







Liver fibrosis improvement assessed by magnetic resonance elastography and Mac-2-binding protein glycosylation isomer in patients with hepatitis C virus infection receiving direct-acting antivirals

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Abstract

Aim: Fibrosis regression has been observed in patients with chronic hepatitis C virus (HCV) infection treated with direct-acting antivirals. This study was aimed at evaluating dynamic changes of serum Mac-2-binding protein glycosylation isomer (M2BPGi) in patients with HCV genotype 1 receiving elbasvir/grazoprevir.

Methods: M2BPGi were serially measured at baseline, during and after therapy. Its diagnostic performance at baseline and sustained virological response at 24 weeks after treatment (SVR24) were compared with transient elastography (TE) and the aspartate aminotransferase/platelet ratio index (APRI) using magnetic resonance elastography (MRE) as a reference.

Results: Overall, 60 HCV mono-infected and 36 HCV/HIV co-infected patients were included with SVR24 rates of 93.3% and 97.2%, respectively. At baseline, TE, M2BPGi and APRI were correlated with MRE ($r = 0.788$, $r = 0.703$ and $r = 0.564$, respectively, $p < 0.001$). The area under the receiver operator characteristics curves for TE, M2BPGi and APRI in differentiating significant fibrosis were 0.88 (95% confidence interval; 0.81–0.95, $p < 0.001$), 0.86 (0.79–0.94, $p < 0.001$) and 0.74 (0.64–0.83, $p < 0.001$), respectively. The corresponding figures for cirrhosis were 0.95 (0.90–1.00, $p < 0.001$), 0.96 (0.92–1.00, $p < 0.001$) and 0.88 (0.79–0.97, $p < 0.001$), respectively. Compared with baseline, all fibrosis markers significantly declined after achieving SVR24. The correlations of TE, M2BPGi and APRI with MRE at time of SVR24 were $r = 0.587$ ($p < 0.001$), $r = 0.457$ ($p < 0.001$) and $r = 0.293$ ($p = 0.004$), respectively. In multivariate analysis, high baseline alanine aminotransferase level, HCV mono-infection and advanced fibrosis were factors associated with M2BPGi reduction.

Abbreviations: ALT, alanine aminotransferase; APRI, AST/platelet ratio index; AST, aspartate aminotransferase; AUROCs, area under the ROC curves; CI, confidence interval; COI, cutoff index; DAAs, direct-acting antivirals; EBR/GZR, elbasvir/grazoprevir; EOT, end of treatment; GT1, HCV genotype 1; HCC, hepatocellular carcinoma; HCV, hepatitis C virus infection; HIV, human immunodeficiency virus; MRE, magnetic resonance elastography; M2BPGi, Mac-2 binding protein glycosylation isomer; PEG-IFN, pegylated interferon; RBV, ribavirin; ROC, receiver operator characteristics; SVR, sustained virological response; SVR24, sustained virological response at 24 weeks after treatment; TE, transient elastography.

Conclusions: HCV eradication is associated with liver fibrosis improvement. M2BPGi has a better performance than APRI in monitoring liver fibrosis in patients treated with direct-acting antivirals. This marker is applicable in resource-limited settings where imaging-based modalities are not widely accessible.

KEYWORDS

DAA, fibrosis, HCV, HIV, M2BPGi, MR elastography

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a worldwide public health concern, with an estimated 180 million people chronically infected with the virus.¹ Currently, the prevalence of chronic HCV infection in Thailand is approximately 2%.² Based on the natural history of chronic HCV infection, it is estimated that 10%–20% of patients will develop cirrhosis and these cirrhotic patients will develop hepatocellular carcinoma (HCC) at an annual rate of 2%–7%.³ The risk of cirrhosis is increased in individuals with older age at infection, heavy alcohol intake, concomitant obesity and co-infection with human immunodeficiency virus (HIV).¹ HCV/HIV co-infected patients are at three-fold greater risk for progression to cirrhosis or liver decompensation and at 10-fold greater risk for liver-related mortality compared with HCV mono-infected patients.⁴ Our recent cross-sectional data showed that advanced liver fibrosis was more commonly found in HCV/HIV co-infected individuals than in the mono-infected group (41% vs. 25%).⁵ Previously, a standard of care for chronic HCV infection was the combination of pegylated interferon (PEG-IFN) and ribavirin (RBV). However, the response rate of this regimen was poor, particularly among HCV/HIV co-infected patients with genotype 1 (GT1) and advanced fibrosis.⁶ With the introduction of direct-acting antivirals (DAAs), HCV treatment has been revolutionized, as HCV mono- and HCV/HIV co-infected individuals could achieve sustained virological response (SVR) rates >90%. It has been shown that SVR is associated with a very low risk of HCV reactivation and a reduced risk of the progression of cirrhosis and HCC development.⁷

An accurate assessment of liver fibrosis stages is necessary for the management of patients with chronic HCV infection. Although liver biopsy has been considered a gold standard in assessing liver histopathology, this procedure has some limitations because of its potential of life-threatening complications and sampling errors.⁸ In addition, the longitudinal evaluation of liver fibrosis is restricted due to the invasiveness of the procedure. An alternative non-invasive tool to measure the severity of liver fibrosis is transient elastography (TE), which has been validated in both HCV mono-infected and HIV/HCV co-infected patients.⁹ Besides ultrasound-based techniques, magnetic resonance elastography (MRE) has been shown to be a highly accurate, non-invasive technique for the diagnosis and staging of liver fibrosis, regardless of inflammation and etiology of liver diseases.¹⁰ Compared with TE, MRE provides a better diagnostic

performance in the identification of liver fibrosis and cirrhosis in patients with chronic viral hepatitis or non-alcoholic fatty liver disease.^{11–13} In addition, patients with chronic HCV infection who achieved SVR after DAA therapy showed subsequent improvement of liver stiffness based on MRE.¹⁴ With the advantages of its accuracy and reproducibility, MRE is now considered as the most reliable non-invasive technique for the assessment of liver fibrosis.^{15,16}

Besides imaging-based techniques, several serum fibrosis markers have been applied to evaluate the extent of liver fibrosis. Recently, the serum Mac-2-binding protein glycosylation isomer (M2BPGi) has emerged as a novel non-invasive marker for liver fibrosis, particularly in patients with chronic HCV infection.^{17,18} In addition, M2BPGi can also be used to predict HCV treatment outcome, including regimens with PEG-IFN- or DAA-based therapies.^{19–21} Despite these data, there are limited studies that directly examine the usefulness of serial M2BPGi in monitoring fibrosis regression following DAA treatment. Therefore, this study was aimed at investigating the clinical utility of serum M2BPGi kinetics during elbasvir/grazoprevir (EBR/GZR) therapy in HCV mono-infected and HIV/HCV co-infected patients. Moreover, the diagnostic performance of serum M2BPGi at baseline and after therapy was compared with TE and aspartate aminotransferase/platelet ratio index (APRI) score by using MRE as a reference.

