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Sofosbuvir-based therapies associated with regression of liver fibrosis in patients with hepatitis C virus infection
A prospective observational study

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Abstract

Oral direct-acting antiviral (DAA) treatment leads to >95% sustained virological response (SVR) and could be clinically useful in regression of liver fibrosis in chronic hepatitis C virus (HCV) infection. We evaluated if ledipasvir/sofosbuvir or sofosbuvir + ribavirin is associated with regression of fibrosis in HCV patients who achieved SVR.

In this prospective cohort study performed at 3 sites in Japan, patients with genotype 1 and genotype 2 were given standard treatment of ledipasvir 90 mg/sofosbuvir 400 mg and sofosbuvir 400 mg + 200–1000 mg/day ribavirin, respectively, for 12 weeks. Liver fibrosis was assessed using Mac-2-binding protein glycosylation isomer (M2BPGi) and other fibrosis markers (platelet count, Fib-4 index, liver stiffness measurement [LSM]) in patients who achieved SVR.

A total of 98.1% of (n = 101/103) patients in genotype 1 cohort and 100% (n = 16/16) in the genotype 2 cohort achieved SVR12. Based on per-protocol analysis, M2BPGi levels showed a significant decrease (–2.2 cut-off index [COI], $P < .0001$) at week 48 after treatment initiation. Forty-three patients showed a significant decrease in Fib-4 index (–1.2, $P < .0001$), and 44 patients showed improvement in LSM (–5.9 kPa, $P < .0001$).

Achievement of SVR after antiviral therapy was associated with fibrosis regression. M2BPGi correlated well with LSM at week 48 after treatment initiation, supporting the sustainable benefit of HCV therapy.

Abbreviations: BMI = body mass index, CHC = chronic hepatitis C, DAA = oral direct-acting antiviral, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, LDV = ledipasvir, LSM = liver stiffness measurement, M2BPGi = Mac-2-binding protein glycosylation isomer, SOF = sofosbuvir, SVR = sustained virological response, TE = transient elastography.

Keywords: fibrosis, hepatitis C, liver, ribavirin, sofosbuvir

1. Introduction

Treatment with highly effective oral direct-acting antivirals (DAAs) has resulted in sustained virologic response (SVR) rate of >95% in patients with advanced liver disease.[1,2] Sofosbuvir or ledipasvir/sofosbuvir therapy provides better SVR achievement than interferon-based therapy, and is therefore expected to offer more clinical benefits to patients through regression of fibrosis. However, to date, there have been no data to support this.

Hepatitis C virus (HCV) infection can cause progressive liver injury, leading to fibrosis and hepatocellular carcinoma (HCC). The extent of histologic fibrosis is an important marker for defining the staging of the disease, as advanced fibrosis has been considered a high-risk factor for the development of HCC.[3] HCC is the fifth leading cause of mortality in Japan.[4] Chronic infection with HCV is a leading cause of HCC in developed countries including Japan.

The association between DAA-based therapy and occurrence of HCC in patients with cirrhosis, and recurrence of HCC after successful treatment in patients with HCV infection is controversial. Pivotal clinical trials assessing antiviral efficacy of DAAs have not included successfully treated patients with HCC or with active disease. Although there are data suggesting improvement in liver functions following DAA therapy, there is limited and controversial information on the incidence of HCC in patients who received DAA therapy.

The therapeutic effect on fibrosis can be evaluated using noninvasive methods including FibroScan and biomarkers; however, there has been no easy and simple procedure with clear assessment. Mac-2 binding protein (M2BP) has been suggested as a biomarker for liver fibrosis, which undergoes fibrosis-related glycosylation and can be detected within 20 minutes by FastLec-Hepa, a glycan-based immunoassay.[5] This method uniquely evaluates severity of the disease through quantitation of M2BP. A glycoproteomic study identified Mac 2-Binding Protein Glycan Isomer (M2BPGi), and suggested it as a reliable marker for assessment of liver fibrosis in patients with viral hepatitis and other liver diseases.[6] Hence, in this study, we evaluated whether ledipasvir/sofosbuvir or sofosbuvir + ribavirin therapy is associated with regression of liver fibrosis, using M2BPGi as well as other fibrosis markers, in chronic hepatitis C (CHC) patients who achieved SVR after treatment.

