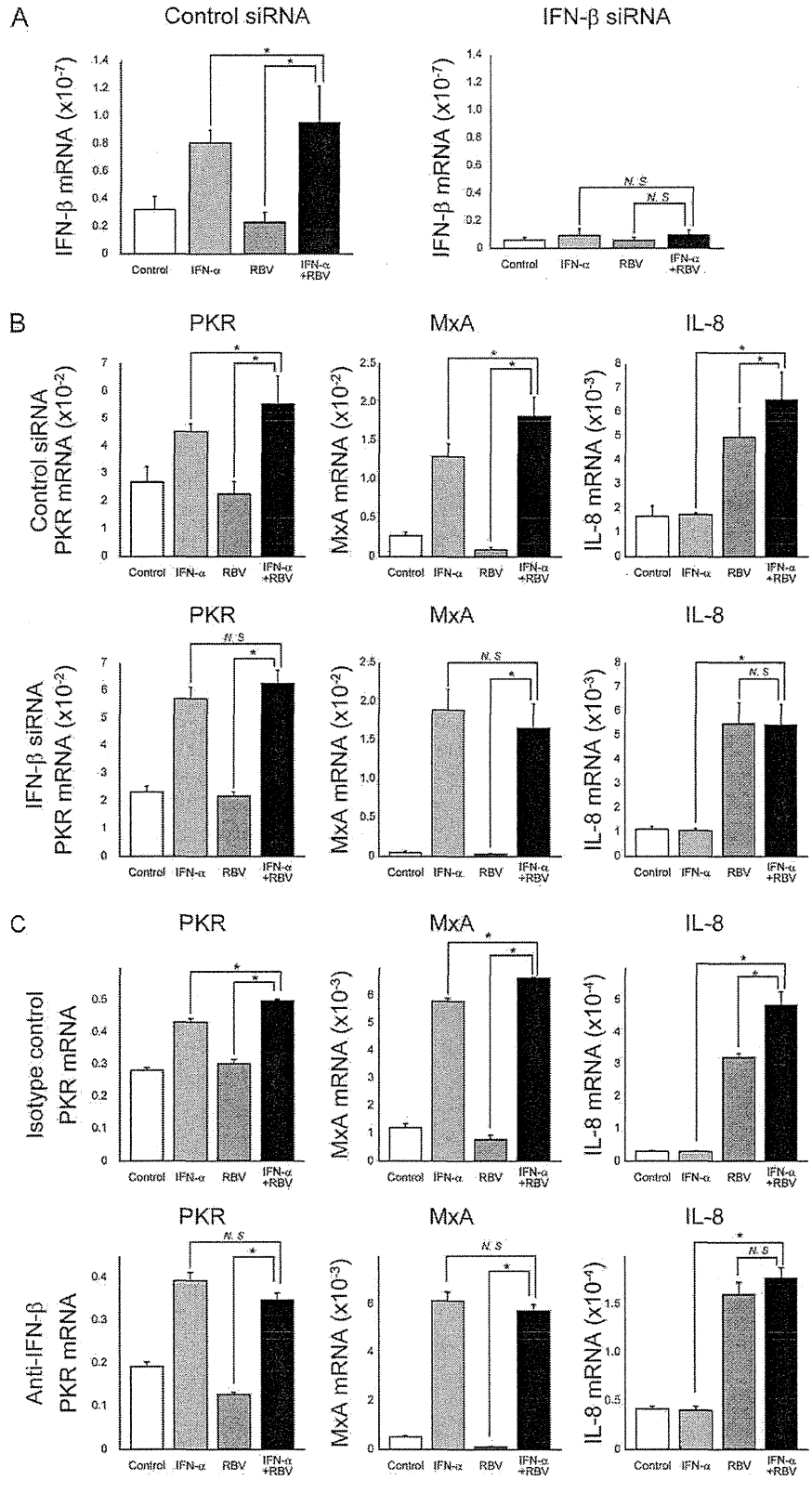
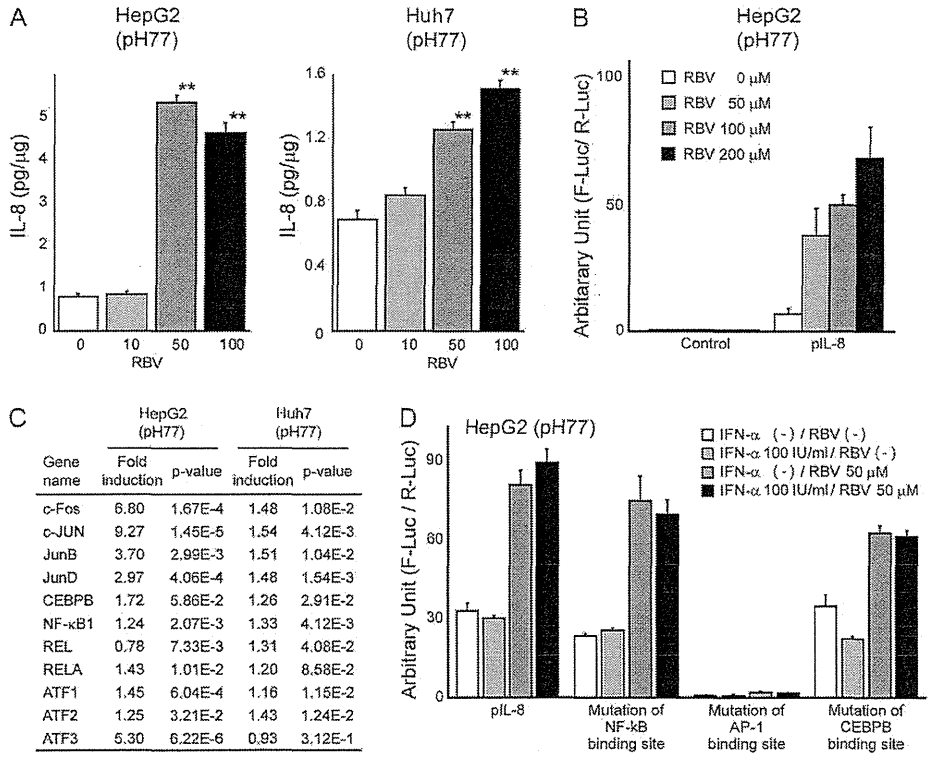


Figure 3. Silencing interferon (IFN)–β diminishes ribavirin (RBV) plus IFN-α–induced upregulation of protein kinase R (PKR) and myxovirus resistance protein A (MxA) but not of interleukin 8 (IL-8). *A,* Transfection with IFN-β-specific small interfering RNA (siRNA) reduced IFN-β mRNA levels by 90% with



Gene name	HepG2 (pH77) Fold induction	HepG2 (pH77) p-value	Huh7 (pH77) Fold induction	Huh7 (pH77) p-value
c-Fos	6.80	1.67E-4	1.48	1.08E-2
c-JUN	9.27	1.45E-5	1.54	4.12E-3
JunB	3.70	2.99E-3	1.51	1.04E-2
JunD	2.97	4.06E-4	1.48	1.54E-3
CEBPB	1.72	5.86E-2	1.26	2.91E-2
NF-κB1	1.24	2.07E-3	1.33	4.12E-3
REL	0.78	7.33E-3	1.31	4.08E-2
RELA	1.43	1.01E-2	1.20	8.58E-2
ATF1	1.45	6.04E-4	1.16	1.15E-2
ATF2	1.25	3.21E-2	1.43	1.24E-2
ATF3	5.30	6.22E-6	0.93	3.12E-1

Figure 4. Ribavirin (RBV) dose-dependently upregulates interleukin 8 (IL-8) expression and IL-8 transcription through AP-1 signaling. *A,* RBV dose dependently increased IL-8 expression in both HepG2 and Huh7 cells. *B,* Results of luciferase assays show that RBV dose-dependently upregulates IL-8 transcription. *C,* Polymerase chain reaction (PCR) array analysis of IL-8 promoter-related genes shows that RBV upregulates AP-1 related genes. *D,* Data from reporter constructs with or without mutations in activator protein 1 (AP-1), CCAAT/enhancer binding protein β (CEBPB), and nuclear factor κ–light-chain-enhancer (NF-κB) sites show that RBV-induced IL-8 transcriptional upregulation is responsible for AP-1. Data are shown as means ± standard error (SE) of 6 replicates . $^{**}P < .01$, compared with absence of RBV; Wilcoxon test.

Neutralization of IFN-β Attenuates ISG Upregulation by RBV Plus IFN-α

We neutralized secreted IFN-β by including an anti–IFN-β antibody in the culture medium and then added IFN-α and/or RBV to the cell cultures to further assay IFN-β inhibition. The upregulation of PKR and MxA induced by RBV plus IFN-α was diminished, compared with that by IFN-α alone, in cells cultured with anti–IFN-β antibody (Figure 3*C*). The amount of the reduction was quite similar to that induced by IFN-β siRNA. These results indicate that RBV plus IFN-α induced these ISGs through an increase in the amount of secreted IFN-β. However, RBV still upregulated IL-8 after neutralization with anti–IFN-β. These results suggest that the main pathway of RBV-induced IL-8 upregulation is not via autocrine IFN-β. These findings corresponded to those of the assay with IFN-β siRNA and confirmed that autocrine IFN-β is responsible for the RBV-induced enhancement of PKR and MxA, but not of IL-8.

