

# Usefulness of Mac-2 Binding Protein Glycosylation Isomer for Prediction of Posthepatectomy Liver Failure in Patients With Hepatocellular Carcinoma

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**Objective:** The aim of this study was to evaluate the usefulness of the Mac-2 binding protein glycosylation isomer (M2BPGi) for the prediction of posthepatectomy liver failure (PHLF) in hepatocellular carcinoma (HCC) patients.

**Summary Background Data:** M2BPGi is a novel serum marker of liver fibrosis. The usefulness of M2BPGi for the prediction of PHLF has not been evaluated.

**Methods:** Clinicopathological data were analyzed in 138 HCC patients who underwent liver resection between August 2011 and November 2014. PHLF was evaluated according to the definition of the International Study Group of Liver Surgery. Performance of preoperative parameters in predicting PHLF was determined using receiver operating characteristic (ROC) analysis.

**Results:** Serum M2BPGi level correlated with the METAVIR fibrosis score. M2BPGi levels of hepatitis C virus (HCV)-positive patients were significantly higher than those of HCV-negative patients, even in the same fibrosis stage. PHLF  $\geq$  Grade B developed in 19 patients (13.8%). The area under the ROC curve (AUROC) of M2BPGi for the prediction of PHLF  $\geq$  Grade B was 0.71. In multivariate analysis, M2BPGi [odds ratio (OR): 2.08, 95% confidence interval (CI) 1.28–3.55], platelet count (OR: 0.39, 95% CI 0.18–0.80), and resection rate (OR: 2.71, 95% CI 1.46–5.40) were the significant factors associated with PHLF  $\geq$  Grade B. The AUROC of the PHLF index defined by these factors was 0.81. Notably, in patients with HCV infection, the predictive ability of M2BPGi for PHLF (AUROC 0.85) was the best among the preoperative parameters.

**Conclusions:** M2BPGi is a useful predictor of PHLF, especially in patients with HCV infection.

**Keywords:** hepatocellular carcinoma, liver fibrosis, Mac-2 binding protein glycosylation isomer, posthepatectomy liver failure

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Hepatocellular carcinoma (HCC) is one of the most common malignancies throughout the world, and liver resection is the mainstay of treatment leading to long-term survival in selected patients.<sup>1,2</sup> With advancements in perioperative patient care and surgical techniques, together with improvements in patient selection criteria, the operative outcomes of liver resection have improved substantially in recent years. However, there still exists postoperative morbidity and mortality, with one of the common causes of hepatectomy-related mortality being the development of posthepatectomy liver failure (PHLF).<sup>3,4</sup>

The incidence of PHLF has been reported as ranging from 1.2% to 32% in different series.<sup>5–13</sup> This wide range in the frequency of PHLF can be attributed to the lack of a universal definition of PHLF. To establish a standardized definition of PHLF, the International Study Group of Liver Surgery (ISGLS) proposed a definition of PHLF in 2010.<sup>13</sup> Moreover, differences between patient populations may also influence the incidence of PHLF. In contrast to a low incidence of PHLF in patients without chronic liver diseases, the rate of PHLF is relatively high in patients with chronic liver diseases or cirrhosis.<sup>5,8,12</sup> Hence, the precise assessment of liver fibrosis and liver function may assist in the prediction and prevention of PHLF. Several parameters, including fibrotic markers, liver function tests, and imaging modalities, have been analyzed to determine their usefulness for the prediction of PHLF.<sup>14–18</sup> However, precise prediction and prevention of PHLF remains difficult, and a more accurate method for predicting PHLF is needed.

Glycoproteins reflect the status of cells. Recently, a glycan sugar chain based immunoassay, named the Mac-2 binding protein glycosylation isomer (M2BPGi) (Sysmex, Kobe, Japan), was developed as a novel marker for liver fibrosis.<sup>19–21</sup> Recent studies have reported the utility of serum M2BPGi as a predictor for hepatic decompensation and HCC development in patients with chronic liver diseases.<sup>22,23</sup> However, the usefulness of M2BPGi for the prediction of PHLF in the setting of preoperative examination has not been evaluated and remains to be elucidated.

The aim of this study was to evaluate the clinical usefulness of the serum M2BPGi value for the prediction of PHLF in HCC patients undergoing liver resection. Moreover, the predictive validity of PHLF was also evaluated in combination with other perioperative parameters.

## METHODS

We retrospectively collected and analyzed clinicopathological data of 138 patients with HCC who underwent liver resection at Kyoto University Hospital between August 2011 and November 2014, and whose frozen serum samples collected before the surgery were available for the measurement of M2BPGi values. These

patients were part of the study population of our previous prospective study that evaluated the usefulness of preoperative liver stiffness measurement by acoustic radiation force impulse (ARFI) imaging in predicting PHLF (unique trial number: UMIN000007172).<sup>24</sup> Patients who underwent extrahepatic bile duct resection and/or preoperative portal vein embolization were excluded from both studies. A frozen serum sample was collected at the time of admission for the liver resection within 1 week before surgery. M2BPGi values were measured using the HISCL M2BPGi Assay Kit (Sysmex, Kobe, Japan). The cutoff index (COI) of serum M2BPGi was calculated according to the equation reported previously.<sup>19-21</sup>

