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Mac-2 Binding Protein Glycosylation Isomer as a Prognostic Marker for Hepatocellular Carcinoma With Sustained Virological Response

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Abstract. *Background/Aim: Mac2-binding protein glycosylation isomer (M2BPGi) is a non-invasive marker for assessing liver fibrosis. This was a retrospective study investigating whether M2BPGi predicts recurrence of hepatocellular carcinoma (HCC) after hepatectomy in patients with HCC who achieved a sustained virological response (SVR). Patients and Methods: We retrospectively reviewed the clinicopathological factors from 60 patients who underwent hepatectomy for HCC after achieving a SVR. We divided all patients into high M2BPGi and low M2BPGi groups and analyzed the clinicopathological and surgical outcomes. Results: High M2BPGi (>1.54, n=23) was significantly associated with lower serum albumin, higher ICGR15, higher Fib-4 index, large blood loss, and worse recurrence-free survival compared to low M2BPGi (≤1.54, n=37). Multivariate analysis identified high M2BPGi and large tumor size as being associated with reduced recurrence-free survival. Multivariate analysis identified lower serum albumin, larger tumor size and higher DCP as associated with reduced overall survival. There was no difference regarding recurrence pattern. Conclusion: Preoperative M2BPGi is a useful biomarker for HCC recurrence after hepatectomy for SVR-HCC.*

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Hepatocellular carcinoma (HCC) is the third most common malignancy worldwide (1). Liver cirrhosis is present in approximately 80-90% of patients with HCC, and thus is the largest single risk factor. The main risk factors for cirrhosis include hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol, and possibly, obesity and diabetes (2). In the modern era, HCV can be eradicated by interferon (IFN) or direct acting antivirals (DAAs). Especially, a sustained viral response (SVR) rate of 90% or more is obtained by DAAs, and it has become possible to widely treat patients who are ineligible/intolerant to IFN or whom IFN is ineffective or who have relapsed. Many studies reported that when SVR for HCV is achieved, even with liver cirrhosis, the liver carcinogenic rate decreases and prognosis improves (3, 4). While SVR is expected to reduce the risk of liver carcinogenesis, liver carcinogenesis after SVR is a problem in recent years. Screening for HCC is considered necessary for a long period of time even if SVR is achieved. Asahina *et al*. reported that in a multivariate analysis high age, sex, liver fibrosis, liver steatosis, total cholesterol level, fasting blood sugar level, alpha-fetoprotein level, and virological response to IFN were independent risk factors associated with HCC after SVR (4).

Recently, Mac2-binding protein glycosylation isomer (M2BPGi) was developed as a reliable and non-invasive marker for assessing liver fibrosis (5, 6). M2BP secreted from hepatic stellate cells is unaltered (7) but its glycosylation structure changes as liver fibrosis progresses. M2BPGi values in HCV-positive patients were significantly higher than those in HCV-negative patients at each stage of liver fibrosis (8). In patients with HCV, serum M2BPGi values were reportedly more sharply elevated during liver

Table I. *Comparison of clinicopathological factors between the two groups classified by M2BPGi.*