2. Methods

2.1. Patients

CHC patients were enrolled in this multicenter, prospective cohort study at 3 centers (Yokohama City University Medical Center, Kanagawa Cancer Center, and Department of Gastroenterology, Yokohama City University Graduate school of Medicine) in Japan during the period of December 2015 to January 2018. Five patients were excluded (2 patients with wrong information at registry and 3 patients did not start therapy).

The HCV therapy consisted of ledipasvir 90 mg/sofosbuvir 400 mg FDC tablet q.d. (genotype 1) and sofosbuvir 400 mg tablet q.d. + 200–1000 mg/day ribavirin (genotype 2) for 12 weeks (Fig. 1). Laboratory data every 2 weeks were excluded if they were >7 days away from the reference date according to the protocol.

2.2. HCV RNA measurement

HCV RNA levels were measured using the commercially available COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan) with the lower detection limit of 1.2 log IU/mL. SVR12 was defined as negative for serum HCV RNA at 12 weeks after the end of treatment.
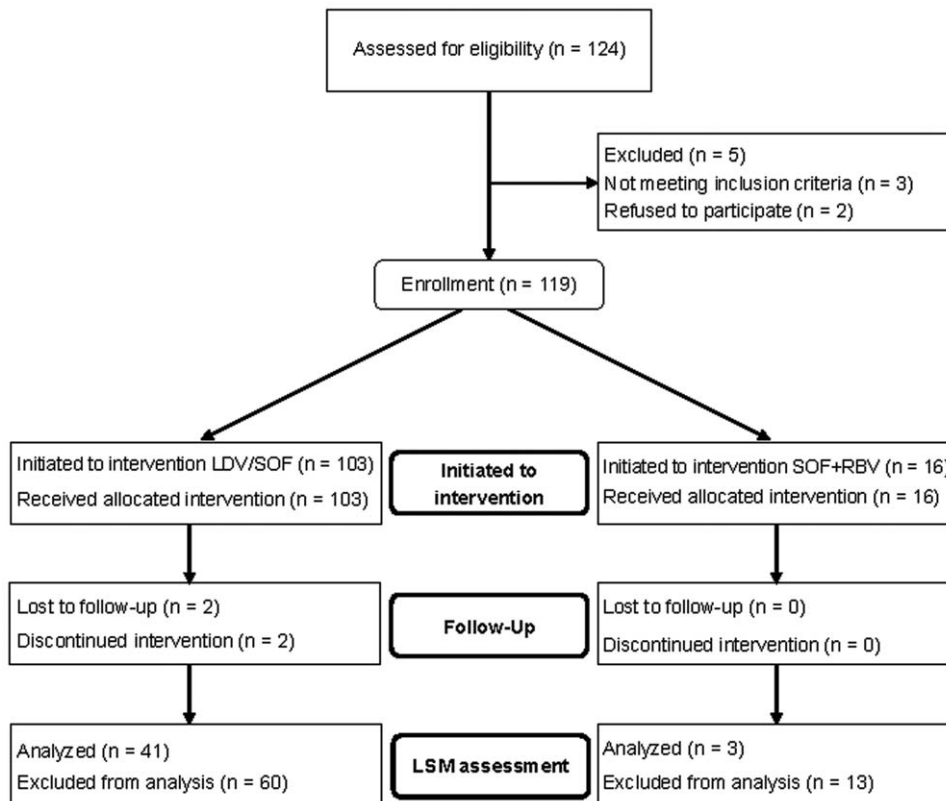

Figure 1. The cohort flowchart.

