The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin

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ABSTRACT

The aim of this study was to assess the association between liver stiffness measured by transient elastography (FibroScan®) and the efficacy of pegylated interferon alpha-2b plus ribavirin combination treatment for patients with chronic hepatitis C virus (HCV) infection. We prospectively studied 145 Japanese patients with chronic HCV infection. FibroScan was done at baseline, at the end of treatment, and at 48 and 96 weeks after the end of treatment. The FibroScan values were significantly decreased for sustained virological response (SVR) patients (the mean rate of change; −16.2%, −32.2% and −43.5%) in comparison with non-SVR patients (−7.2%, −2.1% and +17.3%) at the end of treatment ($P = 0.0127$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after the end of treatment. Among the non-SVR patients, the FibroScan values were significantly decreased for patients with biochemical response (BR) (−17.9%, −30.0% and −27.1%) in comparison with non-BR (−4.1%, +6.4% and +30.6%) at the end of treatment ($P = 0.0270$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after the end of treatment. The FibroScan values may predict a progressively better clinical outcome for patients with successful virological and biochemical responses.

1. Introduction

Hepatitis C virus (HCV) infection is a main cause of chronic viral hepatitis worldwide. Chronic hepatitis can lead to cirrhosis and hepatocellular carcinoma (HCC) (Seeff, 2002; Hayashi et al., 2000). Antiviral treatment with interferon (IFN) for chronic HCV infection can induce viral clearance and biochemical and histological improvement (Davis et al., 1989; Hayashi et al., 1994). Pegylated interferon (PEG-IFN) alpha in combination with ribavirin (RBV), which aims at viral eradication (Poynard et al., 2002a; Furusyo et al., 2008), has contributed to a reduction in the relapse rate and a significant increase in the rate of sustained virological response (SVR) compared with standard IFN monotherapy (Hayashi et al., 1994; Zeuzem et al., 2000; Lindsay et al., 2001). IFN treatment has been reported to be responsible for the regression of liver fibrosis in patients with SVR (Shiratori et al., 2000; Furusyo et al., 1997). Even if a virological response with IFN treatment was not obtained, the deterioration of compensated cirrhosis was prevented and the development of HCC was inhibited (Nishiguchi et al., 1995; Veldt et al., 2004; Kashiwagi et al., 2003), thereby increasing the survival rate (Poynard et al., 2002a).

Liver biopsy had long remained the gold standard for staging fibrosis. However, liver biopsy is no more considered as a perfect methodology because of the invasive nature of the procedure, sampling error and inter-observer variability (Regev et al., 2002; Manning and Afdhal, 2008). Therefore, further testing strategies are needed for assessment of the liver status of patients with liver diseases.

Transient elastography (FibroScan®; Echosens, Paris, France) has been proposed as a promising, rapid, noninvasive and reproducible method for measuring liver stiffness (Sandrin et al., 2002). We previously reported a clinical assessment of FibroScan among patients with chronic hepatitis B and C (Ogawa et al., 2007). The values measured by FibroScan (FibroScan values) have been significantly correlated with histopathological staging of percutaneous liver biopsy and have been shown to be more accurate than biochemical scores such as the aspartate aminotransferase (AST)-to-platelet ratio (APRI) and markers of liver fibrosis (e.g. hyaluronic acid and type IV collagen), which are products of the degradation or synthesis of the extracellular matrix.

It has been repeatedly observed that at least two biomarkers FibroTest® (Biopredictive, Paris, France) and FibroScan (Manning and Afdhal, 2008; Calès et al., 2008; Friedrich-Rust et al., 2008; Poynard et al., 2008; Shaheen et al., 2007) have the same

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diagnostic value as a 20–25 mm liver biopsy. In case of discordance between FibroTest and liver biopsy, half of failures are attributable to FibroTest and half to the biopsy (Poynard et al., 2004; Ngo et al., 2006). Moreover, FibroTest has already demonstrated a similar prognostic value as biopsy (Ngo et al., 2006). In a few countries, biomarkers are recommended by health authorities as first line test in patients with chronic hepatitis C, and biopsy only if biomarkers are not interpretable (Manning and Afdhal, 2008).

The aim of the present long-term prospective study was to evaluate the association between liver stiffness measured by FibroScan and the efficacy of combined PEG-IFN alpha-2b plus RBV treatment for patients with chronic HCV infection.

