Observational Study

M2BPGi for assessing liver fibrosis in patients with hepatitis C treated with direct-acting antivirals

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Abstract

BACKGROUND

Assessing liver fibrosis is important for predicting the efficacy of direct-acting antivirals (DAAs) and patient prognosis. Non-invasive techniques to assess liver fibrosis are becoming important. Recently, serum Mac-2 binding protein glycosylation isomer (M2BPGi) was identified as a non-invasive marker of liver fibrosis.

AIM

To investigate the diagnostic accuracy of M2BPGi in assessing liver fibrosis in patients with chronic hepatitis C (CHC) treated with DAAs.

METHODS

From December 2017 to August 2018, 80 treatment-naïve adult patients with CHC who were eligible for DAAs therapy were consecutively enrolled in this observational cohort study. For 12 weeks, 65 patients were treated with sofosbuvir/daclatasvir, and 15 patients were treated with sofosbuvir/daclatasvir and a weight-based dose of ribavirin at knowledge and technology association for hepatitis C management clinic, Cairo, Egypt. We measured serum M2BPGi levels, PAPAS index, fibrosis-4 (FIB-4) score and liver stiffness measurements (LSM) at baseline and 12 weeks after the end of treatment. Serum M2BPGi levels were measured using enzyme-linked immunosorbent assay.

RESULTS

All patients achieved sustained virologic response (SVR12) (100%). Serum M2BPGi levels, LSM, FIB-4 score and PAPAS index decreased significantly at SVR12 (P < 0.05). Serum M2BPGi levels correlated positively with LSM at baseline and SVR12 (P < 0.001). At baseline, compared with the FIB-4 score and PAPAS index, M2BPGi was the best marker to distinguish patients with grade F4 fibrosis (AUC = 0.801, P < 0.001), patients with grade F2 from grade F0-1 fibrosis (AUC = 0.713, P = 0.012), patients with grade F3-4 from grade F0-2 fibrosis (AUC = 0.730, P < 0.001), and patients with grade F2-4 from grade F0-1 fibrosis (AUC =
INTRODUCTION

Hepatitis C virus (HCV) is considered a public health problem, as approximately 3% of the global population is infected with HCV[1]. It is imperative to assess the degree of liver fibrosis in patients with chronic hepatitis C (CHC) because fibrogenesis causes all the clinical events, including decompensated liver disease and hepatocellular carcinoma (HCC), affecting the prognosis of and treatment strategies used in patients with CHC[2].

Although a liver biopsy is considered the gold standard for stratifying hepatic fibrosis, its clinical utility is substantially limited because of the invasiveness and the sampling variability[3]. Additionally, a liver biopsy is impractical particularly during follow-up due to its invasive nature[4].

Consequently, non-invasive methods have been previously proposed and validated for the assessment of hepatic fibrosis, such as ultrasound or magnetic resonance imaging[5], elastographic techniques[6], serum biomarkers including hyaluronic acid, imaging[7], serum biomarkers including hyaluronic acid, imaging[8], elastographic techniques[6], serum biomarkers including hyaluronic acid, imaging[7], serum biomarkers including hyaluronic acid, imaging[9], and surrogate markers, e.g., the aspartate aminotransferase (AST)-to-platelet ratio index[10], the fibrosis-4 (FIB-4) score[11], AST to alanine aminotransferase (ALT) ratio[12] and PAPAS [platelets/age /phosphatase/alpha fetoprotein (AFP)/AST] index[13].

Mac-2 binding protein glycosylation isomer (M2BPGi) is a glycoprotein that is produced by hepatic stellate cells (HSCs). It functions as a messenger between HSCs and Kupffer cells to promote fibrogenesis[14]. The feasibility of monitoring serum M2BPGi levels to assess hepatic fibrosis was evaluated, and some studies recommended it as an accurate method for staging hepatic fibrosis[15-17].

Subsequently, several investigators validated the usefulness of M2BPGi in various aetiologies of liver diseases, such as viral hepatitis[18-19], mortality in liver cirrhosis[20], biliary atresia[21], non-alcoholic fatty liver disease[22-23], non-alcoholic steatohepatitis[24], primary biliary cirrhosis[25], autoimmune hepatitis[26] and primary sclerosing...
Furthermore, it was investigated as a marker to assess the risk of HCC development\textsuperscript{28,29}. According to recent studies\textsuperscript{17,18,28,30-32}, M2BPGi is a useful marker for monitoring the improvement of patients with liver fibrosis who have achieved a sustained virologic response (SVR) after antiviral therapy. Recently, interferon (IFN)-based treatment has been replaced by direct-acting antivirals (DAAs). The approval of DAAs was a revolution in HCV eradication, with SVR rates exceeding 90\%, good tolerability, and increased efficacy with shorter treatment durations\textsuperscript{33}. However, a few reports have documented the improvement in liver fibrosis in patients treated with IFN-free DAAs\textsuperscript{31,34-37}.

We aimed to investigate the diagnostic accuracy of serum M2BPGi levels in assessing the grade of liver fibrosis in patients with CHC before and after DAAs-based treatment, as well as to compare its diagnostic value with the FIB-4 score and PAPAS index.

**MATERIALS AND METHODS**

From December 2017 to August 2018, 80 treatment-naïve adult patients with CHC who were eligible for DAAs therapy were consecutively enrolled in this observational cohort study. For 12 weeks, 65 patients were treated with sofosbuvir/daclatasvir, and 15 patients were treated with sofosbuvir/daclatasvir and a weight-based dose of ribavirin at Knowledge and Technology Association for Hepatitis C Management Clinic, Cairo, Egypt. The exclusion criteria were (1) positivity for antibodies against human immunodeficiency virus or positivity for hepatitis B surface antigen; (2) other causes of liver disease (autoimmune hepatitis, primary biliary cirrhosis, haemochromatosis, sclerosing cholangitis, Wilson’s disease, or an α1-antitrypsin deficiency); (3) clinical or biochemical evidence of hepatic decompensation (ascites, bleeding varices or encephalopathy); (4) suspected HCC or other cancers; (5) excessive alcohol consumption (> 40 g/d) or intravenous drug abuse; or (6) a previous liver transplantation.