2.3. Liver stiffness measurement (LSM)

Liver fibrosis was measured and staged by transient elastography (TE) (FibroScan 402; Echosens, Paris, France) according to the manufacturer's instructions using an M probe. FibroScan was performed by physicians who had experience of at least 30 TE procedures. Only results with at least 10 valid measurements, a 60% success rate, an interquartile range of <30% of the median elasticity, and a body mass index (BMI) < 30 kg/m^2 were considered reliable.[7] Fibrosis stages were as follows: stage F0–F1 (LSM ≤ 7.0 kPa), F1–F2 (LSM 7.1–8.5 kPa), F2 (LSM 8.6–9.5 kPa), F3 (LSM 9.6–12.5 kPa), F3–F4 (LSM 12.6–14.5 kPa), F4 (LSM > 14.5).[8] Therefore, ≥12.6 kPa was defined as advanced fibrosis.

2.4. Measurement of Wisteria floribunda agglutinin positive human Mac-2 binding protein

Wisteria floribunda agglutinin-positive (WFA$^+$) human Mac-2 binding protein was measured using the HISCLR M2BPGi assay kit (Sysmex Corporation, Hyogo, Japan), according to the manufacturer's instructions, on a fully automatic immunoanalyzer HISCL 2000i (Sysmex Corporation, Hyogo, Japan). The values of WFA$^+$-M2BP conjugated to WFA expressed as cut-off index (COI) units were measured using the following equation:

$$\text{Cutoff index} = \left(\frac{([M2BPGi]sample - [M2BPGi]NC)}{stretchy = \}false\} > ([M2BPGi]PC - [M2BPGi]NC)} \right)$$

where [M2BPGi] sample is the M2BPGi level in the serum sample, PC is the positive control, and NC is negative control.

2.5. Endpoints

The primary endpoint was change of M2BPGi at 24 weeks after SVR achievement (changes from baseline to 48 weeks in M2BPGi). M2BPGi at other time points and other markers such as FIB-4 index, LSM, and platelet count were also evaluated.

2.6. Statistical analysis

Quantitative variables were summarized as median with range, and categorical variables as frequencies and percentages. Primary endpoint and changes from baseline to 48 weeks in fibrosis markers were summarized as mean ± standard deviation, displayed using the boxplot, and analyzed by the paired t test. These analyses were also conducted in subgroups, FibroScan < 12.6 and FibroScan ≥ 12.6. To account for deviation in time of measurement from the planned schedule, fibrosis markers were displayed using a spaghetti plot. P-values < .05 was considered statistically significant. Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) and R 3.5.3 (R Core Team).

2.7. Sample size

A sample size of 84 patients was required in order to detect the change of M2BPGi at 48 weeks from baseline with expected value of 0.1 and standard deviation of 0.25 (two-sided level of 5% and detection power of 95% with a paired t test).

2.8. Ethical approval

This study was approved by Yokohama City University Ethics Committee (Number: D1510010). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008).

This study has also been registered in the UMIN Clinical Trials Registry as UMIN 000020048. The protocol of this multicenter study was compliant with the Helsinki Declaration and was approved by each participating institution. Written informed consent was obtained from each patient.

3. Results

Out of the 124 screened patients, 119 patients were enrolled in the study and provided treatment for CHC (Fig. 1). Table 1 shows baseline characteristics of the study subjects. Approxi-

Table 1

Baseline characteristics (n = 119).