2. Materials and methods

2.1. Patients

We prospectively studied a total of 145 patients infected with chronic HCV infection. Of the 145 patients, 19 (13.1%) refused antiviral treatment because of financial problems or anxiety about the possibility of adverse effects and low initial fibrosis degree (low FibroScan values); however, 4 (21.1%) of them underwent liver biopsy. The 19 untreated patients were followed for the same 3-year period as the treated patients. Of the remaining 126 patients, 118 (93.7%) underwent liver biopsy, all of whom were treated with PEG-IFN alpha-2b plus RBV treatment between January 2005 and July 2006 and followed up for 2 years after the end of treatment.

All patients satisfied the following criteria: (1) chronically infected with HCV and (2) a history of an increased alanine aminotransferase (ALT) level for over 6 months. Exclusion criteria for the study were: (1) positivity for antibody to human immunodeficiency virus (HIV) or positivity for both hepatitis B surface antigen and anti-HCV; (2) clinical or biochemical evidence of hepatic decompensation; (3) excessive active alcohol (i.e. ethanol) consumption (>60 g/day) or drug abuse; (4) suspected hepatocellular carcinoma; or (5) treatment with antiviral or immunosuppressive agents prior to enrollment. Patients who fulfilled the above criteria were recruited at Kyushu University Hospital.

Informed consent was obtained from all patients before enrollment. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of guidelines for good clinical practice.

2.2. Clinical and laboratory assessment

Clinical parameters included aspartate aminotransferase, ALT, platelet count, type IV collagen, prothrombin time, HCV genotype and HCV RNA. We also calculated APRI, using 45 IU/L as the upper limit of the normal AST range (ULN), as previously recommended for evaluating liver fibrosis (Wai et al., 2003):

$$APRI = \frac{AST\ (/ULN) \times 100}{platelet\ count\ (\times 10^9/L)}$$

Body mass index (BMI) was calculated as weight in kilograms/height in square meters. Serum levels of AST, ALT, type IV collagen and HCV RNA, platelet counts, prothrombin time and HCV genotype were measured by standard laboratory techniques at a commercial laboratory (MBC Laboratory, Tokyo, Japan).

2.3. Transient elastography (FibroScan)

FibroScan was done in the right lobe of the liver through the intercostal spaces with the patient lying in the dorsal decubitus position with the right arm in maximal position. The tip of the probe transducer was covered with coupling gel and placed on the skin, between the ribs at the level of the right lobe of the liver. The operator, assisted by an ultrasonic time-motion image, located a liver portion at least 6 cm thick and free of large vascular structures. Once the measurement area had been located, the operator pressed the probe button to start acquisition. The elasticity was automatically calculated by the apparatus and the data were shown as kiloPascal (kPa). All examinations were performed by four accomplished operators (EO, KT, HT, and SO) of our department who individually experienced over 100 examinations. Only liver stiffness measurements obtained with at least six successful acquisitions and a success rate of at least 60% were considered reliable. The validity of FibroScan values depends on an interquartile range of all successful measurements (IQR/M) of less than 30% of median values (Poynard et al., 2008; Lucidarme et al., 2008). The mean IQR/M of the present study was 21.6% and no case with IQR/M > 30% was found. The first measurement of liver stiffness by FibroScan was performed within 2 weeks before liver biopsy examination.

2.4. Liver histology and quantification of liver biopsy

Liver biopsy was done for 122 (84.1%) of the 145 chronic HCV infected patients and was performed by experienced hepatologists with a 16-G disposable needle (Bard® Monopty®; C.R. Bard, Covington, GA) under ultrasound guidance. The median liver biopsy length was 18 mm (minimal length was 15 mm). Liver biopsy specimens were fixed in formalin and paraffin was embedded. All biopsy specimens were analyzed by two experienced pathologists who were blinded to the clinical data. For each specimen, the stage of fibrosis and the grade of activity were established according to the following criteria (Bedossa and Poynard, 1996). Fibrosis was staged on a 0–4 scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. The grading of activity, including the intensity of the necroinflammation, was scored as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

2.5. Therapeutic protocol

All patients were treated with a weight-based, 1.5 μg/kg weekly dose of subcutaneous PEG-IFN alpha-2b (PegIntron A®; Schering-Plough, Osaka, Japan). In combination with PEG-IFN alpha-2b, RBV (Rebetol®; Schering-Plough) was given orally at a daily dose of 600–1000 mg based on bodyweight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing > 80 kg). The length of treatment was 48 weeks for genotype 1b and 24 weeks for genotype 2. The above durations and dosages are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to < 100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had PEG-IFN alpha-2b induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dosage of PEG-IFN alpha-2b was required. Both PEG-IFN alpha-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 85 g/L, 1×10^9/L, or 2.5×10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient desired discontinuation of treatment. All patients received at least 80% or more of the target dosage of PEG-IFN alpha-2b and 60% or over of the RBV, because the condition under the sufficient dosage were needed to estimate between treatment response and liver disease progression accurately.