This study was approved by the Research Ethics Committee of our institution. Written informed consent was obtained from every patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

We evaluated the liver stiffness measurement (LSM), serum M2BPGi levels, FIB-4 score, PAPAS index, biochemical data, haematological data, virologic data and abdominal ultrasound at baseline and 12 weeks after the end of treatment (EOT), namely, the time SVR12 was achieved, of every patient.

**Measurement of HCV RNA levels**

Plasma HCV RNA levels were measured using the Roche TaqMan real-time reverse transcriptase-PCR assay version 2.0, with lower limits of quantification and detection of 15 IU/mL. SVR12 was defined as a lack of detectable HCV RNA at week 12 after EOT.

**Measurement of the serum M2BPGi level**

Serum M2BPGi levels were measured using human M2BPGi enzyme-linked immunosorbent assay kits, with a detection range of 0.625 - 200 ng/mL, sensitivity 0.1 ng/mL, and intra-assay and inter-assay coefficients of variation less than 15%.

**LSM**

The LSM was performed using Fibroscan® (Echosens, 502 Touch, Paris, France). It was conducted by an experienced examiner after the patient had fasted for at least six hours, and 10 valid measurements were recorded. The median LS in kilopascals (kPa) was reported. Only examinations with a success rate > 60\% and IQR < 25\% were included and considered reliable. According to Tschochatzis et al\textsuperscript{38}, the following fibrosis staging cut-off values were used: F0-F1 < 7 kPa; F2 7 - 9.4 kPa; F3 9.5 - 11.9 kPa; and F4 > 12 kPa.

**Non-invasive liver fibrosis assessment**

The PAPAS index and FIB-4 score were calculated using the following formulas:

\[
\text{PAPAS index}^{12} = \log (\text{index} + 1) = 0.0255 + 0.0031 \times \text{age (year)} + 0.1483 \times \log [\text{ALP (U/L)}] + 0.004 \times \log [\text{AST (U/L)}] + 0.0908 \times \log [\text{AFP (ng/L)} + 1] - 0.028 \times \log [\text{platelets count (10^9/L)}].
\]

\[
\text{FIB-4 score}^{39} = \frac{\text{Age (yr)} \times \text{AST (U/L)}}{\text{[platelets count (10^9/L)]} \times [\text{ALT (IU/L)}]^2}. \\
\text{A FIB-4 score < 1.45 indicates no or minimal fibrosis.} \\
\text{A FIB-4 score > 3.25 indicates significant fibrosis.}
\]
Statistical analysis
Statistical analyses were performed using Stata® version 13.1 software (StataCorp. 2013, College Station, TX: StataCorp LP). Patients’ characteristics are presented as mean ± SD, median (IQR) or number (percentage), as appropriate. Accordingly, paired t test, Wilcoxon matched-pairs signed rank test or chi squared test was used, as appropriate. Values were compared between different grades of liver fibrosis using one-way ANOVA test. Pearson’s correlation analysis was used to study the correlation between serum M2BPGi levels and the characteristics of the study population. A receiver operating characteristic (ROC) curve analysis was used to identify the best cut-off value for the serum M2BPGi level with maximum sensitivity and specificity for the differentiation of different grades of fibrosis. A P value < 0.05 was considered significant.

The statistical methods of this study were performed by Hazem M. El-Hariri from Department of Community Medicine, National Research Centre, Cairo, Egypt.

RESULTS

Patients’ characteristics
The studied patients included 40 males (50%) and 40 females (50%), with a mean age of 52.3 ± 10.7 years and BMI (kg/m²) = 28 ± 5.4. Patients’ characteristics at baseline and SVR12 are shown in Table 1.

Safety and adherence to therapy
All patients completed the scheduled course of treatment with follow up until 12 wk after EOT. SVR12 was achieved in all patients (100%). Overall, the treatment was well tolerated. The most commonly reported adverse events were fatigue (5%), followed by pruritus (4.2%), rash (2.3%), headache (2%), and a loss of appetite (1%), all of which were mild in severity.

Impact of SVR12 on the serological data
Haemoglobin levels, WBC, total bilirubin levels and international normalized ratio (INR) did not change significantly after patients achieved SVR12. Platelets count and albumin levels were significantly higher at SVR12. ALT, AST, ALP and creatinine levels decreased significantly after patients achieved SVR12. AFP levels decreased after patients achieved SVR12, but the difference was not statistically significant (Table 1).

Effect of SVR12 on liver fibrosis
Serum M2BPGi levels, LSM, FIB-4 score and PAPAS index decreased significantly after patients achieved SVR12 (Table 1). The improvement in LSM was more noticeable in patients with grade F4 fibrosis (Table 2). Only serum M2BPGi levels were significantly different between patients with different grades of fibrosis at baseline and SVR12 (Table 3).

Correlations with serum M2BPGi levels
At baseline, serum M2BPGi levels correlated positively with total bilirubin levels and negatively with AST levels. At SVR12, serum M2BPGi levels correlated positively with INR and negatively with platelets count (Table 4).

Correlations between serum M2BPGi levels, LSM, FIB-4 score and PAPAS index
At baseline, LSM correlated with serum M2BPGi levels and FIB-4 score. In addition, a significant correlation was observed between FIB-4 score and PAPAS index. At SVR12, LSM correlated with serum M2BPGi levels, FIB-4 score and PAPAS index (Table 5 and Figure 1).

ROC curve analysis for the assessment and differentiation of the grades of liver fibrosis
At baseline, compared with the FIB-4 score and PAPAS index, M2BPGi was the best marker to distinguish patients with grade F4 fibrosis (AUC = 0.801, P < 0.001), patients with grade F2 from grade F0-1 fibrosis (AUC = 0.713, P = 0.012), patients with grade F3-4 from grade F0-2 fibrosis (AUC = 0.730, P < 0.001), and patients with grade F2-4 from grade F0-1 fibrosis (AUC = 0.763, P < 0.001) (Supplementary Table 1, Figures 1 and 3).