	All		SOF · LDV group		SOF · RBV group	
	n	Median (range)	n	Median (range)	n	Median (range)
Age	119	67 (26–82)	103	67 (26–82)	16	60.5 (42–77)
Male/Female	119	51 (42.86%)	103	40 (38.83%)	16	11 (68.75%)
		/		/		/
		68 (57.14%)		63 (61.17%)		5 (31.25%)
WBC, /mm^3	110	4595 (2010–8200)	96	4500 (2010–8200)	14	4740 (3130–7010)
AST, IU/L	110	40 (15–230)	96	41 (15–230)	14	36 (21–192)
ALT, IU/L	110	37 (13–191)	96	38.5 (13–191)	14	35 (19–109)
BUN, mg/dL	110	14 (2–29)	96	14 (6–29)	14	13 (2–24)
T-Bil, mg/dL	110	0.7 (0.2–1.8)	96	0.8 (0.2–1.8)	14	0.7 (0.4–1.4)
Alb, g/dL	109	4.3 (2.9–5.2)	95	4.2 (2.9–5.1)	14	4.3 (3.2–5.2)
PT-INR	100	1 (0.9–1.6)	87	1 (0.9–1.6)	13	1 (0.9–1.2)
AFP	104	3.3 (0.9–114.3)	90	3.5 (0.9–114.3)	14	2.5 (1.5–5.3)
AFP-L3	98	0.5 (0.5–22.3)	86	0.5 (0.5–22.3)	12	0.5 (0.5–0.5)
PIVKA-II	107	19 (10–76)	93	19 (10–76)	14	20 (14–31)
M2BPGi	108	1.7 (0.3–20)	96	1.8 (0.3–20)	12	1 (0.4–20)
FIB-4 index	107	2.9 (0.6–19.5)	93	3.1 (0.6–15.8)	14	2.4 (0.9–19.5)
FibroScan	111	10.1 (0.5–54.2)	99	10.2 (0.5–54.2)	12	8.5 (6.1–22.3)

Data are expressed as median (range); AFP = alpha-fetoprotein, ALT = alanine aminotransferase, AST = Aspartate aminotransferase, M2BPGi = Mac-2-binding protein glycosylation isomer, PIVKA-II = protein induced by vitamin K absence/antagonist-II, PT-INR = Prothrombin time-International normalized ratio, WBC = White blood cell.

mately 98% of the genotype-1 patients and all the genotype-2 patients achieved SVR at 12 weeks. Of the 119 patients enrolled, 51 patients (42.9%) were men, and 103 patients (86.6%) had genotype-1 HCV infection. Patients with data loss were excluded from the table.

3.1. DAA therapy significantly regressed liver fibrosis

Forty-four patients achieved SVR and completed investigations for markers of liver fibrosis at baseline and 48 weeks. We observed that changes in M2BPGi (average [SD]: −2.2 [3.4]; median [range]: −0.8 [−16.2, 0.3]; $P < .0001$), hyaluronic acid (average [SD]: −68.5 [143.2]; median [range]: −23 [−463, 40]; $P = .0332$), FIB-4 index (average [SD]: −1.2 [2]; median [range]: − 0.5 [−9, 0.5]; $P < .0001$), LSM (average [SD]: −5.9 [6.4]; median [range]: −4.9 [−26.5, 3.6]; $P < .0001$), and numbers of platelets (average [SD]: 0.9 [3]; median [range]: 0.9 [−10.2, 8.8]; $P = .0114$) were statistically significant at 48 weeks (Table 2). Patients with data loss were excluded from each analysis.

Spaghetti plots depicted in Fig. 2 show the change in markers of liver fibrosis (M2BPGi, FIB-4 index, LSM, and platelet count) from the start of therapy to 48 weeks in these patients. The general trend showed a marginal change in the individual parameters; there was inter-subject as well as intra-subject variability with time in both magnitude and direction of the change.

3.2. DAA therapy was associated with shift in FibroScan class

Figure 3 shows a shift in FibroScan class from the start of therapy to 48 weeks in the patients. Patients with advanced fibrosis were reduced to 15.9% (n = 7) at 48 weeks from 38.6% (n = 17) at baseline. There were 9 patients with no fibrosis at baseline, 25 patients at 48 weeks showed no fibrosis, indicating the effect of DAA therapy on the regression of liver fibrosis.