Table 1
Baseline characteristics of 145 patients with chronic HCV infection.

Characteristics	Non-treated (n = 19)	PEG-IFN alpha-2b combination with RBV treatment		
		SVR (n = 57)	Non-SVR (n = 69)	P-value[*]
Male/Female (n)	9/10	30/27	25/44	0.0655
Age (years)	63.8 ± 9.2	52.7 ± 13.2	60.3 ± 9.3	0.0003
Body mass index (kg/m^2)	22.2 ± 3.4	23.1 ± 3.1	22.8 ± 3.1	0.5912
Aspartate aminotransferase (AST) (IU/L)	61.2 ± 29.5	65.0 ± 39.2	65.8 ± 42.4	0.9192
Alanine aminotransferase (IU/L)	69.7 ± 50.9	88.3 ± 73.7	72.7 ± 53.2	0.1962
Platelet count (10^9/L)	154 ± 52	155 ± 45	154 ± 51	0.9294
Type IV collagen (ng/mL)	180 ± 68	172 ± 69	192 ± 88	0.1750
AST/Platelet count	1.39 ± 0.89	1.37 ± 0.99	1.35 ± 1.19	0.9100
Prothrombin time (%)	94.4 ± 15.9	90.2 ± 8.7	88.2 ± 13.1	0.3414
HCV genotypes 1b/2 n	19/0	34/23	65/4	<0.0001
Serum HCV RNA level (kIU/mL)	3207 ± 1542	1565 ± 1645	2014 ± 1455	0.1062
Liver histology				
Stage of fibrosis				0.7225
F0 (%)	1 (25.0)	7 (13.2)	7 (10.8)	
F1 (%)	0 (0.0)	10 (18.9)	17 (26.2)	
F2 (%)	1 (25.0)	19 (35.8)	16 (24.6)	
F3 (%)	2 (50.0)	11 (20.8)	15 (23.1)	
F4 (%)	0 (0.0)	6 (11.3)	10 (15.4)	
Grade of activity				0.7243
A0 (%)	0 (0.0)	0 (0.0)	0 (0.0)	
A1 (%)	1 (25.0)	17 (32.1)	21 (32.3)	
A2 (%)	2 (50.0)	34 (64.2)	39 (60.0)	
A3 (%)	1 (25.0)	2 (3.8)	5 (7.7)	
Not determined	15	4	4	

Data are shown by the mean ± standard deviation. PEG-IFN, pegylated interferon; RBV, ribavirin.
[*] P-values were analyzed between SVR and non-SVR patients.

2.6. Efficacy of treatment

SVR was defined as serum HCV RNA undetectable at 24 weeks after the end of treatment. This efficacy variable, SVR, was defined as non-detectable HCV RNA as measured by the COBAS® Amplicor® HCV Monitor Test (version 2.0), and the results were labeled as positive or negative. The lower limit of detection was 50 IU/mL (0.5 kIU/mL) (Lee et al., 2000).

2.7. Assessment of biochemical response (BR) among non-SVR patients

We evaluated BR among non-SVR patients after the end of treatment. Patients who had continuous ALT levels under 30 IU/L every month for 96 weeks after the end of treatment were defined as BR.

2.8. Determination of HCV RNA level and HCV genotype

During the treatment period, HCV RNA was analyzed by the COBAS® Amplicor® HCV Monitor assay (version 2.0; Roche Diagnostics, Tokyo, Japan), with a lower limit of quantitation of 5000 IU/mL and an outer limit of quantitation of 5,100,000 IU (5100 kIU)/mL. The COBAS® Amplicor® HCV Monitor assay (version 2.0) is a semi-automated nucleic acid amplification assay, consisting of manual sample preparation and automated reverse transcription (RT), amplification and detection steps on the COBAS® Amplicor® Analyzer. HCV genotype was determined by RT-PCR using universal and type-specific primers from the putative C gene of the HCV genome, according to Okamoto et al. (1992) and the genotype was classified into the type 1b or type 2a or 2b based on Simmonds et al. (1994).

2.9. Statistical analysis

Statistical analysis was done with BMDP statistical software for the IBM 3090 system computer (BMBD Statistical Software,

Inc., Los Angeles, CA). Continuous data were expressed as mean values, mean ± standard deviation (SD) of the mean. The paired t-test, unpaired t-test, Mann–Whitney U-test or Kruskal–Wallis non-parametric analysis of variance was used for the analysis. The area under the receiver operating characteristic curve (AUROC) analysis was done to evaluate the relationship between histological findings and FibroScan values. The cutoff values were selected from the receiver operating characteristic (ROC) curve to maximize total sensitivity and specificity. A P-value less than 0.05 was regarded as statistically significant.