IL-8 Is Enhanced by RBV Through AP-1 Signaling

The ELISA results showed that RBV increased IL-8 expression in both pH77-HepG2 and pH77-Huh7 cells (Figure 4*A*), but to a greater extent in the former. The results of luciferase assays using a reporter construct of the promoter region of the IL-8 gene confirmed that RBV dose-dependently upregulated the level of IL-8 transcription, thus confirming a direct effect of RBV (Figure 4*B*).

Figure 3 continued. or without IFN-α and RBV in HepG2 cells expressing hepatitis C virus (HCV). *B,* Transfection with IFN-β siRNA diminished additive upregulation of PKR, MxA by RBV plus IFN-α compared with IFN-α alone, indicating that enhanced IFN-β is associated with additive upregulation of IFN stimulating genes by RBV. *C,* Neutralization of secreted IFN-β using anti-IFN-β antibody diminished additive upregulation of PKR and MxA by RBV, indicating that secreted IFN-β is responsible for their upregulation. However, silencing and neutralizing IFN-β did not modify RBV-induced IL-8 upregulation (*B, C*). Samples prepared from HepG2 cells transfected with pH77 and infected with Ad-T7 at day 2 after adding reagents. Data are shown as means ± standard error (SE) of 6 replicates. $^{*}P < .05$.

We examined the mechanism of RBV-induced IL-8 upregulation using a PCR array for transcription factors (Supplementary Table 2) and identified genes associated with the IL-8 promoter (Figure 4*C*). RBV upregulated the AP-1-related genes c-Jun, c-Fos, JunB, JunD, and ATF3, but not CEBPB or NF-κB.

We further analyzed the mechanism of RBV-induced IL-8 upregulation. The results of the luciferase assays using reporter constructs with or without mutations in the AP-1, CEBPB, and NF-κB sites confirmed that AP-1 is responsible for RBV-induced IL-8 transcriptional upregulation (Figure 4*D*).

DISCUSSION

IFN is considered to be essential for eliminating HCV, and RBV has additive anti-HCV effects only when combined with IFN-α. Here, we investigated modifications of the ISGs (PKR, MxA, OAS, and IL-8) that are reportedly associated with HCV replication to determine how RBV plus IFN-α affects HCV. We found that RBV upregulates PKR activity as well as MxA and IL-8 expression through enhancing autocrine IFN-β. Moreover, RBV significantly upregulated IL-8 without IFN-α, which is responsible for AP-1 upregulation. The benefits and disadvantages of ISG modification by RBV must be understood before HCV can be eliminated with or without IFN. Moreover, such understanding might lead to a discovery of novel and more-powerful RBV-like compounds against HCV.

Our findings in vivo showed that RBV upregulates PKR and MxA mRNA in peripheral T lymphocytes from HCV-infected patients treated with IFN-α. These findings are compatible with a report describing that RBV induces ISGs in the human liver and in PBMCs [24]. The ISGs in PBMCs could be predictive factors for evaluating the effectiveness of anti-HCV therapy [24, 25]. However, this strategy is somewhat limited, because ISG expression and/or mechanisms regulating ISG expression might differ between PBMCs and liver cells [26]. We were also concerned that the PBMC population might be altered during IFN-based therapy. We therefore isolated T lymphocytes for this analysis. However, the significantly different distribution of the HCV genotype in patients with or without RBV still imposed limitations. We confirmed the alterations of ISGs by IFN and/or RBV using assays of hepatocytes in vitro because of these limitations.

Thomas et al found that RBV induced ISGs in a culture model comprising the JFH-1 HCV genotype 2a strain and the Huh7.5.1 cell line [27]. We also used the JFH-1 strain with Huh7 cell lines and the H77 HCV genotype 1a strain with Huh7 and HepG2 cell lines. The additive upregulation of autocrine IFN-β was evident in HepG2 cells with H77 but undetectable in Huh7 cells with JFH-1 and H77. Our findings from a replication system using HepG2 cell lines indicated that RBV upregulates some ISGs through IFN-β enhancement. The basis for HCV replication permissiveness in Huh7 and Huh7.5.1 cells has not been fully explained. However, it might be associated with a low response of autocrine IFN-β induction triggered by viral double-stranded RNA (dsRNA) [28] and insufficient expression of Toll-like receptor 3, which recognizes dsRNA [29]. Therefore, interactions between host cellular proteins and HCV using systems based strictly on Huh7 and Huh7.5.1 cells might be confounded by the relative IFN-β deficiency in these cells. The pH77-based HCV expression system permitted HCV replication in both Huh7 and HepG2 cells. We found that autocrine IFN-β and ISGs are more upregulated in HepG2 than in Huh7. We then examined the roles of RBV in the regulation of these factors in HepG2 cells and found that RBV induced the upregulation of IFN-β and of related ISGs. The kinetics of PKR and MxA among the ISGs were similar to those of IFN-β. The increase on day 1 of IFN-β might be affected by transfection and infection, but the similar kinetics of IFN-β and ISGs after adding RBV indicated that these are associated.

We assayed IFN-β inhibition using IFN-β siRNA to knock down IFN-β expression and an anti–IFN-β antibody to block secreted IFN-β. The results of both assays showed that PKR and MxA upregulation was inhibited. The RBV-induced enhancement of these key factors for anti-HCV effects depends on the induction and secretion of autocrine IFN-β. However, RBV only affected PKR, MxA, and autocrine IFN-β, when combined with IFN-α. This phenomenon would be in accord with clinical evidence showing that RBV can only eliminate HCV when combined with IFN-α [30], because the upregulation of autocrine IFN-β by RBV alone is insufficient. Moreover, the less dramatic induction of autocrine IFN-β by RBV would be comparable to the clinical significance of the anti-HCV effects elicited by RBV.

The upregulation of PKR and MxA through IFN-β enhancement will be important for the anti-HCV effects of RBV, but RBV also dose-dependently elevated IL-8 in a process that partially depends on the enhancement of autocrine IFN-β. However, the main mechanism of IL-8 enhancement was independent of autocrine IFN-β. IL-8 is a chemokine that serves as a chemical signal to attract neutrophils at sites of inflammation, and therefore, it is also known as neutrophil chemotactic factor [31]. One possible benefit of RBV-induced IL-8 upregulation is that neutrophils recruited by IL-8 would phagocytose target antigens, which would trigger Toll-like receptors and result in increased autocrine IFN-β and ISG production in vivo. On the other hand, the negative regulation of anti-HCV activity by IL-8 [32] suggests that the upregulation of IL-8 induced by RBV would not benefit HCV elimination [33, 34]. Moreover, IL-8 is related to inflammation including viral hepatitis and would increase oxidant stress [35], which might weaken the effects of anti-HCV drugs. Whether the IL-8 upregulation by RBV would positively or negatively affect the anti-HCV activity of IFN in the body should be evaluated in a future study in vivo that would include the immune systems.

Autocrine IFN-β partially affected RBV-induced IL-8 enhancement, but it mostly depended on the activation of transcription by AP-1, and not by NF-κB or CEBPB. Activator protein-1 is a family of transcription factors comprising homodimers or heterodimers of Jun and Fos or activating transcription factor proteins [36]. Jun-Jun and Jun-Fos dimers preferentially bind to the phorbol 12-O-tetradecanoate-13-acetate–responsive element located in the IL-8 promoter. The results of the PCR array showed that RBV upregulates the Fos and Jun families. These AP-1 proteins are regulated by mitogen-activated protein kinases, which in turn are partially regulated by Toll-like receptor signaling pathways [36]. To clarify the role of RBV-induced AP-1 activation in the context of HCV infection would be a target of further investigation.

In summary, we showed that RBV additively upregulates PKR and MxA through enhancing autocrine IFN-β and IL-8 through AP-1 activation. These properties of RBV could both positively and negatively affect HCV elimination. Understanding the precise effects of RBV in the liver infected with HCV would be essential to developing a suitable therapeutic strategy with which to eliminate HCV in the clinical setting.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Original Article

Occurrence of clinical depression during combination therapy with pegylated interferon alpha or natural human interferon beta plus ribavirin

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Aim: The onset of depression symptoms during pegylated interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to treatment discontinuation in some cases. In the present study, we conducted a questionnaire survey during treatment to determine whether natural human interferon β plus ribavirin (IFNβ/RBV) therapy is associated with a lower incidence of depression symptom onset compared with PEG-IFN/RBV therapy.

Methods: Seventy-seven patients with chronic hepatitis C received PEG-IFN/RBV (PR) or IFNβ/RBV (FR) therapy. A questionnaire survey was administered at the start of treatment, and at 4 and 12 weeks, using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).

Results: BDI-II scores in the PR group increased at 4 and 12 weeks, but remained unchanged in the FR group. At 12 weeks, the mean BDI-II score and incidence of abnormalities with a BDI-II score of ≥14 were significantly lower in the FR group than in the PR group. BDI-II scores during IFNβ/RBV therapy in 11 patients currently using antidepressants remained unchanged up to 12 weeks. None of these 11 patients required addition or dose increases of antidepressants, and there was no evidence of worsened depression symptoms. Nine PR patients had BDI-II scores of ≥14 and PSQI scores of ≥11 at 12 weeks.