3.3. DAA therapy reduced fibrosis in patients with advanced fibrosis

There were 17 patients who had advanced fibrosis before the start of therapy. There was a significant decrease in M2BPGi (average [SD]: −3.6 [4.6]; median [range]: −1.8 [−16.2, −0.2]; $P < .0001$), FIB-4 index (average [SD]: −2.3 [2.3]; median [range]: −1.6 [− 9.0]; $P < .0001$), and LSM (average [SD]: −10.8 [7.6]; median [range]: −9.3 [−26.5, 3.6]; $P < .0001$), and an increase in platelet count (average [SD]: 2.1 [2.2]; median [range]: 1.5 [−2.4, 7.5]; $P = .0006$) at 48 weeks compared with baseline (Fig. 4).

Patients with an LS of 12.6 kPa or higher at the start of treatment and no LSM improvement of ≥30% after 48 weeks were defined as an insufficient fibrosis improvement group. As a result, only 2 female patients were extracted.

3.4. Adverse events (AEs)

Overall, AEs were observed during the treatment period 46.2% (55/119) in all patients, 43.7% (45/103) in SOF/LDV group, 62.5% (10/16) in SOF/RBV group: anemia, 17 (14.2%); eruption, 11 (9.2%); fatigue, 8 (6.7%); headache, 8 (6.7%); ALT elevation, 5 (4.2%); AST elevation, 3 (2.5%); nasopharyngitis, 3 (2.5%) total bilirubin elevation, 2 (1.7%); and diarrhea, 1 (0.8%) (Table 3). Anemia, an AE of RBV, was frequently seen in the SOF/RBV group. AEs leading to death or treatment discontinuation were not found.

4. Discussion

The goals of HCV therapy are to achieve SVR following treatment completion, prevention or reversal of development of liver fibrosis and extrahepatic manifestations of HCV. Interferon-based therapy has been found to be associated with long-term improvement in liver fibrosis associated with SVR in previous reports.[9,10] Long-term clinical outcomes in patients treated with DAAs are currently under investigation.

The present study showed SVR achievement with DAAs was associated with improvement in liver stiffness at 48 weeks suggesting improvement in liver fibrosis in CHC patients. Our study adds to the current literature showing reduced fibrosis following DAA therapy.[11–13] Necroinflammatory activity in addition to the extent of liver fibrosis also influences liver stiffness.[4,14] Kobayashi et al[15] showed a significant decrease in LSM at SVR 24 following DAA therapy; however, LSM at SVR 48 was not statistically significant compared with LSM at SVR 24. In the present study, we also observed a decrease in the number of patients with advanced fibrosis up to 24 weeks compared with patients with advanced fibrosis at the start of therapy showing a shift in FibroScan class following DAA therapy. Even at 48 weeks, the number of patients with advanced fibrosis was reduced compared with baseline. However, we could not carry out a statistical comparison of these groups due to unequal distribution of patients at different time points. After initiation of DAA therapy, inflammation may be resolved until the end of therapy, showing mild improvement in liver stiffness. Improvement in fibrosis at 48 weeks may involve resolution of inflammation as well as improvement in fibrosis. Further, insufficient improvement in fibrosis was observed in 2 female

Table 2

Comparison of markers of liver fibrosis.

	Baseline		48 weeks		Change		
	n	**Mean (SD)**	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**P value**
M2BPGi	44	3.7 (4.4)	44	1.5 (1.4)	44	-2.2 (3.4)	<.0001
Hyaluronic acid, ng/mL	12	255.3 (360.1)	10	120.9 (193.4)	10	-68.5 (143.2)	.0332
IV type collagen, ng/mL	11	162.3 (60.9)	9	138.1 (31.2)	9	−13 (23.1)	.1641
FIB-4 index	43	4.2 (3.6)	43	2.9 (1.9)	43	−1.2 (2)	<.0001
LSM	44	14.1 (9.7)	44	8.2 (5.1)	44	−5.9 (6.4)	<.0001
PLT (×10^4/mm^3)	44	15.2 (5.3)	44	16.1 (4.6)	44	0.9 (3)	.0114

