.23–3.71	0.004	–	–	NS	–	–	NS
Fibrosis	F0–2/F3–4	5.079	2.53–10.0	<0.001	10.306	2.17–48.9	0.003	18.482	2.5–136.2	0.004
HCV-RNA	By 1 log	1.371	0.93–2.02	0.111						
WBC		1.000	1.00–1.00	0.004	–	–	NS	–	–	NS
Hb		0.947	0.81–1.10	0.498						
PLT		0.887	0.85–0.93	<0.001	0.844	0.74–0.96	0.01	–	–	NS
ALT		0.985	0.99–1.01	0.985						
γGTP		1.003	1.00–1.01	0.035	–	–	NS	–	–	NS
IL28	TT/TG or GG	12.088	5.4–26.9	<0.001	39.750	10.3–153.5	<0.001	–	–	NS
Change of HCV RNA at 4 weeks	By 1 log decrease	6.717	4.56–9.88	<0.001				9.292	3.95–21.8	<0.001

Model 1 consisted of pre-treatment factors. Model 2 consisted of pre-treatment factors and virologic response, that is the magnitude of the decrease in HCV RNA from baseline at treatment week 4

OR Odds ratio, *SVR* sustained virologic response, *WBC* white blood cells, *Hb* hemoglobin, *PLT* platelets, *ALT* alanine aminotransferase, *γGTP* γ-glutamyltransferase

Discussion

The importance of the timing of HCV RNA negativiation for the prediction of SVR has been well recognized [12–16]. In this study, all of the patients with RVR achieved SVR and those with c-EVR achieved 90 % of SVR by 48 weeks of treatment. However, only 8 % of the patients achieved RVR and about 40 % achieved c-EVR. This means that there is a need for another predictor of the effects of the response-guided therapy for the remaining population in which RVR or c-EVR were not attained. At present, triple therapy with Peg-IFN, ribavirin plus TVR, which can improve the antiviral outcome, is an option and the next generation of direct-acting antivirals is coming [1–8, 26–29]. Thus, the decision of "to treat or not to treat" during Peg-IFN plus ribavirin combination therapy should be carefully made with consideration of predictable antiviral outcomes and the degree of the adverse effect. In the present study, we focused on the early HCV RNA dynamics during the first 12 weeks of Peg-IFN plus ribavirin combination therapy.

First, we assessed the relationship between the magnitude of the decrease in HCV RNA from the baseline and SVR. At week 4, the patients without RVR were categorized into five groups, almost equally (Fig. 1a). The SVR rate increased step-by-step in proportion to the magnitude of the HCV RNA decrease at week 4, 8 and 12 (Fig. 1b). The SVR rates were very low (0–8 %) in patients with <1 $\log_{10}$ decrease at week 4, <2 $\log_{10}$ decrease at week 8 or <4 $\log_{10}$ decrease at week 12; NR rates were very high (63–100 %) in these patient groups (Fig. 2). All patients with <1 $\log_{10}$ decrease at week 8 or with <2 $\log_{10}$ decrease at week 12 were NR. These results could be useful for preparing guidelines on when to stop the response-guided therapy. Similar results were obtained from stratified analysis according to the IL28B genotype; SVR rates increased step-by-step in proportion to HCV RNA decrease in both the IL28B TT group and the non-TT group (Figs. 4, 5). Both groups showed almost the same SVR rates with the same level of HCV RNA decrease over the same number of weeks after the start of treatment, although few patients with non-TT showed a good response (marked HCV RNA decrease) to the treatment. This means that early HCV RNA decrease can predict SVR irrespective of the IL28B status.

In order to examine in more detail whether early HCV RNA dynamics can predict antiviral outcome irrespective of IL28B status, we investigated the predictive factors for treatment response before and after the start of treatment by multivariate logistic regression analysis. As a result, the IL28B genotype was found to be the strongest predictive factor for pre-treatment prediction in response-guided therapy (Table 2, model 1). However, on analysis including the HCV RNA decrease from the baseline at week 4, the magnitude of the HCV RNA decrease at week 4 was shown to be the best predictor for SVR (Table 2, model 2). Multivariate analysis for NR gave similar results; the IL28B genotype was the strong predictive factor for pre-treatment prediction and the HCV RNA decease at week 4 for post-treatment (Table 3). These results indicate that the information of the IL28B genotype is very useful for predicting the antiviral response before treatment, however, once the treatment is initiated, that of the HCV RNA decrease at week 4 can replace the IL28B status for predicting the antiviral outcome.

