Original Research

Association of Splenic MR Elastographic Findings With Gastroesophageal Varices in Patients With Chronic Liver Disease

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Purpose: To identify magnetic resonance imaging (MRI)-based parameters associated with gastroesophageal varices (GEVs) in patients with chronic liver disease.

Materials and Methods: Ninety-three patients were divided into three groups based on endoscopic findings: group 1 with no GEVs ($n=49$), group 2 with mild GEVs ($n=30$), and group 3 with severe GEVs ($n=14$). We used a multivariate logistic regression analysis to assess liver stiffness, aspartate aminotransferase-to-platelet ratio index, spleen stiffness and volume, portal vein velocity, cross-sectional area, and flow volumes potential independent associators of any (mild and severe) GEVs or severe GEVs.

Results: The analysis showed that spleen and liver stiffness and spleen volume were independently associated with any GEVs (spleen stiffness, odds ratio [95% confidence interval], 1.25 [1.04–1.68], $P=0.018$; liver stiffness, 1.52 [1.13–2.17], $P=0.006$; spleen volume, 1.01 [1.00–1.01], $P=0.016$), whereas spleen stiffness was associated with severe GEVs (1.82 [1.25–2.95]; $P=0.005$).

Conclusion:: Liver and spleen stiffness and spleen volume are associated with GEVs in patients with chronic liver disease. Compared with liver stiffness and spleen volume, spleen stiffness is more strongly associated with severe GEVs.

Key Words: MR elastography; liver cirrhosis; portal hypertension; spleen stiffness; phase-contrast MRI
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GASTROESOPHAGEAL VARICES (GEVs) and GEV-related bleeding are severe complications of portal hypertension (PH) in cirrhosis (1). Screening is indicated in patients with newly diagnosed cirrhosis, and medical treatment must be considered as soon as varices are detected to prevent the first bleeding (2). Upper endoscopy is the first-line investigation to identify GEVs. The examination itself is not associated with serious complications but, if it has to be repeated during follow-up in cirrhotic patients, it is time-consuming and costly. Therefore, a number of noninvasive imaging and biochemical methods for the assessment of GEVs have been developed. In particular, ultrasound techniques for the measurement of liver stiffness (LS) (3), spleen diameter (4,5), portal vein (PV) flow (6,7), and hepatic venous waveforms (8) as well as for the morphological assessment of the portal venous system (7) have been investigated and validated. Other noninvasive methods using computed tomography and magnetic resonance imaging (MRI) have also been reported (9,10).

Liver fibrosis plays a fundamental role in the development of PH in patients with cirrhosis. Therefore, patients at a high risk of GEVs can be identified using the same parameters as those for evaluating liver fibrosis, including LS (measured by elastography) and blood biochemical indices (eg, the aspartate aminotransferase [AST]-to-platelet ratio index [APRI]) (11). It has been previously shown that LS, measured using ultrasound transient elastography, correlates with the hepatic vein pressure gradient (12).

Splenomegaly also plays an important role in the pathophysiology of PH by increasing splanchnic inflow (13). Recently, spleen stiffness (SS) assessed with ultrasound transient elastography has been shown to be a more effective parameter for evaluating the hepatic vein pressure gradient and GEVs than LS (14,15).

LS can be evaluated by magnetic resonance elastography (MRE), which has been recently shown to have considerably high reproducibility, repeatability, and validity for assessing liver fibrosis (16–19). MRE can also be used to evaluate SS (20). Recent studies with animal models have revealed that SS measured by MRE is well correlated with the hepatic venous

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Table 1
Acquisition Parameters for MRE and Phase-Contrast MRI

Parameters	MR elastography	Phase-contrast MRI
Sequence	Gradient echo	Gradient echo
TR/TE (msec)	100/27	12.5/5.0
Flip angle (degrees)	30	20
Slice thickness (mm)	10	10
FOV (cm)	30–34 × 40–45	40 × 28
Matrix	256 × 64	256 × 128
Number of slices	2	1
Other	Frequency of pneumatic driver: 60 Hz Phase offsets: 4	VENC parallel to flow direction: 30 cm/s No. of phases: 32

TR/TE = repetition time/echo time, FOV = field of view, VENC = velocity encoding.

pressure gradient and can be a better predictor of PH than LS (21,22). It is hypothesized that increased PH may directly reflect SS, whereas LS represents both liver fibrosis and portal pressure. As with ultrasound-based portal flow measurement (6,7), another MRI-based approach for evaluating PH and GEVs is phase-contrast MRI (PC-MRI). PV flow measured by PC-MRI was shown to correlate with PH and to be useful in predicting GEVs (23,24).

Thus, the aim of this study was to identify useful MRI parameters that are associated with the severity of GEVs in patients with chronic liver disease (CLD).

MATERIALS AND METHODS

Subjects

This retrospective study was approved by our Institutional Review Board. Between September 2011 and March 2012, 210 patients with CLD (age range, 27–87 years; mean and standard deviation, 68 ± 11 years; male-to-female ratio, 138:82) underwent MRE of the liver and spleen and PC-MRI of the PV in a single MRI examination, which was performed as a routine clinical practice (eg, search for hepatocellular carcinoma). Patients who met at least one of the following criteria were excluded from the study ($n=117$): 1) no upper endoscopic examination within 6 months preceding the MRI examination ($n=86$); 2) a history of interventional treatment for GEVs, including endoscopic ligation ($n=13$) and balloon-occluded retrograde transvenous obliteration ($n=2$) that might have altered the hemodynamics of the portal venous system; 3) failure of MRE image due to high iron deposition ($n=2$ for the liver; $n=2$ for the spleen); 4) failure of PC-MRI due to motion artifacts ($n=7$); 5) presence of a PV thrombus confirmed by contrast-enhanced computed tomography ($n=2$) or a history of partial splenic embolization ($n=1$); or 6) a history of lobectomy or more than one segmentectomy of the liver ($n=2$). After exclusion, the study cohort comprised 93 patients with CLD, all of whom underwent at least one upper endoscopic examination within 6 months of the MRI examination. The mean and standard deviation of the time between MRI and upper endoscopy was 1.4 ± 2.7 months; range, −3.3 to 5.9 months (negative value indicates that MRI was performed followed by upper endoscopy). We included some patients who underwent upper endoscopy after MRI examination because endoscopic examinations were scheduled as a clinical follow-up for the development of GEVs and we were not able to properly coordinate and control the schedule of examination.

The types and distribution of CLD were as follows: hepatitis C virus infection ($n=59$), hepatitis B virus infection ($n=12$), alcoholic liver disease ($n=6$), autoimmune liver disease ($n=3$), and others ($n=13$). Patient characteristics were as follows: age, 69 ± 8 years; body mass index (BMI), 20.8 ± 7 kg/m^2; male-to-female ratio, 59:34; and Child-Pugh classes A ($n=74$), B ($n=17$), and C ($n=2$). Liver tissue samples were available for pathological assessment of liver fibrosis in 36 of 93 patients (31 biopsy and 5 surgically resected samples). Twelve patients had mild bridging fibrosis, 10 had moderate bridging fibrosis, and 14 had cirrhosis.

Reference Standards for GEVs

All upper endoscopic examinations were performed by gastroenterologists who specialized in endoscopy. They recorded the presence or absence of GEVs and, if present, noted their forms and any red-color signs, according to the previously described guidelines (25). GEVs were categorized as straight and small (F1), moderately enlarged and beady (F2), or markedly enlarged with a nodular or tumor-like shape (F3). Patients were divided into three groups according to the types of GEVs and red-color signs: 1) a group with no GEVs ($n=49$); 2) a group with mild GEVs (grade F1 and no red-color sign; $n=30$); and 3) a group with severe GEVs (grades F2–F3 or the presence of the red-color sign; $n=14$). Interventional treatment was recommended for patients with severe GEVs.

MRE and Stiffness Measurement

MRI examination was performed in all subjects after fasting for at least 3 hours to avoid the confounding effects of an overestimated stiffness value (26) and increased mesenteric blood flow after a meal (27). A 1.5 T MRI scanner (Signa, GE Healthcare, Milwaukee, WI) was used for all MRI tests. MRE was performed using a cylindrical passive driver to deliver transcostal vibrations, which was placed across the patient's right anterior chest wall for LS measurement and across the left posterolateral chest wall for SS measurement (28). The generator, placed outside the MRI examination room, produced a pneumatic vibration that was delivered to the passive driver via a plastic cylinder. The generator and passive driver were developed at the Mayo Clinic (Rochester, MN).

A breath-hold 2D gradient-echo MRE sequence was used to acquire two axial wave images. Our imaging parameters for MRE are shown in Table 1. The total acquisition time was 64 seconds (four 16-sec breath-

holds). The same MRE parameters were used for the liver and spleen. The MRI scanner automatically generated liver and spleen elastograms by processing the acquired propagating shear wave images using a previously described 2D direct inversion algorithm (29). The shear stiffness (kPa) of the tissue was presented as a pixel map.

The regions of interest (ROIs) were selected in the right lobe of the liver and near the surface of the spleen on the respective elastograms. In each ROI, the MRE slice proximal to the center of the passive driver on the chest wall was chosen for evaluation. As a rule, the ROIs were 1.0–1.5 cm^2 in area and placed near the edge of the liver or spleen next to the passive driver, where the penetrating wave was well-visualized and no interference was observed in the phase image. LS and SS were measured separately and independently by two radiologists (with 10 and 12 years of experience in abdominal radiology) who were blinded to clinical data and results of upper endoscopy. They selected two ROIs on the respective elastograms, and the average value of the results was used as the respective reviewer's stiffness values.

PC-MRI and Flow Measurements

Coronal slices (4-mm thickness) of steady-state acquisition images of the portal venous system were obtained for reference. All patients underwent breath-hold 2D PC-MRI for flow measurements in the PV. The slice acquired was a cross-section perpendicular to the flow of the PV. Our imaging parameters for PC-MRI are shown in Table 1. We chose velocity encoding of 30 cm/s for the measurement of the PV flow because the recently reported mean and maximum velocity of the PV were about 10 cm/s and 20 cm/s, respectively (30,31). Images were obtained with prospective cardiac gating using a pulse oximeter probe placed on the finger tip. The ROI was selected by a radiologist (7 years of experience in abdominal radiology) first on the magnitude image, and then it was copied onto the phase image. The mean velocity (V, cm/s), area (S, mm^2), and flow volume (Q, mL/min) of the PV were calculated using a workstation software (Advantage Workstation, GE Healthcare). The flow volume per minute was calculated by integrating over the flow volume during a cardiac cycle and multiplying by the number of heartbeats.

