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Original Article

Reduction of liver stiffness by interferon treatment in the patients with chronic hepatitis C

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Aim: To assess the regression of liver fibrosis after interferon (IFN) treatment in patients with chronic hepatitis C, liver stiffness (LS) was measured repeatedly and the factors associated with reduction of LS were assessed.

Methods: LS was measured by transient elastography before treatment, at end of treatment (EOT), and 1 year and 2 years after EOT in 145 patients with chronic hepatitis C treated by IFN with or without ribavirin.

Results: In the patients with sustained virological response (SVR) ($n = 93$) and relapsers ($n = 28$), LS significantly decreased at EOT (median, 5.4 [interquartile range, 4.0–8.6] kilopascals [kPa], $P < 0.0001$ and 6.8 [4.5–8.9] kPa, $P = 0.0023$) and 1 year after EOT (5.3 [4.2–7.0] kPa, $P < 0.0001$ and 6.8 [4.5–9.3] kPa, $P = 0.0204$) compared with baseline (8.0 [5.0–11.9] kPa and 10.6 [7.0–16.6] kPa). In SVR patients, LS significantly decreased 2 years after EOT (5.3 [4.1–6.3] kPa) compared with baseline ($P < 0.0001$) and LS at EOT ($P = 0.0034$). Two points or greater reduction of deduced stage at last LS measurement was observed in 78% of SVR patients, 59% of relapsers and 15% of patients with non-virological response whose pretreatment deduced stages were F3–F4. Fibrosis stage, hyaluronic acid levels, duration of treatment, response to treatment and alanine aminotransferase levels were associated with a 2-point or greater decrease of deduced fibrosis stage.

Conclusion: IFN treatment reduced LS in SVR patients and relapsers. Significant reduction of LS is associated with milder fibrosis stage, lower hyaluronic acid levels, longer IFN treatment, virological response of SVR or relapse and higher alanine aminotransferase levels.

Key words: non-virological response, relapser, ribavirin, sustained virological response, transient elastography

INTRODUCTION

HEPATITIS C VIRUS (HCV) is one of the most frequent and important causes of chronic viral hepatitis and approximately 170 million people are infected with the virus worldwide.[1] HCV usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC).[2,3]

Interferon (IFN) monotherapy and combination therapy of IFN and ribavirin (RBV) have been shown to be effective for eradicating chronic HCV infection.[4–6] Although IFN treatment has been reported to improve liver fibrosis,[7–18] the frequency and extent of improvement and the correlation with response to treatment have not been fully elucidated. The improvement of liver fibrosis is of critical importance, because the ultimate goal of anti-HCV therapy is prevention of cirrhosis, HCC and death from liver disease which correlate with liver fibrosis.[19–21] However, it is difficult to perform liver biopsies repeatedly after IFN treatment to assess the improvement of liver fibrosis.

Liver biopsy is considered the gold standard for assessing liver fibrosis in chronic hepatitis C and other chronic liver disorders. Although liver biopsy is generally safe, it is an invasive and costly procedure, with rare but potentially life-threatening complications, limiting its

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acceptance and repetition. In addition, the accuracy of liver biopsy in assessing fibrosis has limitations because of sampling error and both inter- and intra-observer variability in interpretation.[22,23] Therefore, there is a need to develop and validate non-invasive tests that can accurately reflect the full spectrum of liver fibrosis.

Recently, transient elastography (TE) with use of a new apparatus, FibroScan (EchoSens, Paris, France), for non-invasive measurement of liver stiffness (LS) was developed.[24] LS measured by a FibroScan has been reported to correlate with stage of liver fibrosis in various liver diseases.[25–29]

To assess the regression of liver fibrosis after IFN treatment in patients with chronic hepatitis C, LS was measured repeatedly and the factors associated with reduction of LS were assessed.

METHODS

Patients

ONE HUNDRED AND forty-five patients with chronic hepatitis C were treated by various IFN with or without RBV consecutively in Fujita Health University Hospital from January 2005 to February 2008 (Table 1). One hundred and eighteen patients were treated with pegylated (peg)-IFN-α2b (1.5 μg/kg per week) and RBV (600–1000 mg/day), 16 patients with peg-IFN-α2a (180 μg/week), seven patients with IFN-α2b (6 MU/day, 6 days per week for the first 2 weeks and 3 days per week for the rest of the treatment period) and RBV (600–1000 mg/day), and four patients with consensus IFN (9 MU/day, 3 days/week). The planned treatment duration was 24, 48 or 72 weeks according to HCV genotype, viral load and response to treatment in the first 12 weeks. The responses to IFN treatment were categorized into three types, sustained virological response (SVR) where negativity of HCV RNA sustained 6 months after the end of treatment (EOT), relapse where HCV RNA became negative during treatment but returned to positive after EOT, and non-virological response (NVR) where HCV RNA remained positive throughout treatment. In the study period, we followed up 35 patients with chronic hepatitis C without IFN treatment in whom LS was measured more than twice. These patients were also studied.