The clinicopathological data analyzed in the present study included sex, age, hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, liver function indicators [platelet count, international normalized ratio of prothrombin time (PT-INR), total bilirubin, alanine transaminase (ALT), albumin, and ammonia], fibrosis markers (hyaluronic acid and type 4 collagen), the disappearance rate of indocyanine green (KICG), tumor markers (alpha-fetoprotein and protein induced by vitamin K absence or antagonist-2), liver fibrosis indicators [aspartate aminotransferase to platelet ratio index (APRI) and fibrosis (FIB)-4 index], liver stiffness measurement by ARFI imaging, computed tomography for preoperative assessment, type of hepatectomy, and liver resection rate. The total liver volume and estimated resection volume of anatomical resection ( $n = 87$ ) was calculated using computed tomography volumetry with a volume analyzer system (Synapse Vincent, Fujifilm, Japan).<sup>25,26</sup> In non-anatomical minor resection, resection volume was substituted by the weight of the resected specimen ( $n = 51$ ). Resection rate was calculated by resection volume (mL)/total liver volume (mL). The APRI and FIB-4 index were calculated according to the equations reported previously.<sup>27,28</sup> Liver stiffness was evaluated using an ACUSON S2000 (Mochida Siemens Medical Systems, Tokyo, Japan) and expressed as shear wave velocity (Vs), as previously reported.<sup>24</sup> The pathological liver fibrosis stage of the resected specimen was evaluated according to the METAVIR fibrosis score.<sup>29</sup> Oncological tumor status was evaluated radiologically and pathologically on the basis of the general rules for the clinical and pathological study of primary liver cancer.<sup>30</sup>

The study protocol was approved by our institutional ethics committee and was registered with the University Hospital Medical Information Network (unique trial number: UMIN R000021249).

PHLF was diagnosed on the basis of the ISGLS definition.<sup>13</sup> Namely, elevated PT-INR and concomitant hyperbilirubinemia on or after postoperative day 5 was diagnosed as PHLF. The severity of PHLF was graded as follows: Grade A, PHLF that required no change in patient's clinical management; Grade B, PHLF that required a deviation from the regular course but did not require invasive therapy; Grade C, PHLF that required invasive treatment.

### Statistical Analysis

All statistical analyses were performed using SAS software (JMP 11.0.1; SAS Institute, Inc., Cary, NC). Continuous variables were expressed as mean values  $\pm$  standard deviation or medians with ranges, and compared using Student *t* test between PHLF and non-PHLF group. Categorical variables were compared between PHLF and non-PHLF group using the Chi-square test or Fisher exact test as demanded. The difference among the fibrosis stages and between the abutting stages was compared using the Kruskal-Wallis test and the Wilcoxon rank-sum test, respectively.

The predictive values of PHLF  $\geq$  Grade B were assessed using receiver operating characteristic (ROC) analysis, and the area under the ROC curve (AUROC) was calculated. ROC curves were compared using the DeLong test. In multivariate logistic regression analysis, the predictors were selected through a stepwise procedure

using the minimum Bayesian information criterion (BIC) method among the preoperative liver function indicators (platelet count, PT-INR, total bilirubin, ALT, albumin, ammonia), KICG, Vs, hyaluronic acid, M2BPGi, and resection rate. A risk index of PHLF  $\geq$  Grade B was developed as the sum of products of regression coefficients from the final logistic regression model and the predictors in the model. The final logistic regression model included all the selected predictors. Interaction between the variables was tested by Spearman rank correlation coefficient. A *P* value less than 0.05 was considered statistically significant.

## RESULTS

### Patient Characteristics and Tumor Status

Patient characteristics and tumor status are summarized in Table 1. The study population consisted of 114 men and 24 women with a median age of 68 years (range 41 to 89 yrs). Among these patients, 28 were positive for HBV surface antigen, and 53 were positive for HCV antibody. Three patients were positive for both HBV surface antigen and HCV antibody, and 60 were negative for both HBV and HCV infection (non-B non-C). Child-Pugh classification was Grade A in 123 patients and Grade B in 15 patients. There was no patient with Child-Pugh Grade C classification.

The type of hepatectomy was nonanatomical partial resection in 51 patients, segmentectomy in 11, sectionectomy in 29, bisectonectomy in 5, right and left hemihepatectomy in 20 each, and trisectionectomy in 2. The median operative time was 411 minutes (range 120 to 1044 min), with median blood loss of 587 g (range 0 to 8650 g). The median resection rate was 18.6% (range 0.3% to 70.4%). Intraoperative transfusion was performed in 41 patients (30%). Three-month postoperative mortality was 2.2% ( $n = 3$ ).

The median diameter of the largest tumor was 3.5 cm (range 0.8 to 17 cm), and the median tumor number was 1 (range 1 to 7). Tumor differentiation was categorized as well in 14 patients, moderate in 82, poor in 37, and undifferentiated in 2. Tumor differentiation was undetermined in 3 patients due to necrosis induced by preoperative therapy. Portal, venous, arterial, and biliary tract invasions were observed in 18, 6, 1, and 9 patients, respectively. The distribution of tumor stage was I in 33 patients, II in 55, III in 30, IV-A in 16, and IV-B in 4.

The METAVIR fibrosis score of the background liver was F0 in 20 patients, F1 in 17, F2 in 42, F3 in 22 and F4 in 37. PHLF occurred in 34 patients (25%): Grade A in 15 (11%), Grade B in 14 (10%), and Grade C in 5 (4%).