Variables	M2BPGi ≤1.54 (n=37)	M2BPGi >1.54 (n=23)	*p*-Value
Host related factor			
Age	71.3±6.6	70.2±7.8	0.56
Male/Female	24/13	15/8	1.00
BMI (kg/m^2)	23.0±2.9	24.6±4.1	0.09
Diabetes mellitus	12 (32.4%)	10 (43.5%)	0.42
Albumin (g/dl)	4.3±0.4	3.8±0.4	<0.01
Total bilirubin (mg/dl)	0.9±0.4	0.9±0.4	0.66
Platelet count (/μl)	17.4±6.0	15.7±7.4	0.33
Prothrombin time (%)	97.9±9.3	92.7±13.3	0.08
Creatinine (mg/dl)	0.8±0.3	0.8±0.2	0.47
ICGR15(%)	11.6±5.8	19.4±11.0	<0.01
Fib4	2.84±1.48	3.88±1.77	0.02
ChildB	0 (0%)	3 (13.0%)	0.05
Liver cirrhosis (F3+4)	16 (44.4%)	10 (43.5%)	1.00
Portal hypertension	11 (29.7%)	9 (39.1%)	0.58
IFN/DAA	16/21	10/13	1.00
Tumor related factor			
Tumor size (cm)	3.4±2.5	3.8±3.5	0.53
Multiple tumor number	4 (10.8%)	7 (30.4%)	0.09
AFP (ng/ml)	2,209±12,677	127±376	0.44
DCP (mAU/ml)	1,185±4,718	9,028±33,700	0.17
Poor differentiation (+)	8 (21.6%)	5 (21.7%)	1.00
mvi (+)	7 (18.9%)	7 (30.4%)	0.36
Operative procedures			
Partial hepatic resection	20 (54.1%)	18 (78.3%)	0.09
Operative time (min)	277±117	292±149	0.68
Estimated blood loss (g)	236±345	502±671	0.04
Blood transfusion (+)	2 (5.4%)	5 (21.7%)	0.09
Postoperative complications (+)	1 (2.7%)	3 (14.3%)	0.13

Data are expressed as means±standard deviations or number of patients (percentage) as appropriate. M2BPGi: Mac-2 binding protein glycosylation isomer; BMI: body mass index; ICGR15: indocyanine green dye retension test at 15 min; IFN: interferon; DAA: direct acting antivirals; AFP: alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin; poor differentiation: poorly differentiated HCC; mvi, microvascular invasion.

fibrosis and were significantly decreased in patients who achieved SVR (9). Evaluating the degree of liver fibrosis before surgery is important. Liver biopsy is not only a very high-risk procedure for diagnosing liver fibrosis, but also has a limited indication. Several reports have focused on the relationship between M2BPGi and liver fibrosis. M2BPGi was reported to predict liver fibrosis precisely compared to markers such as the FIB-4 index, hyaluronic acid and type IV collagen (5, 6).

In patients with HCV, M2BPGi was reported to predict the development of HCC and a helpful screening tool for assessing the risk of HCC (8-10), but there were few reports about the relationship between the prognostic impact of M2BPGi and SVR-HCC and then the significance of M2BPGi in post-SVR HCC is unclear.

This was a retrospective study to investigate whether M2BPGi predicts HCC recurrence following hepatic resection for post-SVR HCC.

Patients and Methods

Data from 60 patients who underwent hepatectomy for HCC after achieving SVR at two institutions, the Division of Hepatobiliary and Pancreatic Surgery, Department of General Surgical Science, Gunma University and the Department of Surgical Science, Kyushu University from January 2016 to July 2020 were retrospectively collected. Patients with recurrent HCC and patients with insufficient data were excluded. The patients' clinicopathological factors, recurrence free survival rate and overall survival rate were analyzed. The study protocol was approved by our institutional ethics committee (approval number: HS2020-215) and met the guidelines of the Declaration of Helsinki.

FIB-4 index was calculated as follows: =(age×aspartate aminotransferase)/[platelet count (10^9/l)×(alanine aminotransferase)$^{1/2}$]. Portal hypertension was defined as splenomegaly with a platelet count of <100,000/μl or presence of esophageal-gastric varices, or portosystemic shunts secondary to liver cirrhosis. Splenomegaly was diagnosed when the largest spleen diameter was over 12 cm as measured by CT (11). Esophageal-gastric varices was diagnosed when the varices exceeded F1 (12). Postoperative complications were defined

Table II. *Cox proportional hazard model of all clinical characteristics of recurrence-free survival using univariable and multivariable analysis.*