Conclusions: IFNβ/RBV therapy was associated with a lower incidence of depression symptom onset during treatment. In patients already diagnosed with depression, there was no evidence that IFNβ/RBV therapy caused any worsening of symptoms, indicating that IFNβ/RBV therapy is safe for patients with depression.

Key words: Beck Depression Inventory II, chronic hepatitis C, depression, natural interferon β, pegylated interferon α, Pittsburgh Sleep Quality Index.

INTRODUCTION

INTRODUCTION OF PEGYLATED interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to an improved sustained virological response (SVR) in patients with chronic hepatitis C who are receiving interferon therapy.[1–6] An additional new treatment regimen has been introduced by adding Telaprevir to this PEG-IFN/RBV therapy.[7,8] However, adverse effects of PEG-IFN/RBV include the onset of symptoms of depression.[9–11] Thus, there are some difficulties in treating patients with depression or sleep disorders with PEG-IFN/RBV therapy.

In Japan, natural human interferon β (IFNβ), which has a low association with the onset of symptoms of depression, has been used in interferon therapy for chronic hepatitis C.[12,13] IFNβ plus ribavirin (IFNβ/RBV) combination therapy is now used.[14] However, there are no existing reports on the relationship between PEG-IFN/RBV or IFNβ/RBV therapy and the onset of depression symptoms. Therefore, in the present study, in order to determine if IFNβ/RBV therapy is associated with a lower incidence of the onset of symptoms of depression compared to PEG-IFN/RBV therapy, and to evaluate the safety of the IFNβ/RBV therapy in patients with depression, we conducted a questionnaire survey during PEG-IFN/RBV or IFNβ/RBV therapy to investigate the frequency, timing, and intensity of depression symptoms.

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METHODS

Study population

A TOTAL OF 77 Shinkokura Hospital patients with chronic hepatitis C who received IFN therapy for at least 12 weeks between January 2010 and April 2011 were included in the study. The study protocol was in compliance with both the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Patients were assigned to one of the following three groups: (1) the PEG-IFN/RBV (PR) group, consisting of 41 patients who received PR therapy for a period of 24 to 48 weeks; (2) the IFNβ/RBV (FR) group, consisting of 25 patients who received the FR therapy for a period of 24 to 48 weeks; and (3) the FR-d group, consisting of 11 patients with depression who were on antidepressants and who received the FR therapy for a period of 24 to 48 weeks. Patients in the FR-d group received regular psychiatric consultation and experienced dose reduction, dose increase, or addition of antidepressants during treatment. Patients with depression, those with a previous history of depression, those who were on antidepressants, or those who were on sleep-inducing drugs were excluded from the PR and FR groups. Patients reporting some type of sleep disorder during treatment were given sleep-inducing drugs at the discretion of their primary physician. Treatment regimens of PR or FR therapy were determined by the physician. None of the patients required dose reduction of IFN due to neutropenia or thrombocytopenia prior to 12 weeks. This study is a prospective, non-randomized open trial.

Criteria for exclusion from the study were as follows: (i) clinical or biochemical evidence of hepatic decomposition and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) IFNβ/RBV: white blood cell count of less than 3000/mm^3 and platelet count of less than 50 000/mm^3, PEG-IFN/RBV: white blood cell count of less than 4000/mm^3 and platelet count of less than 80 000/mm^3; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within the 12 months prior to enrollment.

Interferon treatment

Patients in the PR group received the following treatment regimen. In brief, PEG-IFNα-2b (PEG-Intron; MSD Co., Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 lg/kg (range: 1.3–2.0 lg/kg) once a week. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Patients in the FR and FR-d groups received the following treatment regimen. Briefly, IFNβ (Feron; Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 20–44 weeks. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Hepatitis C virus (HCV) RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/mL. Patients were considered to have an SVR if HCV RNA remained undetectable at 24 weeks after the completion of treatment. Urinalysis and measurement of serum albumin levels were performed once every 4 weeks, from the start of treatment to Week 24.

Questionnaire

A questionnaire survey was conducted immediately before the start of treatment and at 4 weeks and 12 weeks using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).[15,16] The questionnaire survey was administered by one expert investigator, who remained blinded to the treatment regimens prescribed to patients, the timing of treatment, and other information. Patients with a BDI-II score of 14 or more were considered to have the onset of depression symptoms. Patients with a PSQI score of 11 or more were identified as having sleep disorder. All patients were given a questionnaire at 12 weeks, while a questionnaire was administered to 58 subjects at the baseline and at 4 weeks, including 28, 19, and 11 patients in the PR, FR, and FR-d groups, respectively.

Statistical analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups, as well as the BDI-II score and the PSQI score at 12 weeks. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to the onset of symptoms of depression. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values less than 0.05, as determined by the two-tailed test, were considered significant. Variables were entered into

multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with patients having the onset of depression included the following variables: age, sex, HCV genotype, type of IFN, hemoglobin, platelet count, alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γ-GTP), total cholesterol, fasting blood sugar, and HCV RNA level.

RESULTS

Baseline background and IFN treatment

TABLE 1 SHOWS THE background of patients in the PR and FR groups. The mean age was significantly higher in the FR group (64.1 years) than in the PR group (52.5 years; $P < 0.001$). The PR group had more men than the FR group, although statistical significance was not reached. Baseline laboratory data showed a significantly lower platelet count in the FR group ($P < 0.05$). Significantly lower γ-GTP values were observed in the FR group ($P < 0.05$). The other laboratory parameters were comparable between the two groups. More patients with genotype 1 were in the PR group than the FR group, although no statistical significance was found. A total of 59 of 66 patients were evaluable for SVR. The proportion of patients with genotype 1 achieving an SVR was 33% (3/9) in the FR group and 48% (12/25) in the PR group. The PR group had a higher SVR rate, although statistical significance was not reached. The SVR rate among patients with genotype 2 was similar in the FR (85%, 11/13) and PR (83%, 10/12) groups. Over 24 weeks of treatment, 8% of patients (3/36) experienced at least one proteinuria event. None of the patients had a serum albumin level of ≤3.3 g/dL.

Change in the BDI-II score and the PSQI score during IFN treatment

Changes in the BDI-II score over time are shown in Figure 1. BDI-II scores in the PR group were increased relative to baseline at 4 and 12 weeks. Corresponding scores in the FR group remained unchanged. At 12 weeks, BDI-II scores were significantly lower in the FR group (5.8) than in the PR group (12.6; $P < 0.05$). The FR-d group had already high BDI-II scores of 23.0 at baseline, but BDI-II scores remained unchanged during treatment. No patients required dose increase or addition of antidepressants during treatment. There was no evidence of worsened depression symptoms during FR therapy.

In the PR group, the incidence of the onset of symptoms of depression, defined as a BDI-II score of 14 or more, increased from 0% at baseline to 21% at 4 weeks

Table 1 Clinical background before combination therapy of interferon β plus ribavirin (IFNβ/RBV) or pegylated interferon α plus ribavirin (PEG-IFN/RBV) in chronic hepatitis C patients

		IFNβ /RBV $n = 25$		PEG-IFN/RBV $n = 41$		IFNβ /RBV with depression $n = 11$	
Study variables		Mean	(SD)	Mean	(SD)	Mean	(SD)
Age	years	64.1	(12.7)**	52.5	(10.2)**	49.2	(9.7)
Gender							
Male		13	(52%)	30	(73%)	5	(45%)
Female		12	(48%)	11	(27%)	6	(55%)
Baseline hemoglobin	g/dL	14.0	(1.4)	14.7	(1.4)	14.0	(2.0)
Baseline platelet	10^9/L	165	(57)*	192	(59)*	202	(78)
Baseline ALT	IU/L	81.2	(81.1)	73.4	(64.0)	65	(43.1)
Baseline γ-GTP	IU/L	47.9	(36.5)*	92.0	(58.5)*	92.1	(96.3)
Baseline total cholesterol	mg/dL	177.1	(23.3)	177.5	(43)	201.5	(38.3)
Baseline fasting blood sugar	mg/dL	118.7	(58.4)	117.5	(33)	10.5.0	(30.8)
Baseline HCV	log IU/mL	5.8	(1.1)	6.1	(0.9)	5.9	(1.1)
HCV genotype							
1		12	(48%)	28	(68%)	5	(45%)
2		13	(52%)	13	(32%)	6	(55%)

*$P < 0.05$ (IFNβ/RBV vs. PEG-IFN/RBV).
**$P < 0.001$ (IFNβ/RBV vs. PEG-IFN/RBV).
ALT, alanine aminotransferase; HCV, hepatitis C virus; γ-GTP, albumin, gamma-glutamyl transpeptidase.

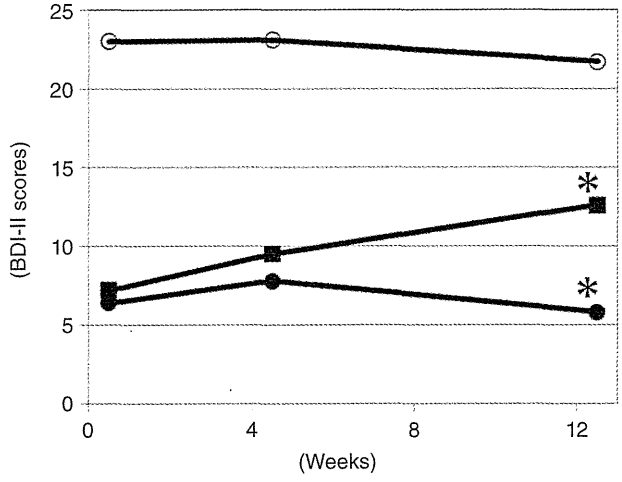

Figure 1 Changes in Beck Depression Inventory II (BDI-II) score for pegylated interferon α plus ribavirin (PEG-IFN/RBV) or interferon β plus ribavirin (IFNβ/RBV) therapy (●: IFNβ/RBV [FR] group, ○: FR-d group [FR patients with depression], ■: PEG-IFN/RBV [PR] group. *$P < 0.05$, FR vs. PR at week 12).