### Performance of M2BPGi as a Marker for Liver Fibrosis

The median COI value of serum M2BPGi was 1.36 (range 0.29 to 14.73). Serum M2BPGi level was positively correlated with the METAVIR fibrosis score (Fig. 1A). The mean COI values were  $0.89 \pm 0.46$ ,  $1.08 \pm 0.53$ ,  $1.56 \pm 0.31$ ,  $2.10 \pm 0.43$ , and  $4.51 \pm 0.33$  in stage F0, F1, F2, F3, and F4 patients, respectively, resulting in statistically significant difference by the Kruskal-Wallis test ( $P < 0.0001$ ). The differences between the abutting stages were significantly between stage F2 and stage F3 ( $P = 0.04$ ), and stage F3 and stage F4 ( $P = 0.007$ ) by the Wilcoxon rank-sum test. The performance of M2BPGi for assessing fibrosis stage was analyzed. The cutoff values of M2BPGi, sensitivities, specificities, positive predictive values, negative predictive values, and AUROC values of each fibrosis stage are summarized in Table 2. The AUROC values of other fibrosis markers or indicators in predicting each fibrosis stage are summarized in Supplemental Table 1, <http://links.lww.com/SLA/B40>. The power of M2BPGi in predicting liver fibrosis was superior or equal to that of the other predictors.

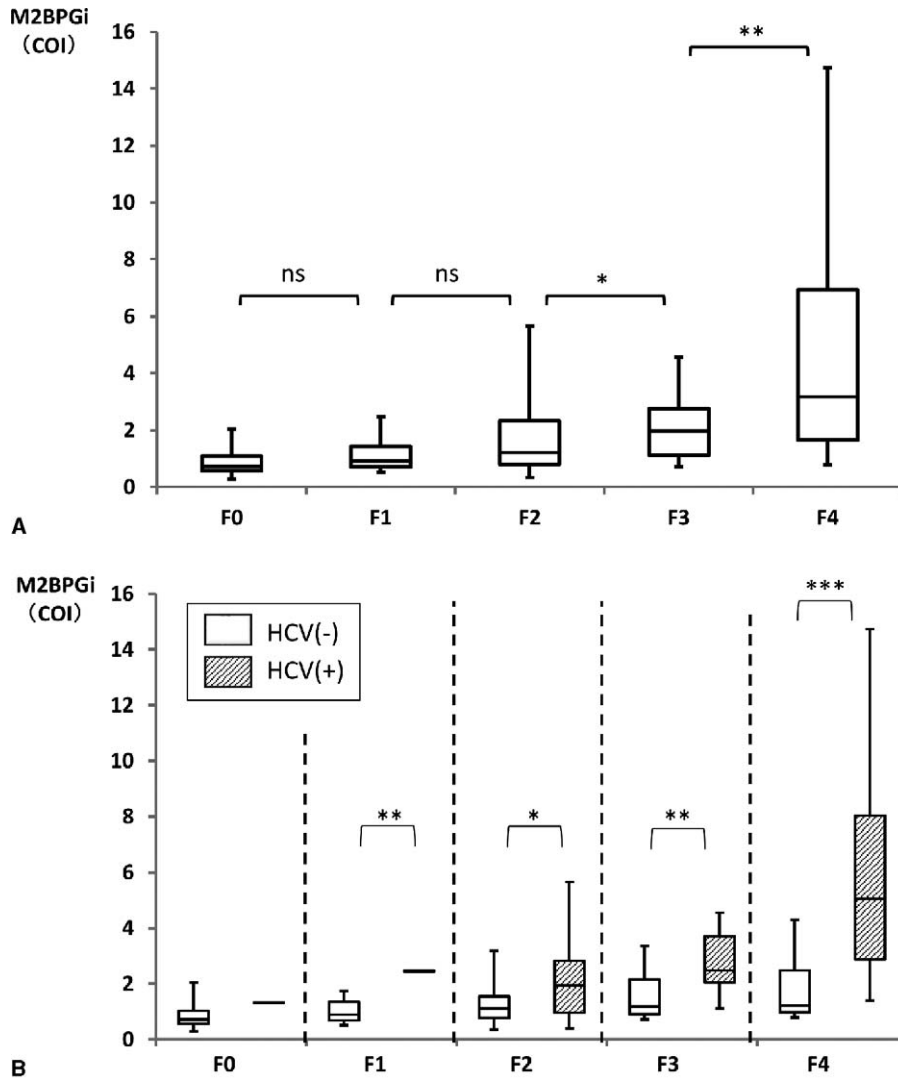
**TABLE 1.** Patients' Characteristics of Study Population