	Univariate analysis		Multivariate analysis	
Variables	Hazard ratio	*p*-Value	Hazard ratio	*p*-Value
Host related factor				
Age	0.99 (0.93, 1.07)	0.98		
Gender (male)	1.98 (0.85, 4.65)	0.11		
BMI (kg/m^2)	1.03 (0.91, 1.16)	0.63		
Diabetes mellitus	0.82 (0.31, 2.91)	0.69		
Albumin (g/dl)	0.44 (0.16, 1.23)	0.11		
Total bilirubin (mg/dl)	2.28 (0.87, 5.27)	0.07		
Platelet count (/μl)	1.04 (0.96, 1.13)	0.27		
Prothrombin time (%)	0.99 (0.94, 1.03)	0.59		
Creatinine (mg/dl)	1.39 (0.26, 4.26)	0.63		
ICG15R (%)	1.07 (1.00, 1.15)	0.02		
M2BPGi >1.54	4.67 (1.46, 14.92)	<0.01	5.54 (1.05, 29.14)	0.04
Child B	7.5 (0.73, 77.56)	0.06		
Liver cirrhosis (F3+4)	0.71 (0.23, 2.16)	0.54		
Fib4	2.19 (0.82, 5,92)	0.12		
Portal hypertension	0.62 (0.19, 2.06)	0.44		
DAA	0.42 (0.14, 1.27)	0.13		
Tumor related factors				
Tumor size (cm)	1.49 (1.28, 1.74)	<0.01	1.74 (1.29, 2.60)	<0.01
Multiple tumor number	5.75 (2.18, 15.20)	<0.01		
Poor differentiation (+)	0.56 (0.13, 2.43)	0.43		
mvi (+)	4.92 (1.92, 12.56)	<0.01		
AFP (ng/ml)	1.00 (0.99, 1.00)	0.50		
DCP (mAU/ml)	1.00 (1.00, 1.00)	0.03		
Operative procedures				
Partial hepatic resection	0.81 (0.32, 2.06)	0.69		
Operative time (min)	1.00 (1.00, 1.00)	0.02		
Estimated blood loss (g)	1.00 (1.00, 1.00)	<0.01		
Blood transfusion (+)	3.02 (0.85, 10.06)	0.08		
Postoperative complications (+)	12.45 (3.15, 49.31)	<0.01		

Values in parentheses are 95% confidence intervals. BMI: Body mass index; ICGR15: indocyanine green dye retension test at 15 min; M2BPGi: Mac-2 binding protein glycosylation isomer; IFN: interferon; DAA: direct acting antivirals; mvi, microvascular invasion; poor differentiation: poorly differentiated HCC; AFP: alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin.

as Clavien–Dindo grade IIIa and higher within 1 month after hepatectomy (13).

Details of operative indication and surgical procedure have been reported previously (14). Patients for hepatectomy had no ascites or controllable ascites with diuretics. Liver volume was calculated by CT and EOB-MRI and the future remnant liver volume was more than 615 ml/m^2 after hepatectomy of more than one segment (15). Liver parenchymal transection was performed using an ultrasonic dissector and a Cavitron Ultrasonic Surgical Aspirator (AMCO Inc., Tokyo, Japan) under the Pringle maneuver. All sizable vessels were ligated. Fibrin glue was routinely applied to the cut surface of the liver.

After discharge, all patients were examined for recurrence by using tumor markers, and by computed tomography every 3 or 6 months. Recurrent HCC was treated by repeat hepatectomy, ablation therapy, and trans-arterial chemoembolization, according to previous studies (14).

Histological data was assessed by the pathologist according to the criteria of the Liver Cancer Study Group of Japan (16). Fibrosis staging was scored using the METAVIR classification on a scale of 0-4 as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis (17).

Associations between continuous and categorical variables and the relevant outcome variables were assessed using Student's *t*-test and χ^2 test, respectively. We also performed a logistic stepwise regression analysis to predict HCC recurrence, using variables with a *p*-value of <0.05 in the univariate analyses. The best cut-off value of M2BPGi was investigated using receiver operating characteristic (ROC) curves and the area under the curve (AUC) was calculated.

All analyses were performed using JMP version 14 software (SAS Institute, Inc., Cary, NC). $p<0.05$ was considered statistically significant.