3. Results

3.1. Characteristics of patients

The major clinical and biochemical parameters of the patients at entry (baseline) are summarized in Table 1. The mean FibroScan values were significantly higher in the treated group (10.2 kPa) than in the non-treated group (7.6 kPa) (P = 0.0406). Of the 126 treated patients, 57 (45.2%) achieved SVR. The median age was significantly younger in the SVR group (52.7 years) than in the non-SVR group (60.3 years) (P = 0.0003). The rate of SVR was higher for patients with genotype 2 (85.2%) than for those with genotype 1b (34.3%) (P < 0.0001). No significant differences between the SVR and non-SVR groups were found for gender, BMI, AST, ALT, platelet count, type IV collagen, APRI, prothrombin time, or serum HCV RNA level.

3.2. Relationship between liver fibrosis and FibroScan values

Fig. 1a and b shows the distribution of FibroScan values according to fibrosis stage and activity grade, respectively. The median values of the patients were 4.8 kPa, 7.1 kPa, 8.5 kPa, 12.8 kPa and 19.5 kPa for F0, F1, F2, F3 and F4, respectively. The FibroScan values were significantly correlated with fibrosis stage (r = 0.807, P < 0.0001) and were also significantly increased in accordance with

Table 2
Optimal cutoff of FibroScan values for the determination of histological fibrosis stage in 122 biopsy-received patients with chronic HCV infection.

	Histological fibrosis stage by liver biopsy			
	$F = 1$ (F0 vs. F1)	$F = 2$ (F1 vs. F2)	$F = 3$ (F2 vs. F3)	$F = 4$ (F3 vs. F4)
Number	15/27	27/36	36/28	28/16
Cutoff value[a] (kPa)	6.1	7.2	10.3	14.9
AUROC	0.81	0.66	0.88	0.94
Sensitivity (%)	63.0	66.7	89.3	100.0
Specificity (%)	86.7	66.7	77.8	82.1
Positive predictive value (%)	89.5	66.7	75.8	76.2
Negative predictive value (%)	56.5	60.0	90.3	100.0
Positive likelihood ratio	4.72	2.00	4.02	5.60

AUROC, area under the receiver operating characteristic curve.

[a] The opitimal cutoff value is the one that gives the higher total sensitivity and specificity.

the grade of activity for the patients ($r = 0.343$, $P < 0.0001$). Table 2 shows the optimal liver stiffness cutoff values obtained for sensitivity and specificity. In analyses of adjacent fibrosis stages (F0 vs. F1, F1 vs. F2, F2 vs. F3 and F3 vs. F4), four threshold FibroScan values were identified: ≥6.1 kPa for F1 (sensitivity 63.0%, specificity 86.7%); ≥7.2 kPa for F2 (sensitivity 66.7%, specificity 66.7%); ≥10.3 kPa for F3 (sensitivity 89.3%, specificity 77.8%) and 14.9 kPa for F4 (sensitivity 100%, specificity 82.1%). The corresponding AUROC were 0.81 for F0 vs. F1, 0.66 for F1 vs. F2, 0.88 for F2 vs. F3 and 0.94 for F3 vs. F4.

3.3. The longitudinal FibroScan values for the PEG-IFN alpha-2b plus RBV combination treatment (Fig. 2a and b)

The baseline mean FibroScan values were 10.3 ± 4.8 kPa, 10.0 ± 5.5 kPa, and 7.6 ± 3.9 kPa for SVR ($n = 57$), non-SVR ($n = 69$), and non-treated patients ($n = 19$), respectively. For SVR patients, the mean FibroScan values were 8.3 kPa, 6.6 kPa and 5.4 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively. For non-SVR patients, the mean FibroScan values were 9.0 kPa, 9.5 kPa and 11.4 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively.

The changes of the FibroScan values of each patient at the end of treatment, 48 weeks and 96 weeks after the end of treatment were calculated with the entry values as the estimated standard. Significant differences were found between SVR (-16.2%, -32.2% and -43.5%) and non-SVR patients (-7.2%, -2.1% and $+17.3\%$) in the mean rate of change of FibroScan values between each testing point ($P = 0.0127$, $P < 0.0001$ and $P < 0.0001$, respectively). For the untreated patients, the mean FibroScan values increased to 8.3 kPa, 9.8 kPa and 10.6 kPa ($+12.9\%$, $+38.0\%$ and $+49.1\%$, respectively) at each testing point. Significant differences were also found between treated patients (SVR and non-SVR) and untreated patients in the mean rate of change of FibroScan values.