Data are expressed as mean (SD); LSM = liver stiffness measurement, M2BPGi = Mac-2-binding protein glycosylation isomer, PLT = platelet.
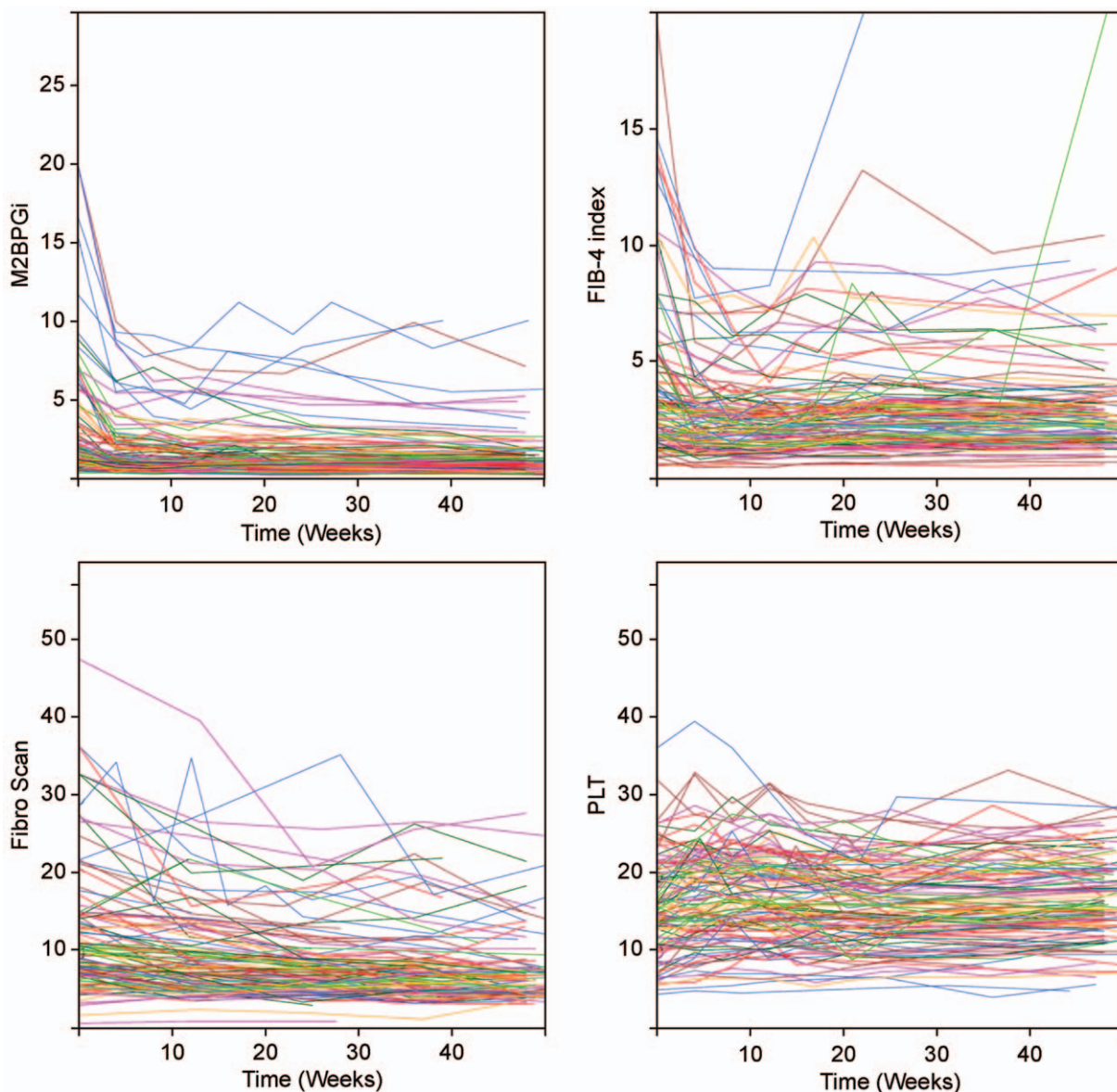
Figure 2. Spaghetti plot showing the individual change in M2BPGi, FIB-4 index, LSM, and platelet count (n=44). Each colored line corresponds to the change in fibrosis marker from the start of therapy to 48 weeks. LSM=liver stiffness measurement; M2BPGi=Mac-2-binding protein glycosylation isomer.

patients with advanced fibrosis. One case was found to have been treated with steroids for autoimmune hepatitis 7 years ago with high antinuclear antibody levels (320×). The other woman has no hematological features but she was treated for hepatocellular carcinoma 96 weeks after the start of treatment, and it is possible that the cancer prevented the improvement of fibrosis.