		Total	PHLF < Grade B	PHLF ≥ Grade B	P
		n = 138	n = 119	n = 19	
General background					
Age	yrs	68 ± 10	68 ± 10	68 ± 9	n.s.
Gender	Male	114 (83%)	97 (82%)	17 (89%)	n.s.
	Female	24 (17%)	22 (18%)	2 (11%)	
Underlying liver disease	HB	28 (20%)	27 (23%)	1 (5%)	n.s.
	HC	53 (38%)	44 (37%)	9 (47%)	n.s.
	HB and HC	3 (2%)	3 (2%)	0 (0%)	n.s.
	non-B non-C	60 (43%)	51 (43%)	9 (47%)	n.s.
Child-Pugh classification	A	123 (89%)	108 (91%)	15 (80%)	n.s.
	B	15 (11%)	11 (9%)	4 (21%)	
Blood examination					
Platelet count	×10 <sup>3</sup> /μL	150 ± 58	156 ± 57	114 ± 50	0.003
PT-INR		1.08 ± 0.10	1.08 ± 0.10	1.10 ± 0.10	n.s.
Alanine transaminase	IU/L	39 ± 40	36 ± 40	54 ± 38	n.s.
Total bilirubin	mg/dL	0.9 ± 0.4	0.87 ± 0.36	1.03 ± 0.41	n.s.
Albumin	g/dL	3.8 ± 0.4	3.8 ± 0.4	3.5 ± 0.5	0.009
Ammonia	μg/dL	45 ± 18	46 ± 20	49 ± 14	n.s.
Fibrosis maker and indicators					
Hyaluronic acid	ng/mL	182 ± 620	171 ± 569	202 ± 170	n.s.
Typ 4 collagen	ng/mL	5.6 ± 2.8	5.4 ± 2.6	7.0 ± 4.0	0.03
M2BPGi	COI	1.36 (0.29–14.73)	1.23 (0.29–10.82)	2.61 (0.71–14.73)	<0.0001
APRI		1.2 ± 2.4	1.1 ± 2.6	1.8 ± 1.4	n.s.
FIB-4 index		4.0 ± 3.2	3.8 ± 3.2	5.3 ± 3.0	n.s.
Indocyanine green test					
K-ICG		0.13 ± 0.04	0.14 ± 0.04	0.12 ± 0.04	0.03
Liver stiffness					
Vs by ARFI	m/s	1.88 ± 0.80	1.79 ± 0.74	2.48 ± 0.92	0.0004
Tumor marker					
AFP	ng/mL	7.3 (0.9–29,499)	6.8 (0.9–18,709)	28.9 (2.0–29,499)	n.s.
PIVKA-2	AU/mL	110 (1–431,000)	87 (1–431,000)	383 (16–35,700)	n.s.
Surgical factor					
Type of hepatectomy	Anatomical	87 (63%)	72 (61%)	15 (79%)	n.s.
	Nonanatomical	51 (37%)	47 (39%)	4 (21%)	
Operative time	min	411 (120–1044)	396 (120–1044)	479 (343–961)	0.003
Blood loss	g	587 (0–8650)	482 (0–7610)	1535 (120–8650)	0.002
Resection rate	%	18.6 (0.3–70.4)	16.7 (0.3–70.4)	34.6 (0.7–55.0)	n.s.
Perioperative transfusion		41 (30%)	28 (24%)	13 (68%)	0.0002
Tumor factor					
Tumor size	cm	3.5 (0.8–17)	3.5 (0.8–17)	3.4 (1.2–12)	n.s.
Tumor number		1 (1–7)	1 (1–7)	1 (1–5)	n.s.
Portal invasion		19 (14%)	15 (10%)	4 (21%)	n.s.
Arterial invasion		6 (4%)	5 (4%)	1 (5%)	n.s.
Venous invasion		1 (1%)	1 (1%)	0 (0%)	n.s.
Biliary invasion		9 (7%)	7 (6%)	2 (11%)	n.s.
Differentiation	Well	14 (10%)	13 (11%)	1 (5%)	n.s.
	Moderate	82 (59%)	73 (61%)	9 (47%)	
	Poor	37 (27%)	30 (25%)	7 (37%)	
	Undifferentiated	2 (1%)	2 (2%)	0 (0%)	
Stage	Unknown	3 (2%)	1 (1%)	2 (11%)	
	I	33 (24%)	27 (23%)	6 (32%)	n.s.
	II	55 (40%)	49 (41%)	6 (32%)	
	III	30 (22%)	26 (22%)	4 (21%)	
IV-A		16 (12%)	13 (11%)	3 (16%)	
	IV-B	4 (3%)	4 (3%)	0 (0%)	
Fibrosis stage					
Metavir fibrosis score	F0	20 (14%)	20 (17%)	0 (0%)	n.s.
	F1	17 (12%)	14 (12%)	3 (16%)	
	F2	42 (30%)	37 (31%)	5 (26%)	
	F3	22 (16%)	20 (17%)	2 (11%)	
	F4	37 (27%)	28 (24%)	9 (47%)	
Surgical outcome					
PHLF	Grade A	15 (11%)			
	Grade B	14 (10%)			
	Grade C	5 (4%)			

Continuous variables are expressed as mean values ± standard deviations or medians with ranges.

Categorical variables are expressed as number of patients.

AFP indicates alpha-fetoprotein; ARFI, acoustic radiation force impulse; COI, cutoff index; HB, hepatitis B; HC, hepatitis C; K-ICG, plasma disappearance rate of indocyanine green; M2BPGi, Mac 2 binding protein glycosylation isomer; n.s., not significant; non-B non-C, nonhepatitis B and nonhepatitis C; PHLF, posthepatectomy liver failure; PIVKA-2, protein induced by vitamin K absence or antagonist-2; PT-INR, international normalized ratio of prothrombin time; Vs, shear wave velocity.