($n = 6$) and 34% at 12 weeks ($n = 14$). In the FR group, the incidence of the onset of symptoms of depression was 10% at 4 weeks ($n = 2$) and 8% at 12 weeks ($n = 2$), compared with 0% at baseline, indicating that the incidence did not change between 4 and 12 weeks. The incidence of the onset of depressive symptoms at 4 weeks was lower, but not significantly, in the FR group than in the PR group. Figure 2 shows the BDI-II score with a treatment regimen of IFN therapy at 12 weeks. The incidence of the onset of depressive symptoms (BDI-II score of 14 or more) was significantly lower in

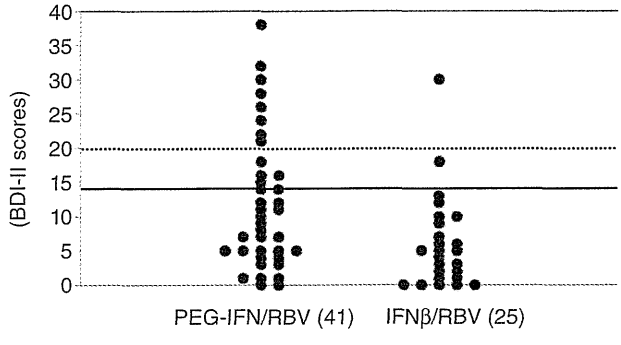

Figure 2 Distribution of Beck Depression Inventory II (BDI-II) scores for treatment regimens of interferon (IFN) therapy at 12 weeks (solid line: BDI-II score of 14, dotted line: BDI-II score of 20).

the FR group (8%, $n = 2$) than in the PR group (34%, $n = 14$; $P < 0.05$). The incidence of the onset of moderate depression symptoms (a BDI-II score of 20 or more) was higher in the PR group (20%, $n = 8$) than in the FR group (4%, $n = 1$). Mean PSQI scores at baseline, 4 weeks, and 12 weeks were 5.44, 6.62, and 7.37 in the PR group and 5.69, 6.01, and 6.88 in the FR group, respectively, indicating higher scores in the PR group than in the FR group from Week 4 onward. The incidence of sleep disorder, defined as a PSQI score of 11 or more, was higher in the PR group at both 4 and 12 weeks (18% and 27%, respectively) than in the FR group (0% and 8%, respectively).

BDI-II score and PSQI score at 12 weeks

Figure 3 shows the correlation between the BDI-II score and the PSQI score at 12 weeks. Some correlation was found between these scores with an overall coefficient of correlation (r) of 0.6755 ($P < 0.0001$). A strong correlation was noted between the BDI-II score and the PSQI score in the PR group, with an r-value of 0.7586 ($P < 0.0001$). In contrast, no correlation was observed in the FR group, with an r-value of 0.3589 ($P = 0.0786$). The incidence of sleep disorder (a PSQI score of 11 or more) at 12 weeks was lower in the FR group (8%, $n = 2$) than in the PR group (27%, $n = 11$). Only nine patients in the PR group had a BDI-II score of 14 or more and a PSQI score of 11 or more, whereas there were no such patients in the FR group, with the difference reaching statistical significance ($P < 0.05$). Three of the nine patients with a BDI-II score of 14 or more

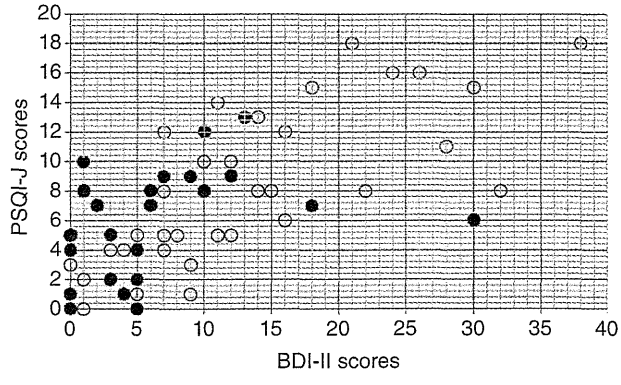

Figure 3 Graph showing correlation between Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI) scores at 12 weeks (correlation coefficient, Total: r = 0.6755, $P < 0.0001$; pegylated interferon α plus ribavirin [PEG-IFN/RBV]: r = 0.7586, $P < 0.0001$; interferon β plus ribavirin [IFNβ/RBV]: r = 0.3589, $P = 0.0786$).

and a PSQI score of 11 or more at 12 weeks discontinued treatment prior to 24 weeks due to depression symptoms.

Predictive factors contributing to the onset of depression symptoms during IFN therapy

Results from univariate and multivariate logistic regression analyses of the factors contributing to the onset of depression symptoms during IFN therapy are shown in Table 2. The univariate regression analysis showed that the type of IFN (PEG-IFNα) was the only factor that contributed to the onset of depressive symptoms ($P < 0.027$). The multivariate logistic regression analysis confirmed that the type of IFN (PEG-IFNα/RBV) was the only contributing significant independent predictive factor.

DISCUSSION

PR THERAPY FOR chronic hepatitis C involves long-term treatment, ranging from 24 to 48 weeks. The duration of treatment in patients with HCV genotype 1 and a high viral load may range from 48 and 72 weeks.[17] Currently available PR therapy yields only a low SVR rate in patients who discontinue treatment early. Thus, it is important to complete treatment as prescribed. The onset of depression symptoms associated with PEG-IFNα treatment is one of the reasons for early discontinuation of treatment due to adverse effects. In Japan, IFNβ, which is associated with a low incidence of the onset of depression symptoms, has been used in patients with depression.[12–14] In addition, due to the milder side effects of IFNβ, we have used it in IFN therapy for hemodialyzed patients with chronic hepatitis C.[18] The SVR rate among patients with HCV genotype 1 who were treated with IFNβ/RBV was lower (approximately 40%) than that among those treated with PEG-IFN/RBV[11], while patients with HCV genotype 2 who were treated with IFNβ/RBV had an SVR rate of approximately 87%, which was similar to that observed in those treated with PEG-IFN/RBV [19].

There have been no reported studies on the relationship between FR therapy and the onset of depression symptoms. In the present study, we demonstrated that FR therapy produced a significantly lower frequency of depression symptoms than PR therapy. We also found no evidence of worsened depression symptoms during the FR therapy in patients with depression.

In the present study, a questionnaire was conducted using BDI-II and PSQI scores to assess depression symptoms and sleep disorder. The BDI-II is way to measure the severity of depression symptoms and consists of 21 questions. Symptoms with a total score of ≥14, ≥20, and ≥29 are considered mild, moderate, and severe, respectively.[15] The PSQI is a questionnaire that is used to measure the quality of sleep. Original versions of both questionnaires have been translated into Japanese, and the translated versions were used in our study.

In the present study, we found that the percentage of patients with a BDI-II score of 14 or more in the PR group was approximately 20% as early as 4 weeks after

Table 2 Results from univariate and multivariate logistic regression analyses of the factors contributing to the onset of depressive symptoms

			Simple regression		Multiple logistic regression	
Factor	Range		Odds ratio	*P*-value	Odds ratio	*P*-value
Age	≧60 / <60	(years)	0.308	0.066	–	–
Sex	Male / Female		0.808	0.728	–	–
Genotype	1 / 2		0.900	0.859	–	–
Type of IFN	PEG-IFN/IFNβ		0.168	0.027	0.168	0.027
Hemoglobin	<14 / ≧14	(g/dL)	1.310	0.647	–	–
Platelet	<15 / ≧15	(10^4/μL)	3.294	0.143	–	–
ALT	≧50 / <50	(IU/L)	1.269	0.682	–	–
γ-GTP	≧45 / <45	(IU/L)	0.990	0.986	–	–
Total cholesterol	≧220 / <220	(mg/dL)	1.667	0.652	–	–
FBS	<110 / ≧110	(mg/dL)	0.682	0.531	–	–
Viral load	≧6.0 / <6.0	(LogIU/mL)	0.829	0.750	–	–

ALT, alanine aminotransferase; FBs, fasting blood sugar; IFN, interferon; γ-GTP, gamma-glutamyl transpeptidase; PEG-IFN/RBV, pegylated interferon α plus ribavirin.

the start of treatment and increased to 34% within the first 12 weeks. However, in the FR group, 10% or less of patients only experienced the onset of mild depressive symptoms and the percentage was comparable at 4 and 12 weeks, after which no patients discontinued treatment due to depression symptoms. At 12 weeks particularly, both the mean BDI-II score and the incidence of abnormalities (a BDI-II score of 14 or more) were significantly lower in the FR group than in the PR group, indicating that FR therapy was less likely to induce the onset of depression symptoms than PR therapy. It appears that assessing the onset of depressive symptoms is useful at 12 weeks of IFN treatment. However, assessment at 4 weeks of treatment also appears to be necessary, when possible, because the onset of depression symptoms may be observed as early as 4 weeks.