MATERIALS AND METHODS

Patients

PATIENTS TREATED WITH EBR/GZR and recruited via clinicaltrials.gov (NCT03037151) at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand between August 2018 and June 2019, were enrolled in this study. Inclusion criteria for EBR/GZR therapy were patients with anti-HCV positive levels for at least 6 months, detectable HCV RNA levels >10 000 IU/ml and infected with HCV GT1, irrespective of liver fibrosis. For patients with HCV/HIV co-infection, each individual received antiretroviral therapy with undetectable plasma HIV-RNA levels. Exclusion criteria were hepatitis B virus co-infection, previous treatment with DAAs, clinical evidence suggestive of decompensated cirrhosis or HCC by imaging studies. Treatment-naïve patients assigned to treatment with EBR/GZR for 12 weeks, while treatment-experienced patients, including

null responders, partial responders or post-treatment relapsers following PEG-IFN/RBV, were treated with EBR/GZR plus weight-based RBV for 16 weeks. Patients were followed up to assess SVR (defined by HCV RNA level <12 IU/ml) at weeks 12 and 24 after therapy cessation (SVR12 and SVR24, respectively).

The study protocol, conducted in compliance with the Helsinki Declaration and Good Clinical Practice guidelines, was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Written informed consent was obtained from all patients for the use of their clinical data and sample specimens.

Laboratory assays

Routine laboratory tests such as complete blood count (CBC) and biochemical tests (e.g., aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were determined at the central laboratory of the King Chulalongkorn Memorial Hospital. APRI score was calculated by the formula (AST/upper limit of normal considered as 40 IU/L)/platelet count ($10^9/L$) \times 100.²²

HCV RNA quantification was performed using real-time quantitative reverse-transcription polymerase chain reaction (Abbott Molecular Inc.) in accordance with the manufacturer's instructions. The lower detection limit of the assay was <12 IU/ml. HCV genotypes were determined by nucleotide sequencing of the core and NS5B regions as described previously.⁵ Plasma HIV RNA level is assessed using the Abbott RealTime HIV-1 Assay (Abbott Molecular Inc.).

Serum samples for M2BPGi testing were collected from the participants and stored at -70°C until analysis. Serial serum M2BPGi measurements at baseline, week 4 of treatment, end of treatment (EOT), 12 weeks after EOT (SVR12) and 24 weeks after EOT (SVR24) were assessed by lectin-antibody sandwich immunoassay using a fully automatic immune-analyzer HISCL-2000i (Sysmex). The measured values of M2BPGi were calculated by the following equation: cutoff index (COI) = $([\text{M2BPGi}]_{\text{sample}} - [\text{M2BPGi}]_{\text{negative controls}}) / ([\text{M2BPGi}]_{\text{positive controls}} - [\text{M2BPGi}]_{\text{negative control}})$.²³

Transient elastography

Liver stiffness was measured at baseline and SVR24 using TE (Echosens) with M-probe and XL-probe as appropriate. The procedure was based on at least 10 validated measurements with a success rate >60% and interquartile range <30%. Results were recorded in kilopascals (kPa) as the median value of all measurements.²⁴

Magnetic resonance elastography

MRE was performed within 2 weeks before starting DAAs and within 2 weeks of SVR24, using the MR imaging system Philips Ingenia at

3.0T (Philips Healthcare) according to the method described previously²⁵ and the examinations were performed by the hardware and software leased from Resoundant. Briefly, at the beginning of each examination, a passive driver was placed on the patient's right upper abdomen to transmit low-amplitude 60-Hz vibrations to the liver for the generation of shear waves using a 2D GRE MRE pulse sequence. Liver stiffness was measured in three slices, and an average value expressed in kPa was obtained. Based on a previous study in patients with chronic HCV infection, liver stiffness cutoff values for significant fibrosis ($\geq\text{F2}$), advanced fibrosis ($\geq\text{F3}$) and cirrhosis (F4) were 3.2, 4.0 and 4.6 kPa, respectively.²⁶

Statistical analyses

Data were expressed as percentages or mean \pm standard deviation. Comparisons between groups were analyzed by the χ^2 or Fisher's exact test for categorical variables and by two-sample *t*-tests for continuous variables as appropriate. Changes in liver stiffness and serum fibrosis markers post-treatment were compared with pre-treatment levels by paired *t*-tests. The diagnostic performances of TE, M2BPGi and APRI were evaluated by calculation of the receiver operator characteristics (ROC) curves using MRE as the reference. Correlations between each parameter were tested by the Spearman's rank test. The logistic regression analysis was performed to evaluate parameters associated with serum M2BPGi reduction. For regression analysis, the median value of each continuous variable was used as the cutoff point. $p < 0.05$ was designated as a statistical significance. Statistical analyses were performed by the IBM SPSS software version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline clinical characteristics and treatment outcome

Initially, 101 patients with HCV GT1, who treated with EBR/GZR were recruited from clinical trial (NCT03037151). However, three patients did not perform MRE at baseline and post-treatment due to claustrophobia and another two patients did not perform MRE after completing the therapy. Apart from these five patients, 96 patients with complete clinical data and liver stiffness measurement were included in this study. Baseline clinical characteristics regarding HIV status are summarized in Table 1. The patients consisted of 74 (77.1%) men and 22 (22.9%) women with a mean age of 47.0 ± 10.8 years. Among these patients, there were 60 (62.5%) individuals with HCV mono-infection and 36 (37.5%) with HCV/HIV co-infection. Compared with the co-infected group, patients with HCV mono-infection had significantly higher average age, body mass index (BMI), APRI score and the distribution of HCV GT1b. There was no significant difference between the two groups in terms of biochemical parameters, previous PEG-IFN/RBV therapy, serum HCV