Results

The best cut-off value of M2BPGi for HCC recurrence was 1.54 (AUC=0.655, $p<0.03$). A comparison of the clinicopathological characteristics of patients with high M2BPGi (>1.54) and low

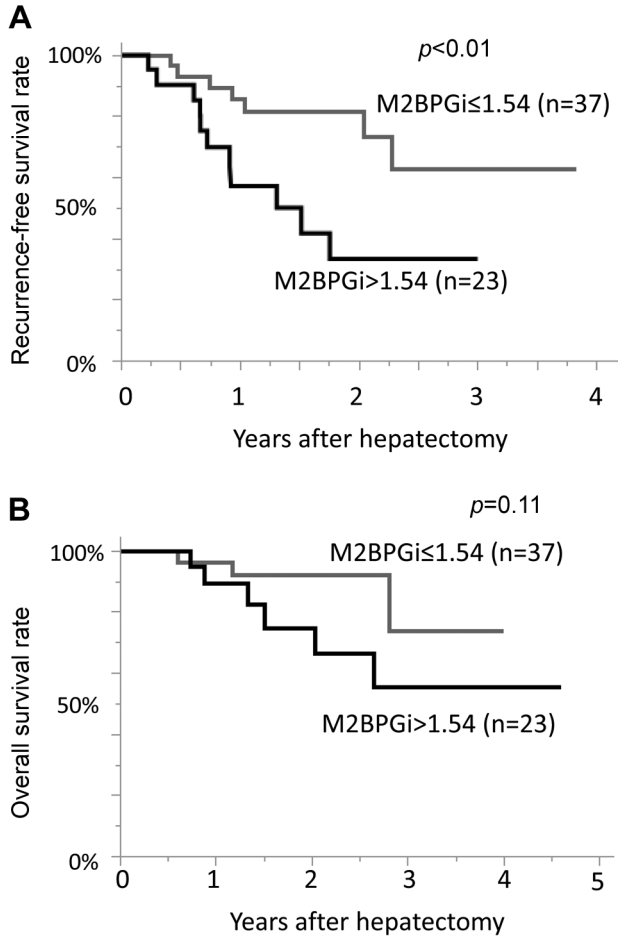

Figure 1. *Kaplan–Meier analysis of (A) recurrence-free survival and (B) overall survival curves in patients with high M2BPGi (thick black lines) and low M2BPGi (thin grey lines). Recurrence-free survival was significantly worse in patients with M2BPGi. M2BPGi, Mac2-binding protein glycosylation isomer.*

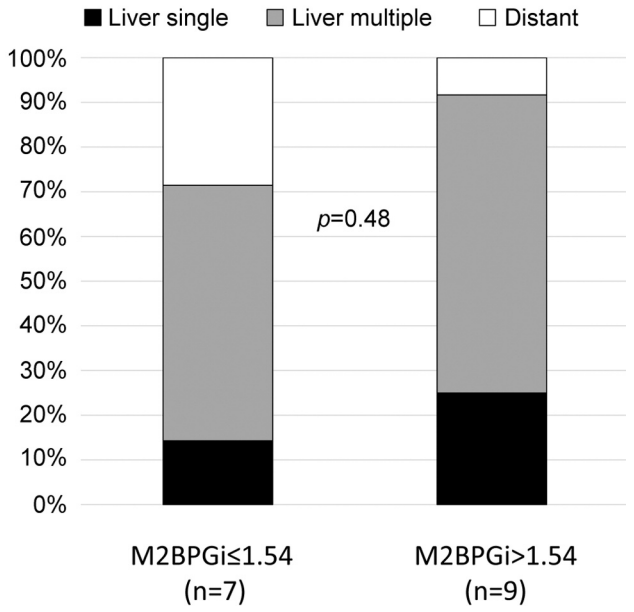

Figure 2. *The pattern of initial recurrence according to M2BPGi. Recurrence patterns were divided into three types: single liver recurrence, multiple liver recurrences and distant metastasis. There was no significance between high and low M2BPGi. M2BPGi, Mac2-binding protein glycosylation isomer.*