The onset of depression symptoms during PR therapy has been associated with sleep disorder. In the present study, there was a strong association between the BDI-II scores and PSQI scores. Careful management is required in patients reporting sleep disorder, which is one of the early symptoms of depression.

Some of the patients receiving PR therapy, who had a BDI-II score of 14 or more and a PSQI score of 11 or more at 12 weeks, discontinued treatment due to the subsequent onset of depressive symptoms; more careful management is required in these patients.

Patients with depression were also included in the present study (FR-d group). There was no increase over time in the BDI-II score of patients with depression and none of the patients with depression required additional or an increased dose of antidepressants; there was no evidence that the depression symptoms worsened. This suggests that FR therapy is safe in both patients with depression and patients at risk for symptoms of depression.

The BDI-II and the PSQI, which were used in the present study, are simple questionnaires, which take several minutes to complete and appear to be useful instruments in assessing the onset of depressive symptoms during IFN therapy. IFNβ/RBV therapy should be used in patients with depression or sleep disorder. Patients showing the onset of depression or sleep disorder during PEG-IFN/RBV therapy should be switched to IFNβ/RBV therapy to continue IFN therapy, having given due consideration to the discontinuation of therapy.

IFNB/RBV THERAPY WAS associated with a low incidence of the onset of depression symptoms during treatment, and was also safe in patients with depression, who showed no evidence of worsening of symptoms during treatment. Depression symptoms during PEG-IFN/RBV therapy were strongly associated with sleep disorders and commonly occurred within the first 12 weeks of treatment. Patients with the onset of both symptoms of depression and sleep disorders should be closely monitored, as they are more likely to discontinue treatment after these conditions develop.

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HEPATOLOGY

Increase in platelet count based on inosine triphosphatase genotype during interferon beta plus ribavirin combination therapy

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Key words
chronic hepatitis C, inosine triphosphatase, natural interferon β, platelet count, ribavirin.

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Abstract

Background and Aim: The inosine triphosphatase (*ITPA*) genotype is associated with ribavirin-induced anemia and pegylated interferon α (PEG IFN-α)-induced platelet reduction during PEG IFN-α plus ribavirin combination therapy. Natural IFN-β plus ribavirin therapy is associated with increases in platelet counts during treatment. We investigated decreases in platelet counts according to *ITPA* genotype during natural IFN-β/ribavirin therapy to determine if patients with low platelet counts were eligible for this combination therapy.

Methods: A total of 187 patients with chronic hepatitis C received PEG IFN-α/ribavirin or natural IFN-β/ribavirin therapy. Decreases in platelet counts based on *ITPA* genotype were investigated during treatment through 24 weeks.

Results: Platelet counts decreased during week 1 of PEG IFN-α/ribavirin therapy, but increased during week 2, after which platelet counts decreased gradually. Platelet counts decreased until week 4 of natural IFN-β/ribavirin therapy, after which platelet counts increased. Platelet counts after week 8 were higher relative to pretreatment platelet counts. Patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during natural IFN-β/ribavirin therapy than those with the *ITPA*-CA/AA genotype; platelet counts after week 8 of this therapy were higher than pretreatment platelet counts, regardless of pretreatment platelet counts. Multivariate logistic regression analyses showed that natural INF-β/ribavirin therapy was the only significant independent predictor for an increase in platelets through week 8.

Conclusion: Natural IFN-β/ribavirin therapy is safe for patients with the *ITPA*-CC genotype, even if their pretreatment platelet counts are low.

Introduction

The introduction of pegylated interferon-α (PEG IFN-α) plus ribavirin (PEG-IFN/RBV) combination therapy has led to an improved sustained virological response (SVR) rate in patients with chronic hepatitis C receiving IFN therapy.[1–6] However, cytopenia has been observed during PEG-IFN/RBV therapy. Specifically, cases of RBV-induced anemia and PEG-IFN-induced thrombocytopenia or neutropenia have been reported, and we have previously described cases of RBV-induced anemia.[7] A genome-wide association study (GWAS) identified the inosine triphosphatase gene (*ITPA*) single nucleotide polymorphism (SNP) as being strongly associated with RBV-induced anemia.[8–10] This *ITPA* SNP was also reported to play a role in the decreases in platelet counts that occur during PEG-IFN/RBV therapy.[11,12] In Japan, natural INF-β plus ribavirin (IFN-β/RBV) therapy has been indicated for the treatment of chronic hepatitis C. This therapy is associated with greater increases in platelet counts than seen with PEG-IFN/RBV therapy.[13] Therefore, we investigated the association between the *ITPA* genotype and decreases in platelet count during IFN-β/RBV therapy to determine if patients with a low platelet count were eligible for IFN-β/RBV therapy.

Methods

Patients. A total of 187 patients with chronic hepatitis C who received IFN therapy for at least 24 weeks at the Shinkokura Hospital between January 2009 and April 2011 were included in the study. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each

patient provided informed consent before participating in this trial.

Criteria for exclusion were as follows: (i) clinical or biochemical evidence of hepatic decomposition or advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) IFN-β/RBV: a white blood cell count of less than 3×10^9/L and a platelet count of less than 50×10^9/L, PEG-IFN/RBV: a white blood cell count of less than 4×10^9/L and a platelet count of less than 80×10^9/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy in the 12 months prior to enrollment.

IFN-β/RBV combination therapy. Interferon-β (Feron; Toray Industries, Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 20–44 weeks. The ribavirin (Rebetol; MSD, Tokyo, Japan) dose was adjusted according to body weight (600 mg for ≤ 60 kg; 800 mg for > 60 to ≤ 80 kg; and 1000 mg for > 80 kg), based on the guidelines of the Ministry of Health, Labor and Welfare of Japan.[5] The drug was administered orally after breakfast and dinner.

PEG-IFN/RBV combination therapy. Pegylated interferon-α-2B (PEG-Intron; MSD) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.3–1.5 μg/kg) once a week. Ribavirin was administered twice a day according to body weight, as described for IFN-β/RBV combination therapy.

This study was a prospective, nonrandomized open trial. Platelet counts and hemoglobin levels were measured at baseline and at weeks 1, 2, 4, 8, 12, and 24.

We genotyped each patient for two SNPs: rs8099917, an *IL28B* SNP previously reported to be associated with therapy outcome, and rs1127354 (14), an *ITPA* SNP reported to be associated with ribavirin-induced anemia[14] and decreases in platelet counts.[11] Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or with the Invader or TaqMan assay, as described elsewhere.[15–17]

Statistical analysis. Statistical analysis was performed using PASW Statistics, version 18 (SPSS, Chicago, IL, USA) and R, version 2.11. Categorical data were analyzed using the χ^2 test and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann–Whitney *U*-test. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to the increase in platelets $> 0 \times 10^9$/L from week 0 through week 8. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values found to be less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.1$) on univariate analysis were entered into a multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with increases in platelets $> 0 \times 10^9$/L from week 0 to week 8 included the following variables: age, sex, method of IFN treatment, hepatitis C virus (HCV) genotype, *ITPA* genotype, *IL28B* genotype, hemoglobin, platelet count, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), and HCV RNA level.

Results

The clinical backgrounds of chronic hepatitis C patients before combination therapy with IFN-β/RBV or PEG-IFN/RBV are shown in Table 1. The mean age of patients receiving IFN-β/RBV therapy was 59.3 years and that of patients receiving PEG-IFN/RBV therapy was 57.9 years, with no difference between the two patient groups. The PEG-IFN/RBV group had more men, although the number was not significantly higher. All baseline laboratory parameters, including hemoglobin levels, platelet counts, ALT levels, γ-GTP levels, and HCV loads, showed no differences

Table 1 Clinical background before combination therapy with interferon β plus ribavirin (IFN-β/RBV) or pegylated interferon plus ribavirin (PEG-IFN/RBV) in chronic hepatitis C patients

		IFN-β/RBV	PEG-IFN/RBV	*P*-value
		n = 45	*n* = 137	
Age	Year (SD)	59.3 (14.3)	57.9 (10.4)	ns
Sex	M/F	22/23	73/64	ns
Hb	g/dL (SD)	14 (1.5)	14.2 (1.4)	ns
Platelet	10^9/L (SD)	178 (59)	183 (59)	ns
ALT	IU/L (SD)	84.1 (63.3)	76.5 (64)	ns
γ-GTP	IU/L (SD)	79.1 (56.29)	69.5 (58.5)	ns
HCV	logIU/mL (SD)	6.7 (1.1)	6.4 (0.9)	ns
HCV genotype	1/2	21/24	102/35	< 0.001
ITPA (rs1127354)	CC/CA or AA	36/9	99/38	ns
IL28B (rs8099917)	TT/TG or GG	35/10	96/41	ns
Decrease in platelet count at week 1	10^9/L (SD)	−47 (32)	−47 (43)	ns
Decrease in platelet count at week 4	10^9/L (SD)	−42 (33)	−28 (33)	< 0.05
Decrease in platelet count at week 8	10^9/L (SD)	19 (36)	−35 (43)	< 0.0001

ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ITPA, inosine triphosphate pyrophosphatase; ns, not significant; SD, standard deviation.

