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ORIGINAL ARTICLE

Serum Mac-2 binding protein level predicts the development of liver-related events and colorectal cancer in patients with NAFLD

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Abstract

We previously demonstrated that Mac-2 binding protein (M2BP) is a useful biomarker for nonalcoholic fatty liver disease (NAFLD), particularly NAFLD fibrosis prediction. In the present study, we investigated the prognostic value of M2BP in patients with NAFLD. A total of 506 patients with biopsy-confirmed NAFLD from 2002 to 2013 were enrolled in this study in Japan. Three hundred fifty-three of these patients with NAFLD were available for follow-up for more than 100 days and showed no liver-related events at the time of entry. Liver-related events were defined as hepatocellular carcinoma (HCC), decompensation, and gastroesophageal varices with variceal treatment. The mean follow-up duration of all the subjects was 2716 ± 1621 days (102–7483 days). Eighteen patients developed new liver-related events (HCC, 8; decompensation, 11; varices, 8). Nine patients developed cardiovascular disease (CVD), and 24 patients developed new cancers in other organs. The median serum M2BP level was 1.603 μg/mL, and we divided our cohort into two

groups according to the serum M2BP level: M2BP low group (M2BP Low) and M2BP high group (M2BP Hi). The incidence of HCC was significantly higher in M2BP Hi (n = 8) than in M2BP Low (n = 0). The incidence of liver-related events was significantly higher in M2BP Hi (n = 16) than in M2BP Low (n = 2). The incidences of death, CVD events, and cancer in other organs were not different between the groups. Interestingly, the incidence of colorectal cancer was significantly higher in M2BP Hi (n = 5) than in M2BP Low (n = 0). *Conclusion*: M2BP is a useful biomarker to predict liver-related events, particularly HCC. Additionally, M2BP is a potential predictive biomarker of colorectal cancer development.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and a growing medical problem worldwide.[1] Diverse hepatic histological changes occur in patients with NAFLD, ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH). Thus, assessing the degree of liver fibrosis during the clinical progression of NAFLD is critical to predict disease progression and formulate therapeutic management decisions.[2,3] Liver biopsy remains the gold standard to assess liver fibrosis.[4,5] However, liver biopsy has significant limitations such as pain, the risk of severe complications, sampling error,[6] cost,[7] and patient unwillingness to undergo invasive testing. A recent study reported that liver fibrosis was independently associated with the long-term outcome in patients with NAFLD.[8] Therefore, a reliable and noninvasive test is required that accurately assesses the degree of liver fibrosis, and predicts the prognosis of patients with NAFLD.

The prognosis of patients with NAFLD depends on liver-related diseases and diseases of other organs, such as cancers in other organs and cardiovascular disease (CVD).[8,9] These findings were from studies performed in the United States and Europe that primarily evaluated Caucasian patients with NAFLD, and CVD was the leading cause of death and event occurrence. Simon et al. investigated the prognosis of patients with NAFLD over a median of 13.6 years and demonstrated the incidence of major adverse cardiovascular events as 24.3/1000 person-years (PY); this incidence was higher than that in control subjects (8.3/1000 PY).[9] A Korean study investigated more than 25,000 patients with NAFLD with a 7.5-year follow-up and found that patients with NAFLD showed a higher association with the development of three cancers: hepatocellular carcinoma (HCC; hazard ratio [HR]: 16.73), colorectal cancer in male patients (HR: 2.01), and breast cancer in female patients (HR: 1.92).[10] These findings indicated that NAFLD is closely associated with disease development in other organs.

