

Original

Factors Related to Liver Stiffness in Patients with Hepatitis C During Direct-acting Antiviral Agent Treatment

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SUMMARY

Background and Aim : The purpose of this study was to observe the changes over time in liver stiffness measured by shear wave elastography (SWE) in hepatitis C patients during direct-acting antiviral agent (DAA) treatment and evaluate the factors affecting the liver stiffness.

Methods : The subjects were 206 consecutive patients diagnosed with hepatitis C in the Dokkyo Medical University Saitama Medical Center treated with DAAs. SWE was performed to measure the propagation velocity of shear waves (V_s) before starting treatment (baseline), at the end of treatment (EOT), and 12 weeks after EOT (follow-up 12). The change in V_s (ΔV_s) was calculated for the difference between baseline and follow-up 12. Clinical parameters were obtained on the same day as SWE. Multiple regression analysis was used to identify factors related to ΔV_s .

Results : Data from 149 patients were used ; all 149 patients achieved sustained virological response. Mean V_s decreased significantly, from 1.58 ± 0.92 m/s at baseline to 1.46 ± 0.27 m/s at EOT ($P=0.00045$). Mean V_s at follow-up 12 was 1.42 ± 0.28 m/s, significantly lower than at EOT ($P=0.00002$). The mean ΔV_s was 0.147 ± 0.164 m/s. On multiple regression analysis, prothrombin time% (PT%) and the change in alanine aminotransferase (Δ ALT) from baseline were significantly related to ΔV_s . Baseline ALT and the FIB4-index tended to affect ΔV_s .

Conclusions : In hepatitis C patients, V_s measured by SWE improved with 12 weeks of DAA therapy and continued to improve to follow-up 12. Baseline PT% and Δ ALT contributed significantly to the improvement of V_s during DAA treatment, and the FIB-4 index also had a great effect.

Key words : direct-acting antiviral agents, hepatitis C, liver stiffness, shear wave elastography, sustained virological response

INTRODUCTION

In recent years, methods that measure liver stiffness non-invasively with ultrasound, such as acoustic radiation force impulse imaging (ARFI)¹, transient elastography (TE)², and strain elastography (SE)³, have been reported to be useful in evaluating hepatic fibrosis. Shear wave elastography (SWE) is a new

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technology that gauges liver stiffness by measuring the propagation velocity of shear waves (Vs) generated in liver tissue. Vs measurements with SWE are reported to be useful in diagnosing the fibrosis in hepatitis C patients⁴.

It has been previously reported that, in hepatitis C patients, liver stiffness measured by SWE was higher in the naïve group than in the sustained virological response (SVR) group, presumably due to hepatitis activity⁵. Improvement of liver stiffness compared with the pre-treatment level was reported in hepatitis C patients who achieved SVR by treatment with direct-acting antiviral agents (DAAs)⁶, and this improvement of liver stiffness was shown to be more prominent in hepatitis C patients with advanced fibrosis^{7,8}.

The purpose of this study was to observe the changes over time in liver stiffness measured by SWE in hepatitis C patients during DAA treatment and evaluate the factors affecting liver stiffness.

METHODS

Patients

The subjects were 206 consecutive patients diagnosed with hepatitis C patients in the Dokkyo Medical University Saitama Medical Center who were treated with DAAs between July 2015 and February 2016. Patients with decompensated liver cirrhosis, hepatocellular carcinoma, autoimmune disease, collagen disease, or chronic heart disease were excluded. Patients with a history of drinking ≥ 20 g alcohol per day and those diagnosed with obvious fatty liver on abdominal ultrasound were also excluded.

This prospective study was approved by the Ethics Committee of the Dokkyo Medical University Saitama Medical Center, and written, informed consent was obtained from all participants. This study conformed to the ethical guidelines of the 2008 Declaration of Helsinki.