3.4. The longitudinal FibroScan values classified according to the BR and non-BR of non-SVR patients (Fig. 3a and b)

Of the 69 non-SVR group patients, 16 (23.2%) achieved BR. The baseline mean FibroScan values were 9.3 ± 5.2 kPa and 10.2 ± 5.6 kPa for BR ($n = 16$) and non-BR ($n = 53$) patients, respectively. For BR patients, the mean FibroScan values were 7.4 kPa, 6.2 kPa and 6.7 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively. For non-BR patients, the mean FibroScan
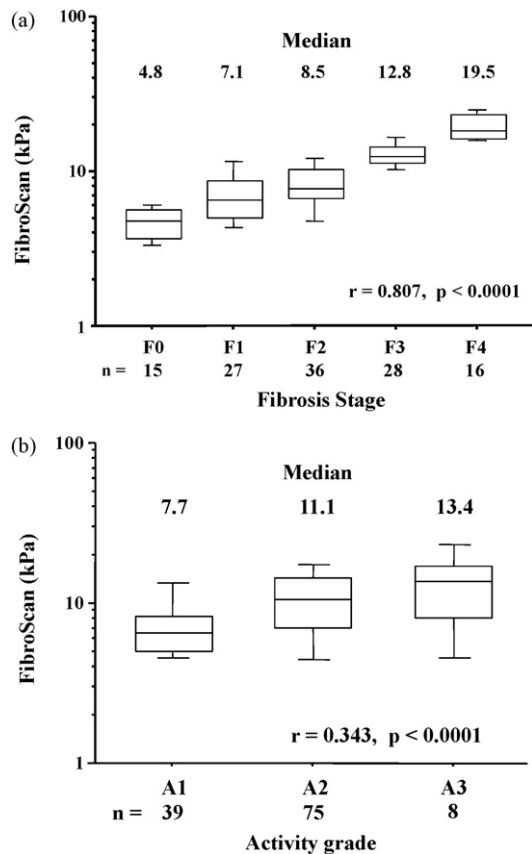
Table 3
Differences of the mean rate of changes of FibroScan values after PEG-IFN alpha-2b plus RBV treatment, classified by age group and gender.

	n	PEG-IFN alpha-2b combination with RBV treatment					
		Week 0	P-value[*]	Week 48	P-value[*]	Week 96	P-value[*]
SVR patients							
Age group			0.7995		0.4128		0.3874
<60 years	22	−16.3		−30.7		−42.1	
≥60 years	35	−15.9		−34.6		−45.9	
Gender			0.4517		0.6278		0.1740
Men	30	−17.7		−31.1		−46.3	
Women	27	−14.4		−33.4		−40.5	
Fibrosis stage			0.6653		0.2369		0.0927
≤F2	36	−16.1		−31.1		−42.0	
≥F3	17	−18.3		−37.4		−50.0	
Non-SVR patients							
Age group			0.7995		0.3478		0.3998
<60 years	27	−6.4		−6.9		11.5	
≥60 years	42	−7.8		1.1		20.9	
Gender			0.3218		0.1625		0.4507
Men	25	−10.8		−9.7		11.8	
Women	44	−5.3		2.3		20.4	
Fibrosis stage			0.6720		0.4893		0.4309
≤F2	40	−7.3		−5.0		13.9	
≥F3	25	−9.7		1.1		23.2	

PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained vilorogical response; week 0, at the end of treatment; week 48, 48 weeks after the end of treatment; week 96, 96 weeks after the end of treatment. The rates of changes of FibroScan values of each patient at the end of treatment, 48 weeks and 96 weeks after the end of treatment were calculated by the entry values as the estimated standard.

[*] P-values were analyzed at each point between age groups, between genders and between fibrosis stages.

Fig. 1. FibroScan values for each fibrosis stage (a) and activity grade (b) of 122 biopsy-received patients with chronic hepatitis C virus infection. The vertical axis is a logarithmic scale. The bottom and the top boxes are the first and third quartiles, respectively. The length of the box represents the interquartile range within which 50% of the values are located. The lines through the middle of the boxes represent the median. The error bars are the minimum and maximum values.