**FIGURE 1.** Mac-2 binding protein glycosylation isomer (M2BPGi) values for each liver fibrosis stage. The differences between the abutting stages were evaluated by the Wilcoxon rank-sum test. Comparisons of serum M2BPGi values between hepatitis C virus (HCV)-negative and HCV-positive patients in the same fibrosis stages. COI, cutoff index, ns:  $P \geq 0.05$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**TABLE 2.** M2BPGi Values for the Assessment of Liver Fibrosis

	Cutoff Value (COI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUROC	95% CI
A, All cases (n = 138)							
≥F1 (n = 118)	1.10	69	80	95	30	0.80	0.69–0.88
≥F2 (n = 101)	2.10	49	97	98	41	0.80	0.72–0.87
≥F3 (n = 59)	1.74	69	80	72	78	0.82	0.74–0.88
F4 (n = 37)	2.59	65	87	65	87	0.83	0.73–0.89
B, HCV (-) (n = 85)							
≥F1 (n = 66)	0.72	86	53	86	52	0.72	0.57–0.84
≥F2 (n = 50)	1.04	64	71	76	58	0.72	0.60–0.81
≥F3 (n = 24)	1.04	75	61	43	86	0.72	0.59–0.82
F4 (n = 12)	0.78	100	34	20	100	0.70	0.54–0.82
HCV (+) (n = 53)							
≥F1 (n = 52)	1.40	85	100	100	12	0.85	0.85–0.85
≥F2 (n = 51)	2.61	61	100	100	10	0.73	0.43–0.90
≥F3 (n = 35)	3.03	66	83	88	56	0.81	0.66–0.90
F4 (n = 25)	4.44	60	93	88	72	0.83	0.70–0.92

AUROC indicates area under the receiver operating characteristic curve; CI, confidence interval; COI, cutoff index; HCV, hepatitis C virus; M2BPGi, Mac 2 binding protein glycosylation isomer; NPV, negative predictive value; PPV, positive predictive value.

### Impact of Underlying Liver Disease on M2BPGi Value

The impact of underlying liver disease on M2BPGi values was analyzed. The M2BPGi values in HCV-positive patients ( $4.06 \pm 3.24$ ) were significantly higher than those in HBV-positive ( $1.24 \pm 0.74$ ) ( $P < 0.0001$ ) and in non-B non-C patients ( $1.23 \pm 0.78$ ) ( $P < 0.0001$ ). There was no significant difference in M2BPGi values between the patients with HCV mono-infection and HBV/HCV coinfection.

Even when stratified according to fibrosis stage, the higher COI values in HCV-positive patients were observed in all fibrosis stage except for F0. The COI value of the HCV-positive group was significantly higher than that of the HCV-negative group in stage F1 ( $P = 0.003$ ), stage F2 ( $P = 0.02$ ), stage F3 ( $P = 0.006$ ), and stage F4 ( $P = 0.0006$ ) (Fig. 1B). Apparently, the COI value in HCV-positive patients became elevated more sharply with progression of fibrosis and showed more wide-ranging distribution than in HCV-negative patients. Reflecting this phenomenon, the performance of M2BPGi for assessing liver fibrosis was superior in the HCV-positive patients, as demonstrated by higher AUROC values than in the HCV-negative patients ( $P = 0.07$ ), although the difference was not statistically significant (Table 2).

### Relationship Between Fibrosis Stage and PHLF $\geq$ Grade B

PHLF  $\geq$  Grade B did not develop in the F0 patients. There was no significant difference in the incidence of PHLF  $\geq$  Grade B between the patients with stage  $\geq$  F2 and stage  $\geq$  F3 (10.1% and 18.6%,  $P = 0.21$ ), and stage  $\leq$  F1 and stage  $\geq$  F2 (8.1% and 15.8%,  $P = 0.40$ ), respectively. The incidence of PHLF  $\geq$  Grade B in stage F4 patients was significantly higher than in stage  $\leq$  F3 patients (24.3% and 9.9%,  $P = 0.04$ ).

### Performance of Preoperative Parameters as Predictors of PHLF

Univariate analysis and multivariate analysis of preoperative parameters in predicting PHLF  $\geq$  Grade B were performed. The predictive power by single factor is summarized in Table 3. The parameters with AUROC value  $>0.70$  were Vs (0.77, 95% CI 0.66–0.85), platelet count (0.72, 95% CI 0.57–0.83), M2BPGi (0.71, 95% CI 0.56–0.82), and ALT (0.70, 95% CI 0.55–0.82). The cutoff value, sensitivity, specificity, positive predictive value, and negative predictive value of M2BPGi in predicting PHLF  $\geq$  Grade B were 2.27, 58%, 71%, 24%, and 91%, respectively (Table 4).

For multivariate analysis, the interactions of preoperative parameters and resection rate were assessed by Spearman rank correlation coefficient. All of the coefficient values were  $<0.50$ , and there was no strong correlation between the variables.

Ten preoperative factors and resection rate were included in a stepwise procedure (minimum BIC method) to select variables for multivariate logistic regression analysis. M2BPGi, platelet count, and resection rate were selected as independent predictors for PHLF  $\geq$  Grade B: M2BPGi [odds ratio (OR): 2.08, 95% CI 1.28–3.55,  $P = 0.004$ ], platelet count (OR: 0.39, 95% CI 0.18–0.80,  $P = 0.02$ ), and resection rate (OR: 2.71, 95% CI 1.46–5.40,  $P = 0.002$ ) (Table 5).