Measurement of the Velocity of the Shear Wave

Measurement of Vs by SWE was performed using a LOGIQ E9 (GE Healthcare, Milwaukee, WI, USA). The right lobe of the liver was visualized through an intercostal space while the patient was lying in a supine position with the right arm in maximum

abduction. Measurements were taken while subjects held their breath during spontaneous breathing. The visual depth of the system was fixed at 8 cm, and the region of interest (ROI) was 1–2 cm below the surface of the liver. The system was adjusted so that sample volume depth was 4 cm or less. The apparatus automatically calculated the Vs, and the results are expressed in m/s. The result was considered reliable only when 10 successful shots and a measurement success rate $>80\%$ were obtained.

The duration of DAAs treatment of all patients was 12 weeks. SWE was performed before starting treatment (baseline), at the end of treatment (EOT), and 12 weeks after EOT (follow-up 12). The change in Vs (Δ Vs) was calculated for the difference between baseline and follow-up 12.

Clinical parameters

Clinical parameters, which were obtained on the same day that SWE was performed, were compared. Clinical parameters other than Vs were the following: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), albumin (Alb), leukocytes (WBC), hemoglobin (Hb), platelets (Plt), prothrombin time% (PT%), α -fetoprotein (AFP), and Mac-2 binding protein glycosylation-isomer (M2BPGi). These parameters were measured on the same days as SWE. The FIB-4 index was estimated using the values of serum AST, ALT, Plt, and age. Δ ALT, Δ AFP, and Δ M2BPGi were calculated with differences between baseline and follow-up 12, similar to Δ Vs.

Each parameter at baseline, Δ ALT, Δ AFP, and Δ M2BPGi were used as explanatory variables in multiple regression analysis.

Statistical analysis

Continuous data for liver stiffness and other clinical parameters are expressed as means \pm standard deviation (SD). A paired Wilcoxon test was used to test for differences in each parameter before and after the start of treatment. Friedman's test was used to test multiple comparisons of each parameter. Multiple regression analysis was used to examine the factors that contributed to Δ Vs. Values of $P < 0.05$ were regarded as significant.

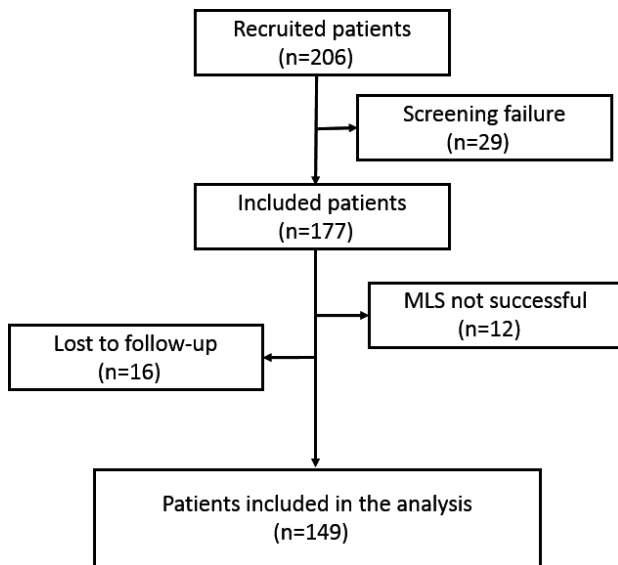


Figure 1 Flowchart of the study

A total of 206 consecutive hepatitis C patients were recruited. Twenty-nine of these patients, including six patients with a history of HCC, 20 patients diagnosed with fatty liver by ultrasound, and 9 patients with a history of drinking ≥ 20 g/day of alcohol, were excluded. Another 12 patients in whom liver stiffness could not be measured were also excluded from the evaluation. In addition, another 16 patients who were lost to follow-up were withdrawn from the study. Data from 149 patients were finally used for the analysis. MLS : measure liver stiffness.