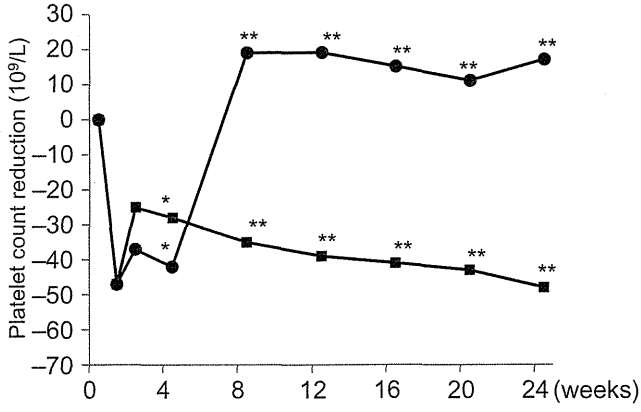

Figure 1 Decreases in platelet count during combination therapy with IFN-β/RBV or PEG-IFN/RBV (closed circle, IFN-β/RBV; closed square, PEG-IFN/RBV; $*P < 0.05$, IFN-β/RBV *versus* PEG-IFN/RBV at week 2; $**P < 0.0001$, IFN-β/RBV *versus* PEG-IFN/RBV at weeks 8, 12, 16, 20, and 24). IFN-β, interferon β; RBV, ribavirin; PEG-IFN, pegylated interferon.

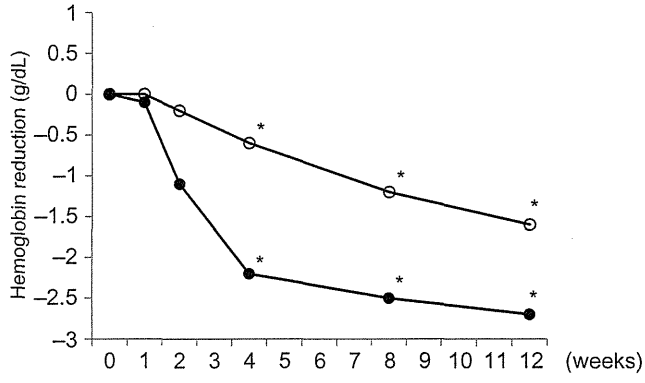

Figure 2 Decreases in hemoglobin levels according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA; $*P < 0.01$, *ITPA*-CC *versus ITPA*-CA/AA (rs1127354) at weeks 4, 8, and 12). IFN-β, interferon β; RBV, ribavirin.

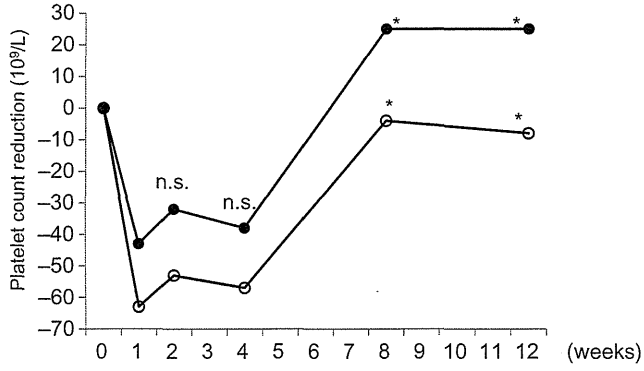

Figure 3 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA; $*P < 0.05$, *ITPA*-CC *versus ITPA*-CA/AA (rs1127354) at weeks 8 and 12). IFN-β, interferon β; RBV, ribavirin.

between the two patient groups. Significantly more patients with HCV genotype 1 were in the PEG-IFN/RBV group ($P < 0.001$). A total of 74% (135/182) patients had the *ITPA*-CC genotype, while 72% of patients had the *IL28B* TT genotype. The frequencies of the *ITPA*-CC genotype and the *IL28B* TT genotype were comparable between the two patient groups. There was no difference in the decreases in platelet counts at week 1; however, at weeks 4 and 8, decreases in platelet counts differed significantly between the two patient groups ($P < 0.05$, $P < 0.0001$).

Platelet count decreases that occurred during combination therapy with IFN-β/RBV or PEG-IFN/RBV are depicted in Figure 1. A decrease in platelet counts of 47×10^9/L was observed at week 1 during IFN-β/RBV therapy. Subsequently, platelet counts transiently increased at week 2, but reduced again at week 4. Platelet counts reduced for 4 weeks after the start of treatment, as IFN-β/RBV therapy involved continuous, daily dosing with IFN-β for 4 weeks after the start of treatment. As per the treatment protocol, IFN-β administration was subsequently reduced to thrice-weekly dosing. At week 8, platelet counts increased and were significantly higher than the pretreatment platelet counts ($P < 0.001$). Platelet counts remained unchanged after week 8. A reduction of 47×10^9/L was observed at week 1 during PEG-IFN/RBV therapy, similar to the reduction that was observed during IFN-β/RBV therapy. Subsequently, platelet counts increased at week 2, decreased at week 4, and gradually decreased further after week 8. The decrease in platelet counts at week 4 during IFN-β/RBV therapy was significantly larger than the decrease observed during PEG-IFN/RBV therapy ($P < 0.05$). However, platelet counts after week 8 of IFN-β/RBV treatment were significantly higher than those during PEG-IFN/RBV therapy ($P < 0.0001$), due to a rapid increase in platelet counts after week 4 of the IFN-β/RBV regimen.

Decreases in hemoglobin levels in relation to the *ITPA* genotype (rs1127354: CC, CA/AA) are shown in Figure 2. At week 2, a large decrease in hemoglobin levels was observed in patients with the *ITPA*-CC genotype. There was no difference in hemoglobin levels based on *ITPA* genotype up to week 2 in patients receiving IFN-β/RBV therapy. Patients with the *ITPA*-CC genotype showed a significantly larger decrease in hemoglobin levels at weeks 4, 8, and 12 than those with the *ITPA*-CA/AA genotype ($P < 0.01$).

Platelet counts during combination therapy with IFN-β/RBV according to the *ITPA* genotype is shown in Figure 3. Similar changes in platelet count decreases were observed in patients with the *ITPA*-CC and *ITPA*-CA/AA genotypes. Patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts at weeks 1, 2, 4, 8, 12, 24 during therapy compared to those with the *ITPA*-CA/AA genotype. Specifically, patients with the *ITPA*-CC genotype showed a statistically lower degree of platelet decrease at weeks 8, 12, and 24 than those with the *ITPA*-CA/AA genotype ($P < 0.05$). Patients with the *ITPA*-CC genotype had significantly increased platelet counts at week 8 compared with the pretreatment platelet counts ($P < 0.0001$).

Decreases in platelet counts during combination therapy with PEG-IFN/RBV in relation to the *ITPA* genotype are shown in

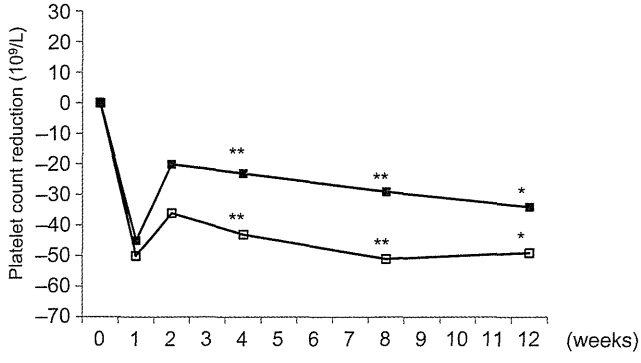

Figure 4 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with PEG-IFN/RBV (closed square, *ITPA*-CC; open square, *ITPA*-CA/AA; $*P < 0.05$, *ITPA*-CC *versus* *ITPA*-CA/AA (rs1127354) at week 12; $**P < 0.01$, *ITPA*-CC *versus* *ITPA*-CA/AA (rs1127354) at weeks 4 and 8). PEG-IFN, pegylated interferon; RBV, ribavirin.

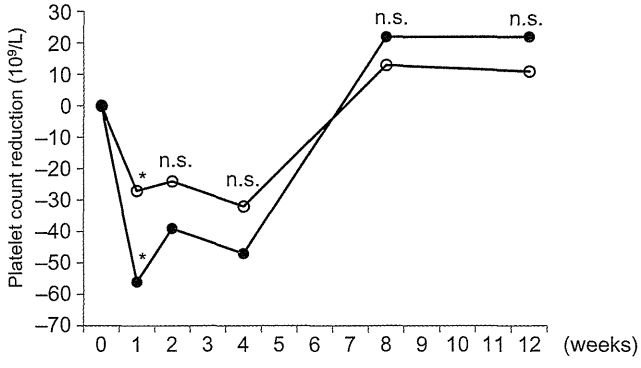

Figure 5 Decreases in platelet count relative to pretreatment platelet counts during combination therapy with IFN-β/RBV (closed circle, $\geq 150 \times 10^9$/L; open circle, $< 150 \times 10^9$/L; $*P < 0.05$, $\geq 150 \times 10^9$/L *versus* $< 150 \times 10^9$/L at week 1). IFN-β, interferon β; RBV, ribavirin.