Mac-2 binding protein (M2BP) is a glycoprotein comprised of seven potential *N*-glycosylation sites involved in cellular differentiation and immune regulation.[11,12] We previously identified M2BP as a major fucosylated glycoprotein secreted from the HuCCT-1 liver bile duct cancer cell line.[13] Serum M2BP concentrations increase in patients with various cancers (e.g., pancreatic, breast, and lung cancer), viral hepatitis, and autoimmune disease.[11] Low M2BP expression is observed in normal liver tissue but is easily detected in hepatocytes from patients with chronic hepatitis type C (CHC) during the progression of liver fibrosis.[14,15] Previously, we developed an enzyme-linked immunosorbent assay (ELISA) kit to measure total serum M2BP levels in humans,[13] revealing that serum M2BP levels could be used to predict histological severity of hepatic fibrosis in 506 patients with biopsy-confirmed NAFLD.[13,16]

Recently, we conducted a 7-year longitudinal study to investigate the significance of serum M2BP levels and found that baseline serum M2BP levels predicted future changes in liver fibrosis.[17] In the present study, following an interval since the serum M2BP measurement of 506 patients with NAFLD at liver biopsy, the prognosis of these patients was followed up. This study aimed to investigate the prognostic value of M2BP level in patients with NAFLD.

PATIENTS AND METHODS

Patients with biopsy-proven NAFLD and histological evaluation

A total of 506 patients with biopsy-confirmed NAFLD from 2002 to 2013 were enrolled in this study from the following six hepatology centers in Japan: Osaka University Hospital, Kochi Medical School Hospital, Hiroshima University Hospital, Ikeda Municipal Hospital, Otemae Hospital, and Osaka City University Hospital. This study included 364 patients with NAFLD who were available for follow-up after more than 100 days at the hepatology

centers (Figure 1). Among the 364 patients, 11 were excluded because of liver-related diseases at the time of entry, and 353 patients were enrolled in this study.

All of the patients with biopsy-proven NAFLD had received percutaneous liver needle biopsies. The biopsied liver samples were embedded in paraffin blocks according to standard procedures and were stained with hematoxylin and eosin and Masson's trichrome stains. All biopsy specimens were centrally evaluated by two experienced hepatic pathologists (Y.K. and H.F.) who were blinded to the clinical data. Adequate liver samples were defined as >1.5 cm long and/or having more than six portal tracts. NASH was confirmed according to Matteoni's classification.[18] Patients with NAFLD with ballooning hepatocytes (Matteoni type 3) and with liver fibrosis (Matteoni type 4) were placed in the NASH cohort. Patients whose liver biopsy specimens showed simple steatosis or steatosis with nonspecific inflammation were placed in the NAFL cohort. Samples were also investigated and quantified according to the NAFLD activity scoring system.[19] Steatosis (0–3), lobular inflammation (0–2), and hepatocellular ballooning (0–2) were quantified. The individual parameters of fibrosis were scored independently according to the NASH Clinical Research Network scoring system.[19] The exclusion criteria for this study included a history of liver-related diseases (HCC, decompensated liver cirrhosis [LC], and gastroesophageal varices) at the time of entry and a history of other hepatic diseases, a substance abuse-induced hepatic disorder, and a history of alcohol abuse (defined as >20 g of alcohol consumption daily).

Definition of HCC, decompensated LC, and gastroesophageal varices

All clinical events were collected and defined using data from the patients' electronic medical records. Liver-related events (HCC, decompensation, and gastroesophageal varices) were defined as follows. HCC was confirmed by (1) histology or (2) typical features on at least one dynamic test (triphasic computed tomography [CT] or magnetic resonance imaging [MRI]) according to the guideline proposed by Japan Society of Hepatology.[20] LC was defined as decompensated cirrhosis. The date of first hospitalization for ascites and/or hepatic encephalopathy was recorded. Ascites was confirmed by (1) the detection of ascitic fluid by aspiration or (2) radiology (ultrasonography, CT, or MRI). Gastroesophageal varices diagnosed as the date of first hospitalization for variceal treatment were recorded.

Anthropometry and laboratory measurements

Anthropometric variables (height and weight) were measured in the standing position, and the body mass index (BMI) was calculated as weight (in kilograms) divided by the height squared in meters. Serum biochemical variables (aspartate aminotransferase [AST], alanine aminotransferase [ALT], γ-glutamyltransferase [GGT], alkaline phosphatase [ALP], total cholesterol, triglyceride, high-density lipoprotein cholesterol [HDL-C], fasting blood glucose, immunoreactive insulin (IRI), albumin, ferritin, hyaluronic acid, and platelet count) were measured using a conventional automated analyzer.