**TABLE 3.** Receiver Operating Characteristic Analysis of Preoperative Factors in Predicting the PHLF  $\geq$  Grade B

	Prediction of PHLF $\geq$ Grade B	
	AUROC	95% CI
Blood examination		
Platelet count ( $\times 10^3/\mu\text{L}$ )	0.72	0.57–0.83
PT-INR	0.58	0.42–0.73
Alanine transaminase, IU/L	0.70	0.55–0.82
Total bilirubin, mg/dL	0.65	0.51–0.77
Albumin, g/dL	0.65	0.50–0.78
Ammonia, $\mu\text{g/dL}$	0.57	0.44–0.69
Fibrosis maker		
Hyaluronic acid, ng/mL	0.69	0.56–0.80
M2BPGi (COI)	0.71	0.66–0.85
Indocyanine green test		
K-ICG	0.64	0.50–0.76
Liver stiffness		
Vs by ARFI, m/s	0.77	0.66–0.85
Surgical factor		
Resection rate (%)	0.60	0.44–0.73

PHLF indicates posthepatectomy liver failure; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; PT-INR, international normalized ratio of prothrombin time; M2BPGi, Mac 2 binding protein glycosylation isomer; COI, cutoff index; K-ICG, plasma disappearance rate of indocyanine green; Vs, shear wave velocity; ARFI, acoustic radiation force impulse

A PHLF risk index incorporating these 3 parameters was generated as follows:

$$[\text{PHLF risk index}] = 0.299 \times \text{M2BPGi} - 0.016 \times \text{platelet count} + 0.052 \times \text{resection rate}$$

The cutoff value, sensitivity, specificity, positive predictive value, and negative predictive value of this index were 1.23, 63%, 83%, 38%, and 93%, respectively. The AUROC of the PHLF risk index was 0.81 (95% CI 0.69–0.89) (Fig. 2).

When stratified according to status of HCV infection, PHLF  $\geq$  Grade B was found in 9 patients (17%) in the HCV-positive group, and in 10 patients (11.8%) in the HCV-negative group. The predictive power by single factor in predicting PHLF  $\geq$  Grade B was evaluated by ROC analysis in each group (Table 6), and the cutoff values, sensitivities, specificities, positive predictive values, and negative predictive values in predicting PHLF  $\geq$  Grade B in each group are summarized in Table 7. The AUROC of M2BPGi in the HCV-positive group was 0.85 (95% CI 0.68–0.94), whereas the AUROC in the HCV-negative group was 0.65 (95% CI 0.46–0.80) (Fig. 3). In the HCV-negative group, although not statistically different, the predictive power of Vs (AUROC; 0.79, 95% CI 0.67–0.87) was better than that of M2BPGi ( $P = 0.09$ ), and Vs was the best predictor of PHLF  $\geq$  Grade B. On the contrary, in the HCV-positive group, M2BPGi had the strongest power in predicting PHLF  $\geq$  Grade B, although there was no significant difference between M2BPGi and Vs ( $P = 0.40$ ). When incorporating M2BPGi with resection rate, the predictive power was improved (AUROC: 0.86).

**TABLE 4.** Cutoff Value and Performance of M2BPGi in Predicting the PHLF  $\geq$  Grade B

	PHLF $\geq$ Grade B	Cutoff Value (COI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All cases (n = 138)	n = 19	2.27	58	71	24	91

**TABLE 5.** Multivariate Analysis by Stepwise Selection in Predicting PHLF  $\geq$  Grade B

	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	P	OR	95% CI	P
Platelet count ( $\times 10^3/\mu\text{L}$ )	0.39	0.20–0.72	0.002	0.39	0.18–0.80	0.02
PT-INR	1.22	0.76–1.88	0.38	—		
Alanine Transaminase, IU/L	1.35	0.91–2.20	0.12	—		
Total bilirubin, mg/dL	1.46	0.94–2.26	0.09	—		
Albumin, g/dL	0.54	0.32–0.86	0.01	—		
Ammonia, $\mu\text{g/dL}$	1.14	0.70–1.77	0.58	—		
Hyaluronic acid, ng/mL	1.05	0.49–1.53	0.82	—		
M2BPGi (COI)	2.05	1.37–3.23	0.0004	2.08	1.28–3.55	0.004
K-ICG	0.54	0.30–0.94	0.03	—		
Vs by ARFI, m/s	2.08	1.34–3.29	0.001	—		
Resection rate (%)	1.37	0.85–2.18	0.19	2.71	1.46–5.40	0.002

Preoperative liver function indicators and resection rate were included in a stepwise selection (BIC method) to select accurate indicators for the PHLF  $\geq$  grade B prediction model. The odds ratios are adjusted to the rate per unit of standard deviation of each factor.

ARFI indicates acoustic radiation force impulse; CI, confidence interval; COI, cutoff index; K-ICG, plasma disappearance rate of indocyanine green; M2BPGi, Mac 2 binding protein glycosylation isomer; PHLF, posthepatectomy liver failure; OR, odds ratio; PT-INR, international normalized ratio of prothrombin time; Vs, shear wave velocity.