values were 9.5 kPa, 10.5 kPa and 12.8 kPa at week 0 and at 48 and 96 weeks after the end of treatment, respectively. For BR patients, the mean rate of change of FibroScan values indicated improvement (−17.9%, −30.0% and −27.1% at weeks 0, 48 and 96 after the end of treatment, respectively) with antiviral treatment, in spite of there not being a virological effect. On the other hand, for non-BR patients, the mean rate of change of FibroScan values showed worsening over time (−4.1%, +6.4% and +30.6% at weeks 0, 48 and 96 after the end of treatment, respectively). A significant difference was found between BR and non-BR patients in the mean rate of change of FibroScan values in each period ($P = 0.0270$, $P < 0.0001$ and $P < 0.0001$, respectively). Moreover, although a significant difference was found between non-BR (+6.4%) and non-treated patients (+38.0%) at week 48 after the end of treatment ($P < 0.0001$), no significant difference was found between non-BR (+30.6%) and non-treated patients (+49.1%) at week 96 after the end of treatment ($P = 0.0835$) in the mean rate of change of FibroScan values. In other analyses, no significant difference was found between the longitudinal rate of change of FibroScan values and age (<60 and ≥60), gender or fibrosis stage (≤F2 and ≥F3) for both SVR and non-SVR patients, as shown in Table 3.

4. Discussion

The present prospective study consists of a demonstration of the association between liver stiffness measured by FibroScan and the efficacy of PEG-IFN alpha-2b plus RBV treatment. Recent reports have shown that treatment for chronic hepatitis C is associated with
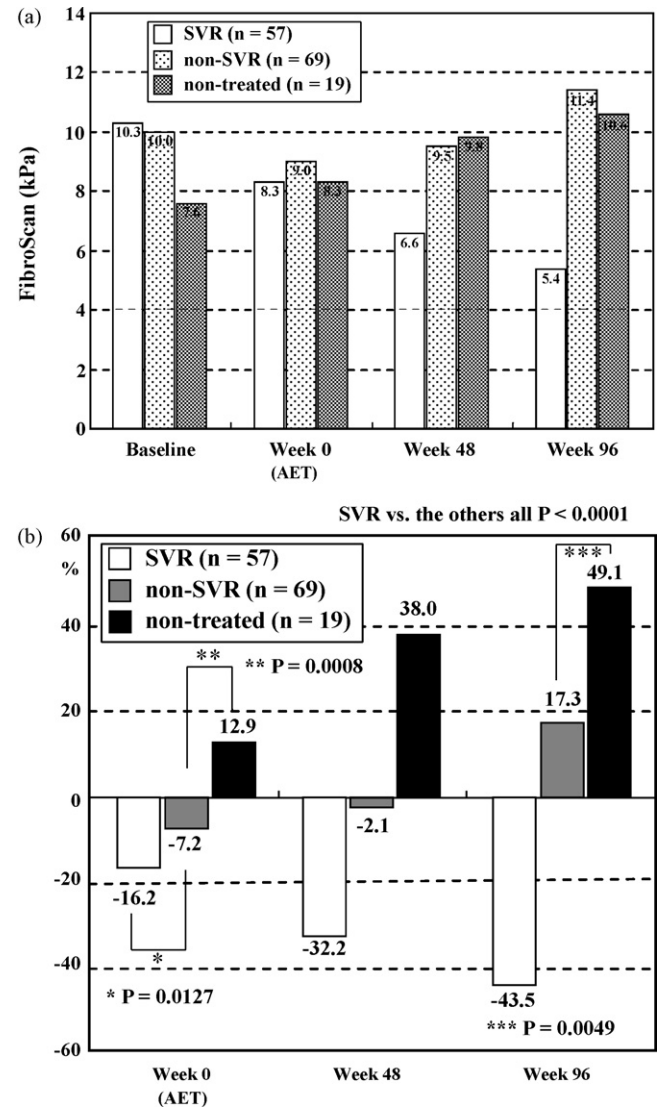

Fig. 2. The longitudinal mean FibroScan values (a) and the mean rate of change of FibroScan values (b) of 126 treated patients after pegylated interferon alpha-2b plus ribavirin combination treatment and 19 non-treated patients. The rates of change of the FibroScan values of each patient at the end of treatment and at 48 and 96 weeks after the end of treatment were calculated using the entry values as the estimated standard. The length of treatment was 48 and 24 weeks for HCV genotypes 1 and 2 patients, respectively. Weeks 0, 48 and 96 for the treated patients indicate the end of treatment and 48 and 96 weeks after the end of treatment, respectively. Weeks 0, 48 and 96 for the non-treated patients indicate 48, 96 and 144 weeks from entry, respectively. SVR, sustained virological response; AET, at the end of treatment.

an improvement of FibroScan values at the end of treatment and 6 months later, whatever the virological response (Vergniol et al., 2008, 2009). However, we investigated the association between an efficacy of the antiviral treatment and FibroScan values related to both virological and biochemical response for a longer period after the treatment than the previous reports. Consequently, we demonstrated that the liver elasticity of SVR patients markedly improved over time and that the liver elasticity of non-SVR patients with biochemical response also improved.