Splenic Volume Measurement

Splenomegaly has been observed as a major manifestation of PH and evaluated by a clinical manipulation and various cross-sectional imaging methods. We measured the spleen volume because of its possible association with the presence of GEVs; an abdominal radiologist who was blinded to clinical data and imaging findings measured the spleen volume in all subjects on fast spin echo T2-weighted images (imaging parameters were as follows: TR/TE 8000/65 msec, field of view 32 × 28, imaging matrix 256 × 192, slice thickness of 6 mm [no intersection gap], echo train length of 16), which was performed as a routine MRI examination in our institution. Spleen volume was estimated by using the following formula (32):

$$\text{spleen volume} = 0.524 \times W \times T \times L \ (cm^3)$$

where W and T are the maximum width (cm) and thickness (cm) of the spleen measured on the maximum cross-section, respectively, and L is the craniocaudal length (cm) of the spleen.

Biochemical Marker of Liver Fibrosis

The APRI was used as a blood-based marker of liver fibrosis. It was calculated using the following formula (11):

$$\text{APRI} = (\text{AST level}/\text{upper limit of AST}/\text{platelet count}/10^6 \times 100)$$

where the upper limit of AST was set at 32 IU/L, which was the reference value of our institutional laboratory. AST increases and platelet count decreases as hepatic fibrosis develops; therefore, the APRI can amplify the reverse relationship between the stage of liver fibrosis and AST level and platelet count (11).

Statistical Analysis

Interreader variability of the LS and SS values measured by the two reviewers was assessed using Pearson's correlation coefficient analysis. Both measurements showed good interreader reproducibility; therefore, we used an average value of the two results as a respective liver and spleen stiffness value in the subsequent analysis.

A univariate analysis in all study groups was performed using the Kruskal–Wallis test or chi-square test for patient characteristics (age, sex, BMI, and Child-Pugh grade), LS (using MRE and APRI), SS (using MRE), spleen volume, and flow-related parameters. A multivariate analysis was performed to discriminate any GEVs from no GEVs and severe GEVs from no GEVs or mild GEVs using a logistic regression model. Before the multivariate analysis, collinearity was assessed by calculating the correlation coefficient between the variables. A receiver operating characteristic (ROC) analysis was performed to estimate the efficacy of discrimination. The cutoff value for liver and spleen stiffness values were defined by the maximum positive and negative likelihood ratios. All statistical analyses were performed using the JMP software v. 9 (SAS Institute Japan, Tokyo, Japan) with the statistical significance for all analyses set at $P < 0.05$.

RESULTS

Patient Characteristics

No statistically significant differences in age, BMI, sex, or Child-Pugh grade were found between the three groups (Table 2).

Interreader Variability

Pearson's correlation coefficients of LS and SS measurements were 0.872 and 0.898, respectively.

Table 2
Comparison of Clinical Characteristics and Measured Parameters Between the Study Groups

	No GEVs $n=49$	Mild GEVs $n=30$	Severe GEVs $n=14$	P value
Age (years)	68 ± 8	70 ± 9	71 ± 6	0.261
M:F	34:15	19:11	6:8	0.713
BMI (kg/m^2)	19 ± 6	23 ± 8	18 ± 7	0.206
Child-Pugh A/B/C	42/7/0	22/7/1	10/3/1	0.362
MRE				
Liver stiffness (kPa)	4.0 ± 1.4	5.6 ± 2.3	6.1 ± 1.6	0.001
Spleen stiffness (kPa)	6.5 ± 2.1	8.5 ± 2.9	9.8 ± 1.7	0.001
APRI	1.4 ± 2.1	3.8 ± 2.4	2.0 ± 0.5	0.001
PC-MRI				
V_{PV} (cm/s)	8.6 ± 1.5	7.9 ± 1.8	7.5 ± 1.8	0.047
S_{PV} (mm^2)	135 ± 42	160 ± 51	147 ± 55	0.067
Q_{PV} (mL/min)	691 ± 264	765 ± 334	631 ± 298	0.515
Spleen volume (cm^3)	157 ± 76	240 ± 128	240 ± 81	0.005

Values are shown as the mean ± standard deviation.
GEV = gastroesophageal varices, BMI = body mass index, V_{PV} = mean portal vein velocity, S_{PV} = portal vein cross-sectional area, Q_{PV} = portal vein flow volume, APRI = aspartate aminotransferase-to-platelet ratio index.

Therefore, we used an average value of the two reviewer's results as a respective liver and spleen stiffness value in the subsequent analysis.

Results of Each Variable: Univariate Analysis

There were significant differences between the three groups in LS, APRI, and spleen volume. A significant difference was also observed in SS measurements, with the highest SS observed in the groups with severe GEVs (Table 2, Fig. 1). Of the PV flow measurements, only mean velocity (V_{PV}) showed a significant difference between the three groups.

Independent Associations: Multivariate Analysis

Moderate collinearity was observed between SS and spleen volume (correlation coefficient, 0.64) but not between any of the other parameters in a multiple regression model. In the multivariate analysis for discriminating any GEVs (mild or severe) from no GEVs, if both SS and spleen volume were included in a model, neither parameter showed a significant association ($P > 0.05$). When SS and spleen volume were included in a model separately, both parameters showed significant associations with the presence of any GEVs ($P = 0.018$ for SS, and 0.016 for spleen volume). Therefore, we chose both parameters as independent associators. LS, SS, and spleen volume showed independent associations with odds ratios (ORs) of 1.52, 1.35, and 1.01, respectively (Table 3). Areas under the ROC curve (AUC) for predicting any GEVs were 0.75 for LS, 0.76 for SS, and 0.73 for spleen volume. In the multivariate analysis for discriminating severe GEVs from no GEVs or mild GEVs, only SS showed a significant association (OR, 1.82) with an AUC of 0.81 (Table 3). Whether spleen volume was included or excluded, this result was stable. MRE of the liver and spleen and endoscopic findings of representative cases from the three groups are shown in Fig. 2.

Cutoff Values

Cutoff values with the maximum positive likelihood ratio for discriminating any GEVs from no GEVs were

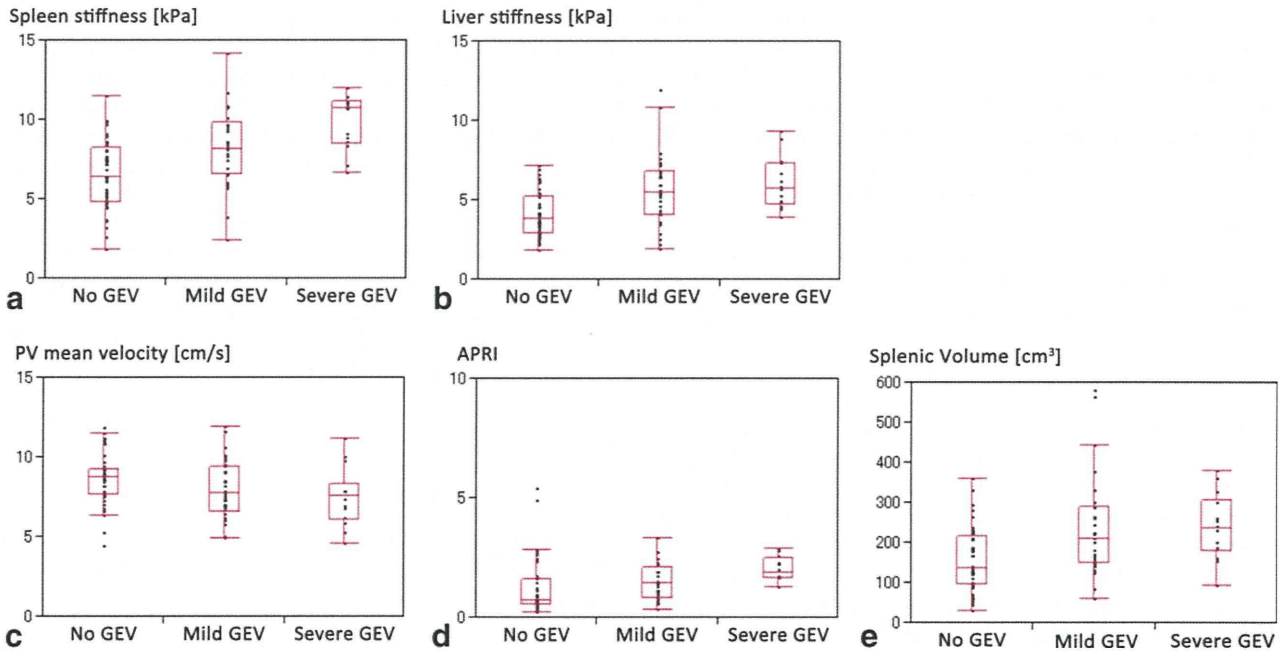

Figure 1. Box-and-whiskers plots of (a) spleen stiffness, (b) liver stiffness, (c) mean portal vein velocity, (d) APRI, and (e) spleen volume. The middle line in the box shows the median, the bottom and top lines of the box show the 25th and 75th percentiles, and the upper and lower whiskers show the minimum and maximum values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3
Multivariate Analysis for Discrimination of Any GEVs From No GEVs and Severe GEVs From No or Mild GEVs

	Diagnosis of any (mild and severe) GEVs			Diagnosis of severe GEVs		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Spleen stiffness (kPa)	1.25	1.04–1.68	0.018	1.82	1.25–2.95	0.005
Liver stiffness (kPa)	1.52	1.13–2.17	0.006	1.42	0.96–2.61	0.078
V_{PV} (cm/s)	0.77	0.56–1.05	0.106	0.69	0.44–1.08	0.144
APRI	0.99	0.78–1.26	0.919	0.84	0.42–1.14	0.254
Spleen volume (cm^3)	1.01	1.00–1.01	0.016	0.99	0.98–1.01	0.809

GEVs = gastroesophageal varices, V_{PV} = mean portal vein velocity, OR = odds ratio, CI = confidence interval, APRI = aspartate aminotransferase platelet ratio index.