Recently, the relationship between the timing of HCV RNA negativiation and IL28B status for predicting the antiviral effect has been reported [21, 24]. No significant difference of the SVR rates was shown among patients with the same on-treatment virologic response, such as RVR or c-EVR, regardless of the IL28B genotype. However, the IL28B status was shown to affect the antiviral outcome among patients who could not attain RVR or c-EVR; higher SVR rates were obtained for patients with a favorable IL28B genotype. With the cohort enrolled in this study, we assessed stratified analysis according to the IL28B genotype and the timing of HCV RNA negativiation in order to determine whether the IL28B genotype directly affects the antiviral outcome or whether the difference of HCV RNA decrease caused by IL28B status affects the antiviral outcome (Supplementary Figure 1). The results showed that the IL28B genotype was associated with SVR among the patients without RVR or c-EVR, while nearly equal SVR rates were attained regardless of the IL28B genotype among the patients with HCV RNA negativiation over the same period of time. These results correspond to those of a previous study [21, 24], and the IL28B genotype was concluded to affect the antiviral outcome among the patients without RVR or c-EVR. However, examination of the HCV RNA decrease among the patients without RVR or c-EVR in our cohort showed that HCV RNA significantly decreased in the IL28 TT group compared with the non-TT group. These results suggest that the IL28B status does not directly affect the antiviral outcome and that the difference of HCV RNA decrease caused by the IL28B status affects the antiviral outcome among the patients without RVR or c-EVR. In sum, the HCV decrease at week 4 can be used in place of the IL28B status for predicting the antiviral outcome after the start of treatment, as shown by the multivariate analysis in the present study.

The limitation of this study was that pre-treatment viral factors were not assessed due to the small numbers of patients. Among the patients enrolled in this study, the amino acid (aa) sequences at position 70 in the HCV core protein were examined in some cases (126 patients). The analysis for SVR and NR including the factor of aa

substitution of core 70, the same results were obtained. That is, the results of the factor of IL28B in model 1 and the HCV RNA decrease at week 4 in model 2 were the significant predictive factors for SVR and NR but the factor of aa substitution of core 70 was not significantly associated with SVR by univariate analysis and NR by multivariate logistic regression analysis. Further examination is needed to clarify the usefulness of the factor of aa substitution of core 70 as a predictor for SVR or NR in this combination therapy.

In conclusion, the IL28B genotype is a very strong predictive factor in pre-treatment prediction. After the start of treatment, the magnitude of the HCV RNA decrease at week 4 seems to be the most reliable marker for predicting the antiviral outcome among patients treated with response-guided Peg-IFN plus ribavirin combination therapy.

Acknowledgments Other institutions and participants in the Osaka Liver Forum are: National Hospital Organization Minami Wakayama Medical Center, M. Kato; Kinki Central Hospital of Mutual Aid Association of Public School Teachers, E. Hayashi; Yao Municipal Hospital, H. Fukui; Osaka Koseinenkin Hospital, Y. Ito; Kaizuka City Hospital, Y. Yamada; Sumitomo Hospital, A. Yamada; Suita Municipal Hospital, T. Nagase; NTT West Osaka Hospital, A. Kaneko; Otemae Hospital, Y. Doi; Itami City Hospital, Y. Saji; Ashiya Municipal Hospital, A. Takeda; Nishinomiya Municipal Central Hospital, H. Ogawa; Saiseikai Senri Hospital, K. Suzuki; Izumiotsu Municipal Hospital, S. Yamagata; Osaka Kaisei Hospital, N. Imaizumi; Kano General Hospital, S. Kubota; Saso Hospital, M. Nishiuchi; and Meiwa Hospotal, Y. Hayakawa. This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from the Ministry of Health, Labour and Welfare of Japan, and Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Conflict of interest Professor Tetsuo Takehara received scholarship funds from Merck Sharp & Dohme K.K. Co., Ltd. and Chugai Pharmaceutical Co., Ltd. Dr. Tatsuya Kanto has an affiliation with a department funded with donations from Merck Sharp & Dohme K.K. Co., Ltd.

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J Gastroenterol (2014) 49:1175–1182
DOI 10.1007/s00535-013-0877-z

The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Liver stiffness measurement by acoustic radiation force impulse is useful in predicting the presence of esophageal varices or high-risk esophageal varices among patients with HCV-related cirrhosis

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Received: 5 August 2013 / Accepted: 23 August 2013 / Published online: 5 September 2013