## DISCUSSION

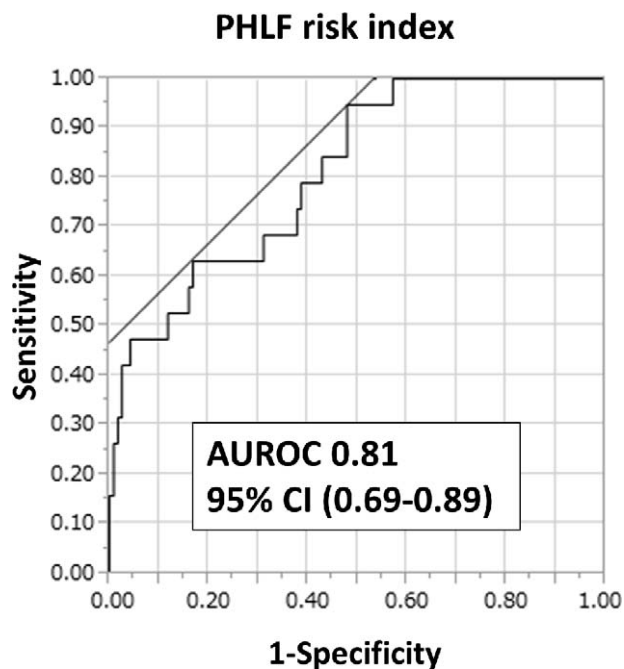
PHLF is a serious complication of liver resection. The prevention of PHLF, as based on the precise evaluation of preoperative liver function, is essential in minimizing postoperative morbidity and mortality.<sup>31</sup> Because liver fibrosis is the end-stage pathological change seen in chronic liver diseases and is directly linked to liver dysfunction, accurate assessment of liver fibrosis may contribute to the prediction of PHLF.

Although liver biopsy has been considered as the gold standard for the assessment of liver fibrosis, an alternative method is sought due to the invasiveness and serious complications of liver

biopsy.<sup>32,33</sup> Recently, several less invasive or noninvasive methods for evaluating liver fibrosis have been reported as having high accuracy in diagnosing liver fibrosis.<sup>34,35</sup> Among those methods, measurement of liver stiffness has been broadly evaluated; however, this method requires specialized equipment, and the result of the examination depends on the patient's condition and the operator's experience. A more simple and universal method for assessing liver fibrosis would be ideal. Serum M2BPGi, which serves as a fibrotic marker by detecting the alteration of glycoprotein caused by liver fibrosis, can be measured within 20 minutes using only the HISCL M2BPGi Assay Kit (Sysmex, Kobe, Japan).<sup>19</sup> The convenience of this examination is a great advantage over other the modalities in the preoperative setting. Moreover, unlike liver stiffness measurement that requires a specialized equipment and operator's experience, the simplified procedure does not accompany inter-facility discrepancies and therefore may facilitate performance of a multicenter study.

Recently, the efficacy of M2BPGi in assessing liver fibrosis has been reported in several series.<sup>21,36</sup> The present study used M2BPGi as a means of assessing liver fibrosis, evaluating the efficacy of M2BPGi in predicting PHLF. To the best of our knowledge, this is the first study to assess the usefulness of M2BPGi for the prediction of PHLF. The present study compared the predictive power of M2BPGi with other preoperative parameters, including Vs by ARFI. Furthermore, because Fujiyoshi et al<sup>36</sup> reported that HCV-positive patients had higher M2BPGi values than HCV-negative patients, even among patients in the same fibrosis stage, we separately evaluated the efficacy of M2BPGi in predicting PHLF according to HCV infection status.

Similar to the previous reports,<sup>21,36</sup> the M2BPGi value in the present study showed a significant correlation with liver fibrosis. M2BPGi was superior to other fibrotic markers and indicators in predicting each stage of liver fibrosis. Compared with Vs by ARFI imaging, M2BPGi demonstrated high performance in predicting early fibrotic changes (F1 to F3), whereas the predictive power of M2BPGi in predicting liver cirrhosis (F4) was almost equal to that of Vs by ARFI imaging. With respect to the prediction of PHLF  $\geq$  Grade B, we evaluated the predictive power of M2BPGi compared with that of Vs by ARFI, other preoperative parameters, and resection rate. In the univariate ROC analysis, Vs by ARFI had the highest AUROC value, whereas, in multivariate analysis, M2BPGi, platelet count, and resection rate were selected as significant factors for the prediction of PHLF  $\geq$  Grade B. When these latter 3 factors, as



**FIGURE 2.** Receiver operating characteristic (ROC) analysis of the PHLF risk index for the prediction of posthepatectomy liver failure (PHLF)  $\geq$  Grade B.

**TABLE 6.** Receiver Operating Characteristic Analysis of Preoperative Factors in Predicting PHLF  $\geq$  Grade B Categorized by the Status of HCV Infection

	HCV (-) (n = 85)		HCV (+) (n = 53)	
	AUROC	(95% CI)	AUROC	(95% CI)
Platelet count ( $\times 10^3/\mu\text{L}$ )	0.70	(0.48–0.85)	0.74	(0.52–0.88)
PT-INR	0.42	(0.23–0.65)	0.75	(0.55–0.88)
Alanine transaminase, IU/L	0.67	(0.46–0.83)	0.74	(0.51–0.89)
Total bilirubin, mg/dL	0.69	(0.52–0.82)	0.60	(0.38–0.79)
Albumin, g/dL	0.51	(0.31–0.70)	0.78	(0.60–0.89)
Ammonia, $\mu\text{g/dL}$	0.51	(0.35–0.64)	0.66	(0.44–0.82)
Hyaluronic acid, ng/mL	0.65	(0.48–0.79)	0.73	(0.54–0.86)
M2BPGi (COI)	0.65	(0.46–0.80)	0.85	(0.68–0.94)
K-ICG	0.64	(0.44–0.80)	0.64	(0.41–0.82)
Vs by ARFI, m/s	0.79	(0.67–0.87)	0.79	(0.58–0.91)
Resection rate (%)	0.65	(0.46–0.79)	0.56	(0.31–0.78)

ARFI, indicates acoustic radiation force impulse; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; COI, cutoff index; HCV, hepatitis C virus; K-ICG, plasma disappearance rate of indocyanine green; M2BPGi, Mac 2 binding protein glycosylation isomer; PHLF, posthepatectomy liver failure; PT-INR, international normalized ratio of prothrombin time; Vs, shear wave velocity.