At SVR12, M2BPGi had the greatest AUCs for differentiating patients with grade F4 fibrosis (AUC = 0.844, P < 0.001), patients with grade F3 from grade F0-2 fibrosis (AUC = 0.893, P = 0.002), patients with grade F3-4 from grade F0-2 fibrosis (AUC =
Table 1 Patients’ characteristics at baseline and sustained virologic response 12

| Measures                      | Baseline | SVR12       | P value |
|-------------------------------|----------|-------------|---------|
| Age (yr)                      | 52.3 ± 10.7 | Non-detectable | 0.000b |
| Gender 40 (50%) males and 40 (50%) females |          |             |         |
| BMI                           | 28 ± 5.4 | 245.3 ± 77.8 | 0.005b |
| HCV RNA (log copies/mL)       | 6.0 ± 0.7 | Non-detectable |         |
| Platelets count (× 10³/mL)    | 220.3 ± 65.3 | 245.3 ± 77.8 | < 0.001b |
| ALT (IU/L)                    | 40.5 (29-54) | 32 (26-38) | < 0.001b |
| AST (IU/L)                    | 39 (29-51) | 30 (24-37) | < 0.001b |
| ALP (IU/L)                    | 138.5 (95.3-196) | 107 (86-141) | < 0.001b |
| Albumin (g/dL)                | 3.8 ± 0.3 | 4.2 ± 0.4 | < 0.001b |
| Total bilirubin (mg/dL)       | 0.7 (0.5-0.8) | 0.7 (0.5-0.95) | 0.8 |
| Creatinine (mg/dL)            | 0.86 ± 0.19 | 0.75 ± 0.23 | 0.002a |
| INR                           | 1.06 ± 0.09 | 1.08 ± 0.15 | 0.222b |
| AFP (ng/mL)                   | 4.5 ± 2.1 | 3.7 ± 1.9 | 0.128 |
| LSM (kPa)                     | 11.4 ± 4.5 | 9.5 ± 3.3 | 0.002b |
| FIB-4 score                   | 1.8 ± 0.5 | 1.3 ± 0.7 | < 0.001b |
| PAPAS index                   | 2.2 ± 0.5 | 2.1 ± 0.3 | 0.010a |
| Serum M2BPGi (ng/mL)          | 9 ± 3.8 | 6.7 ± 2.3 | < 0.001b |

The values are expressed as mean ± SD or median (IQR).

*p < 0.05.

**p < 0.01.

SVR: Sustained virologic response; WBC: White blood cell count; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; INR: International normalized ratio; AFP: Alpha fetoprotein; LSM: Liver stiffness measurement; M2BPGi: Mac-2 binding protein glycosylation isomer.

0.891, P < 0.001), and patients with grade F2-4 from grade F0-1 fibrosis (AUC = 0.750, P < 0.001) (Supplementary Table 1, Figures 2 and 3).

DISCUSSION

Currently, non-invasive methods for detecting liver fibrosis are used much more frequently than liver biopsies[40]. M2BPGi has been shown to be a useful predictor of liver fibrosis[14,17,41]. Previous reports have documented a substantial improvement in liver fibrosis after patients achieve SVR12 through treatment with DAAs for HCV[42-44]. Here, we aimed to investigate the diagnostic accuracy of serum M2BPGi levels for assessing the grade of liver fibrosis in patients with CHC before and after DAAs-based treatment, as well as to compare its diagnostic value with the FIB-4 score and PAPAS index.

The present study confirms the efficacy and safety of sofosbuvir + daclatasvir ± ribavirin in real-world situations. The SVR12 is 100%.

Similar to previous studies[3,16,31,30,53], we observed a significantly increasing trend of serum M2BPGi levels with the progression of liver fibrosis both at baseline and SVR12 (P < 0.001). Moreover, in accordance with Akahane et al[43] and Ishikawa et al[44], serum M2BPGi levels decreased significantly at SVR12 (P < 0.001). In addition, in a study by Miyaki et al[31], serum M2BPGi levels did not change in the non-SVR group (P = 0.715), but decreased significantly in the SVR group (P < 0.0001). These results suggest that serum M2BPGi would be a good surrogate marker for predicting and differentiating liver fibrosis stages.

In the present study, pre-treatment serum M2BPGi levels correlated with bilirubin and AST levels, while serum M2BPGi levels correlated with platelets count and INR at SVR12. These findings suggest that M2BPGi reflects not only the severity of liver fibrosis but also the severity of liver inflammation in CHC patients[41]. This may be
Table 2  Liver stiffness measurements of all patients at baseline and sustained virologic response 12

| Total baseline | Changes in the fibrosis grade at SVR12 |
|---------------|----------------------------------------|
|               | F0-1 (%) | F2 (%) | F3 (%) | F4 (%) |
| F0-1 29 (36.3%) | 23 (79.3) | 5 (17.2) | 1 (3.4) | 0 (0.0) |
| F2 20 (25%) | 10 (50) | 10 (50) | 0 (0.0) | 0 (10) |
| F3 5 (6.3%) | 1 (20) | 2 (40) | 1 (20) | 1 (20) |
| F4 26 (32.5%) | 1 (3.8) | 6 (23.1) | 4 (15.4) | 15 (57.7) |
| Total SVR12 | 35 (43.7) | 23 (28.7) | 6 (7.5) | 16 (20) |

Total patients number = 80. The values are expressed as numbers (%). SVR: Sustained virologic response.

attributed to the role of M2BPGi as a messenger between HSCs and Kupffer cells and its accompanying inflammation[13].

Similar to our results, Ura et al[30] reported a significant negative correlation between serum M2BPGi levels and platelets count (r = -0.47, P < 0.0001). Additionally, Yamasaki et al[54] observed a significant positive correlation between serum M2BPGi and bilirubin levels (r = 0.091, P = 0.001) and a significant negative correlation with platelets count (r = -0.147, P < 0.001). However, in contrast to our results, Yasui et al[55] observed a positive correlation between serum M2BPGi and AFP levels (r = 0.428, P < 0.001), and a negative correlation with albumin levels (r = -0.471, P < 0.001).