In PEG-IFN alpha plus RBV treatment for chronic hepatitis C, we previously reported that it was necessary to administer ≥80% of the target dosage of PEG-IFN alpha-2b plus ≥60% of the target dosage of RBV throughout the treatment in order to achieve virological efficacy in Japanese patients (Furusyo et al., 2008). Each patient in the present study was analyzed under this treatment dosage.
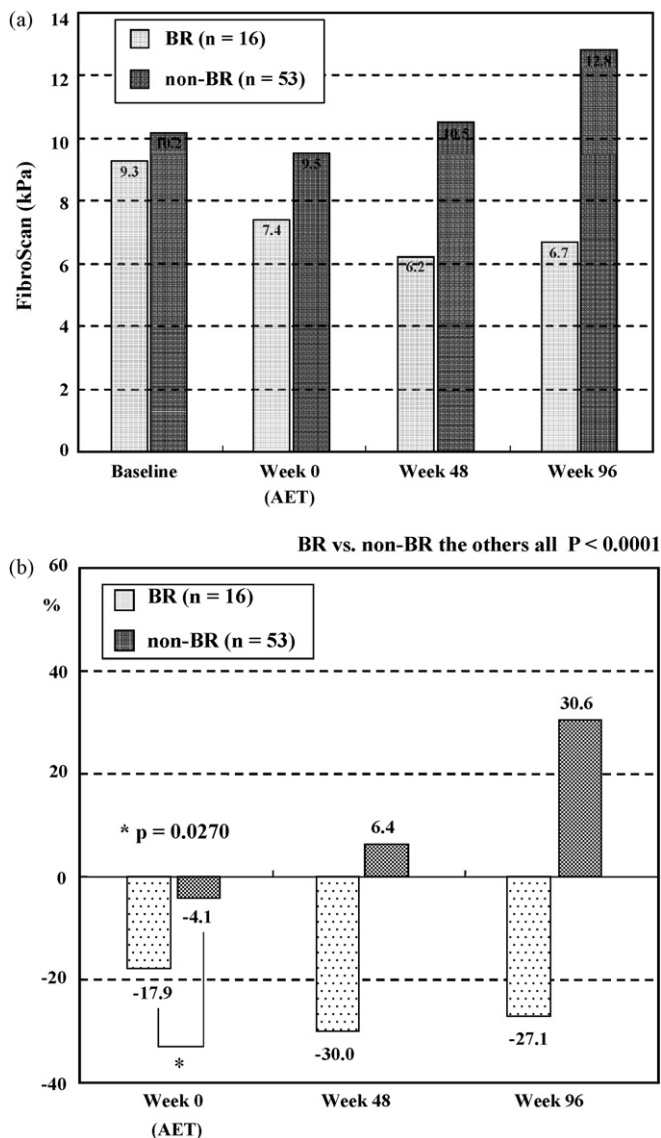
Fig. 3. The longitudinal mean FibroScan values (a) and the mean rate of change of FibroScan values (b) of the non-sustained virological response patients after pegylated interferon alpha-2b plus ribavirin combination treatment, classified by biological response (BR). The rates of change of the FibroScan values of each patient at the end of treatment and at 48 and 96 weeks after the end of treatment were calculated using the entry values as the estimated standard. Weeks 48 and 96 indicate 48 and 96 weeks after the end of treatment, respectively. AET, at the end of treatment.

FibroScan represents a novel clinical methodology based on ultrasound. This can survey a larger sample of the liver parenchyma than liver biopsy and thus more accurately estimates liver fibrosis across a wide range of liver disorders (Ziol et al., 2005; Ogawa et al., 2007). According to meta-analysis, FibroScan could be performed with excellent diagnostic accuracy at distinguishing liver cirrhosis (F4) or no liver cirrhosis (≤F3), with a mean AUROC of 0.94, and at distinguishing ≥F2 and ≤F1 with a mean AUROC of 0.84 (Friedrich-Rust et al., 2008). The optimal cutoff values for the diagnosis of F4 and ≥F2 suggested from the summary ROC are 13.01 kPa and 7.65 kPa, respectively (Friedrich-Rust et al., 2008). Thus, prior studies have already shown that FibroScan can distinguish absent or mild fibrosis from advanced fibrosis in both HCV-mono-infected and HIV-/HCV-co-infected individuals, but it seems to be less accurate at differentiating between intermediate stages. However, Macías et al. (2008) suggested that the usefulness

of FibroScan could be enhanced using two different cutoff values (6.0 kPa and 9.0 kPa) to identify with ≤F1 and ≥F2, respectively, in HIV- and HCV-co-infected patients.