TABLE 1 Baseline characteristics of patients in this study

Baseline characteristics	HCV mono-infection (N = 60)	HCV/human immunodeficiency virus co-infection (N = 36)	p
Age (years)	50.5 ± 10.7	41.4 ± 8.5	<0.001*
Sex (male)	44 (73.3)	30 (63.3)	0.321
Body mass index (kg/m ²)	24.2 ± 3.6	22.3 ± 3.5	0.013*
Aspartate aminotransferase (IU/L)	52.3 ± 37.8	44.0 ± 21.5	0.229
Alanine aminotransferase (IU/L)	62.5 ± 52.1	61.9 ± 61.2	0.959
Platelet count (10 ⁹ /L)	199.3 ± 76.0	209.0 ± 68.0	0.530
Log ₁₀ HCV RNA (IU/ml)	6.2 ± 0.7	6.2 ± 0.9	0.844
HCV genotype			0.005*
GT1a	32 (53.3)	31 (86.1)	
GT1b	24 (40.0)	4 (11.1)	
GT1: not subtyped	4 (6.7)	1 (2.8)	
APRI score	1.0 ± 1.5	0.6 ± 0.3	0.032*
Mac-2 binding protein glycosylation isomer (cutoff index)	2.2 ± 2.0	1.9 ± 2.2	0.508
Transient elastography (kPa)	12.1 ± 10.5	11.3 ± 12.8	0.725
Magnetic resonance elastography (kPa)	3.4 ± 1.1	3.0 ± 1.1	0.080
Previous pegylated interferon/ribavirin therapy	13 (21.7)	6 (16.7)	0.607

Note: Data expressed as mean ± SD or n (%).

Abbreviations: APRI, alanine aminotransferase/platelet ratio index; GT1, genotype 1; HCV, hepatitis C virus.

**p* < 0.05.

RNA level, serum M2MPGi and elastography assessed by TE and MRE.

Regarding treatment outcome, SVR12 rate of the cohort was 97.9% (94 of 96). Of them, three patients experienced virological relapse at week 24 of follow-up and thus overall SVR24 was 94.8% (91 of 96). The HCV mono-infected and co-infected groups achieved comparable SVR12 (98.3% [59 of 60] vs. 97.2% [35 of 36], *p* = 1.000) and SVR24 (93.3% [56 of 60] vs. 97.2% [35 of 36], *p* = 0.647). Moreover, treatment-naïve and treatment-experience patients achieved similar SVR12 (97.4% [75 of 77] vs. 100% [19 of 19], *p* = 1.000) and SVR24 (93.5% [72 of 77] vs. 100% [19 of 19], *p* = 0.579).

Relationship between baseline Mac-2-binding protein glycosylation isomer levels and other clinical parameters

The relationship between serum M2BPGi levels and clinical parameters at baseline was examined. A positive correlation was found between M2BPGi and age (*r* = 0.460, *p* < 0.001), AST (*r* = 0.543, *p* < 0.001) and ALT (*r* = 0.293, *p* = 0.004). A negative correlation was found between M2BPGi and platelet counts (*r* = -0.368, *p* < 0.001). There was no correlation between M2BPGi and other clinical parameters (sex, BMI and HCV RNA level).

Among fibrosis markers at baseline, M2BPGi level was strongly correlated with MRE (*r* = 0.702, *p* < 0.001) with a moderate correlation with TE (*r* = 0.668, *p* < 0.001) and APRI (*r* = 0.527, *p* < 0.001). APRI were moderately correlated with MRE (*r* = 0.476, *p* < 0.001) and TE (*r* = 0.475, *p* < 0.001). Moreover, there was a strong correlation between TE and MRE (*r* = 0.759, *p* < 0.001; Figure 1a-c).

Diagnostic performance of fibrosis markers at baseline

Using MRE as the reference methodology, the diagnostic performance of TE, M2BPGi and APRI at baseline were calculated by the area under the ROC curves (AUROCs; Figure 2). Based on MRE, AUROCs for TE in distinguishing significant fibrosis (≥F2), advanced fibrosis (≥F3) and cirrhosis (F4) were 0.88 (95% confidence interval: 0.81–0.95, *p* < 0.001), 0.94 (0.89–0.99, *p* < 0.001) and 0.95 (0.90–1.00, *p* < 0.001), respectively. The corresponding figures for M2BPGi were 0.86 (0.79–0.94, *p* < 0.001), 0.93 (0.87–0.98, *p* < 0.001) and 0.96 (0.92–1.00, *p* < 0.001), respectively. For APRI, the corresponding values were 0.73 (0.64–0.83, *p* < 0.001), 0.80 (0.70–0.90, *p* < 0.001) and 0.88 (0.78–0.97, *p* < 0.001), respectively.