Figure 4. Similar changes in platelet count decreases were observed in patients with the *ITPA*-CC and *ITPA*-CA/AA genotypes. Patients with the *ITPA*-CC genotype showed a lower degree of platelet reduction at weeks 1, 2, 4, 8, 12, 24 during therapy compared to those with the CA/AA genotype. Specifically, patients with the *ITPA*-CC genotype had a significantly smaller decrease in platelet counts at weeks 4, 8, and 12 than those with the *ITPA*-CA/AA genotype ($P < 0.01$, $P < 0.05$).

Platelet reduction during combination therapy with IFN-β/RBV compared with pretreatment platelet counts is shown in Figure 5. At week 1, patients with a low pretreatment platelet count ($< 150 \times 10^9$/L) showed a significantly smaller decrease in platelet counts than those with a high pretreatment platelet count ($\geq 150 \times 10^9$/L; $P < 0.01$). Five patients had pretreatment platelet counts of $\leq 100 \times 10^9$/L, and a decrease in platelet counts of $\leq 40 \times 10^9$/L was observed in these patients at week 1. Patients with low pretreatment platelet counts showed a small decrease in platelet counts at week 1, after which there was no difference in platelet counts between the groups of patients with high and low

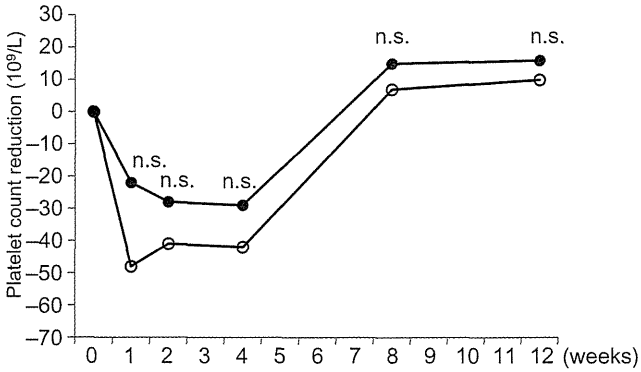

Figure 6 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV in patients with pretreatment platelet counts ($< 150 \times 10^9$/L) (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA). IFN-β, interferon β; RBV, ribavirin.

pretreatment platelet counts. Among patients with both high and low pretreatment platelet counts, platelet counts at week 8 were significantly increased compared with pretreatment platelet counts ($P < 0.01$, $P < 0.05$).

Decreases in platelet counts according to *ITPA* genotype during combination therapy with IFN-β/RBV for patients with pretreatment platelet counts ($< 150 \times 10^9$/L) are shown in Figure 6. For patients with pretreatment platelet counts of $< 150 \times 10^9$/L, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts than those with the *ITPA*-CA/AA genotype.

The results of univariate and multivariate logistic regression analyses of factors associated with the increase in platelets $> 0 \times 10^9$/L from week 0 to 8 are shown in Table 2. Univariate and multivariate logistic regression analyses revealed that IFN-β/RBV therapy was the only significant independent predictor for the increase in platelets $> 0 \times 10^9$/L from week 0 to week 8.

Only one patient in the IFN-β/RBV group was withdrawn from the study by week 24. The reason for discontinuation was proteinuria. The dose of IFN was reduced only in the one patient. The dose of ribavirin was reduced in four of 45 patients, all of whom had the *ITPA*-CC genotype.

Discussion

This study showed that the platelet counts of patients undergoing IFN-β/RBV combination therapy for chronic hepatitis C infection after week 8 are higher than those before treatment. Moreover, patients with the *ITPA*-CC genotype showed a smaller decrease in their platelet counts not only during IFN-β/RBV, but also with PEG-IFN/RBV therapy, compared to those with the *ITPA*-CA/AA genotype. In particular, the results demonstrated that platelet counts after week 8 during IFN-β/RBV therapy were higher than pretreatment platelet counts, regardless of pretreatment platelet counts. Compared with pretreatment platelet counts, patients with the *ITPA*-CC genotype had markedly increased platelet counts after week 8 of IFN-β/RBV therapy. Multivariate logistic regression analyses showed that IFN-β/RBV therapy was the factor that contributed to increased platelet counts at week 8 relative to pretreatment platelet counts.

Table 2 Results of univariate and multivariate logistic regression analyses of factors associated with the increase in platelets > 0 (10^9/L) from week 0 to week 8

			Simple regression		Multiple logistic regression	
Factor	Range		Odds ratio	*P*-value	Odds ratio	*P*-value
Age (years)	≥ 60/< 60		1.219	0.389	–	–
Sex	Male/Female		1.219	0.554	–	–
Genotype	1/2		1.303	0.451	–	–
Method of IFN therapy	IFN-β/RBV/PEG-IFN/RBV		20.797	< 0.0001	23.596	< 0.0001
ITPA	CC/CA or AA		0.468	0.073	–	–
IL28B	TT/TG or GG		0.569	0.153	–	–
Baseline hemoglobin	< 14/≥ 14	g/dL	0.569	0.153	–	–
Baseline platelet count	< 150/≥ 150	10^9/L	0.737	0.399	–	–
Baseline ALT	≥ 50/< 50	IU/L	1.646	0.140	–	–
Baseline γ-GTP	≥ 45/< 45	IU/L	1.603	0.166	–	–
Baseline viral load	≥ 6.0/< 6.0	LogIU/mL	1.833	0.091	–	–

ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; IFN-β, interferon-β; ITPA, inosine triphosphate pyrophosphatase; RBV, ribavirin; PEG-IFN, pegylated interferon.

A GWAS identified several new host genetic variants that may be important for PEG-IFN/RBV therapy in chronic hepatitis C. One of these was the SNP in the *IL28B* gene that was strongly associated with therapy outcome,[18–21] and another was the *ITPA* gene that was associated with RBV-induced anemia during PEG-IFN/RBV therapy in chronic hepatitis C.[8–10]

Tanaka *et al.* reported that one SNP (rs11697186) located on the *DDRGK1* gene on chromosome 20 showed strong associations with a decrease in platelet counts in response to PEG-IFN/RBV therapy, and fine mapping with 22 SNPs around the *DDRGK1* and *ITPA* genes showed that rs11697186 had strong linkage disequilibrium with rs1127354, known as a functional variant of the *ITPA* gene.[11] We investigated the changes in platelet count decreases during IFN-β/RBV or PEG-IFN/RBV therapy relative to the *ITPA* genotype (CC, CA/AA). PEG-IFN/RBV therapy was associated with a larger decrease in hemoglobin levels among patients with the *ITPA*-CC genotype than those with the *ITPA*-CA/AA genotype.[8–10] A reactive increase in platelet counts was observed from week 1 through week 4 of treatment, with patients with the *ITPA*-CC genotype showing a higher degree of a reactive increase in platelet counts. This trend was similar to findings reported by Tanaka *et al.*, who reported that a reactive increase in platelet counts occurred secondary to RBV-induced anemia through week 4.[11]

In this investigation, decreases in hemoglobin levels were also observed from weeks 2 through 4 during IFN-β/RBV therapy. Secondarily, a temporary reactive increase in platelet counts occurred. IFN-β/RBV therapy involves continuous daily dosing of IFN-β for 4 weeks, and therefore, platelet counts typically decrease up until week 4, after which platelet counts rapidly increase following a reduction in the dosing frequency of IFN-β to thrice-weekly dosing. However, patients receiving IFN-β/RBV therapy had higher platelet counts at week 8 than pretreatment platelet counts. Arase *et al.* reported that platelet counts increased following a reduction in the dosing frequency of IFN-β from continuous daily dosing to thrice-weekly dosing.[13] We could demonstrate evidence of a relationship between the reduction of the dosing frequency of IFN-β and increases in platelet counts because we developed a treatment protocol using a 4-week continuous daily dosing of IFN-β and complied strictly with the protocol-defined duration of continuous daily dosing of 4 weeks. A higher degree of these recurrent increases in platelet counts was observed in patients with the *ITPA*-CC genotype than in those with the *ITPA*-CA/AA genotype. As with PEG-IFN/RBV therapy, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during IFN-β/RBV therapy. In the present study, our results demonstrated that the *ITPA* genotype was strongly involved in platelet reduction during IFN therapy, in both PEG-IFN RBV and IFN-β/RBV therapy.

The *ITPA* genotype is strongly associated with ribavirin-induced anemia and IFN-induced platelet reduction, although the reasons for these associations are not clear. Erythropoietin (EPO) is produced when hemoglobin reduction occurs as a result of ribavirin-induced anemia. The sequence homology of thrombopoietin (TPO) and EPO may explain the synergy of the physiological roles of TPO and EPO in platelet production. When EPO is elevated, as in iron deficiency anemia, an amino acid sequence similar to TPO may increase the platelet count.[22]

In Japan, the IFN-β/RBV regimen used in the present study has been indicated for chronic hepatitis C patients receiving IFN-based therapy. The SVR rate among patients with HCV genotype 1 who were treated with IFN-β/RBV was lower (approximately 40%) than that among those treated with PEG-IFN/RBV.[13] We reported that IFN-β/RBV therapy was associated with a lower incidence of depressive symptoms or sleep disorders than PEG-IFN/RBV therapy.[23] Therefore, we have also used IFN-β/RBV therapy in elderly patients or patients with concurrent depression. Patients with HCV genotype 2 who were treated with IFN-β/RBV had an SVR rate of approximately 87%, which was similar to that observed in those treated with PEG-IFN/RBV.[24] This study is a prospective, nonrandomized open trial. Thus, the SVR rate among patients with HCV genotype 1 who were treated with PEG-IFN/RBV was higher than the SVR rate of those treated with IFN-β/RBV. IFN-β/RBV therapy was performed only in patients with depression or sleep disorder, thus the number of enrolled patients with HCV genotype 1 who were treated with IFN-β/RBV was small. As for patients with HCV genotype 2, since there was no difference in the SVR rate between IFN-β/RBV and PEG-IFN/RBV therapies, the number of enrolled patients was not different.