Biomarkers such as FibroTest demonstrated similar results as FibroScan in patients treated with IFN and RBV (Poynard et al., 2002b, 2003; Ngo et al., 2006; Patel et al., 2009) and the combination of FibroTest and ActiTest® (which is a modification of the FibroTest) give not only the fibrosis estimate but also the activity estimate (Poynard et al., 2003). FibroScan has never been demonstrated better than FibroTest for F4 vs. ≤F3 according to evidence-based data (Castéra et al., 2005; Shaheen et al., 2007; Manning and Afdhal, 2008; Poynard et al., 2008; Calès et al., 2008).

While natural history of liver fibrosis progression can vary depending on gender and alcohol consumption, age is the main risk factor for liver fibrosis (Poynard et al., 1997). We recommended that all our patients stop drinking alcohol while under treatment and during follow-up. In our study, the baseline mean value of FibroScan was 7.6 kPa, almost the same as the F2 stage, with a value of 10.5 kPa, nearly equal to the F3 stage, after 3 years in non-treated patients (median age 63.8 years). Poynard et al. (1997) suggested that the rate of liver fibrosis progression of untreated patients was highest in individuals older than 50 years (at a rate of 0.333 stage/year). The findings obtained in the present study were similar to those of the above report.

Many previous reports have shown that IFN treatment achieves biochemical and histological improvement with viral suppression by patients with chronic hepatitis C (Poynard et al., 2002a; Furusyo et al., 1997; Cammá et al., 1998; Bruno et al., 2007; Furusyo et al., 2008). Several potential mechanisms have been hypothesized for the anti-fibrotic effect of IFN, including that IFN alpha can directly reduce fibrogenesis. Shiratori et al. (2000) showed that the fibrosis stage improved from −0.60 at <3 years of follow-up to −0.88 at >3 years follow-up (a rate of −0.28/years) for SVR patients treated with non-pegylated IFN monotherapy. However, Everson et al. (2008) showed that the fibrosis stage improved −1.00 between before treatment and 6 months after the end of treatment of SVR patients receiving PEG-IFN alpha monotherapy. Poynard et al. (2002a) showed that the mean fibrosis stage 6 months after the end of treatment was 1.9 ± 0.9 (SD) for SVR patients with compensated cirrhosis treated with PEG-IFN alpha monotherapy or in combination with RBV. There was no significant difference between PEG-IFN alpha plus RBV and PEG-IFN alpha alone for the 48-week regimen as to an anti-fibrotic effect (Poynard et al., 2002a). Therefore, PEG-IFN alpha itself may have a stronger anti-fibrotic effect than non-pegylated IFN and RBV, due to a pharmaco-dynamic advantage.

In the present study, we demonstrated a dramatic reduction of FibroScan values in both SVR and BR patients. Firstly, our results confirmed, from the viewpoint of liver fibrosis, that IFN treatment is of long-term benefit for chronic hepatitis C patients. Moreover, such reduction was observed at an early stage of PEG-IFN alpha plus RBV treatment by SVR and BR patients. It is probable that such early reduction of FibroScan values means not only the improvement of fibrosis but also of inflammation in the liver as a result of treatment, because FibroScan values were significantly correlated with the grade of activity in the liver in the present study. Compared with the rate of change of FibroScan values between non-advanced fibrosis (F ≤ 2) and advanced fibrosis (F ≥ 3), no significant differences were found at each testing point. Secondly, another important result was that FibroScan values decreased even for BR patients without HCV clearance by treatment. The findings of the present study can explain why the rate of development of HCC is lower in BR patients treated with IFN. Because HCC tends to develop in patients with advanced liver fibrosis (cirrhosis), BR patients who have a reduction of fibrosis probably will not as quickly develop HCC in the future. Thirdly, although the virological treatment itself may produce good

results in terms of a short-term anti-fibrotic effect of the liver, the FibroScan values of non-BR patients became progressively worse in the long-term course after the antiviral treatment. In the case of a high serum ALT level for non-SVR patients, an additional antiviral retreatment has to be considered. In fact, we recommended antiviral treatment for the studied non-treated patients after the completion of this study, and then most of them have started to receive IFN treatment. A long-term IFN treatment regimen was effective in a smaller trial of the anti-fibrotic effect (Arase et al., 2004); therefore, we believe that treatment with careful attention to IFN-related adverse effects could be usefully introduced to help patients avoid progressing to liver fibrosis and HCC. Thus, the FibroScan is a very interesting tool for the follow-up of chronic HCV carriers, as it is easier to use than liver biopsy in clinical settings. These results are of great interest for the understanding of the effects of IFN treatment and of HCV-related liver disease.

In conclusion, our study shows that transient elastography (FibroScan) is a useful tool for the longitudinal assessment of IFN treatment of chronic hepatitis C patients.

Acknowledgements

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