**TABLE 7.** Cutoff Value and Performance of M2BPGi in Predicting the PHLF  $\geq$  Grade B Both in HCV (-) and HCV (+) Patients

	PHLF $\geq$ Grade B	Cutoff Value (COI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HCV (-) (n = 85)	n = 10	1.22	60	69	21	93
HCV (+) (n = 53)	n = 9	5.15	78	86	54	95

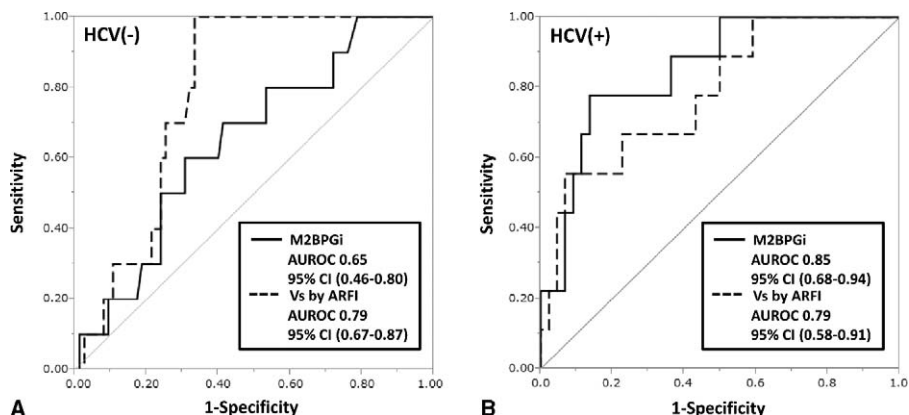
determined by multivariate analysis, were combined, the predictive power became fairly good with AUROC of 0.81. Although addition of M2BPGi to traditional markers did not improve prediction of PHLF significantly, measuring M2BPGi in addition to conventional liver function markers is expected to be useful in clinical setting, as we often encounter a difficult situation that various traditional liver function tests show inconsistent results and judging actual liver function is troublesome. We suppose that M2BPGi is helpful for our judgment especially in such situations.

The present study confirmed that HCV infection status is an indispensable factor when assessing liver fibrosis by M2BPGi. As well as in the previous report,<sup>37</sup> M2BPGi values in HCV-positive patients were significantly higher than those in HCV-negative patients in each stage of liver fibrosis.<sup>36</sup> In HCV patients, serum M2BPGi values become elevated more sharply as liver fibrosis

progresses than in non-HCV patients, showing that M2BPGi is a more sensitive and effective marker for liver fibrosis in HCV patients. Although the mechanism responsible for higher M2BPGi values in HCV-positive patients is still unknown and further molecular biologic investigation is required, the present study clearly showed that the etiology of the patient's condition must be taken into consideration when using M2BPGi for the assessment of liver fibrosis because the cutoff value for each fibrotic stage is different depending on the presence of HCV infection. The efficacy of M2BPGi in predicting PHLF also differs substantially between HCV-negative and HCV-positive patients. Individual preoperative assessment according to the underlying liver disease may be requisite.

The present study had several limitations. First, it was a retrospective study. A multicenter prospective study is needed to

**FIGURE 3.** Impact of hepatitis C virus (HCV) infection on the prediction of post-hepatectomy liver failure (PHLF)  $\geq$  Grade B. A, Receiver operating characteristic analysis for the prediction of PHLF  $\geq$  Grade B in HCV-negative patients. B, Receiver operating characteristic analysis for the prediction of PHLF  $\geq$  Grade B in HCV-positive patients. ARFI, acoustic radiation force impulse; AUROC, area under receiver operating characteristics curve; CI, confidence interval; Vs, shear wave velocity.



assess the efficacy of M2BPGi in predicting PHLF. Second, the sample size and the number of events were relatively small. In particular, when stratified by HCV infection status, because the number of patients with severe PHLF was not greater than 10, multivariate analysis could not be performed in each group.<sup>37</sup> Accumulation of additional cases is required to validate the usefulness of M2BPGi. Third, there is a potential impact of selection bias. The parameters other than M2BPGi, which were measured as a routine preoperative evaluation, might have affected our decision whether or not we should operate on the patients, whereas M2BPGi, which was measured postoperatively, did not. For example, if a certain factor indicates poor liver function and we excluded the patients for operative indication, it might have resulted in underestimation of predictive power of this factor. Underestimation of the parameters other than M2BPGi might have led to the favorable results for M2BPGi.

In conclusion, preoperative measurement of serum M2BPGi can be an effective predictor of PHLF in HCC patients by reflecting the status of liver fibrosis. High M2BPGi values in HCV-positive patients were an especially significant risk factor of PHLF. We hope that this promising method of predicting PHLF in HCC patients will contribute to the refinement of patient selection and improvement in patients' posthepatectomy course.

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