Table 1 Baseline characteristics of the patients with different responses to interferon (IFN) treatment and those without IFN treatment

	SVR patients	Relapsers	NVR patients	Patients without IFN
No. of patients	93	28	24	35
Age (year)	55.0 (55.3–69.8)*	58.0 (55.5–62.5)	56.0 (49.5–62.5)	63.0 (55.3–69.8)*
Sex (female/male)	32/61†	15/13	19/5†	21/14
Previous IFN treatment (+/−)	10/83‡	4/24	10/14‡	4/31‡
AST (IU/L)	48.0 (29.0–70.3)	38.0 (28.5–68.5)	47.5 (31.5–64.0)	53.0 (29.5–75.0)
ALT (IU/L)	65.0 (43.8–97.5)	46.0 (30.5–83.0)	52.0 (31.5–75.0)	55.0 (29.3–87.0)
γ-GTP (IU/L)	42.0 (23.8–79.5)	31.0 (21.0–65.5)	55.0 (40.0–84.0)	38.5 (24.0–85.0)
Hyaluronic acid (ng/mL)	61.0 (23.8–129.5)	84.0 (53.3–176.0)	89.5 (27.0–194.5)	139.0 (61.5–270.5)
Platelet count ($\times 10^4/\mu L$)	16.7 (12.9–19.4)§	15.1 (11.7–17.3)	12.8 (10.9–18.7)	12.2 (8.8–16.7)§
Genotype (1/2/mixed)	44/48/1¶	21/7/0	23/1/0¶	30/5/0¶
Viral load (KIU/mL)	850 (158–2405)††	2350 (1255–3135)††	1550 (645–2850)	1350 (460–2000)††
Fibrosis stage (0/1/2/3/4)	5/40/18/14/13	3/6/6/7/6	4/1/8/7/4	–
Inflammatory grade (0/1/2/3)	7/43/21/19	1/15/8/4	1/11/9/3	–
Liver stiffness (kPa)	8.0 (5.0–11.9)	10.6 (7.0–16.6)	9.9 (5.7–15.7)	10.5 (5.8–15.3)
Duration of IFN treatment (days)	315 (159–326)‡‡	327 (160–446)‡‡	179 (97–267)‡‡	–

*SVR patients were significantly younger than patients without IFN treatment ($P = 0.0016$). †Male sex was significantly more frequently seen in SVR patients than in NVR patients ($P < 0.0001$). ‡NVR patients had previous IFN treatment significantly more frequently than SVR patients and patients without IFN treatment ($P = 0.0066$ and $P = 0.0438$). §Platelet count was significantly higher in SVR patients than in patients without IFN treatment ($P = 0.0016$). ¶Genotype 2 was significantly more frequently seen in SVR patients than in NVR patients and in patients without IFN treatment ($P < 0.0001$ and $P = 0.0048$). ††Viral load was significantly larger in relapsers than in SVR patients ($P = 0.0076$) and in patients without IFN treatment ($P = 0.0394$). ‡‡Duration of treatment was significantly shorter in NVR patients than in SVR patients and in relapsers ($P = 0.0299$ and $P = 0.0018$).
Data are expressed as median (interquartile range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; IFN, interferon; NVR, non-virological response; SVR, sustained virological response.

The study was performed in accordance with the principles of good clinical practice, the principles of the Declaration of Helsinki and its appendices, and local and national laws.

Liver biopsy

Liver biopsies were done in 142 patients before IFN treatment and nine after treatment. Sections were stained with hematoxylin–eosin stain and azan stain. Liver biopsy specimens were assessed by a hepatologist (K. Y.) blinded of clinical data according to Metavir score.[30] Fibrosis was staged as: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Activity was graded as: A0, none; A1, mild; A2, moderate; and A3, severe activity.

LS measurement

Liver stiffness measurement by TE was performed with a FibroScan (EchoSens) before treatment, at EOT (within 6 months after EOT), 1 year after EOT (7–18 months after EOT) and 2 years after EOT (19–30 months after EOT).