In agreement with the present study, Tawara et al[53] reported a correlation coefficient between serum M2BPGi levels and FIB-4 score of less than 0.4, suggesting that the correlation between serum M2BPGi levels and FIB-4 score was weak. In contrast, Ura et al[30] and Yasui et al[55] detected a significant positive correlation between serum M2BPGi levels and FIB-4 score (r = 0.66, P < 0.0001 and r = 0.546, P < 0.001, respectively). This discrepancy can be attributed to the different sample size.

In terms of differentiation of liver fibrosis grades, consistent with the present study, Xu et al[16] reported that the AUC values of M2BPGi for predicting fibrosis grade ≥ F2 and F4 were significantly superior to the values of FIB-4 score (0.774 vs 0.702, P < 0.001 and 0.892 vs 0.818, P < 0.05), respectively. In contrast, Tawara et al[53] reported that the FIB-4 score had a greater AUC value for differentiating of fibrosis grades than M2BPGi (AUC values were 0.768, 0.827 and 0.876 for fibrosis grade F ≥ 2, F ≥ 3 and F4, respectively), while the AUC values of M2BPGi were 0.747, 0.733 and 0.796 for fibrosis grade F ≥ 2, F ≥ 3 and F4, respectively.

The limitations of the present study are the absence of a paired histological evaluation due to the invasiveness of liver biopsy, and the short duration of follow up after completion of treatment. Further large-scale studies with a longer follow-up period should be performed.

In conclusion, M2BPGi is a reliable marker for the non-invasive assessment and prediction of liver fibrosis regression in patients with CHC who achieved an SVR with DAAs therapy.
Table 3  Non-invasive assessment data obtained at baseline and sustained virologic response 12 from patients stratified according to the fibrosis grade

| Parameter                                      | F0-1 (n = 29) | F2 (n = 20) | F3 (n = 5) | F4 (n = 26) | P value |
|-----------------------------------------------|---------------|-------------|------------|-------------|---------|
| Baseline (Total n = 80)                       |               |             |            |             |         |
| LSM (kPa)                                     | 5.9 ± 0.6     | 7.8 ± 0.5   | 10.8 ± 1.2 | 20.6 ± 7.7  | < 0.001b |
| FIB-4 score                                   | 1.7 ± 1.4     | 1.4 ± 0.7   | 1.8 ± 0.4  | 2.3 ± 1.5   | 0.108   |
| PAPAS index                                   | 2.1 ± 0.4     | 2.2 ± 0.6   | 2.3 ± 0.3  | 2.3 ± 0.5   | 0.303   |
| Serum M2BPGi (ng/mL)                          | 4.5 ± 2.2     | 5.3 ± 2.8   | 9.4 ± 4    | 14.5 ± 6.7  | 0.001a  |
| SVR 12 (Total n = 80)                         |               |             |            |             |         |
| LSM (kPa)                                     | 5.8 ± 1.2     | 7.6 ± 0.6   | 9.9 ± 0.8  | 15 ± 2.3    | < 0.001b |
| FIB-4 score                                   | 1.1 ± 0.5     | 1.5 ± 1     | 1.3 ± 0.6  | 1.6 ± 0.5   | 0.075   |
| PAPAS index                                   | 2 ± 0.3       | 2.1 ± 0.3   | 2.1 ± 0.3  | 2.3 ± 0.3   | 0.069   |
| Serum M2BPGi (ng/mL)                          | 3.4 ± 1.6     | 4.9 ± 2.1   | 12.7 ± 5.1 | 13.3 ± 5.2  | 0.001b  |

bP < 0.01. The values are expressed as mean ± SD. SVR: Sustained virologic response; LSM: Liver stiffness measurement; M2BPGi: Mac-2 binding protein glycosylation isomer.

Table 4 Correlations of serum Mac-2 binding protein glycosylation isomer levels with laboratory data

| Variables         | Baseline   | SVR12     | Baseline   | SVR12     |
|-------------------|------------|-----------|------------|-----------|
|                   | r          | P value   | r          | P value   |
| Age               | 0.050      | 0.662     | 0.107      | 0.346     |
| BMI               | -0.007     | 0.951     | 0.057      | 0.617     |
| HCV RNA           | 0.043      | 0.702     | 0.025      | 0.827     |
| Hb                | 0.143      | 0.205     | 0.131      | 0.246     |
| WBC               | 0.047      | 0.677     | -0.152     | 0.178     |
| Platelets count   | -0.118     | 0.297     | -0.299     | 0.007b    |
| ALT               | -0.090     | 0.425     | -0.129     | 0.256     |
| AST               | -0.227     | 0.043a    | 0.006      | 0.961     |
| ALP               | -0.024     | 0.831     | 0.106      | 0.350     |
| Albumin           | 0.016      | 0.888     | 0.070      | 0.535     |
| Total bilirubin   | 0.268      | 0.016b    | 0.111      | 0.326     |
| Creatinine        | 0.158      | 0.162     | 0.088      | 0.439     |
| INR               | 0.063      | 0.582     | 0.220      | 0.049b    |
| AFP               | -0.098     | 0.388     | 0.026      | 0.822     |

aP < 0.05.  
bP < 0.01. SVR: Sustained virologic response; BMI: Body mass index; Hb: Haemoglobin; WBC: White blood cell count; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; INR: International normalized ratio; AFP: Alpha fetoprotein.
Table 5 Correlations between non-invasive assessment methods at baseline and sustained virologic response 12

| Variables      | LSM        | Serum M2BPGi | FIB-4 score |
|----------------|------------|--------------|-------------|
| Serum M2BPGi level | r = 0.453  | P value < 0.001<sup>b</sup> |             |
| FIB-4 score     | r = 0.328  | -0.099       |             |
| P value         | 0.003<sup>b</sup> | 0.384       |             |
| PAPAS index     | r = 0.110  | -0.049       | 0.444       |
| P value         | 0.330      | 0.664        | < 0.001<sup>b</sup> |

At SVR12

| Serum M2BPGi level | r = 0.517  | P value < 0.001<sup>b</sup> |
| FIB-4 score        | r = 0.231  | 0.188         |
| P value            | 0.039<sup>a</sup> | 0.095       |
| PAPAS index        | r = 0.328  | 0.185         | 0.188       |
| P value            | 0.003<sup>b</sup> | 0.100      | 0.095       |

<sup>a</sup>p < 0.05.<br><sup>b</sup>p < 0.01. SVR: Sustained virologic response; LSM: Liver stiffness measurement; M2BPGi: Mac-2 binding protein glycosylation isomer.