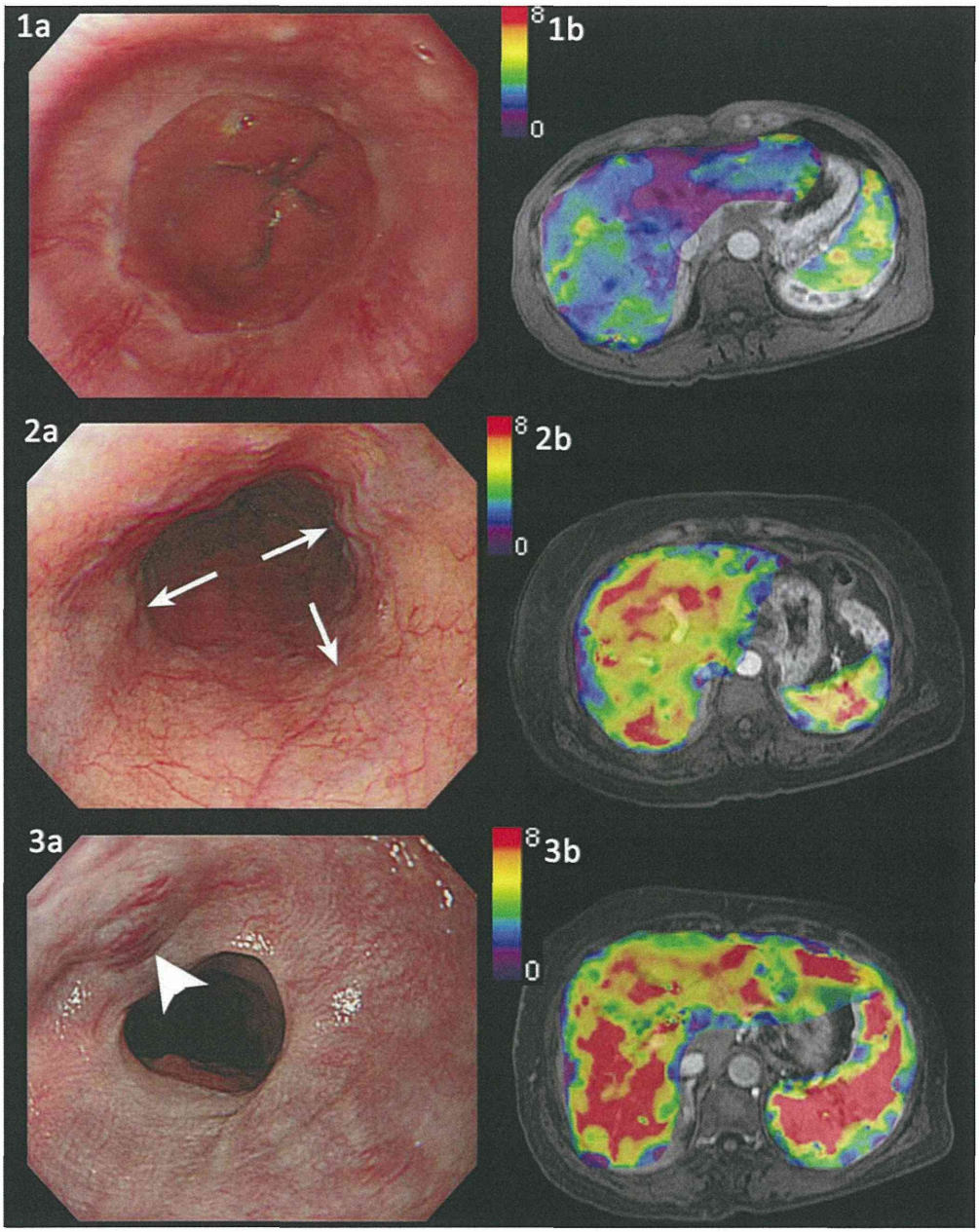

Figure 2. Case 1: A 53-year-old man with CLD without any GEVs (**1a**); liver and spleen elastograms superimposed on a contrast-enhanced MR image (**1b**) show low values for both liver (2.7 kPa) and spleen stiffness (4.7 kPa). Case 2: A 73-year-old woman with CLD with mild GEVs (**2a**, arrows); liver and spleen elastograms superimposed on a contrast-enhanced MR image (**2b**) show a high value for liver stiffness (6.4 kPa), but spleen stiffness remains low (6.6 kPa). Case 3: A 75-year-old woman with CLD with severe GEVs accompanied by the red-color sign (**3a**, arrowhead); liver and spleen elastograms superimposed on a contrast-enhanced MR image (**3b**) show high values for both liver (7.3 kPa) and spleen stiffness (12.0 kPa).

Table 4
Liver and Spleen Stiffness Cut-Off Values for Discriminating Any GEVs From No GEVs and Severe GEVs From No or Mild GEVs

Cut-off value	Sensitivity	Specificity	PPV	NPV	LR+	LR–
Diagnosis of any (mild and severe) GEVs						
Spleen stiffness						
10.1 kPa	34% (15/44)	98% (48/49)	94% (15/16)	62% (48/77)	16.70	1.48
5.6 kPa	95% (42/44)	41% (20/49)	59% (42/71)	91% (20/22)	1.61	8.98
Liver stiffness						
6.7 kPa	29% (13/44)	96% (47/49)	86% (13/15)	60% (47/78)	7.23	1.36
3.4 kPa	91% (40/44)	35% (17/49)	55% (40/72)	81% (17/21)	1.39	3.81
Diagnosis of severe GEVs						
Spleen stiffness						
10.8 kPa	43% (6/14)	95% (75/79)	60% (6/10)	90% (75/83)	8.46	1.66
7.1 kPa	93% (13/14)	45% (35/79)	23% (13/57)	97% (35/36)	1.67	6.20
Liver stiffness						
8.8 kPa	14% (2/14)	98% (77/79)	50%(2/4)	87% (77/89)	5.64	1.14
4.4 kPa	93% (13/14)	52% (41/79)	25% (13/51)	98% (41/42)	1.93	7.27

PPV = positive predictive value, NPV = negative predictive value, LR+ = positive likelihood ratio, LR– = negative likelihood ratio.

10.1 kPa for SS and 6.7 kPa for LS. These cutoff values provided positive predictive values of 94% and 86% for SS and LS, respectively. Maximum negative likelihood ratios were observed with the cutoff values of 5.6 kPa for SS and 3.4 kPa for LS, which resulted in negative predictive values of 91% and 81%, respectively (Table 4).

To distinguish severe GEVs from no or mild GEVs, the maximum positive likelihood ratios were 10.8 kPa for SS and 8.8 kPa for LS, whereas the maximum negative likelihood ratios were obtained with a cutoff value of 7.1 kPa for SS and 4.4 kPa for LS (Table 4).

DISCUSSION

Various factors associated with the development and progression of GEVs have been suggested in patients with cirrhosis and chronic hepatitis (33). Among those factors, liver fibrosis grade, which has been reported to be correlated with LS measured by MRE (19,34), is an important marker of PH and GEVs. In our study, both markers of liver fibrosis, LS and the APRI, were correlated with the severity of GEVs. LS was an independent predictor of mild-to-severe GEVs, which is consistent with the previous studies using ultrasound elastography (3,35) and MR elastography (24). The APRI was not a significant indicator of GEVs, which is also consistent with a recent study (36). Meanwhile, it has been recently reported that the hepatic venous pressure gradient is better correlated with SS than with LS measured by transient elastography (14). The results of the multivariate analyses in our study are in line with the previous reports. Although the precise mechanisms leading to spleen enlargement in PH are poorly understood, splenic congestion has been suggested to play a major role in increasing spleen volume, which is observed in neoangiogenesis and lymphoid hyperplasia in the spleen (13,37). The reason why the stiffness is increased in PH can be explained by increased tissue perfusion; a recent study using a pig model of renal ischemia suggested that decreased perfusion reduced tissue stiffness (38). In a previous study, the mean SS measured by MRE in healthy volunteers was 4.2 kPa (28). In our study, the SS in patients with CLD without GEVs was higher (6.7 kPa) than the previously reported data, indicating that SS might increase in the precirrhotic stage. Although SS was more reliable than the other MRI-based parameters in the assessment of GEVs in our study, a significant overlap between the three groups in SS value was observed. Therefore, we conclude that SS alone cannot be used as an indicator of GEVs and more reliable noninvasive methods should be developed and validated.

We also demonstrated that spleen volume was significantly different among the three groups studied. In the multivariate analysis, spleen volume showed significant association with the presence of any GEVs, but no significant association with severe GEVs. A recent study showed that spleen volume combined with a biochemical marker (eg, platelet count and/or albumin) can predict the presence of varices in patients with CLD (39). Meanwhile, in pediatric patients with extrahepatic portal vein obstruction, spleen volume does not correlate with the grade of varices (40). We speculate that spleen volume will increase with PH development; however, it might not be useful by itself for the assessment of severe GEVs.

We showed excellent interreader variability for both LS and SS measurements. Of these, the reproducibility of LS was consistent with a recent report (41). To our best knowledge, the reproducibility of SS measurement has not been reported so far. Although we did not assess the repeatability of stiffness measurements in our study, a previous report showed excellent repeatability of MRE of the liver. (17,18,41). In our institution, clinical technicians are proficient in the MRE technique and all procedures are conducted without complications. MRE is associated with only slight pneumatic vibration and rarely causes subjective discomfort or pain. Recently, gadoxetic acid-enhanced MRI has become a popular method in patients with cirrhosis to assess hepatocellular carcinoma and a 20-minute waiting period for hepatocyte phase image is sufficient to obtain an MRE image. In

our opinion, MRE of the liver and spleen is well-tolerated by almost all patients and should be implemented as part of a routine MRI examination.