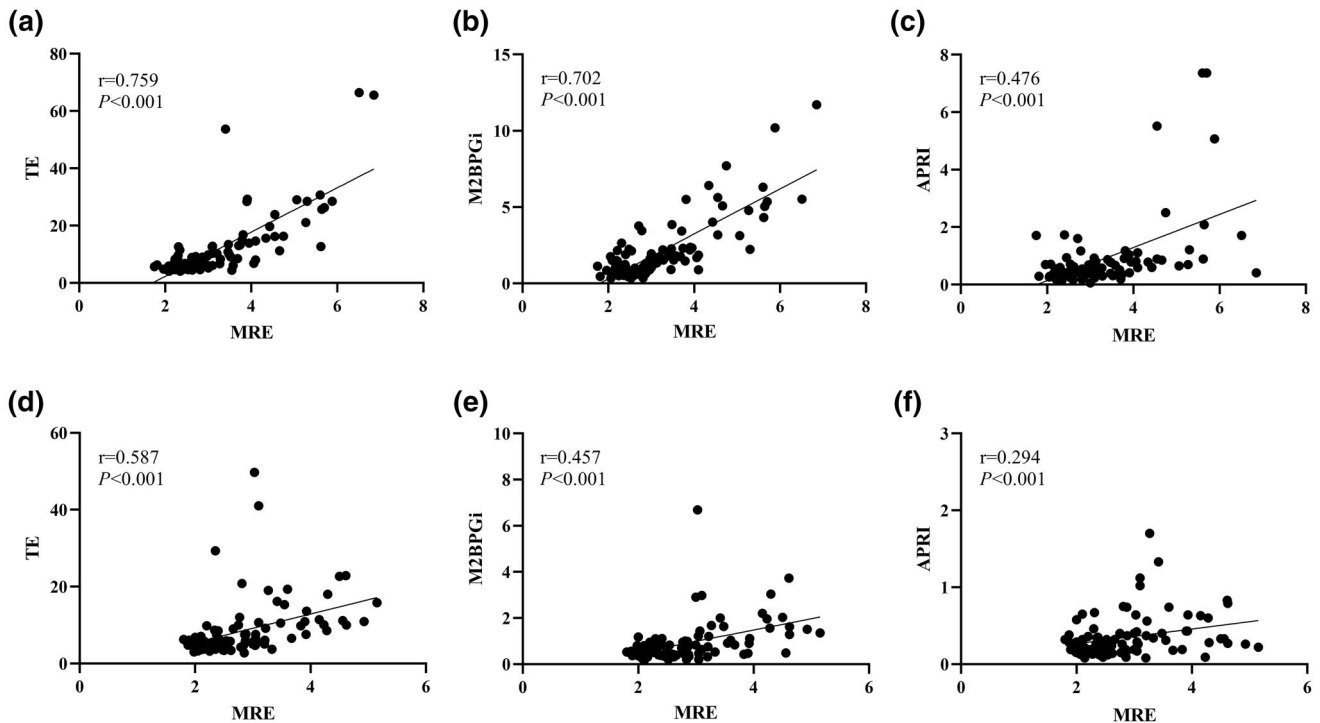


FIGURE 1 Correlation of fibrosis markers with magnetic resonance elastography (MRE): (a-c) at baseline and (d-f) sustained virological response at 24 weeks after treatment. APRI, aspartate aminotransferase/platelet ratio index; M2BPGi, Mac-2 binding protein glycosylation isomer; TE, transient elastography

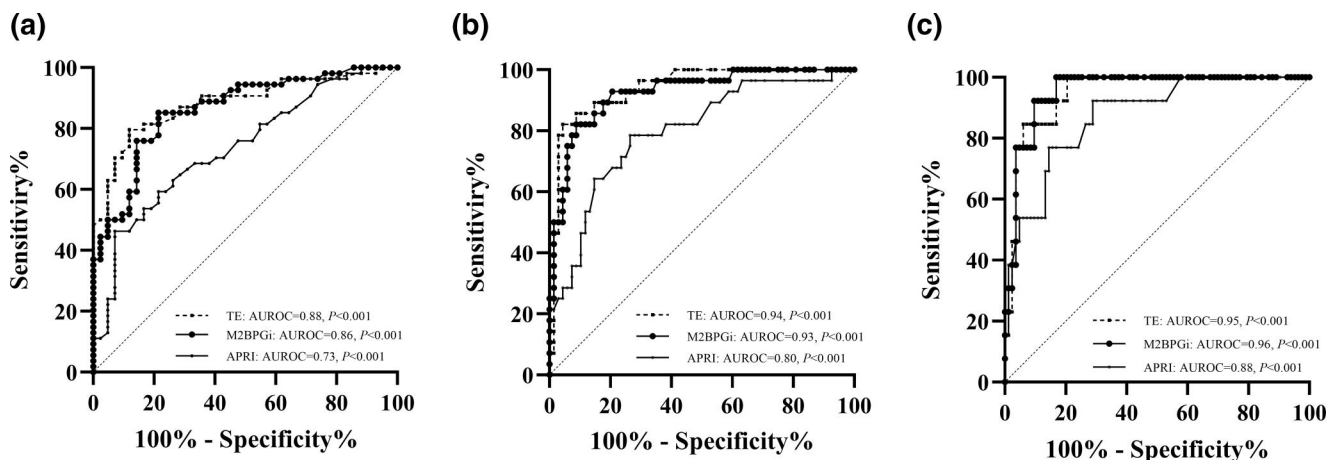


FIGURE 2 AUROC of fibrosis markers using magnetic resonance elastography as the reference (a) F01 vs. F234, (b) F012 vs. F34, and (c) F0123 vs. F4. APRI, aspartate aminotransferase/platelet ratio index; AUROC, area under the receiver operator curves; M2BPGi, Mac-2 binding protein glycosylation isomer; TE, transient elastography

Changes and correlations of fibrosis markers at sustained virological response 24 weeks after treatment

Overall, all fibrosis markers were significantly changed from baseline to the time of SVR24. Specifically, liver stiffness evaluated by MRE decreased significantly from baseline to SVR24 (3.2 ± 1.1 vs. 2.8 ± 0.8 kPa, $p < 0.001$). The corresponding figures for liver stiffness measurement using TE were 11.8 ± 11.3 versus 8.5 ± 7.4 kPa

($p < 0.001$). Regarding serum fibrosis markers, the mean levels of M2BPGi at baseline and SVR24 were 2.1 ± 2.1 and 0.9 ± 0.9 COI, respectively ($p < 0.001$), while the corresponding values for APRI were 0.8 ± 1.2 and 0.3 ± 0.3 , respectively ($p < 0.001$).

The correlations between various fibrosis markers at SVR24 were also determined. M2BPGi level at this time point was moderately correlated with MRE ($r = 0.457$, $p < 0.001$), TE ($r = 0.549$, $p < 0.001$) and APRI ($r = 0.438$, $p < 0.001$). The APRI was weakly correlated with MRE ($r = 0.294$, $p = 0.004$) and had moderate

correlation with TE ($r = 0.441$, $p < 0.001$). In addition, there was a moderate correlation between TE and MRE ($r = 0.587$, $p < 0.001$; Figure 1d-f).

Figure 3 demonstrates the changes of fibrosis markers from baseline to SVR24 in relation to treatment response. Liver stiffness values measured by MRE decreased significantly from baseline to SVR24 in responders (3.3 ± 1.1 vs. 2.8 ± 0.8 kPa, $p < 0.001$) but the significance was not in non-responders (2.7 ± 0.6 vs. 2.5 ± 0.6 kPa, $p = 0.433$) (Figure 3a). For TE measurement, the corresponding figures in responders were 12.1 ± 11.6 versus 8.7 ± 7.5 kPa ($p < 0.001$), while in non-responders were 7.1 ± 2.4 versus 5.6 ± 2.4 kPa ($p = 0.021$; Figure 3b). Regarding M2BPGi, the levels at baseline and SVR24 in responders were 2.2 ± 2.1 versus 0.9 ± 0.9 COI ($p < 0.001$), while in non-responders were 1.3 ± 1.2 versus 1.2 ± 1.0 ($p = 0.480$; Figure 3c). Among responders, there were three patients whose M2BPGi levels increased significantly at SVR24 compared with their baseline levels. Clinical characteristics of these patients are shown in Table S1. For APRI, the levels at baseline and

SVR24 in responders were 0.8 ± 1.3 versus 0.3 ± 0.3 ($p < 0.001$), while in non-responders were 0.8 ± 0.6 versus 0.3 ± 0.2 ($p = 0.134$; Figure 3d).