M2BPGi (≤1.54) is shown in Table I. High M2BPGi was significantly associated with lower serum albumin, higher ICGR15, Child-Pugh B, higher Fib 4 index and large blood loss compared to low M2BPGi. Univariate and multivariate analyses of factors considered prognostic for recurrence-free survival for all patients are shown in Table II. Univariate analysis identified nine prognostic factors for reduced recurrence-free survival. Multivariate analysis identified high M2BPGi and large tumor size for reduced recurrence-free survival. Figure 1A shows that recurrence-free survival in patients with high M2BPGi was significantly worse than in those with low M2BPGi. Univariate and multivariate analysis of factors considered for overall survival of all patients are shown in Table III. Univariate analysis identified nine prognostic factors for reduced overall survival. Multivariate analysis identified lower serum albumin, larger tumor size and higher des-gamma-carboxy prothrombin (DCP) for reduced overall survival. There was no significant difference in overall survival (p=0.11), but overall survival in patients with high M2BPGi had a worse tendency (Figure 1B). Figure 2 demonstrates the recurrence pattern according to the M2BPGi.

A total of 19 patients had recurrence. Recurrence patterns were divided to three types: single liver recurrence, multiple liver recurrences and distant metastasis. Patients with high M2BPGi had 25% single liver recurrence, 66.7% multiple liver recurrences and 8.3%distant metastasis. Patients with low M2BPGi had 14.3% single liver recurrence, 57.1% multiple liver recurrences and 28.6% distant metastasis. There was no significance between high and low M2BPGi.

Discussion

In this study, high M2BPGi was significantly associated with worse liver function, higher FIB4 index, larger blood loss, and worse recurrence-free survival compared to patients with low M2BPGi regarding hepatectomy for HCC patients with SVR.

M2BPGi was first developed as a useful marker for assessing liver fibrosis, but is clinically applied and widely used for various purposes. In hepatectomy, M2BPGi is correlated with liver fibrosis and can predict postoperative ascites (18) or postoperative liver failure (19). High M2BPGi is not only a risk factor of HCC carcinogenesis, but also a

Table III. *Cox proportional hazard model of all clinical characteristics of overall survival using univariable and multivariable analysis*.

	Univariate analysis		Multivariate analysis	
Variables	Hazard ratio	*p*-Value	Hazard ratio	*p*-Value
Host related factor				
Age	1.07 (0.96, 1.19)	0.23		
Gender (male)	4.35 (0.54, 34.92)	0.17		
BMI (kg/m^2)	0.92 (0.74, 1.11)	0.43		
Diabetes mellitus	2.29 (0.61, 8.54)	0.22		
Albumin (g/dl)	0.24 (0.05, 1.00)	0.05	0.02 (0.05, 0.64)	0.02
Total bilirubin (mg/dl)	1.53 (0.34, 5.01)	0.53		
Platelet count (/μl)	1.14 (1.03, 1.27)	0.01		
Prothrombin time (%)	1.05 (0.98, 1.12)	0.15		
Creatinine (mg/dl)	2.12 (0.15, 11.13)	0.48		
ICG15R (%)	1.02 (0.95, 1.08)	0.52		
M2BPGi >1.54	2.92 (0.73, 11.71)	0.13		
Child B	17.9 (2.91, 110.42)	<0.01		
Liver cirrhosis (F3+4)	0.54 (0.13, 2.25)	0.39		
Fib4	0.98 (0.59, 1,45)	0.91		
Portal hypertension	0.39 (0.05, 3.15)	0.38		
DAA	0.65 (0.17, 2.48)	0.52		
Tumor related factors				
Tumor size (cm)	1.49 (1.28, 1.74)	<0.01	2.19 (1.33, 7.48)	<0.01
Multiple tumor number	2.16 (0.53, 8.83)	0.28		
Poor differentiation(+)	0.67 (0.33, 1.53)	0.99		
mvi (+)	7.88 (1.96, 31.60)	<0.01		
AFP (ng/ml)	1.00 (0.99, 1.00)	0.87		
DCP (mAU/ml)	1.00 (1.00, 1.00)	<0.01	1.00 (1.00, 1.00)	<0.01
Operative procedures				
Partial hepatic resection	0.34 (0.09, 1.27)	0.11		
Operative time (min)	1.00 (1.00, 1.00)	0.04		
Estimated blood loss (g)	1.00 (1.00, 1.00)	0.01		
Blood transfusion (+)	6.01 (1.44, 25.22)	0.01		
Postoperative complications (+)	23.45 (3.23, 172.66)	<0.01		