RESULTS

A total of 206 consecutive hepatitis C patients were recruited for the study. Twenty-nine of these patients, including six patients with a history of HCC, 20 patients diagnosed with fatty liver by ultrasound, and 9 patients with a history of drinking ≥ 20 g/day of alcohol, were excluded. Another 4 patients in whom liver stiffness could not be measured because of obesity or hepatic atrophy and 8 patients with large measurement errors were also excluded from the evaluation. In addition, another 16 patients, including 5 patients who discontinued their hospital visits after DAA therapy and 9 patients without periodic SWE measurements, were withdrawn from the study. There were no patients with autoimmune hepatitis or cardiac failure. Therefore, data from 149 patients were finally used for the analysis (Figure 1).

The DAAs used for treatment in 149 of these patients were sofosbuvir (SOF), ledipasvir (LDV) in 52 patients, ombitasvir/paritaprevir/ritonavir in 41

Table 1 Patient characteristics (n = 149)

Age (yr)	66.6 ± 11.8
Sex (male/female)	62/87
Serotype (1/2)	92/57
IFN (Yes/No)	40/109
HCV RNA (LogIU/ml)	5.8 ± 0.9
ALT (IU/L)	56.2 ± 38.7
Total bilirubin (mg/dL)	0.83 ± 0.29
Serum albumin (g/dL)	4.18 ± 0.37
WBC ($\times 10^3/\text{mm}^3$)	5.0 ± 1.7
Hb (g/dL)	13.7 ± 1.4
Platelet ($\times 10^4/\text{mm}^3$)	16.1 ± 5.5
Prothrombin time (%)	98.3 ± 16.2
AFP (ng/mL)	9.7 ± 19.7
Fib4 index	3.6 ± 2.7
M2BPGi (C.O.I.)	3.0 ± 3.3
Vs (m/s)	1.57 ± 0.25

AFP : α -fetoprotein, ALT : alanine aminotransferase, Hb : hemoglobin, HCV : hepatitis C virus, IFN : interferon, M2BPGi : Mac-2 binding protein glycosylation isomer, Vs : velocity of shear wave, WBC : white blood cell.

patients, and SOF/ribavirin in 56 patients. Table 1 shows the characteristics of the 149 patients (62 men, 87 women ; mean age 66.6 ± 11.8 years). Forty patients (26.9%) had previously received interferon (IFN) therapy. Their mean ALT was 56.2 ± 38.7 (10–252) IU/L, including a large number of hepatitis patients with relatively mild active disease. Other biochemical values, AFP, M2BPGi, and Vs, were consistent with chronic hepatitis or compensated liver cirrhosis.

DAA therapy was continued for 12 weeks. All 149 patients achieved SVR at follow-up 12.

Figure 2 shows the changes in Vs. Mean Vs decreased significantly during the 12-week treatment period, from 1.58 ± 0.92 m/s at baseline to 1.46 ± 0.27 m/s at EOT ($P=0.00045$). Mean Vs at follow-up 12 was 1.42 ± 0.28 m/s, a significant decrease from the Vs at EOT ($P=0.00002$). The mean difference between baseline and follow-up 12 of Vs (Δ Vs) was 0.147 ± 0.164 m/s.

Figure 3 shows that Vs was significantly correlated with the FIB-4 index at baseline ($r=0.5483$, $P=0.0000$). Figure 4 shows the comparison of Δ Vs between the group of patients with the FIB-4 index < 3.25 (n=93) and the group of patients with the FIB-4 index ≥ 3.25 (n=56). Although Δ Vs tended to be