Dynamic changes of Mac-2-binding protein glycosylation isomer according to treatment response

Details of serum M2BPGi decline during and after HCV therapy in relation to treatment response are shown in Figure 4. As mentioned, serum M2BPGi levels gradually declined during and after therapy in patients achieving SVR24 but not in patients without SVR. In the responders, mean M2BPGi at baseline, weeks 4, EOT, SVR12 and SVR24 were as follows: 2.2 ± 2.1 , 1.4 ± 1.5 , 1.2 ± 1.3 , 0.9 ± 1.0 and 0.9 ± 0.9 COI, respectively (all $p < 0.001$ compared with baseline). The corresponding figures for non-responders were 1.3 ± 1.2 , 1.0 ± 0.8 , 0.9 ± 0.9 , 1.0 ± 0.9 and 1.2 ± 1.0 COI, respectively (all $p > 0.05$ compared with baseline).

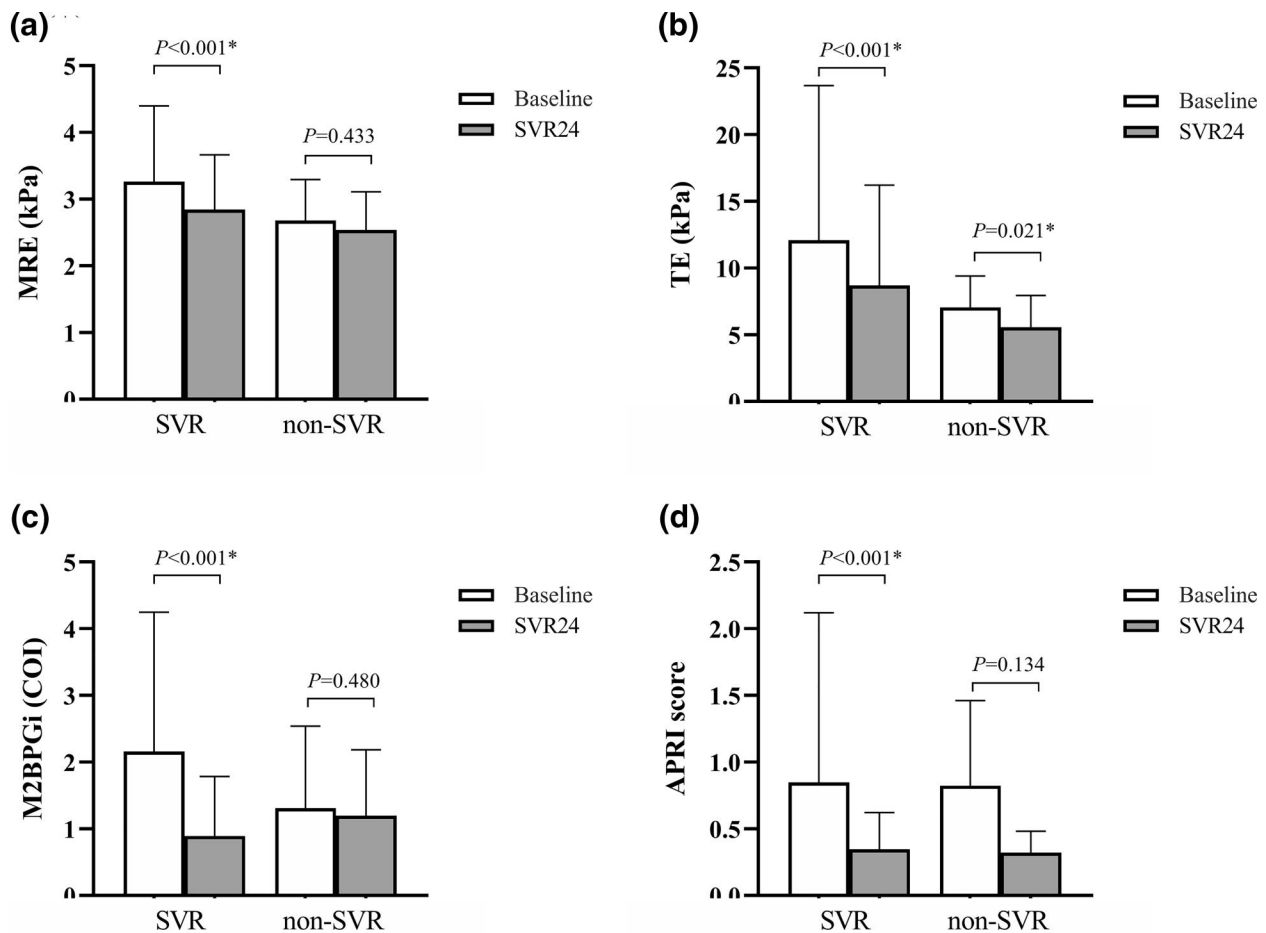


FIGURE 3 Changes of fibrosis markers from baseline to sustained virological response at 24 weeks after treatment (SVR24) according to treatment response: (a) magnetic resonance elastography (MRE), (b) transient elastography (TE), (c) Mac-2 binding protein glycosylation isomer (M2BPGi), (d) aspartate aminotransferase/platelet ratio index (APRI). COI, cutoff index

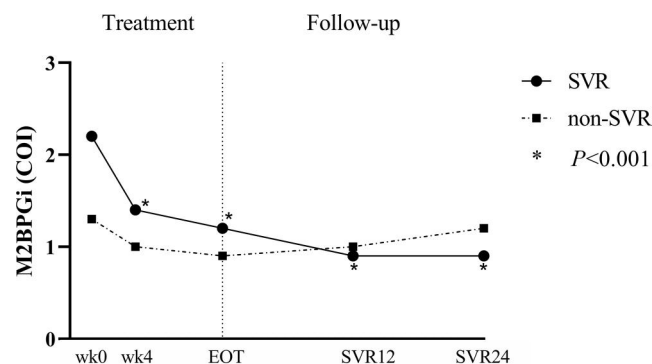


FIGURE 4 Kinetics of Mac-2 binding protein glycosylation isomer (M2BPGi) levels during and after therapy according to treatment response. COI, cutoff index; EOT, end of treatment; SVR12, sustained virological response at 12 weeks after treatment; SVR24, sustained virological response at 24 weeks after treatment; wk, week

Relationship between Mac-2-binding protein glycosylation isomer levels and other clinical parameters at sustained virological response 24 weeks after treatment

The relationship between serum M2BPGi levels and clinical parameters at SVR24 was also analyzed. Again, positive correlation was observed between M2BPGi and AST ($r = 0.462$, $p < 0.001$) and ALT ($r = 0.391$, $p < 0.001$). A negative correlation was detected between M2BPGi and platelet counts ($r = -0.343$, $p = 0.001$). However, there was no correlation between M2BPGi and other clinical parameters, including HCV RNA level.