Values in parentheses are 95% confidence intervals. BMI: Body mass index; ICGR15: indocyanine green dye retension test at 15 min; M2BPGi: Mac-2 binding protein glycosylation isomer; IFN: interferon; DAA: direct acting antivirals; mvi, microvascular invasion; poor differentiation: poorly differentiated HCC; AFP: alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin.

prognostic factor for patients with HCC. M2BPGi is useful as a prognostic factor for both HCV-negative, HCV-positive and HBV related HCC (8-10, 20-24). M2BPGi will be a useful biomarker to predict HCC recurrence, which signifies a new carcinogenesis after hepatectomy for SVR-HCC. Moreover, M2BPGi levels are not affected by tumor-related factors such as tumor number, tumor size, AFP and DCP (24).

There exist some reports that SVR before hepatectomy is essential for improving long-term outcome for HCV-related HCC (25), but there are no reports on the relationship between M2BPGi and prognosis after hepatectomy in patients with SVR-HCC. Nakagawa *et al*. reported that AFP at SVR was an independent marker for HCC among patients with a prior history of HCC (n=84), but M2BPGi was not significant (23). And then, our result is the first report about the significance of M2BPGi regarding hepatectomy for SVR achieving-HCC.

It was believed that there are two recurrence patterns of HCC such as multicentric recurrence and intrahepatic metastasis. Multicentric recurrence is gathered as a new carcinogenesis from poor liver function and more patients presented with solitary recurrent tumors in the multicentric recurrence group than in the intrahepatic metastasis group (26). M2BPGi was associated to poor liver function and then may be related to multicentric recurrence of HCC, but there was no difference regarding with recurrence pattern in this study. On the other hand, Dolgormaa et al reported that M2BPGi induced tumor-promoting effects on HCC in cirrhotic liver by activated mTOR signaling *in vitro* and the growth of xenografted HCC *in vivo* (27). M2BPGi may affect hepatocarcinogenesis through liver fibrosis and intrahepatic metastasis though promoting malignant potential. More detailed research about the interaction between M2BPGi and HCC recurrence will be needed in the future.

Patients who achieve SVR are less likely to develop hepatocellular carcinoma, but there are no clear criteria for follow-up of patients who achieve SVR. In this study, high M2BPGi was associated to poor liver function and could be related to recurrence of HCC. Therefore, long-term and detailed follow-up is required.

There are several limitations of this study. First, the sample size was small. M2BPGi became measurable only recently and we collected data from just two institutions. Seconds, the observation period was relatively short for monitoring HCC. Third, the cut-off values of M2BPGi differed according to the etiology of fibrosis and a history of HCC. We identified a cut-off value of 1.54 using a ROC curve, but cut-off values vary depending on the study (20, 23, 24). Finally, there was selection bias because of the retrospective nature of the study. Further studies with larger sample sizes will lead to a definitive conclusion.

This retrospective analysis indicated that preoperative M2BPGi is a useful biomarker for HCC recurrence after hepatectomy for SVR-HCC. Careful surveillance will be required for patients with high M2BPGi.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Authors' Contributions

Norifumi Harimoto participated in participated in research design, data analysis, and the writing of the paper; Shinji Itoh, Tomoharu Yoshizumi and Ken Shirabe contributed to the discussion and reviewed the manuscript; Takahiro Yamanaka, Kei Hagiwara, Norihiro Ishii and Mariko Tsukagoshi participated in data collection; Akira Watanabe and Kenichiro Araki participated in research design

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