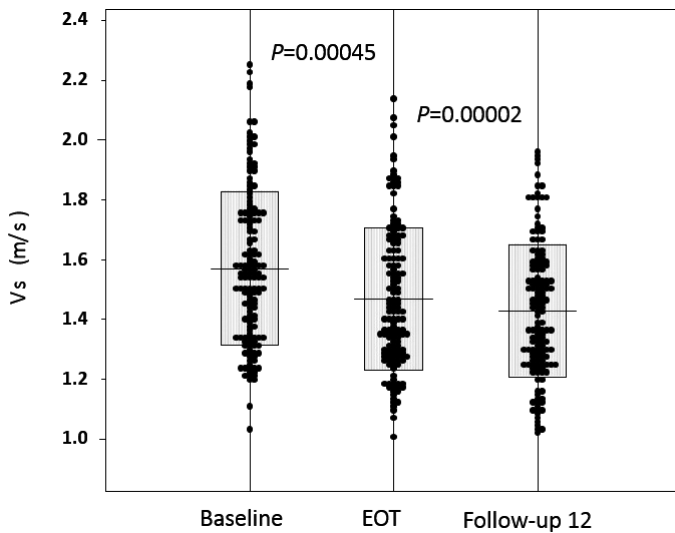


Figure 2 Changes in Vs from baseline to follow-up 12

Mean Vs decreases significantly during the 12-week treatment period, from 1.58 ± 0.92 m/s at baseline to 1.46 ± 0.27 m/s at EOT ($P=0.00045$). Mean Vs at follow-up 12 is 1.42 ± 0.28 m/s, a significant decrease from the Vs at EOT ($P=0.00002$).

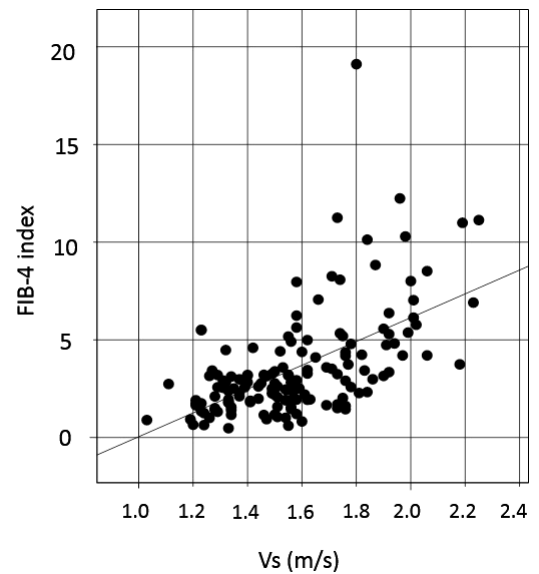


Figure 3 Relationship between Vs and the FIB-4 index at baseline

Vs correlates significantly with the FIB-4 index at baseline ($r=0.5483$, $P=0.0000$).

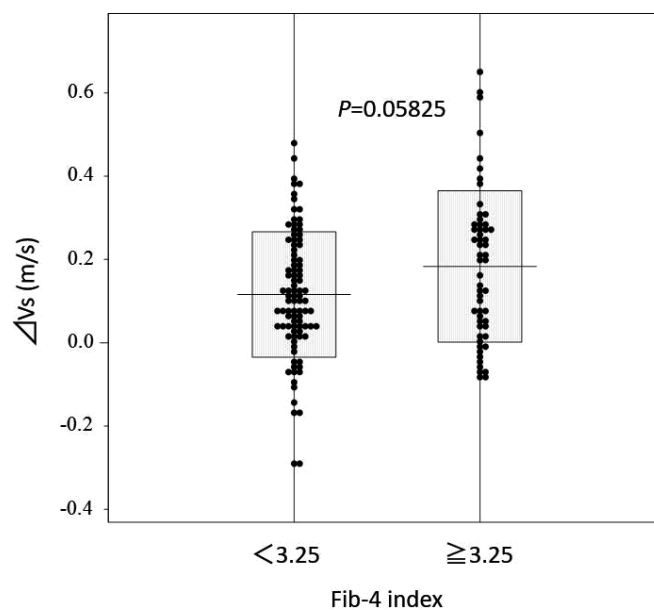


Figure 4 Comparison of ΔV_s between the group with the FIB-4 index < 3.25 and the group with the FIB-4 index ≥ 3.25

Comparison of ΔV_s between the group of patients with FIB-4 index < 3.25 ($n=93$) and the group of patients with FIB-4 index ≥ 3.25 ($n=56$) shows that ΔV_s tends to be greater in the group with a FIB-4 index ≥ 3.25 , but there is no significant difference ($P=0.05825$).