Abstract
Background Screening and periodic surveillance for esophageal varices (EVs) by esophagogastroduodenoscopy (EGD) are recommended for cirrhotic patients. We investigated non-invasive liver stiffness measurement using acoustic radiation force impulse (ARFI) for the diagnosis of EV presence and high-risk EVs among patients with HCV-related cirrhosis.
Methods Among 181 consecutive patients with HCV-related cirrhosis, we studied 135 patients who had received EGD and ARFI. Serum fibrosis markers [platelet count, FIB-4, and aspartate aminotransferase-to-platelet ratio index (APRI)] were measured in a training set of 92 patients and compared with ARFI in the diagnostic performance for EV presence and high-risk EVs. Furthermore, the obtained optimal cutoff values of ARFI were prospectively examined in a validation set of 43 patients.
Results In the training set, the ARFI value increased with the EV grade ($p < 0.001$). The ARFI value for high-risk EVs was significantly higher than that for low-risk EVs ($p < 0.001$). AUROC values for diagnosis of EV presence and high-risk EVs by ARFI were 0.890 and 0.868, which had the highest diagnostic performance among factors including serum fibrosis markers. The optimal cutoff value of ARFI for EV presence was 2.05 m/s with good sensitivity (83 %), specificity (76 %), PPV (78 %), and NPV (81 %), and that for high-risk EVs was 2.39 m/s with good sensitivity (81 %), specificity (82 %), PPV (69 %), and NPV (89 %). These cutoff values obtained in the training cohort also showed excellent performance in the validation set.
Conclusions Liver stiffness measurement by ARFI is useful in predicting EV presence or high-risk EVs among patients with HCV-related cirrhosis.

Keywords Acoustic radiation force impulse · Esophageal varices · HCV-related cirrhosis · Portal hypertension · Liver stiffness

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Introduction

Esophageal varices (EVs) resulting from portal hypertension are present in approximately 50 % of patients with cirrhosis [1], and variceal bleeding is life-threatening with a 14 % mortality for hospitalized patients [2, 3]. The risk of bleeding has been shown to be related to the size of the varices, the presence of red signs, and the stage of liver insufficiency as evaluated by the Child–Pugh score [2, 4, 5]. Patients with high-risk EVs require prophylactic treatment to prevent variceal bleeding [1, 6]. Among patients with cirrhosis, the rate of EV incidence was reported to be 5 % at 1 year, 17 % at 2 years, and 28 % at 3 years, and the rate of EV progression from a small to a large size was found to be 12 % at 1 year, 25 % at 2 years, and 31 % at 3 years [7]. Therefore, cirrhotic patients should undergo periodic surveillance for EVs.

Esophagogastroduodenoscopy (EGD) is the gold standard for the diagnosis of EVs. American Association for the Study of Liver Diseases (AASLD) guidelines and Baveno V consensus strongly recommend screening EGD for all patients who are diagnosed with cirrhosis [1, 6]; the recommended intervals are 2–3 years for patients without varices and 1–2 years for those with small varices [6]. However, EGD causes psychological distress for patients when not performed under sedation, resulting in poor acceptance by patients. Furthermore, repeated EGD examinations can lead to complications and entail high costs. Therefore, instead of EGD, the development of non-invasive methods which can be useful in evaluating EVs is very important.

Several non-invasive markers such as platelet count, FIB-4 index, aspartate aminotransferase-to-platelet ratio index (APRI), and FibroTest have been examined as predictors of the accumulation of fibrosis and have been reported to predict the presence of EVs and large EVs in cirrhotic patients [8–10]. However, their performance values were not sufficiently accurate to support the use of these markers as alternatives to EGD. On the other hand, the platelet count/spleen diameter ratio (PSR) with a cutoff value of 909 has shown to have high sensitivity (100 %) and specificity (93 %) [11]. However, the PSR cutoff value (909) has not shown good accuracy for the prediction of EVs in a recent study [12].

As a non-invasive and ultrasound-based method, transient elastography (TE) has shown excellent diagnostic accuracy for the estimation of liver fibrosis [13, 14]. In addition, TE is useful for predicting portal hypertension [15]. However, TE measurements are difficult to perform with obese patients and when ascites is present, and the interfering structures such as blood vessels can not to be avoided in TE measurement [16]. On the other hand, acoustic radiation force impulse (ARFI), a new ultrasound imaging modality for evaluation of liver stiffness, can be performed for these patients, and a high level of accuracy of ARFI for predicting liver fibrosis has been reported over the last few years [17–19].

The aim of this study was to investigate whether ARFI can be useful in selecting patients with hepatitis C virus (HCV)-related cirrhosis who need screening EGD for the diagnosis of EV and who need EGD at short intervals for the diagnosis of the progression to high-risk EVs.