Ten validated measurements were made on each patient. The results were expressed in kilopascals (kPa). Only procedures with 10 validated measurements and a success rate of at least 60% (ratio of the number of successful acquisitions over the total number of acquisitions) were considered reliable. The median value was considered representative of the liver elastic modulus.

The change ratio of LS was calculated by the formula: $100 - (\text{LS after treatment}) / (\text{pretreatment LS}) \times 100$.

Statistical analysis

The patients were divided according to the responses to IFN treatment. The groups were compared by χ^2-test and Mann–Whitney *U*-test. The changes of LS were assessed by Wilcoxon signed rank sum test. Bonferroni's adjustment was adopted, when multiple comparisons were done. The diagnostic performance of LS was determined in terms of sensitivity, specificity, positive and negative predictive values, positive likelihood ratio, diagnostic accuracy and area under receiver–operator curves (ROC). Optimal cut-off values for fibrosis stages were determined at the maximum total of sensitivity and specificity with negative predictive value of more than 80%. The multivariate analysis for the factors associated with significant reduction of LS after treatment was done by stepwise regression analysis. Data were expressed as median (interquartile range).

RESULTS

Responses to IFN treatment

NINETY-THREE OF 145 patients (64.1%) obtained SVR, 28 (19.3%) had virological relapse and 24 (16.6%) had NVR (Table 1).

Sustained virological response patients were significantly younger than patients without IFN treatment ($P = 0.0016$). Male sex was significantly more frequently seen in SVR patients than in NVR patients ($P < 0.0001$). NVR patients had previous IFN treatment significantly more frequently than SVR patients and patients without IFN treatment ($P = 0.0066$ and $P = 0.0438$). Platelet count was significantly higher in SVR patients than in patients without IFN treatment ($P = 0.0016$). Genotype 2 was seen significantly more frequently in SVR patients than in NVR patients and in patients without IFN treatment ($P < 0.0001$ and $P = 0.0048$). Viral load was significantly larger in relapsers than in SVR patients ($P = 0.0076$) and in patients without IFN treatment ($P = 0.0394$). Duration of treatment was significantly shorter in NVR patients than in SVR patients and in relapsers ($P = 0.0299$ and $P = 0.0018$).

Changes of LS

The intervals between pretreatment LS and LS at EOT, 1 year after EOT or 2 years after EOT in patients treated with IFN were 352 (263–421) days, 690 (529–796) days or 981 (873–1076) days, respectively. The interval between first and second measurement of LS in patients without IFN treatment was 656 (360–922) days.

In SVR patients, LS significantly decreased at EOT ($P < 0.0001$), 1 year after EOT ($P < 0.0001$) and 2 years after EOT ($P < 0.0001$) compared with pretreatment values (Fig. 1a). LS significantly decreased 2 years after EOT compared with the values at EOT ($P = 0.0034$). In relapsers, LS significantly decreased at EOT ($P = 0.0023$) and 1 year after EOT ($P = 0.0204$) compared with pretreatment values (Fig. 1b). In NVR patients, LS at EOT, 1 year after EOT and 2 years after EOT did not significantly differ from pretreatment values (Fig. 1c). LS at second measurement did not differ significantly from LS at first measurement in patients without IFN treatment (Fig. 1d).

Liver stiffness values of last measurement were compared among the patients with different responses to IFN treatment and those without IFN treatment