ELISA for M2BP

We measured serum M2BP using our ELISA kit (Immuno-Biological Laboratory Co., Ltd., Fujioka, Japan; code # 27362) as previously reported.[13]

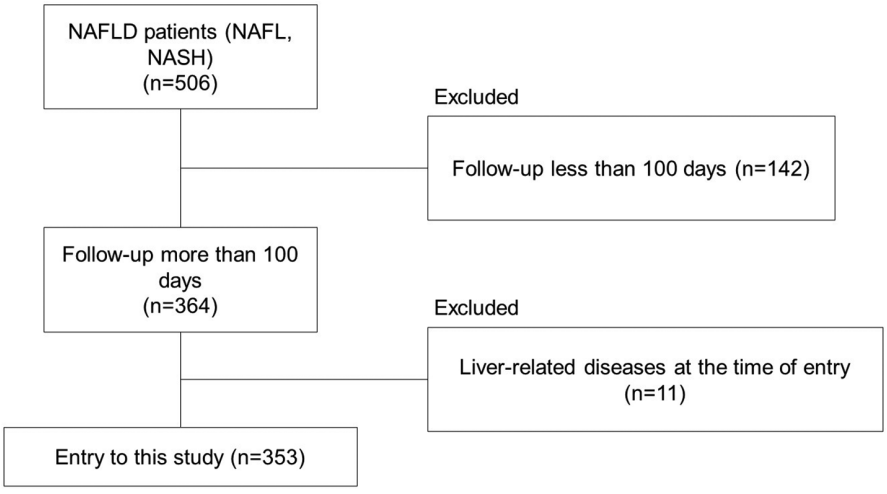

FIGURE 1 Flow diagram of patient enrollment throughout the study. Abbreviations: NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis

TABLE 1 Study subjects at liver biopsy

Variable	NAFL	NASH	*p* value[a]
(A) Patients with NAFL and NASH			
Number	86	267	
Age (y)	48.2 ± 10.9	53.3 ± 13.8	<0.0001
Sex (F/M)	31/54	153/114	<0.001
BMI (kg/m^2)	26.3 ± 4.3	28.0 ± 4.9	<0.001
AST (U/L)	38.1 ± 22.6	64.7 ± 44.3	<0.001
ALT (U/L)	65.3 ± 43.2	99.8 ± 74.3	<0.0001
AST/ALT ratio	0.655 ± 0.214	0.703 ± 0.244	n.s.
GGT (U/L)	88.3 ± 124.7	84.9 ± 69.3	<0.01
ALP (U/L)	226.0 ± 76.9	270.9 ± 115.9	<0.0005
T-Chol (mg/dL)	204.2 ± 38.8	206.0 ± 35.8	n.s.
TG (mg/dL)	129.4 ± 75.2	138.9 ± 60.0	n.s.
HDL-C (mg/dL)	52.5 ± 10.5	49.3 ± 13.0	<0.05
FBS (mg/dL)	107.1 ± 18.5	104.9 ± 19.1	n.s.
IRI (mU/mL)	10.1 ± 5.5	13.4 ± 7.9	n.s.
Albumin (g/dL)	4.53 ± 0.34	4.43 ± 0.45	n.s.
Ferritin (ng/mL)	177.3 ± 134.0	295.5 ± 300.1	<0.005
Hyaluronic acid (ng/mL)	34.6 ± 34.7	106.5 ± 220.8	<0.01
Platelet count (×10^4/μL)	23.8 ± 4.9	22.2 ± 17.6	<0.0001
M2BP (μg/mL)	1.28 ± 0.69	2.16 ± 1.25	<0.0001
	M2BP Low	**M2BP Hi**	***p* value**[b]
(B) M2BP Low and Hi Patients			
Number	177	176	
Age (years)	50.8 ± 12.4	53.3 ± 14.1	<0.05
Sex (F/M)	84/93	100/76	n.s.
BMI (kg/m^2)	26.7 ± 4.3	28.4 ± 5.2	<0.001
AST (U/L)	49.9 ± 32.1	66.5 ± 48.2	<0.0001
ALT (U/L)	82.2 ± 51.4	100.7 ± 83.1	<0.05
AST/ALT ratio	0.649 ± 0.202	0.734 ± 0.263	<0.005
GGT (U/L)	89.2 ± 99.2	82.1 ± 70.3	n.s.
ALP (U/L)	247.5 ± 93.5	272.4 ± 122.2	n.s.
T-Chol (mg/dL)	208.3 ± 40.4	202.7 ± 33.9	n.s.
TG (mg/dL)	127.0 ± 68.8	141.6 ± 65.1	n.s.
HDL-C (mg/dL)	52.6 ± 13.1	49.0 ± 10.8	n.s.
FBS (mg/dL)	106.0 ± 16.5	105.8 ± 20.9	n.s.
IRI (mU/mL)	11.1 ± 7.7	13.7 ± 7.3	n.s.
Albumin (g/dL)	4.51 ± 0.43	4.39 ± 0.41	<0.05
Ferritin (ng/mL)	235.2 ± 192.8	315.0 ± 343.0	n.s.
Hyaluronic acid (ng/mL)	121.4 ± 326.2	75.0 ± 82.7	n.s.
Platelet count (×10^4/μL)	22.3 ± 4.8	22.8 ± 21.4	n.s.
M2BP (μg/mL)	1.11 ± 0.33	2.79 ± 1.18	<0.0001
Stage (0/1/2/3/4)	71/60/31/15/0	27/34/59/51/5	<0.0001