Factors associated with decline of Mac-2-binding protein glycosylation isomer levels after hepatitis C virus therapy

Univariate and multivariate analyses were calculated to identify baseline factors that were associated with decline serum M2BPGi levels (>50% from baseline). These factors included age, sex, BMI, HIV status, previous PEG-IFN/RBV treatment, AST, ALT, platelet counts, HCV sub-genotype, HCV RNA level and liver stiffness staging assessed by MRE. In univariate analysis, parameters associated with reduced M2BPGi were old age, high baseline AST and ALT levels, HCV mono-infection and advanced fibrosis ($\geq F3$). In multivariate analysis, high baseline ALT level, HCV mono-infection and advanced fibrosis were independent factors associated with M2BPGi reduction (Table 2).

DISCUSSION

A NEW PARADIGM of HCV therapy has improved substantially after the development of DAAs that are specifically addressed to viral targets in the HCV life cycle.⁷ The combination of EBR (NS5A inhibitor) and GZR (NS3/4A protease inhibitor) is a once-daily, fixed-dose tablet that has been approved for treating chronic HCV GT1 or GT4 infection. This combination with or without weight-adjusted RBV for 12–16 weeks displays high rates of SVR in GT1-infected individuals, including treatment-naïve or treatment-experienced patients. For instance, a phase 3 randomized controlled open label trial showed that SVR was achieved by 92.4% of patients treated with EBR/GZR for 12 weeks and 98.1% of patients given EBR/GZR plus

TABLE 2 Logistic regression analysis of factors associated with Mac-2-binding protein glycosylation isomer reduction (>50% from baseline)

Factors	Category	Univariate analysis		Multivariate analysis	
		OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Age (years)	≥ 50 vs. < 50	3.76 (1.57–9.03)	0.003*	2.11 (0.72–6.21)	0.174
Sex	Male vs. female	2.26 (0.83–6.19)	0.112		
Body mass index	≥ 24 vs. < 24	1.68 (0.73–3.80)	0.226		
Human immunodeficiency positivity	No vs. yes	5.16 (2.26–13.94)	$< 0.001^*$	4.88 (1.63–14.61)	0.005*
Previous HCV therapy	Yes vs. no	0.98 (0.36–2.67)	0.962		
Aspartate aminotransferase (IU/L)	≥ 50 vs. < 50	3.56 (1.43–8.87)	0.007*	0.73 (0.15–3.56)	0.679
Alanine aminotransferase (IU/L)	≥ 60 vs. < 60	4.81 (1.88–12.33)	0.001*	7.42 (1.42–38.89)	0.018*
Platelet count ($10^9/L$)	≥ 200 vs. < 200	0.94 (0.42–2.10)	0.878		
HCV sub-genotype	GT1b vs. GT1a	1.32 (0.56–3.07)	0.528		
Log ₁₀ HCV RNA (IU/ml)	≥ 6.0 vs. < 6.0	1.32 (0.55–3.16)	0.532		
Liver fibrosis staging	F34 vs. F012	1.87 (1.31–2.69)	0.001*	1.75 (1.10–2.79)	0.019*

Abbreviations: CI, confident interval; HCV, hepatitis C virus; OR, odds ratio.

* $p < 0.05$.

RBV for 16 weeks.²⁷ The corresponding figures in our cohort were 93.5% and 100%, respectively. Regarding HIV positivity, our results also showed that EBR/GZR therapy achieved comparable cure rates among patients with HCV mono-infection and HCV/HIV co-infection (93.3% and 97.2%, respectively). The SVR rate among HCV/HIV co-infected individuals in this cohort was in line with recent reports.^{28,29} Together, our results indicate that the EBR/GZR regimen is highly effective among Thai individuals infected with HCV G1, regardless of previous PEG-IFN/RBV treatment and HIV status.

It has been shown that significant fibrosis regression occurs after HCV eradication following DAA therapy, which is linked to a decreased risk of cirrhosis and HCC development.⁷ As mentioned, serial liver biopsies are associated with an increased risk of procedure-related complications. Thus, monitoring dynamic changes of fibrosis by reliable non-invasive methods is more practical than liver biopsy in clinical settings. In this study, MRE was selected as the reference imaging modality for fibrosis staging due to its high diagnostic accuracy.¹⁰ Based on recent meta-analysis, MRE displays AUROCs of 0.88, 0.93 and 0.92 for the prediction of $\geq F2$, $\geq F3$ and F4 fibrosis stages, respectively.¹⁰ Interestingly, a report of fiber quantitative analyses in patients with chronic HCV infection showed that MRE assessment significantly correlated with quantitative hepatic collagen and elastic fibers.³⁰ Compared with TE, MRE exhibited a greater diagnostic performance for the detection of fibrosis and cirrhosis in prospective cohorts of patients with chronic viral hepatitis.^{11,13} Moreover, MRE provides the advantage over TE by visualizing the entire liver rather than sampling only small hepatic regions. Thus, MRE is considered an alternative method for liver biopsy in monitoring fibrotic changes after HCV therapy. In this report, we found that liver stiffness measured by MRE significantly decreased from baseline to the time of SVR24 in patients achieving SVR, but there was no significant change among non-responders. Of note, liver stiffness at baseline and SVR24 in responders were 3.3 and 2.8 kPa, which were similar to the data of a recent report (3.1 and 2.8 kPa, respectively).¹⁴ Together, these results indicate that DAA therapies result in a significant improvement in liver stiffness assessed by MRE in patients after achieving SVR.