Figure 1 Correlation between serum Mac-2 binding protein glycosylation isomer levels and liver stiffness measurement at baseline and after patients achieved sustained virologic response 12. A: Correlation between serum Mac-2 binding protein glycosylation isomer (M2BPGi) levels and liver stiffness measurement (LSM) at baseline; B: Correlation between serum M2BPGi levels and LSM after patients achieved sustained virologic response 12. M2BPGi: Mac-2 binding protein glycosylation isomer; LSM: Liver stiffness measurement; SVR: Sustained virologic response.
ARTICLE HIGHLIGHTS

Research background
Assessing liver fibrosis is important for predicting the efficacy of direct-acting antivirals (DAAs) and patient prognosis. Non-invasive techniques to assess liver fibrosis are becoming important. Recently, serum Mac-2 binding protein glycosylation isomer (M2BPGi) was identified as a non-invasive marker of liver fibrosis.

Research motivation
The approval of DAAs was a revolution in hepatitis C virus eradication, with sustained virologic response (SVR) rates exceeding 90%. However, a few reports have documented the improvement in liver fibrosis in patients treated with DAAs. Although liver biopsy is considered the gold standard for stratifying hepatic fibrosis, its clinical utility is substantially limited because of the invasiveness and the sampling variability. Accordingly, serum M2BPGi was evaluated as a non-invasive marker for assessing the grade of hepatic fibrosis in patients who have achieved SVR after antiviral therapy.

Research objectives
We aimed to investigate the diagnostic accuracy of serum M2BPGi levels in assessing the grade
of liver fibrosis in patients with chronic hepatitis C (CHC) before and after DAAs-based treatment, as well as to compare its diagnostic value with the FIB-4 score and PAPAS index.

Research methods

Eighty treatment-naïve adult patients with CHC who were eligible for DAAs therapy were consecutively enrolled in this observational cohort study. For 12 weeks, 65 patients were treated with sofosbuvir/daclatasvir, and 15 patients were treated with sofosbuvir/daclatasvir and a weight-based dose of ribavirin. We measured serum M2BPGi levels, PAPAS index, FIB-4 score and liver stiffness measurements (LSM) at baseline and 12 weeks after the end of treatment. Serum M2BPGi levels were measured using enzyme-linked immunosorbent assay.

Research results

All patients achieved SVR12 (100%). Serum M2BPGi levels, LSM, FIB-4 score and PAPAS index decreased significantly at SVR12 (P < 0.05). Serum M2BPGi levels correlated positively with LSM at baseline and SVR12 (P < 0.001). At baseline, compared with the FIB-4 score and PAPAS index, M2BPGi was the best marker to distinguish patients with grade F4 fibrosis (AUC = 0.801, P < 0.001), patients with grade F2 from grade F0-1 fibrosis (AUC = 0.713, P = 0.012), patients with grade F3-4 from grade F0-2 fibrosis (AUC = 0.730, P < 0.001), and patients with grade F2-4 from grade F0-1 fibrosis (AUC = 0.763, P < 0.001). At SVR12, M2BPGi had the greatest AUCs for differentiating patients with grade F4 fibrosis (AUC = 0.844, P < 0.001), patients with grade F3 from grade F0-2 fibrosis (AUC = 0.893, P = 0.002), patients with grade F4 from grade F0-2 fibrosis (AUC = 0.891, P < 0.001), and patients with grade F4 from grade F0-1 fibrosis (AUC = 0.750, P < 0.001).

Research conclusions

M2BPGi is a reliable marker for the non-invasive assessment and prediction of liver fibrosis regression in patients with CHC who achieved an SVR with DAAs therapy.

Research perspectives

Non-invasive methods have been previously proposed and validated for the assessment of hepatic fibrosis. Further studies are needed to investigate their therapeutic potential and ultimate clinical utility.