Patients and methods

The 181 consecutive patients with HCV-related cirrhosis visited in our hospital between April 2009 and January 2013. Among them, 40 patients did not undergo ARFI and 6 patients did not undergo EGD. Finally, 135 patients who had undergone both EGD and ARFI were enrolled in this study. ARFI was measured less than 6 months before or after the EGD examination. HCV infection was diagnosed by a real-time PCR method. Diagnosis of cirrhosis was done by histologic examination or combined physical, laboratory, and radiologic findings. Patients with a history of endoscopic treatment for EVs, portal thrombosis, β-blocker use, post-liver transplantation, co-infection with HBV, or other causes of liver disease [autoimmune hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis (NASH), primary biliary cirrhosis, etc.] were excluded from the study.

The patient characteristics and the following biochemical tests were recorded at the time of ARFI: platelet count, prothrombin time, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and albumin. Data were collected on the presence or absence of ascites, hepatic encephalopathy, and hepatocellular carcinoma (HCC). The Child–Pugh scores were also determined.

The training set comprised 92 patients who had undergone EGD and ARFI measurements between April 2009 and September 2012. The validation set comprised 43 patients who had undergone EGD and ARFI measurements from October 2012 to January 2013. We examined the diagnostic performance of ARFI for the presence of EVs and high-risk EVs and conducted comparative analysis with several serum non-invasive markers in the training set. The obtained optimal cutoff values of ARFI were prospectively examined in the validation set.

This study was approved by the institution's ethics committee and was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki amended in 2008. Written informed consent was obtained from all study patients.

Non-invasive serum markers

The relationships were examined for three established serum markers of liver fibrosis, i.e., platelet count, FIB-4, and APRI, and the presence of EVs or the risk of variceal bleeding. Serum liver fibrosis scores were calculated according to previously published formulas: FIB-4 = [age (years) × AST (IU/L)]/[platelet count (10^9/L) × ALT (IU/L)$^{1/2}$]; APRI = [(AST/ULN) × 100]/platelet count 10^9/L (ULN = the upper limit of normal) [20, 21].

Liver stiffness measurements

Liver stiffness was measured by ARFI, using the Siemens ACUSON S-2000 ultrasound system. ARFI is based on the measurement of the acoustic shear wave induced by an ultrasonic push pulse to assess the elastic properties of target tissues [22]. During real-time B-mode imaging, a region of interest (ROI) was set in the right hepatic lobe at a depth of 2 cm below the liver capsule. Patients were

examined in a supine position with short intervals of breath-holding. The operator performing the ARFI measurements was blinded to the EGD results. The velocity of the shear wave was quantified, and the results are expressed in meters per second (m/s). The mean values of 10 valid measurements for each patient were used for further analyses.

Endoscopic evaluation of EVs

EGD examination was performed by endoscopists who were blinded to the AFRI results. Consensus was reached for discrepancies in the diagnosis of varices during endoscopic conferences. EVs were recorded according to the general rules of the Japanese Society for Portal Hypertension [23]. The grade of EVs was classified as follows: F0, lesions assuming no varicose appearance; F1, straight small-calibered varices; F2, moderately enlarged, beady varices; F3, markedly enlarged, nodular, or tumor-shaped varices. Red color (RC) signs included red wale marking, cherry red spot, or hematocytic spot. We categorized varices as presence (grade $\geq$ F1) or absence (grade = F0). Patients were then divided into two groups according to the risk of EV bleeding; high-risk EVs (grade $\geq$ F2 or F1 with RC signs) and low-risk EVs (F0 or F1 without RC signs).

Statistical analysis

Quantitative demographic data are expressed as mean ± standard deviation. The non-parametric Mann–Whitney test was used to compare various subgroups. The relationship between the ARFI value and the grade of varices was assessed by the Jonckheere–Terpstra test. The diagnostic performance of ARFI, platelet count, APRI, and FIB-4 was assessed by using curves of receiver operating characteristics (ROC) and analysis of the area under the ROC (AUROC) curve. The optimal cutoff values were determined to maximize the sum of sensitivity (Se) and specificity (Sp). Se, Sp, positive predictive value (PPV), and negative predictive value (NPV) were calculated. AUROCs were compared using the DeLong test. The 95 % confidence intervals (95 % CIs) were calculated for each predictive test and a p value less than 0.05 was regarded as significant for each statistical test. All statistical analyses were performed with SPSS software, version 19 (SPSS, Chicago, IL, USA) and MedCalc software (MedCalc, Ostend, Belgium).

Results

The characteristics of the patients in the training and validation sets are presented in Table 1. ARFI was successfully performed in all patients. In the training set, EVs were found on conducting EGD in 47 patients (51.1 %): 19 with F1, 22 with F2, and 6 with F3. RC signs were present in 24 patients (26.1 %). There were 31 (33.7 %) and 61 (66.3 %) patients with high-risk and low-risk EVs, respectively. In the validation set, EVs were present on EGD in 27 (62.8 %) patients. High-risk EVs were present in 12 patients (27.9 %). The median ARFI values of the training set and the validation set were 2.12 m/s [interquartile range (IQR) 1.56–2.69] and 2.28 m/s (IQR 1.69–2.61).