Table 2 Changes of each parameters in baseline and follow-up 12

	Baseline	Follow-up 12	<i>p</i> value
ALT (IU/L)	56.2 ± 38.7	17.8 ± 10.2	0.0000
GGT (IU/L)	43.8 ± 34.7	22.8 ± 14.5	0.0000
Total bilirubin (mg/dL)	0.83 ± 0.29	0.93 ± 0.35	0.0000
Serum albmin (g/dL)	4.18 ± 0.37	4.35 ± 0.30	0.0000
WBC ($\times 10^3/\text{mm}^3$)	5.0 ± 1.7	5.3 ± 1.6	0.0044
Hb (g/dL)	13.7 ± 1.4	13.6 ± 1.8	0.3065
Platelet ($\times 10^4/\text{mm}^3$)	16.1 ± 5.5	17.8 ± 6.0	0.0000
Prothrombin time (%)	98.3 ± 16.2	94.6 ± 16.4	0.0004
AFP (ng/mL)	9.7 ± 19.7	3.6 ± 2.7	0.0000
M2BPGi (C.O.I.)	3.0 ± 3.3	2.5 ± 1.5	0.0000
Vs (m/s)	1.57 ± 0.25	1.42 ± 0.22	0.0000

AFP : α -fetoprotein, ALT : alanine aminotransferase, Hb : hemoglobin, HCV : hepatitis C virus, IFN : interferon, M2BPGi : Mac-2 binding protein glycosylation isomer, Vs : velocity of shear wave, WBC : white blood cell.

Table 3 Multiple regression analysis with Δ Vs as the dependent variable

	β	SE	<i>std</i>	<i>t</i>	<i>P</i>
Age (yr)	-0.0008	0.0013	-0.0565	0.60548	0.54597
Sex	-0.04	0.03094	-0.0119	1.29383	0.19813
ALT (IU/L)	-0.0027	0.00148	-0.6327	1.82865	0.06985
Total bilirubin (mg/dL)	0.0529	0.04799	0.0937	1.10230	0.27247
Serum albmin (g/dL)	-0.0569	0.04383	-0.1256	1.29858	0.19650
WBC ($\times 10^3/\text{mm}^3$)	0.01188	0.01044	0.1230	1.18346	0.23889
Hb (g/dL)	0.02098	0.01277	0.1749	1.64263	0.10299
Platelet ($\times 10^4/\text{mm}^3$)	-0.0001	0.00382	-0.0034	0.02764	0.97800
Prothrombin time (%)	0.00406	0.00092	0.3970	4.39628	0.00002
AFP (ng/mL)	-0.0039	0.00619	-0.4644	0.63013	0.52977
M2BPGi (C.O.I.)	0.00249	0.0156	0.0493	0.15963	0.87343
Fib4 Index	0.01631	0.00927	0.2669	1.75866	0.08108
Δ ALT (IU/L)	0.00346	0.00154	0.7579	2.25017	0.02620
Δ AFP (ng/mL)	0.00331	0.00643	0.3662	0.51387	0.60826
Δ M2BPGi (C.O.I.)	0.02149	0.0225	0.2969	0.95513	0.34137

AFP : α -fetoprotein, ALT : alanine aminotransferase, Hb : hemoglobin, HCV : hepatitis C virus, IFN : interferon, M2BPGi : Mac-2 binding protein glycosylation isomer, Vs : velocity of shear wave, WBC : white blood cell.

greater in the group with a FIB-4 index ≥ 3.25 , which indicates advanced fibrosis, no significant difference was observed ($P=0.05825$).

Table 2 shows the changes in each parameter from baseline to follow-up 12. The ALT, AFP, and M2BPGi levels decreased significantly at follow-up 12 from baseline. There were changes in the reference values, but total bilirubin, serum albumin, WBC and Plt levels increased significantly at follow-up 12 from baseline. Similarly, there was a change in the reference value,

but prothrombin activity decreased significantly at follow-up 12 from baseline.

From these results, we added Δ ALT, Δ AFP, and Δ M2BPGi as explanatory variables in the multiple regression analysis.