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; FBS, fasting blood glucose; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; IRI, immunoreactive insulin; M, male; n.s., not significant; T-Chol, total cholesterol; TG, triglyceride.

[a]Patients with NAFL versus NASH.

[b]M2BP Low versus Hi patients.

Statistical analysis

Statistical analysis was conducted using JMP Pro 16.0 software (SAS Institute Inc., Cary, NC). Variables were expressed as means ± SD). Clinical outcomes were illustrated by Kaplan–Meier curves and were compared using the log-rank test. The diagnostic performances of the markers were assessed by analyzing receiver operating characteristic (ROC) curves. The probabilities of true positive (sensitivity) and true negative (specificity) assessments were determined for selected cutoff values, and the area under the ROC curve (AUROC) was calculated for each index. The Youden index was used to identify the optimal cutoff points. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Characteristics of the study subjects

Of the 506 patients with NAFLD, 353 were monitored for more than 100 days and were included in this study (Figure 1). The characteristics of the study subjects are given in Table 1A. A total of 353 patients with NAFLD (NAFL, 86; NASH, 267) were included in this study. In our cohorts, the patients with NASH had a higher proportion of female patients than the patients with NAFL. Age, BMI, AST, ALT, ALP, IRI, ferritin, and hyaluronic acid were significantly higher in patients with NASH than in patients with NAFL. The serum GGT and HDL-C levels and platelet count were significantly lower in patients with NASH than in patients with NAFL. The serum Mac2bp levels were also increased significantly in patients with NASH compared to those in patients with NAFL, as described previously.[13,16]

Follow-up evaluation

The mean follow-up duration of all subjects was 2716 ± 1621 days (102–7483 days, approximately 7.4 years). The mean follow-up duration of patients with NAFL and NASH was 2761 ± 1564 days and 2700 ± 1642 days, respectively. Our cohort represented 2626.3 PY in all subjects, 650.6 PY in patients with NAFL, and 1,975.7 PY in patients with NASH. Ten patients died. Liver-related death occurred in 1 patient because of cholangiocellular carcinoma; the causes of death in the other 9 patients were wide-ranging (lung cancer, breast cancer, stomach cancer, pneumonia, heart failure, subarachnoid hemorrhage, congestive heart failure, pancreatic cancer, and acute myeloid leukemia).