Table 1 Baseline characteristics of patients in training and validation sets

Factor	Training set	Validation set
Number	93	43
Age (year)	68.8 ± 9.3	72.6 ± 6.9
Sex (male/female)	48/45	26/17
BMI (kg/m^2)	22.7 ± 3.2	22.3 ± 3.2
Child–Pugh class		
A	60 (64.5 %)	31 (72.1 %)
B	31 (33.3 %)	11 (25.6 %)
C	2 (2.2 %)	1 (2.3 %)
HCC		
No	47 (50.5 %)	12 (27.9 %)
Yes	46 (49.5 %)	31 (72.1 %)
Ascites		
No	82 (88.2 %)	35 (81.4 %)
Yes	11 (11.8 %)	8 (18.6 %)
Platelet ($\times 10^4$/mm^3)	9.63 ± 4.94	9.85 ± 4.30
AST (IU/L)	54.0 ± 29.9	38.6 ± 22.6
ALT (IU/L)	48.9 ± 32.9	53.4 ± 38.6
Albumin (g/dl)	3.59 ± 0.59	3.67 ± 0.56
Total bilirubin (mg/dl)	1.06 ± 0.86	1.02 ± 0.61

Results are given as mean ± standard deviation or n (%)

BMI body mass index, *HCC* hepatocellular carcinoma, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

ARFI measurements and EV grades

The median ARFI values for patients without EVs and with EVs were 1.59 m/s (IQR 1.27–2.07) and 2.63 m/s (IQR 2.14–3.03) and the ARFI value for patients with EVs was significantly higher than that without EV ($p < 0.001$) (Fig. 1a). The median ARFI values according to the grade of EVs were as follows: F0, 1.59 m/s (IQR 1.27–2.07); F1, 2.23 m/s (IQR 1.95–2.66); F2, 2.74 m/s (IQR 2.47–3.12); F3, 3.02 m/s (IQR 2.89–3.16) (Fig. 1b). The ARFI value increased with the grade of EVs ($p < 0.001$). The median ARFI value for low-risk and high-risk EVs were 1.74 m/s (IQR 1.40–2.24) and 2.84 m/s (IQR 2.48–3.15), and the