Table 3 shows the results of the multiple regression analysis for factors related to Δ Vs. PT% and Δ ALT from baseline were significantly related to Δ Vs. The ALT at baseline and the FIB4-index at baseline tended to affect Δ Vs, but there were no significant rela-

tions ($P=0.07056$, $P=0.08108$).

DISCUSSION

Significant advances have been made in the treatment of hepatitis C with the advent of DAAs. DAA therapy has milder side effects compared to IFN therapy, and there is a high rate of SVR at 12 weeks^{9~13}. In the present study, SVR was achieved in all 149 patients by 12-week DAA treatment.

It has been previously reported that Vs as measured by SWE is about 1.2 m/s in healthy livers⁵. In the present study, the mean Vs at baseline was 1.58 ± 0.92 m/s, but it decreased significantly to 1.46 ± 0.27 m/s during a short 12-week period of DAA therapy. Vs at follow-up 12 further decreased significantly to 1.42 ± 0.28 m/s. Liver stiffness shown as Vs is influenced not only by the degree of liver stiffness, but also by necroinflammatory activity^{14,15}. Elevated ALT levels in the present study, corresponding to the presence of necroinflammatory activity, decreased significantly from baseline to EOT. Therefore, the reduction of ALT levels from baseline to EOT appeared strongly associated with the reduction of liver stiffness as shown by SWE.

Marcellin et al. who performed multiple liver biopsies in patients with SVR after IFN therapy, reported histologic improvement in inflammation after treatment was completed, but they found no clear evidence of fibrosis improvement even after follow-up for 3 years or longer¹⁶. Therefore, improvement of hepatic fibrosis in the present patients after a short 12-week treatment period was not to be expected. The improvement in Vs should be considered to reflect remission of inflammation, rather than improvement in fibrosis.

Previous studies showed that the FIB-4 index can be used to classify liver disease and monitor HCV-infected patients in longitudinal studies^{17,18}.

Ogasawara et al. showed a positive correlation between baseline liver stiffness and the FIB-4 index, and more prominent improvement of liver stiffness by DAAs in patients with hepatic cirrhosis with a FIB-4 index ≥ 3.25 than in patients with chronic hepatitis with a FIB-4 index < 3.25 ⁷. In the present study, the positive correlation between baseline liver stiffness and the FIB-4 index was confirmed. Although Δ Vs

tended to be greater in the group of patients with a baseline FIB-4 index ≥ 3.25 than in the group of patients with a baseline FIB-4 index < 3.25 , no significant difference was observed ($P=0.05825$).

M2BPGi is a new liver fibrosis marker that quantitatively measures changes in the carbohydrate structure of Mac-2 binding protein¹⁹, and it is also considered useful in predicting carcinogenesis in hepatitis C patients^{20~22}. In the present study, M2BPGi, which at baseline was 2.93 ± 2.62 C.O.I., or about 3-times the upper normal limit, decreased significantly at EOT to 1.58 ± 1.30 C.O.I. Therefore, it is assumed that elevated M2BPGi, like Vs, is not only due to hepatic fibrosis, but also due to effects caused by inflammation²³.

As factors contributing to the improvement of Vs, PT% and Δ ALT were identified as significant in the present study. Pre-treatment ALT and the FIB-4 index were confirmed to greatly affect the improvement of Vs. Thus, the present study showed that DAAs improve liver stiffness in patients with advanced fibrosis whose liver was severely inflamed before treatment and whose protein-synthesizing capability is sufficiently maintained.

LIMITATIONS

This study was conducted at a single institution, and although this was a prospective study in which 206 patients were enrolled, data from a smaller number, only 149 patients, could be used for evaluation. Since no patients underwent liver biopsy in this study, the histopathological findings and Vs could not be compared.

CONCLUSIONS

Vs measured by SWE in hepatitis C patients improved with 12 weeks of DAA therapy. This improvement continued until follow-up 12 (12 weeks after EOT). The significant factors that contribute to the improvement of Vs during DAA treatment are baseline PT% and Δ ALT. The FIB-4 index also has a great effect on the improvement.

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