REFERENCES

1. Morozov VA, Layag S. Hepatitis C virus: Morphogenesis, infection and therapy. World J Hepatol 2018; 10: 186-212 [PMID: 29527250 DOI: 10.4254/wjh.v10.i2.186]
2. Karanja KN, Crosse J, Cox J, Fye H, Njie R, Goldin RD, Taylor-Robinson SD. Hepatic steatosis and fibrosis: Non-invasive assessment. World J Gastroenterol 2016; 22: 9880-9897 [PMID: 28081966 DOI: 10.3748/wjg.v22.i45.9880]
3. Huang CI, Huang CF, Yeh ML, Lin YH, Liang PC, Hsieh MH, Dai CY, Hsieh MY, Lin ZY, Chen SC, Huang IF, Yu ML, Chung WL. Serum Wisteria floribunda agglutinin-positive Mac-2-binding protein expression predicts disease severity in chronic hepatitis C patients. Kaohsiung J Med Sci 2017; 33: 394-399 [PMID: 28811008 DOI: 10.1016/j.kjms.2017.05.017]
4. Mokdad AA, Lopez AD, Shiarzaz S, Lozano R, Mokdad AH, Stanaway J, Murray CJ, Naghavi M. Liver cirrhosis mortality in 187 countries between 1980 and 2010: A systematic analysis. BMJ Med 2014; 12: 145 [PMID: 25242659 DOI: 10.1136/ijome-2014-0145-27]
5. Lurie Y, Webb M, Cytter-Kuiti R, Shteingart S, Lederkremer GZ. Non-invasive diagnosis of liver fibrosis and cirrhosis. World J Gastroenterol 2015; 21: 11567-11583 [PMID: 26556987 DOI: 10.3748/wjg.v21.i41.11567]
6. Wang QB, Zhu H, Liu HL, Zhang B. Performance of magnetic resonance elastography and diffusion-weighted imaging for the staging of hepatic fibrosis: A meta-analysis. Hepatology 2012; 56: 239-247 [PMID: 22278368 DOI: 10.1002/hep.25610]
7. Cui J, Heba E, Hernandez C, Haufe W, Hooker J, Andre MP, Valasek MA, Aryafer H, Sirlin CB, Loomba R. Magnetic resonance elastography is superior to acoustic radiation force impulse for the Diagnosis of liver fibrosis in patients with biopsy-proven nonalcoholic fatty liver disease: A prospective study. Hepatology 2016; 63: 453-461 [PMID: 26506734 DOI: 10.1002/hep.28337]
8. Tatsunami C, Kudo M, Ueshima K, Kitai S, Takahashi S, Inoue T, Minami Y, Chung H, Mackawa K, Fujimoto K, Akiko T, Takeshi M. Noninvasive evaluation of hepatic fibrosis using serum fibrotic markers, transient elastography (FibroScan) and real-time tissue elastography. Intervirology 2008; 51 Suppl 1: 27-33 [PMID: 18544945 DOI: 10.1159/000122602]
9. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003; 38: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
10. Vallet-Flachard A, Maillet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. Hepatology 2007; 46: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]
11. Lin ZH, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, Sun Y, Xuan SY. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology 2011; 53: 726-736 [PMID: 21339199 DOI: 10.1002/hep.24105]
12. Ozel BD, Poyrazoglu OK, Karanam A, Karanam H, Alhinkaya E, Sevinç E, Zararsiz G. The PAPAS index: a novel index for the prediction of hepatitis C-related fibrosis. Eur J Gastroenterol Hepatol 2015; 27: 895-900 [PMID: 25951491 DOI: 10.1097/EJG.0000000000000379]
13. Shirabe K, Bekki Y, Gantumur D, Araki K, Ishii N, Kano A, Narimatsu H, Mizokami M. Mac-2 binding protein glycan isomer (M2BPGi) is a new serum biomarker for assessing liver fibrosis: more than a
bimarker of liver fibrosis. *J Gastroenterol* 2018; 53: 819-826 [PMID: 29318378 DOI: 10.1007/s00535-017-1425-z]

14. Yoshima T, Shirabe K, Iekami T, Yoshizumi T, Kuno A, Togayauchi A, Gotoh M, Narimatsu H, Korenaga M, Mizokami M, Nishie A, Aishima S, Maehara Y. A novel serum marker, glycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA(+)M2BPGi), for assessing liver fibrosis. *J Gastroenterol* 2015; 50: 76-84 [PMID: 24603981 DOI: 10.1007/s00535-014-0946-y]

15. Kuno A, Ikeda Y, Tanaka Y, Ito K, Matsuda A, Sekiya S, Hige S, Sakamoto M, Kage M, Mizokami M, Narimatsu H. A serum “sweet-doughnut” protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. *Sci Rep* 2013; 3: 1065 [PMID: 23232209 DOI: 10.1038/srep01065]

16. Xu H, Kong W, Liu L, Chi X, Wang X, Wu R, Gao X, Wang H, Qu L, Qi Y, Pan Y, Niu J. Accuracy of M2BPGi, compared with FibroScan®, in analysis of liver fibrosis in patients with hepatitis C. *BMC Gastroenterol* 2017; 17: 62 [PMID: 28486931 DOI: 10.1186/s12876-017-0618-2]

17. Zou X, Zhu MY, Li W, Zhang DH, Li FJ, Gong QM, Liu F, Jiang JH, Zhang MH, Kuno A, Narimatsu H, Zhang Y, Zhang X. Serum WFA(+)M2BPGi levels for the evaluation of early stages of liver fibrosis in patients with chronic hepatitis B virus infection. *Liver Int* 2017; 37: 35-44 [PMID: 27300763 DOI: 10.1111/liv.13188]

18. Ishii A, Nishikawa H, Enomoto M, Iwata Y, Kishino K, Shimono Y, Hasegawa K, Nakano C, Takata R, Nishimura T, Yoh K, Aizawa N, Sakai Y, Ikeda N, Takashima T, Iijima H, Nishiguchi S. Clinical implications of serum Wisteria floribunda agglutinin-positive Mac-2-binding protein in treatment-naïve chronic hepatitis B. *Hepatol Res* 2017; 47: 204-215 [PMID: 26990490 DOI: 10.1111.hepr.12703]

19. Nakamura M, Kanda T, Ito J, Haga Y, Takanashi K, Wu S, Yasaki S, Nakamoto S, Yokosuka O. Serum microRNA-122 and Wisteria floribunda agglutinin-positive Mac-2 binding protein are useful tools for liquid biopsy of the patients with hepatitis B virus and advanced liver fibrosis. *Plos One* 2017; 12: e0177302 [PMID: 28475652 DOI: 10.1371/journal.pone.0177302]

20. Hanai T, Shiraki M, Ohsishi S, Miyazaki T, Ieda T, Kochi T, Imai K, Suetsugu A, Takai K, Shimizu M, Moriwaki H. Impact of serum glycosylated Wisteria floribunda agglutinin positive Mac-2 binding protein levels on liver functional reserves and mortality in patients with liver cirrhosis. *Hepatol Res* 2015; 45: 1083-1090 [PMID: 2556570 DOI: 10.1111/hepr.12473]

21. Yamada N, Sanada Y, Tashiro M, Hirata Y, Okada N, Iihara Y, Uralashis T, Mizuta K. Serum Mac-2 binding protein glycosylation isoform predicts grade F4 liver fibrosis in patients with biliary atresia. *J Gastroenterol* 2017; 52: 245-252 [PMID: 27349650 DOI: 10.1111/jgs.14255]

22. Lai L.L., Chan WK, Shihanelevar P, Nik Mustapha NR, Goh KL, Mahadeva S. Serum Wisteria floribunda agglutinin-positive Mac-2 binding protein in non-alcoholic fatty liver disease. *Plos One* 2017; 12: e0174982 [PMID: 28369190 DOI: 10.1371/journal.pone.0174982]