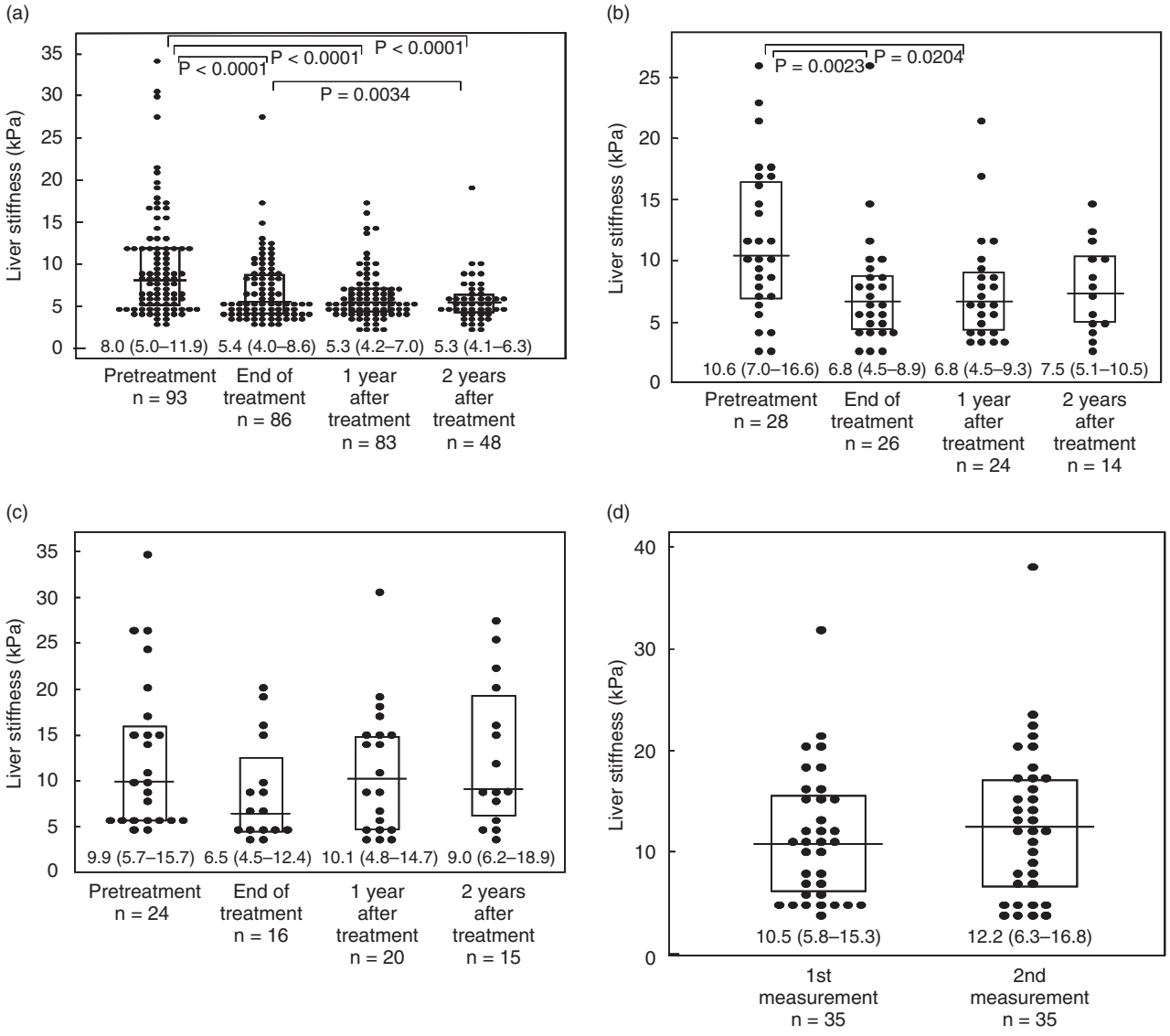


Figure 1 Changes of liver stiffness (LS) of the patients with different responses to interferon (IFN) treatment and those without IFN treatment. LS was measured before treatment, at the end of treatment (EOT), 1 year after EOT and 2 years after EOT. Horizontal bars and boxes represent median values and interquartile ranges, respectively. (a) Patients with sustained virological response (SVR). LS significantly decreased at EOT ($P < 0.0001$), 1 year after EOT ($P < 0.0001$) and 2 years after EOT ($P < 0.0001$) compared with pretreatment values. LS significantly decreased 2 years after EOT compared with the values at EOT ($P = 0.0034$). (b) In relapsers, LS significantly decreased at EOT ($P = 0.0023$) and 1 year after EOT ($P = 0.0204$) compared with pretreatment values. (c) In patients with non-virological response (NVR), LS at EOT, 1 year after EOT and 2 years after EOT did not significantly differ from pretreatment values. (d) In patients without IFN treatment, LS at second measurement did not differ significantly from LS at first measurement.

(Table 2). The last LS values were significantly lower in SVR patients than in NVR patients and in patients without IFN treatment ($P = 0.0008$ and $P < 0.0001$). The last LS values were significantly lower in relapsers than in patients without IFN treatment ($P = 0.0134$). The change ratios of LS were significantly higher in SVR patients than in NVR patients and patients without IFN treatment ($P = 0.0052$ and $P < 0.0001$). The change ratios of LS were significantly higher in relapsers than in NVR patients and patients without IFN treatment