Previous studies have demonstrated that serum M2BPGi is a reliable non-invasive fibrosis marker that might predict the efficacy of IFN-based and IFN-free DAA therapy.^{19,20} However, the dynamic changes of M2BPGi following antiviral therapy have not been well described. To our knowledge, this study is the first with emphases on serial and dynamic changes of M2BPGi levels during and after DAA therapy. At baseline, our data showed that serum M2BPGi displayed diagnostic accuracy superior to that of APRI, which was in line with previous data.³¹ In fact, the AUROCs for assessing several stages of liver fibrosis using serum M2BPGi were almost comparable with those of TE. Beyond cross-sectional observations, M2BPGi could also be used to monitor potential fibrosis regression and predict treatment response in patients receiving DAA therapy. As shown in this study, patients achieving SVR displayed a significant and rapid reduction in serum M2BPGi at week 4 and throughout the follow-up periods as compared with pretreatment values. Moreover, serum

M2BPGi at SVR24 had a better correlation with MRE compared with APRI.

Of note, the extent of M2BPGi decline at SVR24 was greater than that found in MRE measurement. These findings might reflect the improvement in necroinflammatory activities and fibrosis regression after DAA therapy. In this regard, the continuous and rapid decline in M2BPGi values within weeks during therapy could be linked to the reduction of necroinflammatory activities, as this marker was positively correlated with AST and ALT levels. This observation was in line with previous data demonstrating that serum M2BPGi quantification was partly correlated with serum aminotransferase levels.³² Nonetheless, further M2BPGi decline was also observed beyond EOT and throughout the follow-up, which might represent the contribution of ongoing fibrosis regression in patients who achieved SVR.

Several recent studies have demonstrated that HCC occurrence is the most common liver-related complication in patients with advanced fibrosis/cirrhosis who have achieved SVR with DAA therapy. To this end, monitoring simple non-invasive markers after treatment can help to identify individuals at high risk of HCC. Besides its role as a reliable marker of liver fibrosis, serum M2BPGi has another clinical utility as a predictor of future HCC development in patients with chronic HCV infection.³² For example, a recent report demonstrated that M2BPGi values ≥ 1.75 COI after achieving SVR were independently predictive for early HCC occurrence in patients with chronic HCV infection treated with DAAs.³³ In this respect, it seems that M2BPGi at the time of SVR is able to predict the clinical outcome of patients with pre-existing advanced fibrosis/cirrhosis. Although the specific role of M2BPGi that links to HCC progression has not been entirely explored, previous *in vitro* observations suggested that activated stellate cell-derived M2BP might be a key modulator in both fibrogenesis and hepatocarcinogenesis.³⁴ Together, our data and previous reports indicate that serum M2BPGi can be used as a surrogate marker for monitoring the dynamic changes of liver fibrosis during DAA therapy as well as an accurate tool for predicting HCC development after SVR. Its clinical utility may be particularly suitable in resource-limited settings where imaging-based modalities such as MRE and TE are not widely accessible.

This study had some limitations as it included a small number of patients, particularly co-infected individuals, and a relative short period after completion of the therapy. Further large-scale and longer follow-up studies are warranted to confirm these observations. In addition, compared with the co-infected group, patients with HCV mono-infection had a significantly higher APRI score, although there were no significant differences between groups in terms of serum M2BPGi and elastography evaluated by TE and MRE. However, in a recent propensity score-matched cohort, M2BPGi levels were significantly higher in patients with HIV/HCV co-infection compared with levels in the HCV mono-infected group.³⁵ Such a discrepancy probably contributed to the difference in study design, as our report enrolled unmatched patients with or without HIV infection.

In conclusion, our data showed that HCV eradication with EBR/GZR therapy was associated with significant fibrosis improvement, as measured using MRE, TE and serum fibrosis markers. Compared with APRI, serum M2BPGi represented a better non-invasive marker for the assessment of liver fibrosis at baseline and after DAA therapy. In patients achieving SVR24, serum M2BPGi gradually declined during and after the administration of DAA, particularly in individuals with significant fibrosis at baseline.

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CONFLICT OF INTEREST

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REFERENCES

1. Thrift AP, El-Serag HB, Kanwal F. Global epidemiology and burden of HCV infection and HCV-related disease. *Nat Rev Gastroenterol Hepatol.* 2017;14:122–32.
2. Akkarathamrongsin S, Praianantathavorn K, Hacharoen N, Theamboonlers A, Tangkijvanich P, Tanaka Y, et al. Geographic distribution of hepatitis C virus genotype 6 subtypes in Thailand. *J Med Virol.* 2010;82:257–62.
3. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol.* 2013;10:553–62.
4. Lo Re V, 3rd, Kallan MJ, Tate JP, Localio AR, Lim JK, Goetz MB, et al. Hepatic decompensation in antiretroviral-treated patients coinfected with HIV and hepatitis C virus compared with hepatitis C virus-monoinfected patients: a cohort study. *Ann Intern Med.* 2014;160:369–79.
5. Avihingsanon A, Jitmitraprap S, Tangkijvanich P, Ramautarsing RA, Apornpong T, Jirajariyavej S, et al. Advanced liver fibrosis by transient elastography, fibrosis 4, and alanine aminotransferase/platelet ratio index among Asian hepatitis C with and without human immunodeficiency virus infection: role of vitamin D levels. *J Gastroenterol Hepatol.* 2014;29:1706–14.
6. Shire NJ, Welge JA, Sherman KE. Response rates to pegylated interferon and ribavirin in HCV/HIV coinfection: a research synthesis. *J Viral Hepat.* 2007;14:239–48.
7. Ioannou GN, Feld JJ. What are the benefits of a sustained virologic response to direct-acting antiviral therapy for hepatitis C virus infection?. *Gastroenterology.* 2019;156:446–60e2.
8. Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med.* 2001;344:495–500.
9. Kirk GD, Astemborski J, Mehta SH, Spoler C, Fisher C, Allen D, et al. Assessment of liver fibrosis by transient elastography in persons with hepatitis C virus infection or HIV-hepatitis C virus coinfection. *Clin Infect Dis.* 2009;48:963–72.
10. Singh S, Venkatesh SK, Wang Z, Miller FH, Motosugi U, Low RN, et al. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. *Clin Gastroenterol Hepatol.* 2015;13:440–51e6.
11. Dyvorne HA, Jajamovich GH, Bane O, Fiel MI, Chou H, Schiano TD, et al. Prospective comparison of magnetic resonance imaging to transient elastography and serum markers for liver fibrosis detection. *Liver Int.* 2016;36:659–66.
12. Park CC, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, et al. Magnetic resonance elastography vs transient elastography in detection of fibrosis and noninvasive measurement of steatosis in patients with biopsy-proven nonalcoholic fatty liver disease. *Gastroenterology.* 2017;152:598–607e2.
13. Lefebvre T, Wartelle-Bladou C, Wong P, Sebastiani G, Giard JM, Castel H, et al. Prospective comparison of transient, point shear wave, and magnetic resonance elastography for staging liver fibrosis. *Eur Radiol.* 2019.
14. Tada T, Kumada T, Toyoda H, Sone Y, Takeshima K, Ogawa S, et al. Viral eradication reduces both liver stiffness and steatosis in patients with chronic hepatitis C virus infection who received direct-acting anti-viral therapy. *Aliment Pharmacol Ther.* 2018;47:1012–22.
15. Byrne CD, Targher G. Time to replace assessment of liver histology with MR-based imaging tests to assess efficacy of interventions for nonalcoholic fatty liver disease. *Gastroenterology.* 2016;150:7–10.
16. Horowitz JM, Venkatesh SK, Ehman RL, Jhaveri K, Kamath P, Ohliger MA, et al. Evaluation of hepatic fibrosis: a review from the society of abdominal radiology disease focus panel. *Abdom Radiol (NY).* 2017;42:2037–53.
17. Ito K, Murotani K, Nakade Y. Serum WFA+ -M2BP levels predict liver fibrosis, development of hepatocellular carcinoma, and overall survival: a meta-analysis. *J Gastroenterol Hepatol.* 2017;32:1922–30.
18. Shirabe K, Bekki Y, Gantumur D, Araki K, Ishii N, Kuno A, et al. Mac-2 binding protein glycan isomer (M2BPGi) is a new serum biomarker for assessing liver fibrosis: more than a biomarker of liver fibrosis. *J Gastroenterol.* 2018;53:819–26.
19. Nagata H, Nakagawa M, Nishimura-Sakurai Y, Asano Y, Tsunoda T, Miyoshi M, et al. Serial measurement of Wisteria floribunda agglutinin positive Mac-2-binding protein is useful for predicting liver fibrosis and the development of hepatocellular carcinoma in chronic hepatitis C patients treated with IFN-based and IFN-free therapy. *Hepatol Int.* 2016;10:956–64.
20. Ura K, Furusyo N, Ogawa E, Hayashi T, Mukae H, Shimizu M, et al. Serum WFA(+)-M2BP is a non-invasive liver fibrosis marker that can predict the efficacy of direct-acting anti-viral-based triple therapy for chronic hepatitis C. *Aliment Pharmacol Therapeut.* 2016;43:114–24.
21. Watanabe T, Tokumoto Y, Joko K, Michitaka K, Horiike N, Tanaka Y, et al. Predictors of treatment efficacy and ALT non-normalization with sofosbuvir/ribavirin therapy for patients with hepatitis C virus genotype 2. *J Med Virol.* 2017;89:1567–73.

22. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38:518–26.
23. Kuno A, Sato T, Shimazaki H, Unno S, Saitou K, Kiyohara K, et al. Reconstruction of a robust glycodiagnostic agent supported by multiple lectin-assisted glycan profiling. *Proteomics Clin Appl*. 2013;7:642–7.
24. Boursier J, Zarski JP, de Ledinghen V, Rousselet MC, Sturm N, Lebaill B, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. *Hepatology*. 2013;57:1182–91.
25. Trout AT, Serai S, Mahley AD, Wang H, Zhang Y, Zhang B, et al. Liver stiffness measurements with MR elastography: agreement and repeatability across imaging systems, field strengths, and pulse sequences. *Radiology*. 2016;281:793–804.
26. Ichikawa S, Motosugi U, Ichikawa T, Sano K, Morisaka H, Enomoto N, et al. Magnetic resonance elastography for staging liver fibrosis in chronic hepatitis C. *Magn Reson Med Sci*. 2012;11:291–7.
27. Kwo P, Gane EJ, Peng CY, Pearlman B, Vierling JM, Serfaty L, et al. Effectiveness of elbasvir and grazoprevir combination, with or without ribavirin, for treatment-experienced patients with chronic hepatitis C infection. *Gastroenterology*. 2017;152:164–75e4.
28. Braun DL, Hampel B, Kouyos R, Nguyen H, Shah C, Flepp M, et al. High cure rates with grazoprevir-elbasvir with or without ribavirin guided by genotypic resistance testing among human immunodeficiency virus/hepatitis C virus-coinfected men who have sex with men. *Clin Infect Dis*. 2019;68:569–76.
29. Sulkowski M, Hezode C, Gerstoft J, Vierling JM, Mallolas J, Pol S, et al. Efficacy and safety of 8 weeks versus 12 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin in patients with hepatitis C virus genotype 1 mono-infection and HIV/hepatitis C virus co-infection (C-WORTHY): a randomised, open-label phase 2 trial. *Lancet*. 2015;385:1087–97.
30. Yasui Y, Abe T, Kurosaki M, Matsunaga K, Higuchi M, Tamaki N, et al. Non-invasive liver fibrosis assessment correlates with collagen and elastic fiber quantity in patients with hepatitis C virus infection. *Hepatology*. 2019;49:33–41.
31. Toshima T, Shirabe K, Ikegami T, Yoshizumi T, Kuno A, Togayachi A, et al. A novel serum marker, glycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA(+)-M2BP), for assessing liver fibrosis. *J Gastroenterol*. 2015;50:76–84.
32. Yamasaki K, Tateyama M, Abiru S, Komori A, Nagaoka S, Saeki A, et al. Elevated serum levels of Wisteria floribunda agglutinin-positive human Mac-2 binding protein predict the development of hepatocellular carcinoma in hepatitis C patients. *Hepatology*. 2014;60:1563–70.
33. Yasui Y, Kurosaki M, Komiyama Y, Takada H, Tamaki N, Watakabe K, et al. Wisteria floribunda agglutinin-positive Mac-2 binding protein predicts early occurrence of hepatocellular carcinoma after sustained virologic response by direct-acting antivirals for hepatitis C virus. *Hepatology*. 2018;48:1131–9.
34. Bekki Y, Yoshizumi T, Shimoda S, Itoh S, Harimoto N, Ikegami T, et al. Hepatic stellate cells secrete WFA+ -M2BP; its role in biological interactions with Kupffer cells. *J Gastroenterol Hepatol*. 2017;32:1387–93.
35. Takatsuki M, Yamasaki K, Natsuda K, Hidaka M, Ono S, Adachi T, et al. Wisteria floribunda agglutinin-positive human Mac-2-binding protein as a predictive marker of liver fibrosis in human immunodeficiency virus/hepatitis C virus coinfected patients. *Hepatology*. 2020;50:419–25.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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