Liver biopsy remains the gold standard for the assessment of liver fibrosis. However, due to its invasive nature and difficulty, especially in patients unable to visit for follow-up, several other methods have been developed. Our study observed a significant change in hyaluronic acid, FIB-4 index, and platelet count at 48 weeks with a decrease in fibrosis. However, reliability of these markers is of concern due to sensitivity and specificity. FibroScan has been found to be useful for the assessment of fibrosis stage of patients with chronic hepatitis B and C.[16,17] However, cost of the instrument and restricted use in obese patients may limit the
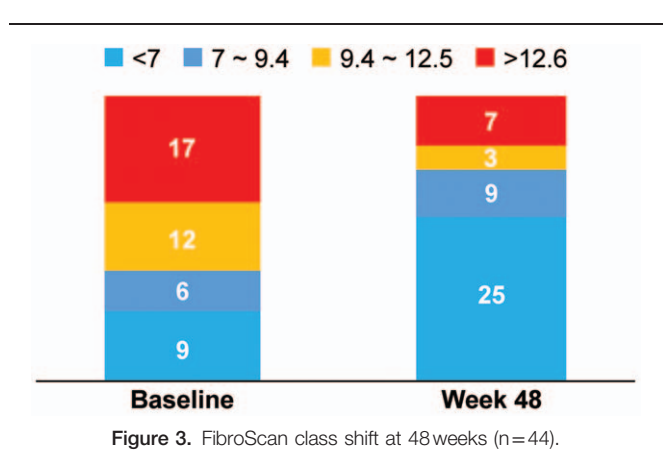

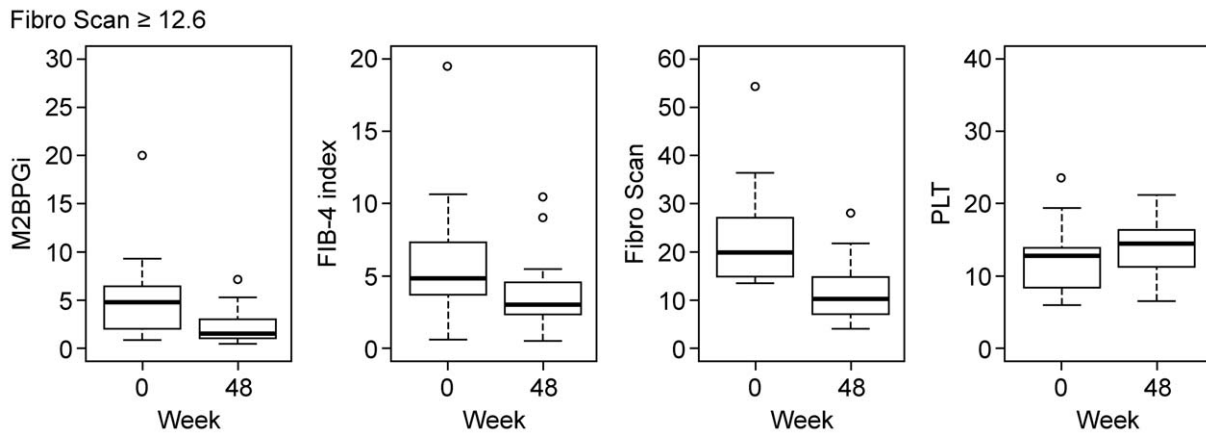**Figure 3.** FibroScan class shift at 48 weeks (n=44).