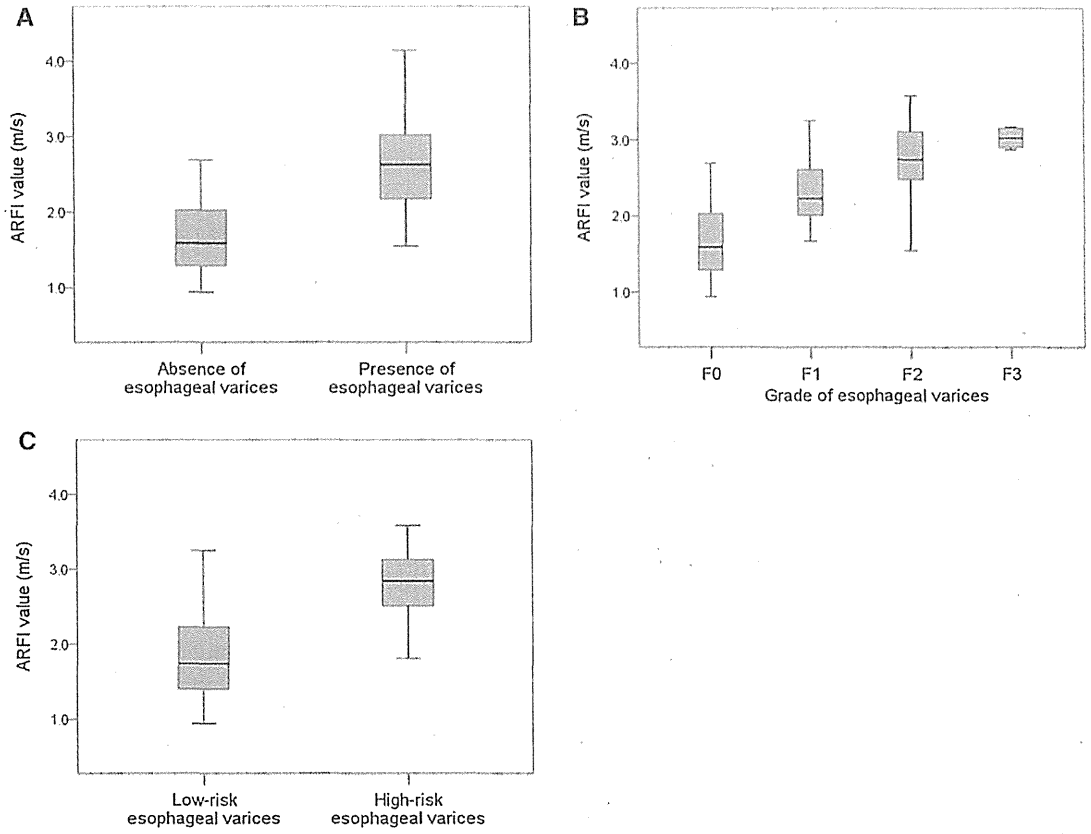

Fig. 1 Correlation between ARFI values and esophageal varices. The *top* and *bottom* of each *box* represent the first and third quartiles, respectively. The *middle line* represents the median. Correlation between ARFI values and **a** the presence or absence of esophageal varices, **b** the grade of esophageal varices, **c** low-risk esophageal varices or high-risk esophageal varices

ARFI value with high-risk EVs was significantly higher than that with low-risk EVs ($p < 0.001$) (Fig. 1c).

Diagnostic performances and optimal cutoff values for the presence of EVs

The ROC curves for diagnosis of the presence of EVs by ARFI, platelet count, FIB-4, and APRI are shown in Fig. 2. AUROCs (95 % CIs) were as follows: ARFI, 0.890 (0.825–0.955); platelet count, 0.735 (0.632–0.838); FIB-4, 0.745 (0.642–0.848); APRI, 0.684 (0.573–0.795). ARFI had the best diagnostic performance for predicting EVs compared with all other parameters (ARFI vs. platelet counts, $p = 0.0047$; ARFI vs. FIB-4, $p = 0.0041$; ARFI vs. APRI, $p = 0.0001$).

Table 2 shows the Se, Sp, NPV, and PPV of the optimal cutoff values for diagnosis of the presence of EVs. The optimal cutoff value of ARFI was 2.05 m/s with the best performance of Se (83 %), Sp (76 %), PPV (78 %), and NPV (81 %) compared with those of platelet count, FIB-4, and APRI.

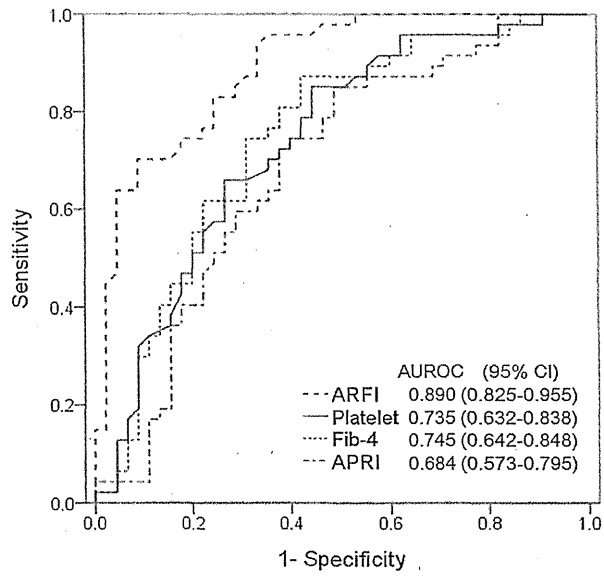

Fig. 2 Receiver operating characteristics curves of ARFI, platelet count, FIB-4, and APRI for detecting the presence of esophageal varices

Table 2 Diagnostic performance of ARFI, platelet count, FIB-4, and APRI for detecting the presence of esophageal varices

	Cutoff	Se (%)	Sp (%)	PPV (%)	NPV (%)
ARFI	2.05	83	76	78	81
Platelet	8.25	67	67	71	67
FIB-4	6.21	71	69	73	69
APRI	1.50	59	64	67	64

Se sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value

Diagnostic performances and optimal cutoff values for high-risk EVs

The ROC curves for diagnosis of high-risk EV by ARFI, platelet count, FIB-4, and APRI are shown in Fig. 3. AUROCs (95 % CIs) were as follows: ARFI, 0.868 (0.792–0.943); platelet count, 0.659 (0.547–0.771); FIB-4, 0.741 (0.635–0.847); APRI, 0.669 (0.555–0.784). ARFI had the best diagnostic performance for predicting EV compared with all other parameters (ARFI vs. platelet count, $p = 0.0004$; ARFI vs. FIB-4, $p = 0.0109$; ARFI vs. APRI, $p = 0.0002$).