TABLE 2 Incidence rate of event per 1000 person-years

	NAFL (n = 86)	NASH (n = 267)
(A) NAFL vs. NASH		
Death	1.54	4.56
HCC	0.00	4.05
Decompensation	0.00	5.57
Varices	0.00	4.05
Liver-related disease	0.00	9.11*
CVD	1.54	6.07
Cancer in other organs	4.61	13.67
	M2BP Low (n = 177)	M2BP Hi (n = 176)
(B) M2BP Low patients vs. M2BP Hi		
Death	5.00	2.80
HCC	0.00	5.61*
Decompensation	1.67	6.31
Varices	0.83	4.91
Liver-related disease	1.67	11.22**
CVD	6.67	3.51
Cancer in other organs	13.33	11.22

Note: HCC was confirmed by histology or computed tomography (CT)/magnetic resonance imaging (MRI); varices was defined as gastroesophageal varices patients who require hospitalization (rupture and/or preventive therapy).

Abbreviation: CVD, cardiovascular disease.

*$p < 0.05$; **$p < 0.01$; log-rank test.

Table 2A lists the major complications and their rates. Eighteen patients (5.1%) developed new liver-related events, 9 patients (2.5%) developed new CVD events, and 24 patients (6.8%) developed new cancers in other organs in our cohort. The incidences of these events are provided in Table 2. The incidences of death, HCC, CVD events, and cancers in other organs were similar between patients with NAFL and NASH. The liver-related event incidence was significantly higher in patients with NASH than in patients with NAFL (0.00 vs. 9.11/1000 PY).

The median value of the serum M2BP level was 1.603 μg/mL, and we divided our cohort into two groups by the serum M2BP levels: M2BP low group (M2BP Low) and M2BP high group (M2BP Hi). The characteristics of the study subjects are given in Table 1B. In our cohorts, the M2BP Hi group had a higher proportion of advanced liver fibrosis (F3–F4) patients than the M2BP Low group. The incidence of death, CVD events, and cancer in other organs was similar between the groups. Interestingly, the incidence of HCC and liver-related events was significantly higher in M2BP Hi than in M2BP Low (0.00 vs. 5.61/1000 PY, 1.67 vs. 11.22/1000 PY, respectively).