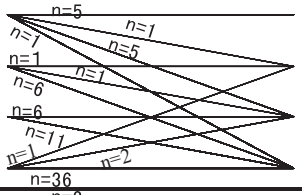
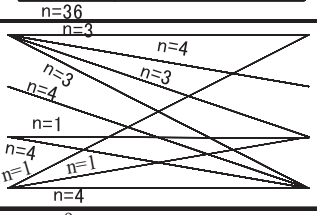
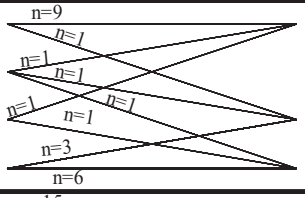
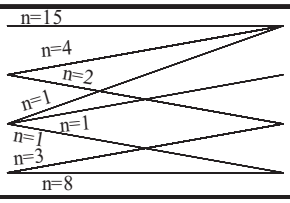
	Fibrosis stage deduced from liver stiffness	1st measurement		Last measurement
SVR patients n=93	F4	29 (31%)		5 (5%)
	F3	8 (9%)		3 (3%)
	F2	17 (18%)		14 (15%)
	F0–1	39 (42%)		71 (76%)
Relapsers n=28	F4	13 (46%)		4 (14%)
	F3	4 (14%)		4 (14%)
	F2	5 (18%)		5 (18%)
	F0–1	6 (21%)		15 (54%)
NVR patients n=24	F4	10 (42%)		11 (46%)
	F3	3 (13%)		0 (0%)
	F2	2 (8%)		5 (21%)
	F0–1	9 (38%)		8 (33%)
Patients without IFN treatment n=35	F4	15 (43%)		20 (57%)
	F3	6 (17%)		1 (3%)
	F2	3 (9%)		5 (14%)
	F0–1	11 (31%)		9 (26%)

Figure 2 Changes of fibrosis stage deduced from liver stiffness (LS) of the patients with different responses to interferon (IFN) treatment and those without IFN treatment. The deduced fibrosis stages of first and last measurements were compared. Twenty-nine of 37 (78%) sustained virological response (SVR) patients, 10 of 17 (59%) relapsers, two of 13 (15%) patients with non-virological response (NVR) and none of 21 patients without IFN treatment with deduced fibrosis stages of F3–F4 at first LS measurement had a 2-point or greater reduction of deduced stage at last LS measurement.

($P = 0.0418$ and $P = 0.0005$). The interval between first and last LS measurement was significantly longer in relapsers than in patients without IFN treatment ($P = 0.0428$).

Changes of fibrosis stages deduced from LS according to cut-off values for fibrosis stages

Based on ROC analysis, the optimal discriminate cut-off values were determined at the maximum total of sensitivity and specificity with negative predictive value of more than 80% (Table 3). The cut-off values were 6.9 kPa for F2 or greater, 9.5 kPa for F3 or greater and 11.4 kPa for F4.

Fibrosis stages were deduced from LS according to cut-off LS values for fibrosis stages. The deduced fibrosis stages of first and last measurements were compared (Fig. 2). Twenty-nine of 37 (78%) SVR patients, 10 of 17 (59%) relapsers, two of 13 (15%) NVR patients and none of 21 patients without IFN treatment with deduced fibrosis stages of F3–F4 at first LS measurement had 2 points or greater reduction of deduced stage at last LS measurement. Six of 13 (46%) of SVR patients with pretreatment biopsy-proven cirrhosis had a deduced fibrosis stage of F0–F1 after treatment, while none of the relapsers, NVR patients or patients without IFN treatment with pretreatment biopsy-proven cirrhosis did (data not shown).

Table 2 Change ratio of LS of the patients with different response to IFN treatment and those without IFN treatment

	1st LS (kPa)	Last LS (kPa)	Change ratio of LS (%)	Interval (days)
SVR patients	8.0 (5.0–11.9)	5.3 (4.0–6.8)*	36.4 (10.9–50.1)‡	811 (526–949)
Relapsers	10.6 (7.0–16.6)	6.8 (4.5–9.9)†	36.1 (8.8–53.0)§	890 (609–1143)¶
NVR patients	9.9 (5.7–15.7)	8.9 (5.1–16.2)*	11.2 (−30.2–29.4)‡§	833 (531–976)
Patients without IFN treatment	10.5 (5.9–15.3)	12.2 (6.3–16.8)*†	−4.3 (−34.5–11.3)‡§	656 (360–922)¶

*The last LS values were significantly lower in SVR patients than in NVR patients and patients without IFN treatment ($P = 0.0008$ and $P < 0.0001$). †The last LS values were significantly lower in relapsers than patients without IFN treatment ($P = 0.0134$). ‡The change ratios of LS were significantly higher in SVR patients than in NVR patients and patients without IFN treatment ($P = 0.0052$ and $P < 0.0001$). §The change ratios of LS were significantly higher in relapsers than in NVR patients and patients without IFN treatment ($P = 0.0418$ and $P = 0.0005$). ¶The interval between first and last LS measurement was significantly longer in relapsers than in patients without IFN treatment ($P = 0.0428$).
Data are expressed as median (interquartile range).
IFN, interferon; LS, liver stiffness; NVR, non-virological response; SVR, sustained virological response.