Therefore, more patients with HCV genotype 1 were included in the PEG-IFN/RBV group.

In this investigation, there were few discontinuations, dose reductions of IFN, and dose reductions of ribavirin in the IFN-β/RBV group. This is likely due to the fact that few patients developed anorexia, no patients showed weight loss, and dietary intake was adequate during the IFN-β/RBV therapy.

In the present study, patients with the *ITPA*-CC genotype showed a higher increase in platelet counts after week 8 during IFN-β/RBV therapy than those with the *ITPA*-CA/AA genotype. Platelet counts after week 8 were increased compared with pretreatment platelet counts, regardless of pretreatment platelet counts. In patients with low pretreatment platelet counts, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet count than those with the CA/AA genotype, and the platelet counts were increased after week 8. The IFN-β/RBV regimen appears to be a safe strategy for IFN therapy for patients with the *ITPA*-CC genotype, even if they have low pretreatment platelet counts.

The present study demonstrated that as with PEG-IFN/RBV therapy, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during IFN-β/RBV therapy. Platelet counts after week 8 of IFN-β/RBV therapy were increased compared with pretreatment platelet counts, regardless of pretreatment platelet counts. Therefore, we concluded that IFN-β/RBV therapy is safe for patients with the *ITPA*-CC genotype, even if their pretreatment platelet counts are low.

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Review Article

Can non-invasive assessment of liver fibrosis replace liver biopsy?

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Transient elastgraphy, acoustic radiation force impulse and real-time elastography are the methods with very good or excellent diagnostic accuracy for the assessment of liver fibrosis stage. They do not provide the information on inflammatory activity, steatosis, iron deposition or other findings derived from liver biopsy. Even on account of fibrosis stage, these non-invasive methods do not give us the estimation completely corresponding to that of liver biopsy. However they provide us useful clinical information that liver biopsy has been providing us, such as appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis. Recently non-invasive methods for assessment of inflammatory activity, steatosis and iron deposition in the liver have been developed. Thus in the near future, non-invasive methods will replace liver biopsy.

Key words: acoustic radiation force impulse, fibrosis stage, inflammatory activity, liver stiffness, real-time elastography, transient elastography

INTRODUCTION

NON-INVASIVE ASSESSMENT OF liver fibrosis has been one of major objectives in the society of hepatologists for a long time. Routine laboratory tests, serum markers of fibrosis[1–7] and apparatuses for measuring liver stiffness (LS) have been tested. The apparatuses include transient elastography (TE),[8,9] acoustic radiation force impulse (ARFI),[10] real-time elastography,[11] and magnetic resonance imaging (MRI).[12]

Liver biopsy is the gold standard for the assessment of fibrosis stage in chronic viral hepatitis. However, liver biopsy is an invasive and expensive procedure, and its accuracy is sometimes questionable because of sampling errors, inadequate specimens and the subjectivity of diagnosis.[13,14]

Infections of hepatitis B virus (HBV) and hepatitis C virus (HCV) are world-wide problems and cause the need of a great number of liver biopsies mainly for assessment of fibrosis stage and inflammatory activity, which sometimes cause serious complications. Thus the replacement of liver biopsies with non-invasive methods is an important subject to be deal with as soon as possible.

In this article, we review the manuscripts that applied non-invasive methods to estimate fibrosis stages for the five different clinical aims in the replacement of liver biopsies. These aims include the determination of appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis. We will discuss whether non-invasive methods can replace liver biopsies for these aims.

We discuss the three methods that have been often reported; TE, ARFI imaging, and real-time elastography. Algorithm of serum fibrosis markers such as FibroTest[2] will be also described. There have been published a lot of manuscripts on non-invasive methods, and we selected the manuscripts that seem to us to be important in discussing whether non-invasive methods can replace liver biopsies.

Transient elastography measures LS with the use of an apparatus, FibroScan (EchoSens, Paris, France).[8] FibroScan is equipped with a probe including an

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ultrasonic transducer and a vibrator. A vibration of mild amplitude and low frequency is transmitted from the vibrator placed on the body surface toward the liver through the intercostal space. The vibration induces an elastic shear wave that propagates through the liver tissue. The pulse-echo ultrasound acquisitions follow the propagation of the shear wave and determine its velocity. The velocity is directly related to tissue stiffness; the harder the tissue, the faster the shear wave propagates. LS is calculated from velocity and expressed in kilopascal (kPa).

Acoustic radiation force impulse imaging is a radiation force-based imaging method that is provided with conventional B-mode ultrasonography (Siemens Acuson S2000, Siemens AG, Germany).[10] In ARFI imaging, an initial ultrasonic pulse is transmitted at diagnostic intensity levels to obtain a baseline signal for later comparison. A short-duration, high-intensity acoustic pushing pulse is transmitted from the probe, and cause shear wave in the liver. A series of diagnostic intensity pulses are used to quantitate shear wave velocity (Vs; m/s). The velocity of the shear wave depends on LS.

Real-time elastography is an imaging technique that can reveal the physical property of tissue using conventional ultrasound probes; the Hitachi EUB-8500 and EUB-900 machines (Hitachi Medical Systems, Tokyo, Japan).[11] The region of interest is divided up in to 30 000 finite elements before compression. During the compression by the probe or heart beats, the displacement of each element is measured. In hard tissue, the amount of displacement is low, whereas in soft tissue, the amount of displacement is high. The calculation of tissue elasticity distribution is performed in real time, and the results are displayed as color-coded images with the conventional B-mode image in the background. In this way, a large number of summarizing variables were obtained to characterize elastography. The final score was based on 10 summarizing variables selected from them to obtain high reproducibility. The variables selected for the final score differ among the investigators.

APPROPRIATE TIME TO START ANTIVIRAL THERAPY: DIAGNOSIS OF SIGNIFICANT FIBROSIS (F> OR =2)

IN CHRONIC VIRAL hepatitis, the presence of significant fibrosis (F> or =2) indicates the need of antiviral therapies both in chronic hepatitis B and in chronic hepatitis C.[15,16]

A meta-analysis of the performance of TE for staging of liver fibrosis demonstrated that the area under the receiver operating characteristic curve (AUROC) for significant fibrosis ranged 0.68–1.0 among different studies with a mean of 0.84 (95% confidence intervals [CI], 0.82–0.86) and an adjusted AUROC of 0.91 and that the optimal cut-off value for the significant fibrosis suggested from the summary ROC techniques was 7.65 kilopascals (kPa).[17]

We published a review article on the investigations of TE for assessment of fibrosis stages and presented the summary table.[18] Thus we do not show the table in the present article.

Friedrich-Rust *et al.* studied 134 patients with chronic liver diseases and reported that the AUROC for the diagnosis of significant fibrosis of real-time elastography, TE and FibroTest was 0.69, 0.84 and 0.85, respectively.[19]

Koizumi measured LS with real-time tissue elastography in 70 patients with chronic hepatitis C[20]. The elastic ratio (ratio of the value in the intrahepatic venous small vessels divided by the value in the hepatic parenchyma) was calculated. The cut-off value and AUROC for significant fibrosis were 2.73 and 0.89, respectively.

Although real-time elastography is a hopeful non-invasive method, the calculations of elastic value differ among the investigators. Thus we think it is inappropriate to present the summary table.

Friedrich-Rust *et al.* studied 86 patients with chronic viral hepatitis and reported that the AUROC for the diagnosis of significant fibrosis of ARFI, TE, and FibroTest was 0.82, 0.84, and 0.82, respectively.[10] The cut-off values for significant fibrosis of ARFI and TE were 1.37 m/s (sensitivity 68.5%, specificity 92.6%) and 6.3 kPa (sensitivity 83.3%, specificity 74.1%), respectively.

Takahashi *et al*[21] studied 55 patients mainly consisting of people with HCV by ARFI. The AUROC and cut-off value of the Vs for significant fibrosis were 0.94 (95% CI, 0.87–0.99) and 1.34 m/s (sensitivity 91.4%, specificity 80%).

Fierbinteanu-Braticevici[22] studied 74 patients with HCV by ARFI. The AUROC and cut-off value of Vs for significant fibrosis were 0.902 (95% CI, 0.831–0.972, $P < 0.001$) and 1.215 m/s (sensitivity 100%, specificity 71%).

The summary of investigations of ARFI for assessment of significant fibrosis is shown in Table 1.[10,21–29]

Generally the diagnostic accuracy of test with AUROC of 0.7–0.8 was considered as good, that of 0.8–0.9 as very good, and that of 0.9–1.0 as excellent. The diagnostic accuracy of TE, ARFI and real-time elastography for significant fibrosis is very good or excellent. They do not give us the estimation completely corresponding to that of liver biopsy; in our study (AUROC 0.88; sensitivity 81%;