Ultrasound parameters such as LS, SS, and spleen size have been widely investigated and reported to be reliable indicators of GEVs (3–8). Ultrasound methods are widely available and inexpensive but they are operator-dependent and less sensitive, especially in obese patients.

In this study we did not assess the visually detectable GEVs on routine MRI because the focus of this study was also on quantitative parameters rather than qualitative assessments, which might be inferior in reproducibility. Furthermore, our study cohort comprised a heterogeneous group in which both patients with and without contrast-enhanced MRI were included. This heterogeneity of contrast-enhanced MRI might influence reliability of diagnostic performance of visually detectable GEVs on MRI (10). In simple assessment by one author of this study, visually detectable GEVs on routine MRI, which was defined as dilated luminal vessels abutting or protruding into the luminal space (23), were observed in three patients with mild GEVs, nine patients with severe GEVs, and not observed in no GEVs. Sensitivity and specificity were 27% (12/44), 100% (49/49) for any GEVs, and 64% (9/14), 96% (76/79) for severe GEVs, respectively. These results of visual assessment were comparable with the results of SS.

PV flow measurements by PC-MRI and duplex Doppler ultrasound in the previous studies showed inconclusive results for assessing PH and GEVs (23,42). The results of our study also suggest that PV flow measurements are less promising for predicting GEVs than SS. This may be due to complex contributing factors such as meal consumption prior to examination, cardiac output, and breath-holding (43), in addition to the inherent instability in measurements by 2D PC-MRI. The recently developed time-resolved 3D PC-MRI or 4D PC-MRI may provide a more comprehensive assessment of PV flow dynamics (44).

Our study has several limitations. First, the cutoff values were based on the retrospective patient cohort; therefore, they could not be directly extrapolated to clinical practice, and further prospective studies are needed to validate these values for the prediction of GEVs. Second, there is no available reference standard for SS or PH. Therefore, the validity and robustness of the SS measurement could not be verified. Third, the time between MRI and upper endoscopy was relatively long and differed between patients, which might have produced biased results. Finally, we could not set the endpoint of bleeding from GEVs, which is the most important clinical outcome. Prospective observation should be conducted to elucidate the utility of noninvasive functional MRI parameters in clinical settings.

In conclusion, MRE of the liver and spleen, and spleen volume measured on routine MRI, are useful for predicting GEVs in patients with CLD. Spleen stiffness is a more reliable predictor of severe GEVs than liver stiffness and spleen volume.

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Original Research

Association of Splenic MR Elastographic Findings With Gastroesophageal Varices in Patients With Chronic Liver Disease

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Purpose: To identify magnetic resonance imaging (MRI)-based parameters associated with gastroesophageal varices (GEVs) in patients with chronic liver disease.

Materials and Methods: Ninety-three patients were divided into three groups based on endoscopic findings: group 1 with no GEVs ($n=49$), group 2 with mild GEVs ($n=30$), and group 3 with severe GEVs ($n=14$). We used a multivariate logistic regression analysis to assess liver stiffness, aspartate aminotransferase-to-platelet ratio index, spleen stiffness and volume, portal vein velocity, cross-sectional area, and flow volumes potential independent associators of any (mild and severe) GEVs or severe GEVs.

Results: The analysis showed that spleen and liver stiffness and spleen volume were independently associated with any GEVs (spleen stiffness, odds ratio [95% confidence interval], 1.25 [1.04–1.68], $P=0.018$; liver stiffness, 1.52 [1.13–2.17], $P=0.006$; spleen volume, 1.01 [1.00–1.01], $P=0.016$), whereas spleen stiffness was associated with severe GEVs (1.82 [1.25–2.95]; $P=0.005$).

Conclusion:: Liver and spleen stiffness and spleen volume are associated with GEVs in patients with chronic liver disease. Compared with liver stiffness and spleen volume, spleen stiffness is more strongly associated with severe GEVs.

Key Words: MR elastography; liver cirrhosis; portal hypertension; spleen stiffness; phase-contrast MRI
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GASTROESOPHAGEAL VARICES (GEVs) and GEV-related bleeding are severe complications of portal hypertension (PH) in cirrhosis (1). Screening is indicated in patients with newly diagnosed cirrhosis, and medical treatment must be considered as soon as varices are detected to prevent the first bleeding (2). Upper endoscopy is the first-line investigation to identify GEVs. The examination itself is not associated with serious complications but, if it has to be repeated during follow-up in cirrhotic patients, it is time-consuming and costly. Therefore, a number of noninvasive imaging and biochemical methods for the assessment of GEVs have been developed. In particular, ultrasound techniques for the measurement of liver stiffness (LS) (3), spleen diameter (4,5), portal vein (PV) flow (6,7), and hepatic venous waveforms (8) as well as for the morphological assessment of the portal venous system (7) have been investigated and validated. Other noninvasive methods using computed tomography and magnetic resonance imaging (MRI) have also been reported (9,10).

Liver fibrosis plays a fundamental role in the development of PH in patients with cirrhosis. Therefore, patients at a high risk of GEVs can be identified using the same parameters as those for evaluating liver fibrosis, including LS (measured by elastography) and blood biochemical indices (eg, the aspartate aminotransferase [AST]-to-platelet ratio index [APRI]) (11). It has been previously shown that LS, measured using ultrasound transient elastography, correlates with the hepatic vein pressure gradient (12).

Splenomegaly also plays an important role in the pathophysiology of PH by increasing splanchnic inflow (13). Recently, spleen stiffness (SS) assessed with ultrasound transient elastography has been shown to be a more effective parameter for evaluating the hepatic vein pressure gradient and GEVs than LS (14,15).

LS can be evaluated by magnetic resonance elastography (MRE), which has been recently shown to have considerably high reproducibility, repeatability, and validity for assessing liver fibrosis (16–19). MRE can also be used to evaluate SS (20). Recent studies with animal models have revealed that SS measured by MRE is well correlated with the hepatic venous

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Table 1
Acquisition Parameters for MRE and Phase-Contrast MRI

Parameters	MR elastography	Phase-contrast MRI
Sequence	Gradient echo	Gradient echo
TR/TE (msec)	100/27	12.5/5.0
Flip angle (degrees)	30	20
Slice thickness (mm)	10	10
FOV (cm)	30–34 × 40–45	40 × 28
Matrix	256 × 64	256 × 128
Number of slices	2	1
Other	Frequency of pneumatic driver: 60 Hz Phase offsets: 4	VENC parallel to flow direction: 30 cm/s No. of phases: 32

TR/TE = repetition time/echo time, FOV = field of view, VENC = velocity encoding.

pressure gradient and can be a better predictor of PH than LS (21,22). It is hypothesized that increased PH may directly reflect SS, whereas LS represents both liver fibrosis and portal pressure. As with ultrasound-based portal flow measurement (6,7), another MRI-based approach for evaluating PH and GEVs is phase-contrast MRI (PC-MRI). PV flow measured by PC-MRI was shown to correlate with PH and to be useful in predicting GEVs (23,24).

Thus, the aim of this study was to identify useful MRI parameters that are associated with the severity of GEVs in patients with chronic liver disease (CLD).

MATERIALS AND METHODS

Subjects

This retrospective study was approved by our Institutional Review Board. Between September 2011 and March 2012, 210 patients with CLD (age range, 27–87 years; mean and standard deviation, 68 ± 11 years; male-to-female ratio, 138:82) underwent MRE of the liver and spleen and PC-MRI of the PV in a single MRI examination, which was performed as a routine clinical practice (eg, search for hepatocellular carcinoma). Patients who met at least one of the following criteria were excluded from the study ($n=117$): 1) no upper endoscopic examination within 6 months preceding the MRI examination ($n=86$); 2) a history of interventional treatment for GEVs, including endoscopic ligation ($n=13$) and balloon-occluded retrograde transvenous obliteration ($n=2$) that might have altered the hemodynamics of the portal venous system; 3) failure of MRE image due to high iron deposition ($n=2$ for the liver; $n=2$ for the spleen); 4) failure of PC-MRI due to motion artifacts ($n=7$); 5) presence of a PV thrombus confirmed by contrast-enhanced computed tomography ($n=2$) or a history of partial splenic embolization ($n=1$); or 6) a history of lobectomy or more than one segmentectomy of the liver ($n=2$). After exclusion, the study cohort comprised 93 patients with CLD, all of whom underwent at least one upper endoscopic examination within 6 months of the MRI examination. The mean and standard deviation of the time between MRI and upper endoscopy was 1.4 ± 2.7 months; range, −3.3 to 5.9 months (negative value indicates that MRI was performed followed by upper endoscopy). We included some patients who underwent upper endoscopy after MRI examination because endoscopic examinations were scheduled as a clinical follow-up for the development of GEVs and we were not able to properly coordinate and control the schedule of examination.

The types and distribution of CLD were as follows: hepatitis C virus infection ($n=59$), hepatitis B virus infection ($n=12$), alcoholic liver disease ($n=6$), autoimmune liver disease ($n=3$), and others ($n=13$). Patient characteristics were as follows: age, 69 ± 8 years; body mass index (BMI), 20.8 ± 7 kg/m^2; male-to-female ratio, 59:34; and Child-Pugh classes A ($n=74$), B ($n=17$), and C ($n=2$). Liver tissue samples were available for pathological assessment of liver fibrosis in 36 of 93 patients (31 biopsy and 5 surgically resected samples). Twelve patients had mild bridging fibrosis, 10 had moderate bridging fibrosis, and 14 had cirrhosis.

Reference Standards for GEVs

All upper endoscopic examinations were performed by gastroenterologists who specialized in endoscopy. They recorded the presence or absence of GEVs and, if present, noted their forms and any red-color signs, according to the previously described guidelines (25). GEVs were categorized as straight and small (F1), moderately enlarged and beady (F2), or markedly enlarged with a nodular or tumor-like shape (F3). Patients were divided into three groups according to the types of GEVs and red-color signs: 1) a group with no GEVs ($n=49$); 2) a group with mild GEVs (grade F1 and no red-color sign; $n=30$); and 3) a group with severe GEVs (grades F2–F3 or the presence of the red-color sign; $n=14$). Interventional treatment was recommended for patients with severe GEVs.