Figure 4. Comparison of fibrosis markers in patients with LSM > 12.6 kPa (n = 17). LSM = liver stiffness measurement.

use of FibroScan in the assessment of fibrosis stage. WFA+-M2BP has been identified as a unique marker of liver fibrosis. Our study observed there was a significant decrease in M2BPGi at 48 weeks compared with baseline. We also found a significant decrease in M2BPGi at 48 weeks in patients with advanced fibrosis. Uojima et al[18] showed that WFA+-M2BP positively correlated with Child-Pugh score. Previous studies have revealed a correlation of WFA+-M2BP with the stage of liver fibrosis as well as the extent of liver inflammation activity.[19–21]

Therefore, it would appear that elevated WFA+-M2BP, accompanied by suppression of liver inflammation following DAA therapy may result in a positive clinical outcome in CHC patients. Nagata et al[22] also showed a decrease in M2BPGi levels in patients achieving SVR following completion of therapy; however, the decrease was not statistically significant compared with patients who did not achieve SVR. M2BPGi has also been found to be useful in the assessment of fibrosis in other liver diseases. Wei et al[23] observed a positive correlation of M2BPGi with the progression of fibrosis in patients with chronic hepatitis B.

Recently, Xu et al[24] showed an increase in M2BPGi levels in patients with hepatitis C with fibrosis progression. M2BPGi was found to be an independent factor associated with fibrosis in CHC patients. Toshima et al[25] compared WFA+-M2BP levels in patients with liver fibrosis and observed cut-off indices of 1.62, 1.82, 3.02, 3.32, and 3.67 of WFA+-M2BP in liver fibrosis grades

F0, F1, F2, F3, and F4, respectively. Compared with other noninvasive methods, M2BPGi measurement is a simple, inexpensive, reflection of fibrosis severity; hence, M2BPGi may be an alternative noninvasive serum marker for assessment of liver fibrosis.

In this study, Sofosbuvir-based regimen improved non-invasive FibroScan measurements, while also improving serum markers Fib-4, M2BPGi, and platelets. However, since there are still few facilities with FibroScan in Japan, it is also important to measure serum markers without FibroScan in order to follow up the improvement of liver stiffness. It has been clarified that M2BPGi is simple and has less shedding than LSM, so it may be recommended as one of the simple approximation methods. Also, even if the liver stiffness improves, the risk of carcinogenesis does not disappear,[26] so regular diagnostic imaging follow-up after HCV elimination is necessary.

Our study has several limitations. First, the study subjects participated on an outpatient basis; therefore, the follow-up data at every 2-week interval could not be collected. Hence, a lower number of patients were studied in the final analysis. It also restricted our analysis of the subgroups. Second, we measured the stage of fibrosis using LSM values without liver biopsy, which may lead to bias. Third, since M2BPGi is affected by inflammation, it will be necessary to compare with the newly developed fibrosis marker that are less susceptible to inflammation such as autotaxin as recently reported[27] in the future.

Table 3

Adverse events (n = 119).

	All		SOF · LDV group		SOF · RBV group	
	n	%	n	%	n	%
Anemia	17	14.3	12	11.7	5	31.3
Eruption	11	9.2	9	8.7	2	12.5
Fatigue	8	6.7	6	5.8	2	12.5
Headache	8	6.7	8	8.7	0	0
ALT elevation	5	4.2	5	4.9	0	0
AST elevation	3	2.5	3	2.9	0	0
Nasopharyngitis	3	2.5	2	1.9	1	6.3
Total bilirubin elevation	2	1.7	1	0.9	1	6.3
Diarrhea	1	0.8	1	0.9	0	0

ALT = alanine aminotransferase, AST = aspartate aminotransferase.

In conclusion, DAA therapy significantly reduced liver fibrosis. M2BPGi correlated well with the LSM at 48 weeks following start of therapy, supporting the comprehensive and sustainable benefit of HCV therapy in reducing liver fibrosis.

Acknowledgments

Author contributions

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