The factors associated with a 2-point or greater reduction of deduced fibrosis stage was examined in 67 patients with a pretreatment deduced fibrosis stage of F3–F4. In univariate analysis, previous IFN treatment ($P = 0.0120$), alanine aminotransferase (ALT) levels ($P = 0.0076$), pretreatment fibrosis stage ($P = 0.0022$), response to IFN treatment ($P = 0.0002$) and duration of IFN treatment ($P = 0.0030$) were significantly associated with a 2-point or greater decrease of deduced fibrosis stage (Table 4). Sex ($P = 0.0904$), aspartate aminotransferase (AST) levels ($P = 0.0706$), hyaluronic acid ($P = 0.0633$) and interval between the first and last measurement of LS ($P = 0.0677$) also tended to be associated with a 2-point or greater decrease of deduced fibrosis stage. Multivariate analysis selected pretreatment fibrosis stage ($P = 0.007$), hyaluronic acid ($P = 0.009$), duration of IFN treatment ($P = 0.014$), response to IFN treatment ($P = 0.027$) and ALT levels ($P = 0.027$) as factors associated with a 2-point or greater decrease of deduced fibrosis stage (Table 5).

Table 3 Optimal cut-off value of liver stiffness for each fibrosis stage was determined at the maximum total of sensitivity and specificity with negative predictive value of more than 80%

	≥F2	≥F3	F4
Cut-off value (kPa)	6.9 kPa	9.5 kPa	11.4 kPa
Positive predictive value (%)	82.8	67.7	41.2
Negative predictive value (%)	80.0	90.9	97.8
Sensitivity (%)	86.7	86.3	91.3
Specificity (%)	74.6	76.9	74.8
Positive likelihood ratio	3.4	3.7	3.6
Diagnostic accuracy (%)	81.7	80.3	77.5
Area under ROC value	0.90	0.89	0.88

ROC, receiver–operator curve.

Changes of ALT levels by IFN treatment

In SVR patients, ALT levels significantly decreased at EOT ($P < 0.0001$), 1 year after EOT ($P < 0.0001$) and 2 years after EOT ($P < 0.0001$) compared with baseline (Table 6). In relapsers, ALT levels significantly decreased at EOT ($P = 0.0015$) and 1 year after EOT ($P = 0.0019$) and 2 years after EOT ($P = 0.0196$) compared with baseline. ALT levels significantly decreased at EOT compared with baseline in NVR patients ($P = 0.0003$). In patients without IFN treatment, ALT levels did not significantly decrease at last measurement compared with baseline.

Liver histology after IFN treatment

Liver biopsies after treatment were done in one SVR patient, five relapsers and three NVR patients. The intervals between first biopsies and second biopsies were 760 (593–968) days. No change of histological fibrosis stage was observed in eight patients, whose deduced fibrosis stage decreased 1 point in three patients, did not change in four patients and increased 1 point in one patient. A 1-point increase of histological fibrosis stage was observed in a patient whose deduced fibrosis stage decreased 1 point.

DISCUSSION

THE PRESENT STUDY demonstrated that LS significantly reduced at EOT and 1 year after EOT in SVR patients and relapsers compared with pretreatment values, and that LS significantly reduced 2 years after EOT compared with pretreatment values and the values at EOT in SVR patients. So far, several studies with

Table 4 Characteristics of the patients with or without a 2-point or greater reduction of liver stiffness among 67 patients with pretreatment deduced fibrosis stage of F3–F4