Table 3 shows the Se, Sp, PPV, and NPV of the optimal cutoff values for diagnosis of high-risk EVs. The optimal cutoff value of ARFI was 2.39 m/s with the best performance of Se (81 %), Sp (82 %), PPV (69 %), and NPV (89 %) compared with those of platelet count, FIB-4, and APRI.

Diagnostic performance of ARFI cutoff value in validation set

In the validation set, the optimal cutoff values of ARFI analyzed by the training set for diagnosis of the presence of EVs (2.05 m/s) and high-risk EVs (2.39 m/s) were prospectively analyzed. The ARFI cutoff value for the presence of EVs showed good performance with Se (85 %), Sp (81 %), PPV (89 %), and NPV (77 %), and that for high-risk EVs with Se (83 %), Sp (77 %), and NPV (92 %), but not PPV (59 %) (Table 4). Thus, the diagnostic performance of ARFI for the presence of EVs and high-risk EVs in the validation set were almost equal to those in the training set.

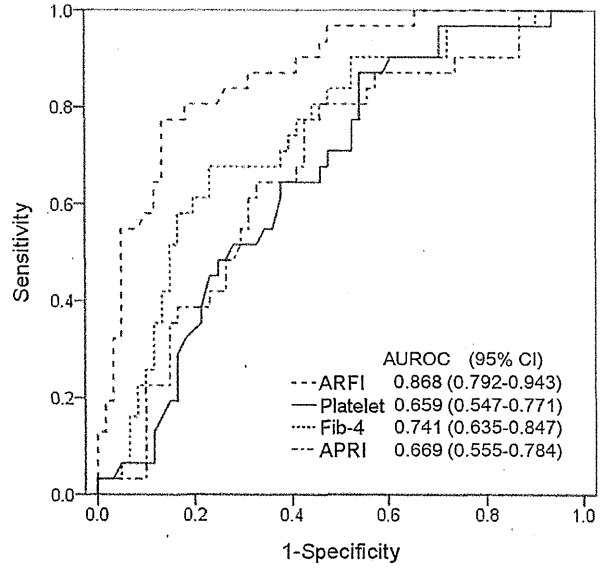

Fig. 3 Receiver operating characteristics curves of ARFI, platelet count, FIB-4, and APRI for diagnosis of high-risk esophageal varices

Table 3 Diagnostic performance of ARFI, platelet count, FIB-4, and APRI for detecting high-risk esophageal varices

	Cutoff	Se (%)	Sp (%)	PPV (%)	NPV (%)
ARFI	2.39	81	82	69	89
Platelet	7.95	64	63	49	63
FIB-4	7.70	67	78	63	78
APRI	1.62	64	68	53	68

Se sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value

Table 4 Diagnostic performance of ARFI for detecting the presence of esophageal varices and high-risk esophageal varices in training and validation sets

	Cutoff	Set	Se (%)	Sp (%)	PPV (%)	NPV (%)
Presence	2.05	Training	83	76	78	81
		Validation	85	81	89	77
High-risk	2.39	Training	81	82	69	89
		Validation	83	77	59	92

Se sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value

Discussion

Screening EGD for EVs is clinically important in the management of patients with cirrhosis. However, EGD is an examination that is not readily accepted by patients [24]. Therefore, there has been increasing interest in developing non-invasive methods for prediction of EVs. Recently, several serum markers and imaging methods have been shown to correlate well with liver fibrosis and can replace liver biopsy in the diagnosis of liver fibrosis [18]. Several of these methods have been tried for non-invasive assessment of EV prediction. Another simple test, the platelet count, has not been a good predictor of the presence of EVs in patients with cirrhosis (AUROC 0.63) [8]. In a large cirrhotic cohort, FIB-4 showed an AUROC of 0.64 for the

presence of EVs and 0.63 for large EVs, and APRI showed an AUROC of 0.57 for the presence of EVs and 0.60 for large EVs [9]. In the present study, the diagnostic performances for the presence of EVs and high-risk EVs by platelet count (AUROC 0.735 and 0.659, respectively), FIB-4 (AUROC 0.745 and 0.741, respectively), and APRI (AUROC 0.684 and 0.669, respectively) were superior to those by previously reported findings [8, 9]. Nevertheless, ARFI showed better diagnostic performances (AUROC 0.890 and 0.868, respectively) than these serum fibrosis markers in this study.