MRE and Stiffness Measurement

MRI examination was performed in all subjects after fasting for at least 3 hours to avoid the confounding effects of an overestimated stiffness value (26) and increased mesenteric blood flow after a meal (27). A 1.5 T MRI scanner (Signa, GE Healthcare, Milwaukee, WI) was used for all MRI tests. MRE was performed using a cylindrical passive driver to deliver transcostal vibrations, which was placed across the patient's right anterior chest wall for LS measurement and across the left posterolateral chest wall for SS measurement (28). The generator, placed outside the MRI examination room, produced a pneumatic vibration that was delivered to the passive driver via a plastic cylinder. The generator and passive driver were developed at the Mayo Clinic (Rochester, MN).

A breath-hold 2D gradient-echo MRE sequence was used to acquire two axial wave images. Our imaging parameters for MRE are shown in Table 1. The total acquisition time was 64 seconds (four 16-sec breath-

holds). The same MRE parameters were used for the liver and spleen. The MRI scanner automatically generated liver and spleen elastograms by processing the acquired propagating shear wave images using a previously described 2D direct inversion algorithm (29). The shear stiffness (kPa) of the tissue was presented as a pixel map.

The regions of interest (ROIs) were selected in the right lobe of the liver and near the surface of the spleen on the respective elastograms. In each ROI, the MRE slice proximal to the center of the passive driver on the chest wall was chosen for evaluation. As a rule, the ROIs were 1.0–1.5 cm^2 in area and placed near the edge of the liver or spleen next to the passive driver, where the penetrating wave was well-visualized and no interference was observed in the phase image. LS and SS were measured separately and independently by two radiologists (with 10 and 12 years of experience in abdominal radiology) who were blinded to clinical data and results of upper endoscopy. They selected two ROIs on the respective elastograms, and the average value of the results was used as the respective reviewer's stiffness values.

PC-MRI and Flow Measurements

Coronal slices (4-mm thickness) of steady-state acquisition images of the portal venous system were obtained for reference. All patients underwent breath-hold 2D PC-MRI for flow measurements in the PV. The slice acquired was a cross-section perpendicular to the flow of the PV. Our imaging parameters for PC-MRI are shown in Table 1. We chose velocity encoding of 30 cm/s for the measurement of the PV flow because the recently reported mean and maximum velocity of the PV were about 10 cm/s and 20 cm/s, respectively (30,31). Images were obtained with prospective cardiac gating using a pulse oximeter probe placed on the finger tip. The ROI was selected by a radiologist (7 years of experience in abdominal radiology) first on the magnitude image, and then it was copied onto the phase image. The mean velocity (V, cm/s), area (S, mm^2), and flow volume (Q, mL/min) of the PV were calculated using a workstation software (Advantage Workstation, GE Healthcare). The flow volume per minute was calculated by integrating over the flow volume during a cardiac cycle and multiplying by the number of heartbeats.

Splenic Volume Measurement

Splenomegaly has been observed as a major manifestation of PH and evaluated by a clinical manipulation and various cross-sectional imaging methods. We measured the spleen volume because of its possible association with the presence of GEVs; an abdominal radiologist who was blinded to clinical data and imaging findings measured the spleen volume in all subjects on fast spin echo T2-weighted images (imaging parameters were as follows: TR/TE 8000/65 msec, field of view 32 × 28, imaging matrix 256 × 192, slice thickness of 6 mm [no intersection gap], echo train length of 16), which was performed as a routine MRI examination in our institution. Spleen volume was estimated by using the following formula (32):

$$\text{spleen volume} = 0.524 \times W \times T \times L \ (cm^3)$$

where W and T are the maximum width (cm) and thickness (cm) of the spleen measured on the maximum cross-section, respectively, and L is the craniocaudal length (cm) of the spleen.

Biochemical Marker of Liver Fibrosis

The APRI was used as a blood-based marker of liver fibrosis. It was calculated using the following formula (11):

$$\text{APRI} = (\text{AST level}/\text{upper limit of AST}/\text{platelet count}/10^6 \times 100)$$

where the upper limit of AST was set at 32 IU/L, which was the reference value of our institutional laboratory. AST increases and platelet count decreases as hepatic fibrosis develops; therefore, the APRI can amplify the reverse relationship between the stage of liver fibrosis and AST level and platelet count (11).

Statistical Analysis

Interreader variability of the LS and SS values measured by the two reviewers was assessed using Pearson's correlation coefficient analysis. Both measurements showed good interreader reproducibility; therefore, we used an average value of the two results as a respective liver and spleen stiffness value in the subsequent analysis.

A univariate analysis in all study groups was performed using the Kruskal–Wallis test or chi-square test for patient characteristics (age, sex, BMI, and Child-Pugh grade), LS (using MRE and APRI), SS (using MRE), spleen volume, and flow-related parameters. A multivariate analysis was performed to discriminate any GEVs from no GEVs and severe GEVs from no GEVs or mild GEVs using a logistic regression model. Before the multivariate analysis, collinearity was assessed by calculating the correlation coefficient between the variables. A receiver operating characteristic (ROC) analysis was performed to estimate the efficacy of discrimination. The cutoff value for liver and spleen stiffness values were defined by the maximum positive and negative likelihood ratios. All statistical analyses were performed using the JMP software v. 9 (SAS Institute Japan, Tokyo, Japan) with the statistical significance for all analyses set at $P < 0.05$.

RESULTS

Patient Characteristics

No statistically significant differences in age, BMI, sex, or Child-Pugh grade were found between the three groups (Table 2).

Interreader Variability

Pearson's correlation coefficients of LS and SS measurements were 0.872 and 0.898, respectively.

Table 2
Comparison of Clinical Characteristics and Measured Parameters Between the Study Groups

	No GEVs $n=49$	Mild GEVs $n=30$	Severe GEVs $n=14$	P value
Age (years)	68 ± 8	70 ± 9	71 ± 6	0.261
M:F	34:15	19:11	6:8	0.713
BMI (kg/m^2)	19 ± 6	23 ± 8	18 ± 7	0.206
Child-Pugh A/B/C	42/7/0	22/7/1	10/3/1	0.362
MRE				
Liver stiffness (kPa)	4.0 ± 1.4	5.6 ± 2.3	6.1 ± 1.6	0.001
Spleen stiffness (kPa)	6.5 ± 2.1	8.5 ± 2.9	9.8 ± 1.7	0.001
APRI	1.4 ± 2.1	3.8 ± 2.4	2.0 ± 0.5	0.001
PC-MRI				
V_{PV} (cm/s)	8.6 ± 1.5	7.9 ± 1.8	7.5 ± 1.8	0.047
S_{PV} (mm^2)	135 ± 42	160 ± 51	147 ± 55	0.067
Q_{PV} (mL/min)	691 ± 264	765 ± 334	631 ± 298	0.515
Spleen volume (cm^3)	157 ± 76	240 ± 128	240 ± 81	0.005

Values are shown as the mean ± standard deviation.
GEV = gastroesophageal varices, BMI = body mass index, V_{PV} = mean portal vein velocity, S_{PV} = portal vein cross-sectional area, Q_{PV} = portal vein flow volume, APRI = aspartate aminotransferase-to-platelet ratio index.

Therefore, we used an average value of the two reviewer's results as a respective liver and spleen stiffness value in the subsequent analysis.

Results of Each Variable: Univariate Analysis

There were significant differences between the three groups in LS, APRI, and spleen volume. A significant difference was also observed in SS measurements, with the highest SS observed in the groups with severe GEVs (Table 2, Fig. 1). Of the PV flow measurements, only mean velocity (V_{PV}) showed a significant difference between the three groups.

Independent Associations: Multivariate Analysis

Moderate collinearity was observed between SS and spleen volume (correlation coefficient, 0.64) but not between any of the other parameters in a multiple regression model. In the multivariate analysis for discriminating any GEVs (mild or severe) from no GEVs, if both SS and spleen volume were included in a model, neither parameter showed a significant association ($P > 0.05$). When SS and spleen volume were included in a model separately, both parameters showed significant associations with the presence of any GEVs ($P = 0.018$ for SS, and 0.016 for spleen volume). Therefore, we chose both parameters as independent associators. LS, SS, and spleen volume showed independent associations with odds ratios (ORs) of 1.52, 1.35, and 1.01, respectively (Table 3). Areas under the ROC curve (AUC) for predicting any GEVs were 0.75 for LS, 0.76 for SS, and 0.73 for spleen volume. In the multivariate analysis for discriminating severe GEVs from no GEVs or mild GEVs, only SS showed a significant association (OR, 1.82) with an AUC of 0.81 (Table 3). Whether spleen volume was included or excluded, this result was stable. MRE of the liver and spleen and endoscopic findings of representative cases from the three groups are shown in Fig. 2.