23. Mizuno M, Shima T, Oya H, Misumoto Y, Mizuno C, Isoda S, Kuramato M, Taniguchi M, Noda M, Sakai K, Koyama N, Okanoue T. Classification of patients with non-alcoholic fatty liver disease using rapid immunoassay of serum type IV collagen compared with liver histology and other fibrosis markers. *Hepatol Res* 2017; 47: 216-225 [PMID: 26997642 DOI: 10.1111/hepr.12710]

24. Nishikawa H, Enomoto H, Iwata Y, Kishino K, Shimono Y, Hasegawa K, Nakano C, Takata R, Yoh K, Nishimura T, Aizawa N, Sakai Y, Ikeda N, Takashima T, Iishii A, Iijima H, Nakamura H, Nishiguchi S. Clinical significance of serum Wisteria floribunda agglutinin positive Mac-2 binding protein level in non-alcoholic steatohepatitis. *Hepatol Res* 2016; 46: 1194-1202 [PMID: 27081262]

25. Nishikawa H, Enomoto H, Iwata Y, Hasegawa K, Nakano C, Takata R, Nishimura T, Yoh K, Aizawa N, Sakai Y, Ikeda N, Takashima T, Iishii A, Iijima H, Nishiguchi S. Impact of serum Wisteria floribunda agglutinin positive Mac-2 binding protein and serum interferon-γ-inducible protein-10 in primary biliary cirrhosis. *Hepatol Res* 2016; 46: 575-583 [PMID: 26418076 DOI: 10.1111/hepr.12951]

26. Nishikawa H, Enomoto H, Iwata Y, Hasegawa K, Nakano C, Takai K, Nishimura T, Yoh K, Aizawa N, Sakai Y, Ikeda N, Takashima T, Iishii A, Iijima H, Nishiguchi S. Clinical significance of serum Wisteria floribunda agglutinin positive Mac-2 binding protein level and high-sensitivity C-reactive protein concentration in autoimmune hepatitis. *Hepatol Res* 2016; 46: 613-621 [PMID: 26400984 DOI: 10.1111/hepr.12956]

27. Umetsu S, Inai A, Sogo T, Komatsu H, Fujisawa T. Usefulness of serum Wisteria floribunda agglutinin-positive Mac-2 binding protein in children with primary sclerosing cholangitis. *Hepatol Res* 2018; 48: 355-363 [PMID: 29168311 DOI: 10.1111/hepr.13004]

28. Nagata K, Nakagawa M, Asahina Y, Sato A, Asano Y, Tsunoda T, Miyoshi M, Kaneko S, Otani S, Kawai-Kitahata F, Murakawa M, Nitta S, Iitsu Y, Azuma S, Sakumina S, Nouchi T, Sakai H, Tomita M, Watanabe M, Ochamizu Liver Conference Study Group. Effect of interferon-based and -free therapy on liver function parameters and reduces liver fibrosis markers in chronic hepatitis C patients. *Hepatol Res* 2016; 46: 758-764 [PMID: 26574180 DOI: 10.1111/hepr.12621]

29. Suda T, Okawa O, Masaoka R, Gyotoku Y, Tokotomi N, Katayama Y, Tamano M. Shear wave elastography in hepatitis C patients before and after antiviral therapy. *World J Hepatol* 2017; 9: 64-68 [PMID: 28105260 DOI: 10.4254/wjh.v9.i1.64]

30. Kusakabe A, Kuroski M, Iikura J, Ito K, Kikuhara S, Tsuji K, Kobashi H, Suhada T, Kimura H, Nair R, Furuta K, Iwami N. Efficacy and safety of glecaprevir/pibrentasvir as retreatment therapy for patients with genotype 2 chronic hepatitis C who failed prior sofosbuvir plus ribavirin regimen. *Hepatol Res* 2019; 49: 1121-1126 [PMID: 31209976 DOI: 10.1111/hepr.13387]

31. Tamori A, Hiai H, Uchida-Kobayashi S, Enomoto M, Kozuka R, Motoyama H, Kawaunara E, Hagihara A, Teranishi Y, Yoshida K, Morikawa H, Murakami Y, Kawaada N. Outcomes for CHC Patients with Hepatitis C Virus 1b Treated with Asunaprevir and Daclatasvir Combination. *Ann Hepatol* 2017; 16: 734-741 [PMID: 28809743 DOI: 10.5041/ah.2017.01732]
Lee HW, Oh SR, Kim DY, Jeong Y, Kim S, Kim BK, Kim SU, Kim DY, Ahn SH, Han KH, Park JY. Daclatasvir Plus Asunaprevir for the Treatment of Patients with Hepatitis C Virus Genotype 1b Infection: Real-World Efficacy, Changes in Liver Stiffness and Fibrosis Markers, and Safety. Gut Liver 2018;12:324-330 [PMID: 29409309 DOI: 10.5009/gl17290]

Ishikawa T, Inami M, Owaki T, Sato H, Nozawa Y, Sano T, Iwanga A, Seki K, Homma T, Toshiki Yoshiada T. Serum Wisteria floribunda Agglutinin Positive Mac-2 Binding Protein and Fib-4 Index on the Clinical Course of Patients with Chronic Hepatitis C Receiving Daclatasvir/Asunaprevir Therapy. Ann Digest Liver Dis 2017; 1: 1001

Eshsharkawy A, Alem SA, Fouad R, El Raziky M, El Akel W, Abdo M, Tantawi O, AbdAllah M, Bourliere M, Esmat G. Changes in liver stiffness measurements and fibrosis scores following sofosbuvir based treatment regimens without interferon. J Gastroenterol Hepatol 2017; 32: 1624-1630 [PMID: 28177545 DOI: 10.1111/jgh.13758]

Tsotchazis EA, Gurusamy KS, Ntoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. J Hepatol 2011; 54: 650-659 [PMID: 21468922 DOI: 10.1016/j.jhep.2010.07.033]

Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M, APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006; 43: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]

Mauss S, Pol S, Buti M, Dufell E, Gore C, Lazarus Jv, de Grient H, Lindgren J, Mozalevskis A, Raben D, Schatz E, Wiktor S, Rockstroh JK, European consensus working group on late presentation for Viral Hepatitis Care. Late presentation of chronic viral hepatitis for medical care: a consensus definition. BMC Med 2017; 15: 92 [PMID: 28644835 DOI: 10.1186/s12916-017-0856-9]