	Patients with a ≥2-point reduction of deduced fibrosis stage ($n = 41$)	Patients without a ≥2-point reduction of deduced fibrosis stage ($n = 26$)	*P*-value
Age (year)	60.0 (57.8–64.0)	60.0 (54.0–66.0)	
Sex (female/male)	15/26	15/11	$P = 0.0904$
Previous IFN treatment (+/−)	5/36	10/16	$P = 0.0120$
AST (IU/L)	66.0 (48.8–107.3)	53.0 (39.0–74.0)	$P = 0.0706$
ALT (IU/L)	90.0 (55.5–143.5)	58.0 (48.0–78.0)	$P = 0.0076$
γ-GTP (IU/L)	49.0 (31.8–95.3)	56.5 (43.0–86.0)	
Hyaluronic acid (ng/mL)	109.0 (54.7–152.5)	141.0 (86.0–303.0)	$P = 0.0633$
Platelet count ($\times 10^4/\mu L$)	13.5 (11.0–16.6)	11.6 (10.0–14.9)	
Genotype (1/2)	30/11	16/10	
Viral load (KIU/mL)	1800 (565–2533)	1395 (400–2600)	
Fibrosis stage (0/1/2/3/4)	1/1/17/14/8	0/1/3/8/14	$P = 0.0022$
Inflammatory grade (1/2/3)	9/15/17	8/10/8	
Liver stiffness (kPa)	13.3 (10.9–17.3)	14.6 (12.0–22.8)	
Response to IFN treatment (NVR/non-NVR)	2/39	11/15	$P = 0.0002$
Duration of IFN treatment (days)	325 (171–351)	174 (157–315)	$P = 0.0030$
Intervals between first and last measurement of LS (days)	912 (690–1068)	721 (510–950)	$P = 0.0677$

Data are expressed as median (interquartile range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; IFN, interferon; LS, liver stiffness; NVR, non-virological response.

paired pre- and post-IFN treatment biopsies reported decreased fibrosis in 29% (mean times between biopsies, 1.6 years),[15] 44% (2.5 years),[16] 59% (3.7 years)[14] or 82% (5.2 years)[17] of SVR patients. George *et al*. reported that 67% of SVR patients with pretreatment cirrhosis or advanced fibrosis had a 2-point or greater decrease in fibrosis score in 5.2 years.[17] In the present study, 78% of SVR patients with pretreatment deduced a fibrosis stage

Table 5 Logistic regression analysis for predicting a 2-point or greater reduction of liver stiffness among 67 patients with pretreatment deduced fibrosis stage of F3–F4

Variable	β	Standard error	*P*-value	Odds ratio	95% confidence interval
Fibrosis stage					
0: F3/F4					
1: F0–F2	3.405	1.264	0.007	30.1	2.5–358.6
Hyaluronic acid					
0: >239 ng/mL					
1: ≤239 ng/mL	3.13	1.196	0.009	22.9	2.2–238.2
Duration of IFN treatment					
0: <320 days					
1: ≥320 days	2.189	0.893	0.014	8.9	1.6–51.4
Response to IFN treatment					
0: NVR					
1: non-NVR	2.73	1.231	0.027	15.3	1.4–171.1
ALT levels					
0: <78 IU/L					
1: ≥78 IU/L	2.061	0.933	0.027	7.9	1.3–48.9

ALT, alanine aminotransferase; IFN, interferon; NVR, non-virological response.

Table 6 Change of ALT levels of the patients with different responses to IFN treatment and those without IFN treatment

	ALT before treatment (IU/L)	ALT at end of treatment (IU/L)	ALT 1 year after treatment (IU/L)	ALT 2 years after treatment (IU/L)	Last ALT (IU/L)
SVR patients	65.0 (43.8–97.5)*	18.0 (11.0–26.3)*	15.0 (11.0–20.0)*	17.0 (11.0–22.5)*	–
Relapsers	46.0 (30.5–83.0)†	18.0 (13.0–39.0)†	22.5 (16.0–33.0)†	24.0 (18.0–33.0)†	–
NVR patients	52.0 (31.5–75.0)‡	33.5 (15.0–52.0)‡	36.0 (27.0–55.0)	46.0 (31.0–58.5)	–
Patients without IFN treatment	55.0 (29.3–87.0)	–	–	–	45.0 (34.5–81.3)

*In SVR patients, ALT levels was significantly lower at end of treatment, 1 year after treatment and 2 years after treatment than before treatment ($P < 0.0001$ for all). †In relapsers, ALT levels was significantly lower at end of treatment, 1 year after treatment and 2 years after treatment than before treatment ($P = 0.0015$, $P = 0.0019$ and $P = 0.0196$). ‡In NVR patients, ALT levels was significantly lower at end of treatment than before treatment ($P = 0.0003$).
Data are expressed as median (interquartile range).
ALT, alanine aminotransferase; IFN, interferon; NVR, non-virological response; SVR, sustained virological response.

of F3–F4 had a 2-point or greater decrease in deduced fibrosis stage in a period of 2.1 years. Additionally, 46% of SVR patients with pretreatment biopsy-proven cirrhosis had deduced fibrosis stage of F0–F1 after treatment. The reduction of LS observed in the present study probably reflects the regression of fibrosis, while the reduction of LS appears faster and more conspicuously than the previously reported regression of fibrosis assessed by biopsies.