Cutoff Values

Cutoff values with the maximum positive likelihood ratio for discriminating any GEVs from no GEVs were

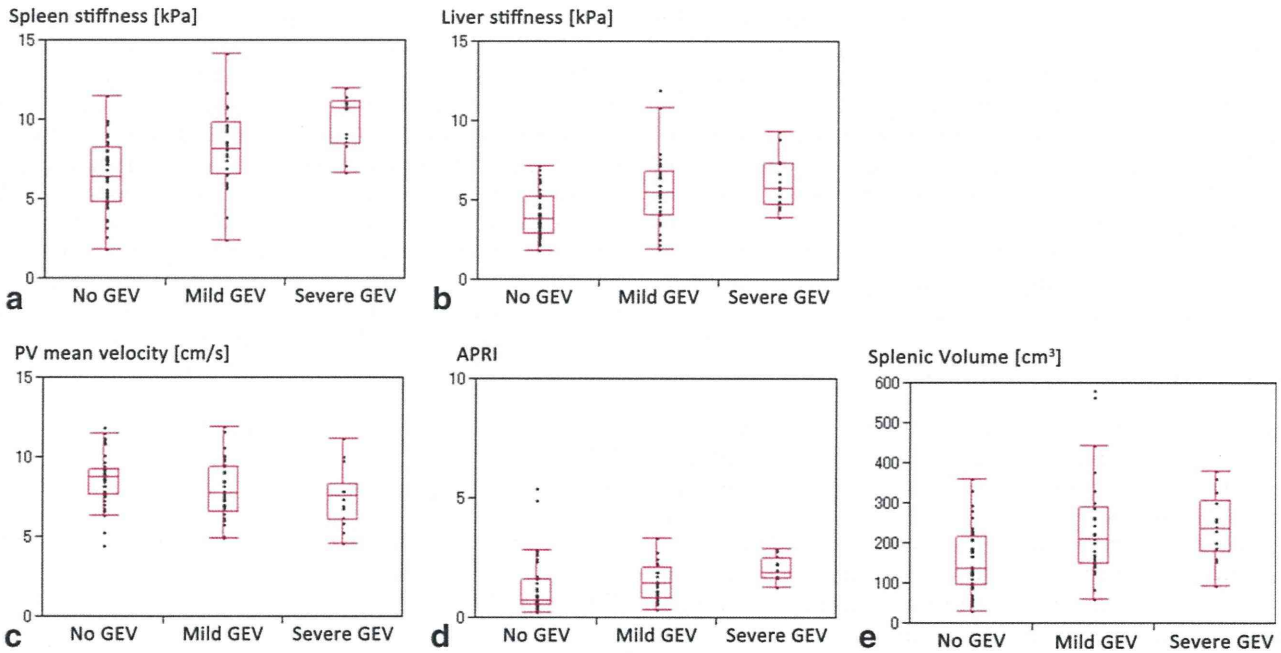

Figure 1. Box-and-whiskers plots of (a) spleen stiffness, (b) liver stiffness, (c) mean portal vein velocity, (d) APRI, and (e) spleen volume. The middle line in the box shows the median, the bottom and top lines of the box show the 25th and 75th percentiles, and the upper and lower whiskers show the minimum and maximum values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3
Multivariate Analysis for Discrimination of Any GEVs From No GEVs and Severe GEVs From No or Mild GEVs

	Diagnosis of any (mild and severe) GEVs			Diagnosis of severe GEVs		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Spleen stiffness (kPa)	1.25	1.04–1.68	0.018	1.82	1.25–2.95	0.005
Liver stiffness (kPa)	1.52	1.13–2.17	0.006	1.42	0.96–2.61	0.078
V_{PV} (cm/s)	0.77	0.56–1.05	0.106	0.69	0.44–1.08	0.144
APRI	0.99	0.78–1.26	0.919	0.84	0.42–1.14	0.254
Spleen volume (cm^3)	1.01	1.00–1.01	0.016	0.99	0.98–1.01	0.809

GEVs = gastroesophageal varices, V_{PV} = mean portal vein velocity, OR = odds ratio, CI = confidence interval, APRI = aspartate aminotransferase platelet ratio index.

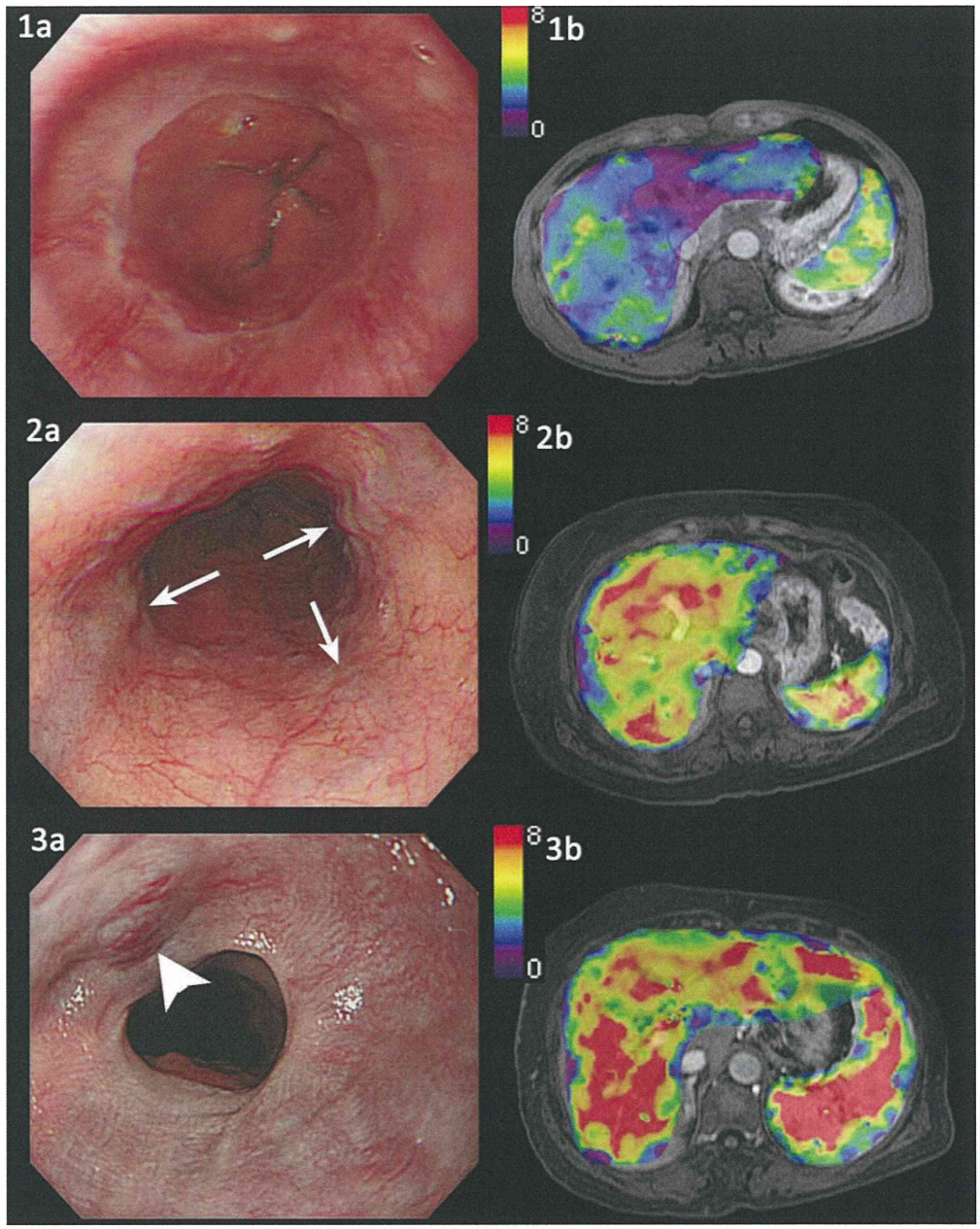

Figure 2. Case 1: A 53-year-old man with CLD without any GEVs (**1a**); liver and spleen elastograms superimposed on a contrast-enhanced MR image (**1b**) show low values for both liver (2.7 kPa) and spleen stiffness (4.7 kPa). Case 2: A 73-year-old woman with CLD with mild GEVs (**2a**, arrows); liver and spleen elastograms superimposed on a contrast-enhanced MR image (**2b**) show a high value for liver stiffness (6.4 kPa), but spleen stiffness remains low (6.6 kPa). Case 3: A 75-year-old woman with CLD with severe GEVs accompanied by the red-color sign (**3a**, arrowhead); liver and spleen elastograms superimposed on a contrast-enhanced MR image (**3b**) show high values for both liver (7.3 kPa) and spleen stiffness (12.0 kPa).

Table 4
Liver and Spleen Stiffness Cut-Off Values for Discriminating Any GEVs From No GEVs and Severe GEVs From No or Mild GEVs

Cut-off value	Sensitivity	Specificity	PPV	NPV	LR+	LR–
Diagnosis of any (mild and severe) GEVs						
Spleen stiffness						
10.1 kPa	34% (15/44)	98% (48/49)	94% (15/16)	62% (48/77)	16.70	1.48
5.6 kPa	95% (42/44)	41% (20/49)	59% (42/71)	91% (20/22)	1.61	8.98
Liver stiffness						
6.7 kPa	29% (13/44)	96% (47/49)	86% (13/15)	60% (47/78)	7.23	1.36
3.4 kPa	91% (40/44)	35% (17/49)	55% (40/72)	81% (17/21)	1.39	3.81
Diagnosis of severe GEVs						
Spleen stiffness						
10.8 kPa	43% (6/14)	95% (75/79)	60% (6/10)	90% (75/83)	8.46	1.66
7.1 kPa	93% (13/14)	45% (35/79)	23% (13/57)	97% (35/36)	1.67	6.20
Liver stiffness						
8.8 kPa	14% (2/14)	98% (77/79)	50%(2/4)	87% (77/89)	5.64	1.14
4.4 kPa	93% (13/14)	52% (41/79)	25% (13/51)	98% (41/42)	1.93	7.27

PPV = positive predictive value, NPV = negative predictive value, LR+ = positive likelihood ratio, LR– = negative likelihood ratio.

10.1 kPa for SS and 6.7 kPa for LS. These cutoff values provided positive predictive values of 94% and 86% for SS and LS, respectively. Maximum negative likelihood ratios were observed with the cutoff values of 5.6 kPa for SS and 3.4 kPa for LS, which resulted in negative predictive values of 91% and 81%, respectively (Table 4).

To distinguish severe GEVs from no or mild GEVs, the maximum positive likelihood ratios were 10.8 kPa for SS and 8.8 kPa for LS, whereas the maximum negative likelihood ratios were obtained with a cutoff value of 7.1 kPa for SS and 4.4 kPa for LS (Table 4).