Chen CC, Huo HT, Chen YL, Chen RC, Wu WP, Chou CT. Diagnostic Accuracy of Acoustic Radiation Force Impulse (ARFI) and Wisteria floribunda Agglutinin-Positive Mac-2-Binding Protein (W-MA2BP) in Patients with Chronic Liver Disease. Med Sci Monit 2019; 25: 7169-7174 [PMID: 31548540 DOI: 10.12659/MSM.916537]

Singh S, Fasciocamso A, Loomba R, Falck-Ytter YT. Magnitude and Kinetics of Decrease in Liver Stiffness After Antiviral Therapy in Patients With Chronic Hepatitis C: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2018; 16: 27-38.e4 [PMID: 28847904 DOI: 10.1016/j.cgh.2017.04.038]

Bachofner JA, Valli PY, Krieger A, Bergamin I, Künzler P, Baserga A, Brun D, Seifert B, Monceux A, Fehr J, Semela D, Magenta L, Müller-Hartmann B, Zerulli Rebera-Piccoli B, Mertens JC. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. Liver Int 2017; 37: 369-376 [PMID: 27878216 DOI: 10.1111/liv.13256]

Dolmanaslihvi E, Abutidze A, Khakhartishvili N, Karchava M, Shvarzvadze L, Tsirtsadze T. Regression of liver fibrosis over a 24-week period after completing direct-acting antiviral therapy in patients with chronic hepatitis C receiving care within the national hepatitis C elimination program in Georgia: results of hepatology clinic HEPA experience. Eur J Gastroenterol Hepatol 2017; 29: 1223-1230 [PMID: 28857900 DOI: 10.1097/MEG.0000000000000964]

Kobayashi Y, Iijima H, Tada T, Kumada T, Yoshida M, Aski T, Nishimura T, Nakano C, Takata R, Yoh K, Ishii A, Takashima T, Sakai Y, Aizawa N, Nishikawa H, Ikeda N, Iwata Y, Eronnito H, Hirota S, Fujimoto I, Nishiguchi S. Changes in liver fibrosis and steatosis among patients with hepatitis C virus infection who received direct-acting antiviral therapy and achieved sustained virological response. J Hepatol 2018; 69: 546-551 [PMID: 29493553 DOI: 10.1016/j.jhep.2018.05.001]

Akhane T, Kurzak M, Akhata J, Tuqui K, Ijoko K. The Impact of Virological Response on the Kinetics of Steatosis and Fibrosis Decline in Patients with Chronic Hepatitis C after Direct Antiviral Therapy. J Viral Hepatol 2018; 25: 1221-1228.e1 [PMID: 18843414 DOI: 10.1111/jvh.12270]

Deterding K, Höner Zu Siederdissen C, Port K, Solbak P, Sollik L, Kirschner J, Mix C, Cornberg J, Höner Zu Siederdissen C, Deterding K. Improvement of liver function parameters in patients with chronic hepatitis C receiving daclatasvir/asoaprevir for hepatitis C genotype 1b infection. Clin Gastroenterol Hepatol 2019; 17: 1320-1330 [PMID: 30411079 DOI: 10.1016/j.cgh.2018.02.048]

Facciorusso A, Loomba R, Falck-Ytter YT. Magnitude and Kinetics of Decrease in Liver Stiffness After Antiviral Therapy in Patients With Chronic Hepatitis C: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2018; 16: 27-38.e4 [PMID: 28847904 DOI: 10.1016/j.cgh.2017.04.038]

Bachofner JA, Valli PY, Krieger A, Bergamin I, Künzler P, Baserga A, Brun D, Seifert B, Monceux A, Fehr J, Semela D, Magenta L, Müller-Hartmann B, Zerulli Rebera-Piccoli B, Mertens JC. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. Liver Int 2017; 37: 369-376 [PMID: 27878216 DOI: 10.1111/liv.13256]

Dolmanaslihvi E, Abutidze A, Khakhartishvili N, Karchava M, Shvarzvadze L, Tsirtsadze T. Regression of liver fibrosis over a 24-week period after completing direct-acting antiviral therapy in patients with chronic hepatitis C receiving care within the national hepatitis C elimination program in Georgia: results of hepatology clinic HEPA experience. Eur J Gastroenterol Hepatol 2017; 29: 1223-1230 [PMID: 28857900 DOI: 10.1097/MEG.0000000000000964]

Kobayashi Y, Iijima H, Tada T, Kumada T, Yoshida M, Aski T, Nishimura T, Nakano C, Takata R, Yoh K, Ishii A, Takashima T, Sakai Y, Aizawa N, Nishikawa H, Ikeda N, Iwata Y, Eronnito H, Hirota S, Fujimoto I, Nishiguchi S. Changes in liver fibrosis and steatosis among patients with hepatitis C virus infection who received direct-acting antiviral therapy and achieved sustained virological response. J Hepatol 2018; 69: 546-551 [PMID: 29493553 DOI: 10.1016/j.jhep.2018.05.001]

Akhane T, Kurzak M, Akhata J, Tuqui K, Ijoko K. The Impact of Virological Response on the Kinetics of Steatosis and Fibrosis Decline in Patients with Chronic Hepatitis C after Direct Antiviral Therapy. J Viral Hepatol 2018; 25: 1221-1228.e1 [PMID: 18843414 DOI: 10.1111/jvh.2018.02.048]
Yatsuhashi H. 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: 1563-1570 [PMID: 25042054 DOI: 10.1002/hep.27305]

Yasui Y, Kurosaki M, Komiyama Y, Takada H, Tamaki N, Watakabe K, Okada M, Wang W, Shimizu T, Kubota Y, Higuchi M, Takaura K, Tsuchiya K, Nakanishi H, Takahashi Y, Itakura J, Enomoto N, Izumi N. 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. *Hepatol Res* 2018; 48: 1131-1139 [PMID: 30030872 DOI: 10.1111/hepr.13233]