In this study, we examined patients with HCV infection. A large study evaluating the performance of TE for diagnosis of cirrhosis showed variation in optimal liver stiffness cutoff values depending on the underlying cause of HCV infection, HBV infection, and alcoholic liver disease or NASH [25]. The factors which contribute to increasing liver stiffness other than fibrosis were reported as follows: severe inflammation which was characterized by ALT elevation often displayed in patients with chronic HBV infection [26–29], perisinusoidal fibrosis which was common in patients with alcoholic liver disease and NASH [25], and alcohol consumption characterized by AST elevation [30]. In addition, pooled meta-analysis suggested lower diagnostic performance for liver fibrosis in patients with chronic HBV infection. That is, the AUROC of diagnosis of the fibrosis (METAVIR fibrosis score $\geq$2) by ARFI in patients with chronic HBV infection was lower than that in those with chronic HCV infection (AUROC 0.79 vs. 0.88) [19]. This result could be related to architectural abnormalities, characterized by inhomogeneous liver fibrosis and macronodular cirrhosis, which is common in patients with HBV-related cirrhosis [25]. Thus, we suggest that liver stiffness measurement (LSM) for diagnosis of liver fibrosis should be evaluated according to the underlying disease, and the patients who were limited to HCV-related cirrhosis were examined in this study.

Recently, prediction of the presence of EVs and large or high-risk EVs by TE or ARFI has been reported in several studies [28, 31–36]. However, the diagnostic performances differed greatly among these reports; AUROC for the presence of EVs was from 0.58 to 0.84 and that for large or high-risk EVs was from 0.58 to 0.83 [28, 31–36]. The reason for these differences of AUROCs is considered to arise as a result of the underlying liver disease. In fact, Pritchett et al. [31] reported that the AUROC for predicting large varices by TE in patients with only HCV-related cirrhosis was higher than those with various cirrhosis etiologies except HCV-related (AUROC 0.78 vs. 0.72). Thus, as well as the evaluation of the LSM for diagnosis of liver fibrosis, the correlation between EVs and the liver stiffness by ARFI should be evaluated according to the specific etiology of liver diseases.

Our study is the first assessment of the prediction of EVs by ARFI for a patient group with homogeneous cirrhotic disease of HCV etiology. This led to the very good diagnostic performance for predicting the presence of EVs or high-risk EVs (AUROC 0.890 or 0.868, respectively) in the training cohort, and these results were also confirmed to prospectively validate another cohort of HCV-related cirrhosis patients whose characteristics differed from the training cohort. The results of our study showed better diagnostic performance than those of the past studies described above. Only one report showed high diagnostic performance of TE for the presence of EVs and high-risk EVs among patients with only HCV (AUROC 0.87 and 0.84, respectively) [37]. TE can show diagnostic performance for EVs comparable to ARFI. However, ARFI may have some advantages over TE for LSM. For one, the rate of unsuccessful TE was reported to be as high as 18.9 %, mostly because of obesity, ascites, and patients with narrow intercostal spaces [16]. On the other hand, ARFI is not limited by these conditions and the rate of unsuccessful results was reported to be 2.9 % overall [19]. In the present study, ARFI could be successfully performed in all patients. Moreover, ARFI is superior in terms of its convenience because it is integrated into a conventional ultrasonography (US) system using conventional probes and can be performed during standard US examinations. In addition, the ROI, which is 10 mm long and 6 mm wide, is smaller than that of TE and can be chosen while performing real-time B-mode imaging. Therefore, we can see the ROI while avoiding nearby interfering structures such as blood vessels and minimize the measurement error. Further study is needed to clarify whether ARFI surpasses TE in LSMs.

In recent studies, the relationship between spleen stiffness and the presence of EVs and high-risk or large EVs has been assessed. Takuma et al. [36] reported the high diagnostic performance of spleen stiffness for the presence of EVs and high-risk EVs (AUROC 0.933 and 0.930, respectively). On the other hand, Vermehren et al. [34] showed that the diagnostic performance of spleen stiffness for predicting large EVs was low (AUROC 0.58). Thus, the diagnostic values of EVs or portal hypertension by spleen stiffness remain controversial. The mechanism of spleen stiffness arising as portal hypertension develops should be investigated.

The limitation of our study is that a serial prospective study by ARFI for the development of EVs for a specific individual was not done. Therefore, the ideal interval for ARFI measurement to follow-up on EVs remains unclear. Further study is needed.

In conclusion, for patients with HCV-related cirrhosis, we showed that LSM by ARFI can non-invasively predict the presence of EVs or high-risk EVs. This indicates that the non-invasive ARFI can be useful in selecting patients with HCV-related cirrhosis who need screening EGD or EGD at short intervals for the diagnosis of the progression to high-risk EVs.

Acknowledgement Dr. Kanto belongs to an endowed department sponsored by MSD. Dr. Takehara received donations from MSD and Chugai Pharmaceutical Co., Ltd.

Conflict of interest The authors declare that they have no conflict of interest.

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