DISCUSSION

Various factors associated with the development and progression of GEVs have been suggested in patients with cirrhosis and chronic hepatitis (33). Among those factors, liver fibrosis grade, which has been reported to be correlated with LS measured by MRE (19,34), is an important marker of PH and GEVs. In our study, both markers of liver fibrosis, LS and the APRI, were correlated with the severity of GEVs. LS was an independent predictor of mild-to-severe GEVs, which is consistent with the previous studies using ultrasound elastography (3,35) and MR elastography (24). The APRI was not a significant indicator of GEVs, which is also consistent with a recent study (36). Meanwhile, it has been recently reported that the hepatic venous pressure gradient is better correlated with SS than with LS measured by transient elastography (14). The results of the multivariate analyses in our study are in line with the previous reports. Although the precise mechanisms leading to spleen enlargement in PH are poorly understood, splenic congestion has been suggested to play a major role in increasing spleen volume, which is observed in neoangiogenesis and lymphoid hyperplasia in the spleen (13,37). The reason why the stiffness is increased in PH can be explained by increased tissue perfusion; a recent study using a pig model of renal ischemia suggested that decreased perfusion reduced tissue stiffness (38). In a previous study, the mean SS measured by MRE in healthy volunteers was 4.2 kPa (28). In our study, the SS in patients with CLD without GEVs was higher (6.7 kPa) than the previously reported data, indicating that SS might increase in the precirrhotic stage. Although SS was more reliable than the other MRI-based parameters in the assessment of GEVs in our study, a significant overlap between the three groups in SS value was observed. Therefore, we conclude that SS alone cannot be used as an indicator of GEVs and more reliable noninvasive methods should be developed and validated.

We also demonstrated that spleen volume was significantly different among the three groups studied. In the multivariate analysis, spleen volume showed significant association with the presence of any GEVs, but no significant association with severe GEVs. A recent study showed that spleen volume combined with a biochemical marker (eg, platelet count and/or albumin) can predict the presence of varices in patients with CLD (39). Meanwhile, in pediatric patients with extrahepatic portal vein obstruction, spleen volume does not correlate with the grade of varices (40). We speculate that spleen volume will increase with PH development; however, it might not be useful by itself for the assessment of severe GEVs.

We showed excellent interreader variability for both LS and SS measurements. Of these, the reproducibility of LS was consistent with a recent report (41). To our best knowledge, the reproducibility of SS measurement has not been reported so far. Although we did not assess the repeatability of stiffness measurements in our study, a previous report showed excellent repeatability of MRE of the liver. (17,18,41). In our institution, clinical technicians are proficient in the MRE technique and all procedures are conducted without complications. MRE is associated with only slight pneumatic vibration and rarely causes subjective discomfort or pain. Recently, gadoxetic acid-enhanced MRI has become a popular method in patients with cirrhosis to assess hepatocellular carcinoma and a 20-minute waiting period for hepatocyte phase image is sufficient to obtain an MRE image. In

our opinion, MRE of the liver and spleen is well-tolerated by almost all patients and should be implemented as part of a routine MRI examination.

Ultrasound parameters such as LS, SS, and spleen size have been widely investigated and reported to be reliable indicators of GEVs (3–8). Ultrasound methods are widely available and inexpensive but they are operator-dependent and less sensitive, especially in obese patients.

In this study we did not assess the visually detectable GEVs on routine MRI because the focus of this study was also on quantitative parameters rather than qualitative assessments, which might be inferior in reproducibility. Furthermore, our study cohort comprised a heterogeneous group in which both patients with and without contrast-enhanced MRI were included. This heterogeneity of contrast-enhanced MRI might influence reliability of diagnostic performance of visually detectable GEVs on MRI (10). In simple assessment by one author of this study, visually detectable GEVs on routine MRI, which was defined as dilated luminal vessels abutting or protruding into the luminal space (23), were observed in three patients with mild GEVs, nine patients with severe GEVs, and not observed in no GEVs. Sensitivity and specificity were 27% (12/44), 100% (49/49) for any GEVs, and 64% (9/14), 96% (76/79) for severe GEVs, respectively. These results of visual assessment were comparable with the results of SS.

PV flow measurements by PC-MRI and duplex Doppler ultrasound in the previous studies showed inconclusive results for assessing PH and GEVs (23,42). The results of our study also suggest that PV flow measurements are less promising for predicting GEVs than SS. This may be due to complex contributing factors such as meal consumption prior to examination, cardiac output, and breath-holding (43), in addition to the inherent instability in measurements by 2D PC-MRI. The recently developed time-resolved 3D PC-MRI or 4D PC-MRI may provide a more comprehensive assessment of PV flow dynamics (44).

Our study has several limitations. First, the cutoff values were based on the retrospective patient cohort; therefore, they could not be directly extrapolated to clinical practice, and further prospective studies are needed to validate these values for the prediction of GEVs. Second, there is no available reference standard for SS or PH. Therefore, the validity and robustness of the SS measurement could not be verified. Third, the time between MRI and upper endoscopy was relatively long and differed between patients, which might have produced biased results. Finally, we could not set the endpoint of bleeding from GEVs, which is the most important clinical outcome. Prospective observation should be conducted to elucidate the utility of noninvasive functional MRI parameters in clinical settings.

In conclusion, MRE of the liver and spleen, and spleen volume measured on routine MRI, are useful for predicting GEVs in patients with CLD. Spleen stiffness is a more reliable predictor of severe GEVs than liver stiffness and spleen volume.

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Original Article

Clinical efficacy of combination therapy with ME3738 and pegylated interferon-alpha-2a in patients with hepatitis C virus genotype 1

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Aim: ME3738, a derivative of soyasapogenol B, enhances the anti-hepatitis C virus (HCV) effect of interferon in an *in vitro* replication system and an *in vivo* mouse model of HCV infection. ME3738 plus pegylated interferon (PEG IFN)-α-2a treatment for 12 weeks decreased HCV RNA levels in enrolled late virus responder (LVR) patients with relapsed HCV. Half of the patients reached undetectable HCV RNA level. The present clinical study of ME3738 was conducted in naïve chronic hepatitis C patients to investigate the sustained virological response (SVR) and safety of 48-week treatment with ME3738 plus PEG IFN-α-2a.

Methods: Subjects (n = 135) with genotype 1b chronic hepatitis C with high viral loads were divided into three groups (ME3738 50 mg b.i.d., 200 mg b.i.d. or 800 mg b.i.d.). ME3738 was administrated p.o. and PEG IFN-α-2a (180 μg/week) s.c. for 48 weeks, and SVR was assessed at 24 weeks of treatment-free follow up.

Results: The viral disappearance rates at 12 and 48 weeks were 23.0% and 48.9%, respectively. SVR was seen in 5.9% of subjects. ME3738 did not worsen the adverse reactions generally seen with PEG IFN-α-2a treatment, and any adverse reactions specific to ME3738 were not observed.

Conclusion: ME3738 plus PEG IFN-α-2a treatment to naïve chronic hepatitis C patients showed an antiviral effect and a good safety profile up to 48 weeks. However, HCV RNA was again detected in many subjects after treatment termination. Even though ME3738 is not enough to suppress HCV reproduction in this treatment. ME3738 was concurrently used with PEG IFN-α-2a treatment; however, a clear additional effect on SVR was not confirmed.

Key words: clinical efficacy, hepatitis C virus, ME3738, pegylated interferon-alpha-2a

INTRODUCTION

HEPATITIS C VIRUS (HCV) causes chronic hepatitis through persistent infection and is a major cause of liver cancer, particularly in Japan.[1,2] The standard therapy for chronic hepatitis C is co-administration of pegylated interferon (PEG IFN) and ribavirin (RBV), with a reported efficacy rate of approximately 50% in refractory cases with genotype 1b and high levels of plasma HCV RNA.[3] In Japan, many patients with chronic hepatitis C are elderly at present, and this standard therapy in these patients can result in unavoidable treatment discontinuation, low therapeutic effect due to a high incidence of adverse reactions, the need for dose reduction of PEG IFN because of decreased neutrophil count, or the need for dose reduction of RBV because of anemia.[4,5]

ME3738 is a derivative of soyasapogenol B derived from soybeans and ME3738 has been shown to

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suppress liver inflammation in various hepatitis mouse models.[6,7] In patients with chronic hepatitis C with elevated alanine aminotransferase (ALT) values, ME3738 monotherapy showed a tendency toward suppression of liver inflammation, and the only major adverse reactions were digestive symptoms and abnormal laboratory data such as 10% of the patients enrolled in frequency.

ME3738 has also shown to enhance the suppressive effect of IFN on HCV in an *in vitro* replication system of HCV and in an *in vivo* HCV-infected mouse model (chimeric mice).[8,9]

A clinical study of ME3738 was conducted in patients with chronic hepatitis C to confirm viral disappearance with 12 weeks of treatment.[10] Patients were those with chronic hepatitis C who had received combination therapy with PEG IFN-α-2b and RBV. Among the patients who achieved viral disappearance after 12–24 weeks of treatment (late virus responders: LVR), those who showed viral reactivation after 48 weeks of treatment were enrolled.

ME3738 was administrated p.o. twice a day at a dose of 50 mg/day or 800 mg/day, and PEG IFN-α-2a was administrated s.c. at a dose of 180 μg once a week. This combination therapy was administrated for 12 weeks. In all subjects enrolled in this study, viral disappearance had not been achieved by previous therapy (co-administration of PEG IFN-α-2b and RBV) within 12 weeks of treatment (HCV RNA, ≥1.7 log IU/mL). On the other hand, when ME3738 was co-administrated, viral disappearance was achieved in approximately 50% of subjects within 12 weeks of treatment. Therefore, it was suggested that ME3738 co-administrated with PEG IFN would contribute to early viral disappearance.

The present clinical study of ME3738 was conducted in naïve chronic hepatitis C patients to investigate the sustained virological response (SVR) and safety of 48-week treatment with ME3738 plus PEG IFN-α-2a.

METHODS

Study design and dosing

THIS STUDY WAS conducted as a multicenter, randomized, single-blind, comparative phase II study (protocol no. ME3738-11).

ME3738 used in the study was provided by Meiji Seika Pharma (Tokyo, Japan).

ME3738 doses were selected as 50 mg/day and 800 mg/day, the safety of which was confirmed in a previous study using 12-week combined administration. In addition, 200 mg/day was selected as an intermediate dose to confirm a dose-dependent effect on SVR, and a total of three doses were selected.