The present study demonstrated significant reduction of LS in relapsers. In relapsers with pretreatment deduced fibrosis stage of F3–4, 59% of patients had a 2-point or greater decrease of deduced stage after treatment. Camma *et al.* reported reduced fibrosis in relapsers 24 weeks after treatment of peg-IFN-α2a.[18] Toccaceli *et al.* reported regressed fibrosis in 14% and progressed fibrosis in 14% of relapsers.[16] Veldt *et al.* reported no regressed fibrosis in biochemical responders (detectable virus but normal ALT levels).[15] Thus, regression of fibrosis in relapsers is ambiguous from the previous histological studies. Further histological studies are needed to elucidate whether the reduced LS in relapsers shown in the present study actually reflects the regression of fibrosis.

The reduction of LS shown in the present study was more prominent or faster in SVR patients and relapsers compared with the previous observations by biopsies. LS might be able to detect more subtle regression of fibrosis compared with liver biopsy. Liver fibrosis is usually semiquantitatively assessed by numerical systems.[31,32] These numerical systems might not be sensitive enough to detect subtle change of liver fibrosis. Recently, direct measurement of the amount of fibrosis in the biopsy specimen by computer-assisted morphometric image analysis has been reported.[33–35] Arima *et al.* reported that 42% of SVR patients of F3–F4 pretreatment had decreased fibrosis assessed by numerical systems, while the fibrosis area measured by morphometric image analysis decreased in 92%.[36] Thus, the conspicuous and early reduction of LS after IFN treatment might reflect the detection of subtle regression of fibrosis which could be measured by morphometric image analysis but not by numerical systems.

Recently, several reports questioned the supposition that LS is determined entirely by liver fibrosis.[37] In those studies, the association between LS and necroinflammatory activity was demonstrated. Coco *et al.* reported that the patients with biochemical remission had lower LS values than those with identical fibrosis stage but elevated ALT.[38] Sagir *et al.* and Arena *et al.* reported that the patients with acute liver damage showed high LS values suggestive of cirrhosis, while none of them had any other signs of cirrhosis.[39,40] Ogawa *et al.* reported that LS values decreased after IFN treatment not only in SVR patients but also biochemical responders.[41] We reported that LS significantly correlates with ALT levels which are the indicators of necroinflammatory activity in the patients with chronic hepatitis C.[42] In the present study, LS significantly decreased in SVR patients and relapsers. ALT levels also significantly decreased in SVR patients and relapsers. Thus, a part of the reduction of LS may be attributed to the regression of necroinflammatory activity. However, the regression of necroinflammatory activity alone could not explain the considerable reduction of LS. Forty-six percent of patients with pretreatment biopsy-proven cirrhosis had LS values corresponding to F0–F1 after treatment, while no patients with cirrhosis had such low LS values before treatment. To compensate the defect of LS which is affected by factors other than fibrosis, Castera *et al.* recommended

the combined use of FibroScan and FibroTest.[25] Vergniol *et al*. reported that both LS and FibroTest values fell in IFN-treated patients.[43] It is necessary to compare LS and FibroTest with liver biopsies. In the present study, biopsies were done in only nine patients without significant reduction of LS after treatment, and no significant regression of histological fibrosis was observed. Further studies are needed to assess the correlation between LS and histological findings after IFN treatment.

A 2-point or greater reduction of deduced stage after treatment was associated with pretreatment fibrosis stage, hyaluronic acid levels, duration of IFN treatment, virological response and ALT levels in multivariate analysis. This indicates that the beneficial effects of IFN treatment on LS are closely associated with milder fibrosis stage which is also indicated by lower hyaluronic acid levels, virological response of SVR or relapse, severer inflammatory activity indicated by higher ALT level and longer IFN treatment.

Vergniol *et al*. reported that LS significantly reduced 6 months after EOT, whatever the virological response.[43] In the present study, LS slightly reduced in NVR patients at EOT without statistical significance, and returned to the pretreatment values 2 years after EOT. Further study is needed to examine whether LS reduces in NVR patients. If LS reduces even in NVR patients, long-term IFN treatment might be beneficial for the patients.

The present study demonstrated that IFN treatment significantly reduced LS in SVR patients and relapsers. The beneficial effects of IFN treatment on LS are closely related to milder fibrosis stage, lower hyaluronic acid levels, longer IFN treatment, virological response of SVR or relapse and higher ALT levels. Further studies are needed to confirm that the reduction of LS is actually associated with the regression of histological fibrosis.

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