Subjects were divided into three groups, with ME3738 administrated p.o. twice a day (after breakfast and dinner) at a dose of 50 mg/day, 200 mg/day or 800 mg/day. PEG IFN-α-2a 180 μg was administrated s.c. to all subjects once a week. This combination therapy was administrated for 48 weeks. Before starting treatment, subjects were assigned to each of the three groups at the central registration center, so that the number of subjects in each group was identical.

Patients were assigned doses based on an allocation chart that was preliminarily prepared in a central registration center (Moss Institute), which was a party independent of the investigative group.

In subjects who achieved disappearance of HCV RNA at the completion of the 48-week treatment (end of treatment: EOT), follow-up observation was performed for an additional 24 weeks (24-week follow-up after EOT) to confirm SVR.

During the study period, blood cell counts were measured every week before administration of PEG IFN-α-2a, and the dose of PEG IFN-α-2a was reduced to 90 μg or withdrawn according to the following criteria:

- Dose reduction: neutrophil count less than 750/mm^3 or platelet count less than 50 000/mm^3
- Dose withdrawal (suspension): neutrophil count less than 500/mm^3, platelet count less than 25 000/mm^3 or hemoglobin less than 8.5 g/dL.

The following study discontinuation criteria were set. When the HCV RNA determined after 12 weeks of treatment was not lower than the baseline level by at least 2.0 Log IU/mL, the study was to be discontinued in the subject concerned. When HCV RNA was detected after 48 weeks of treatment or during the follow-up period, the study was also to be discontinued in the subject concerned at the time point of detection.

Subjects

The study population included patients with naïve chronic hepatitis C.

The target patients were aged 20 years or more, with HCV genotype 1b and a high viral load (≥5.0 log IU/mL).

Patients meeting the following conditions were excluded from this study: patients positive for hepatitis B surface antigen, patients with hepatitis other than chronic hepatitis C, patients with liver cirrhosis or liver cancer, patients with a FibroIndex[11] not less than 2.25 (F4), and patients with a baseline neutrophil count less than 1500/mm^3, a baseline platelet count less

than 90 000/mm^3, or a baseline hemoglobin value less than 10 g/dL. Patients with a complication or anamnesis that could possibly influence the safety or efficacy of ME3738 were also excluded. Concomitant drugs or therapies that could possibly influence the safety and efficacy of ME3738, such as systemic antiviral drug and double-membrane filtration plasmapheresis, were also prohibited during the study period.

This study was conducted in accordance with Good Clinical Practice guidelines, conforming to the Declaration of Helsinki. Written informed consent was obtained from all patients before their enrollment in this study, which was conducted with the approval of the institutional review board of each study site.

Safety and efficacy assessments

Assessments were performed at baseline before starting the study; after 2, 4, 8 and 12 weeks of treatment; every 4 weeks thereafter; at EOT; and during the follow-up period. Assessments included inquiries, vital sign measurements, laboratory tests, adverse event surveys and HCV RNA determinations.

When any adverse event was noted, follow-up examinations were performed in the subject concerned and, if necessary, after study completion.

The amount of HCV RNA during the study period was determined by the TaqMan polymerase chain reaction (PCR) method.

At each determination time point, the disappearance of HCV RNA was judged when no signal was detected with the TaqMan-PCR method. Efficacy was assessed as the HCV disappearance rate after 12 weeks of treatment and at week 24 of the follow-up period. When "HCV RNA undetectability" was maintained until week 24 of the follow-up period, SVR was judged in the subject concerned.

The 16 subjects who participated in the extended treatment study (study ME3738-12) after receiving the treatment in this study were tabulated as subjects without SVR.

Mutations of the HCV core protein (amino acid [a.a.]70 and a.a.91), mutations of the IFN-sensitivity determining region (ISDR) and single nucleotide polymorphisms (SNP) of the *IL28B* gene (five sites: rs8103142, rs11881222, rs8099917, rs12980275 and rs12979860) were determined as background factors of the subjects.

Statistical analysis

Background factors were tabulated by each treatment group, and the intergroup bias was assessed using Student's *t*-test or one-way ANOVA for continuance data and Fisher's exact test for categorical data. A two-sided significance level of 5% was adopted.

In the efficacy evaluation, the rate of subjects with SVR was calculated for each treatment group, and the intergroup difference and the 95% confidence interval was determined. In the safety evaluation, adverse events and adverse reactions were tabulated for each treatment group to determine the incidence rate. The Medical Dictionary for Regulatory Activities (MedDRA) ver. 14.0 was used for tabulation of adverse events.

RESULTS

OF THE 135 subjects (male, 49; female, 86) included in the study, 46 were in the ME3738 50-mg/day group, 45 in the ME3738 200-mg/day group and 44 in the ME3738 800-mg/day group. The mean age was 57.6 years (range, 23–76) with elderly subjects over 60 years occupying more than half of the study population. As for sex, elderly women were dominant.

As per the protocol, 24 subjects in whom the viral amount did not decrease from the baseline level by at least 2 log IU/mL after 12 weeks of treatment were discontinued from the study. An additional three subjects were discontinued for other reasons, resulting in a total of 27 subjects being withdrawn from the study. As a result, study treatment was continued in 108 subjects after 12 weeks of treatment.

Of these 108 subjects, 16 subjects were discontinued from the study for various reasons, including occurrence of an adverse event. A total of 92 subjects completed the 48-week treatment. Among the subjects in whom HCV RNA was positive after 12 weeks of treatment and negative conversion was achieved with 36 weeks of treatment (LVR), 16 subjects from whom consent was obtained participated in the extended treatment study (study ME3738-12) and received 24 weeks of extended treatment in continuation from the 48-week treatment in this study.

Therefore, the follow-up observation was performed in 47 subjects who achieved viral disappearance after 48 weeks of treatment and did not participate in the extended treatment study, and SVR was judged in eight of those subjects after 24 weeks of follow-up observation (Fig. 1).

The major patient background factors showed no biases among the three treatment groups (ME3738 50-mg/day, ME3738 200-mg/day and ME3738 800-mg/day groups) (Table 1). SNP of the *IL28B* gene were determined in 126 subjects who provided consent for

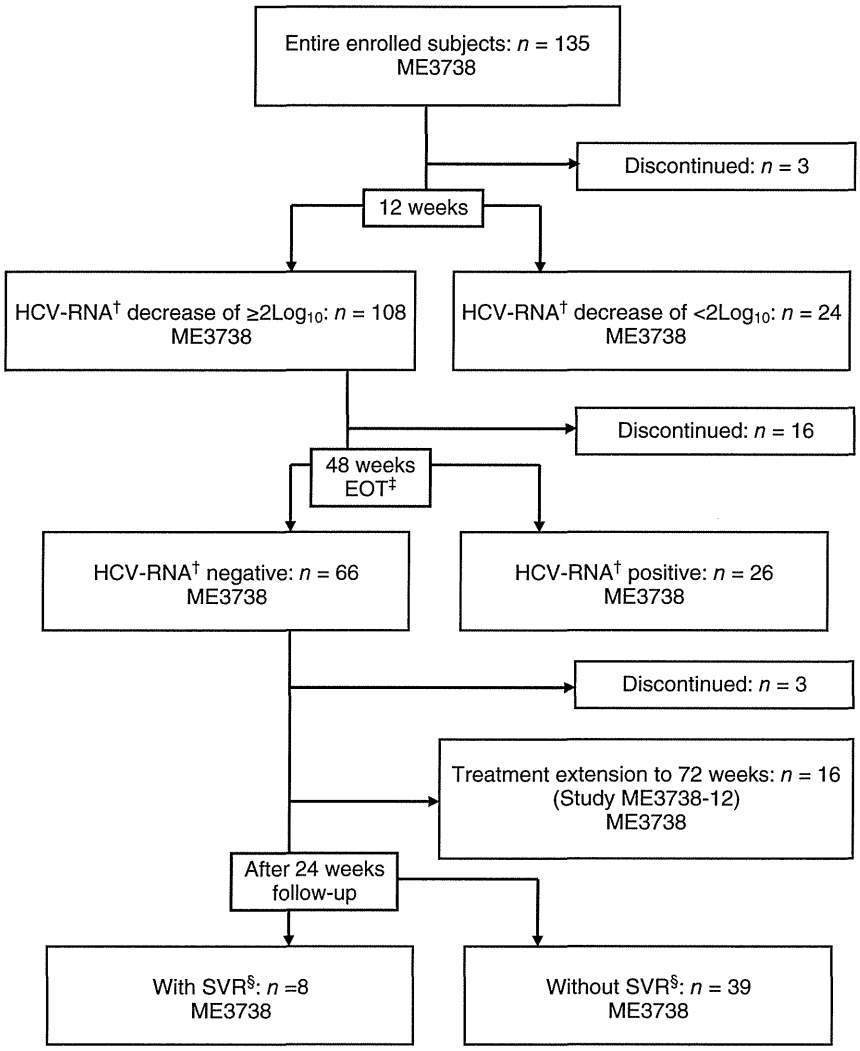

Figure 1 Subject disposition. †Hepatitis C virus. ‡End of treatment. §Sustained virological response.

the analysis. Among the 126 subjects, the result for rs12980275 was different from the results of the other four SNP in three subjects, whereas the results of all five SNP were coincident in the remaining 123 subjects. Even in the three subjects in whom the result for rs12980275 was different, the results for the other four SNP were coincident, allowing the coincident results of four SNP to be included in the tabulation.

The SNP of *IL28B* determined in this study were a major homo allele in 68.1% (92/126) of the subjects, a minor hetero allele in 24.4% (33/126) of the subjects and a minor homo allele in 0.7% (1/126) of the subjects. These rates were similar to the results previously reported in Japan.[11]

Efficacy

Table 2 shows the virological effects at each stage of this study. The viral disappearance rate indicating the proportion of subjects who achieved viral disappearance was 5.9% (8/135) after 4 weeks of treatment, 23.0% (31/135) after 12 weeks of treatment and 48.9% (66/135) at EOT. The rate of subjects with SVR was 5.9% (8/135) after 24 weeks of follow-up observation. The viral reactivation rate, meaning the proportion of subjects in whom viral regrowth was observed during the follow-up observation period from among the total subjects included in the follow-up observation, was 83.0% (39/47), and reactivation occurred within 12 weeks of