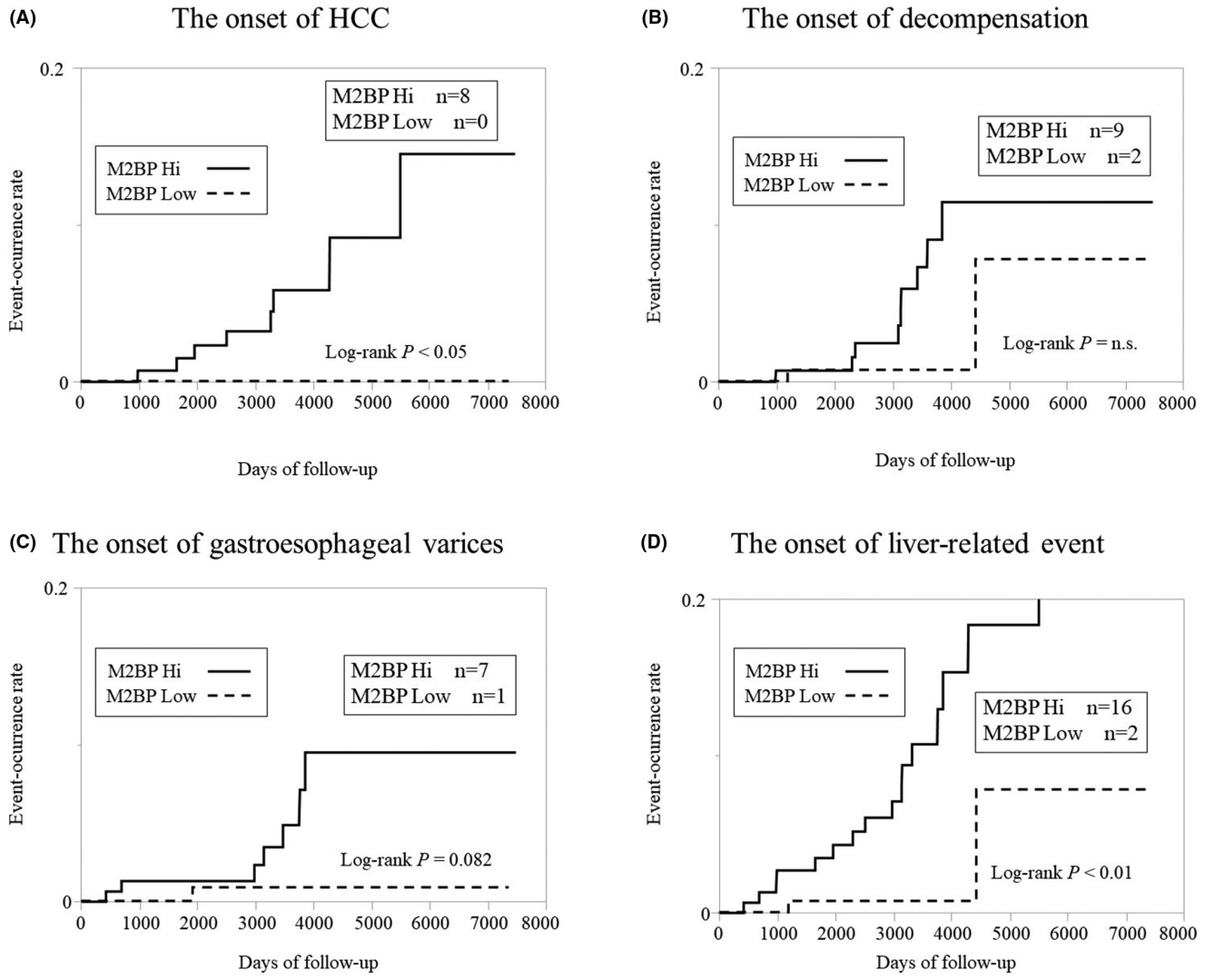

FIGURE 2 Liver-related event development according to serum Mac-2 binding protein (M2BP) levels. (A) Comparison of new hepatocellular carcinoma (HCC) development according to serum M2BP levels. (B) Comparison of new decompensation development according to serum M2BP levels. (C) Comparison of new gastroesophageal varices development according to serum M2BP levels. (D) Comparison of liver-related event development according to the serum M2BP levels. Abbreviations: M2BP Low, M2BP low group; M2BP Hi, M2BP high group

Incidence of liver-related events

The cumulative probability obtained using Kaplan–Meier analysis of liver-related events (HCC, decompensated LC, and varices) between M2BP Low and M2BP Hi is illustrated in Figure 2. The incidence of HCC was significantly higher in M2BP Hi (n = 8) than in M2BP Low (n = 0) (Figure 2A). The incidences of LC and varices were also higher in the M2BP Hi group than in the M2BP Low group, but the differences were not significant (Figure 2B,C). The incidence of liver-related events was significantly higher in M2BP Hi (n = 16) than in M2BP Low (n = 2) (Figure 2D).

Using ROC analyses, we set cutoff values for the serum M2BP levels for HCC, LC, varices, and liver-related events (Figure 3). The cutoff value for HCC occurrence was 1.892 μg/mL, and the AUROC, sensitivity, and specificity of this cutoff value were 0.832, 100%, and 62.6%, respectively (Figure 3A). The cutoff value for LC occurrence was 1.701 μg/mL, and the AUROC, sensitivity, and specificity of this cutoff value were 0.680, 81.8%, and 56.1%, respectively (Figure 3B). The cutoff value for varices occurrence was also 1.701 μg/mL, and the AUROC, sensitivity, and specificity of this cutoff value were 0.706, 87.5%, and 55.9%, respectively (Figure 3C). The cutoff value for liver-related events was 1.859 μg/mL, and the AUROC, sensitivity, and specificity of this cutoff value were 0.738, 83.3%, and 63.0%, respectively (Figure 3D).

We compared the development of liver-related events (HCC, decompensation, and varices) by M2BP level and degree of liver fibrosis (Table S1). There were no relationships between the development of liver-related events and M2BP levels in early stage (F0–F2)

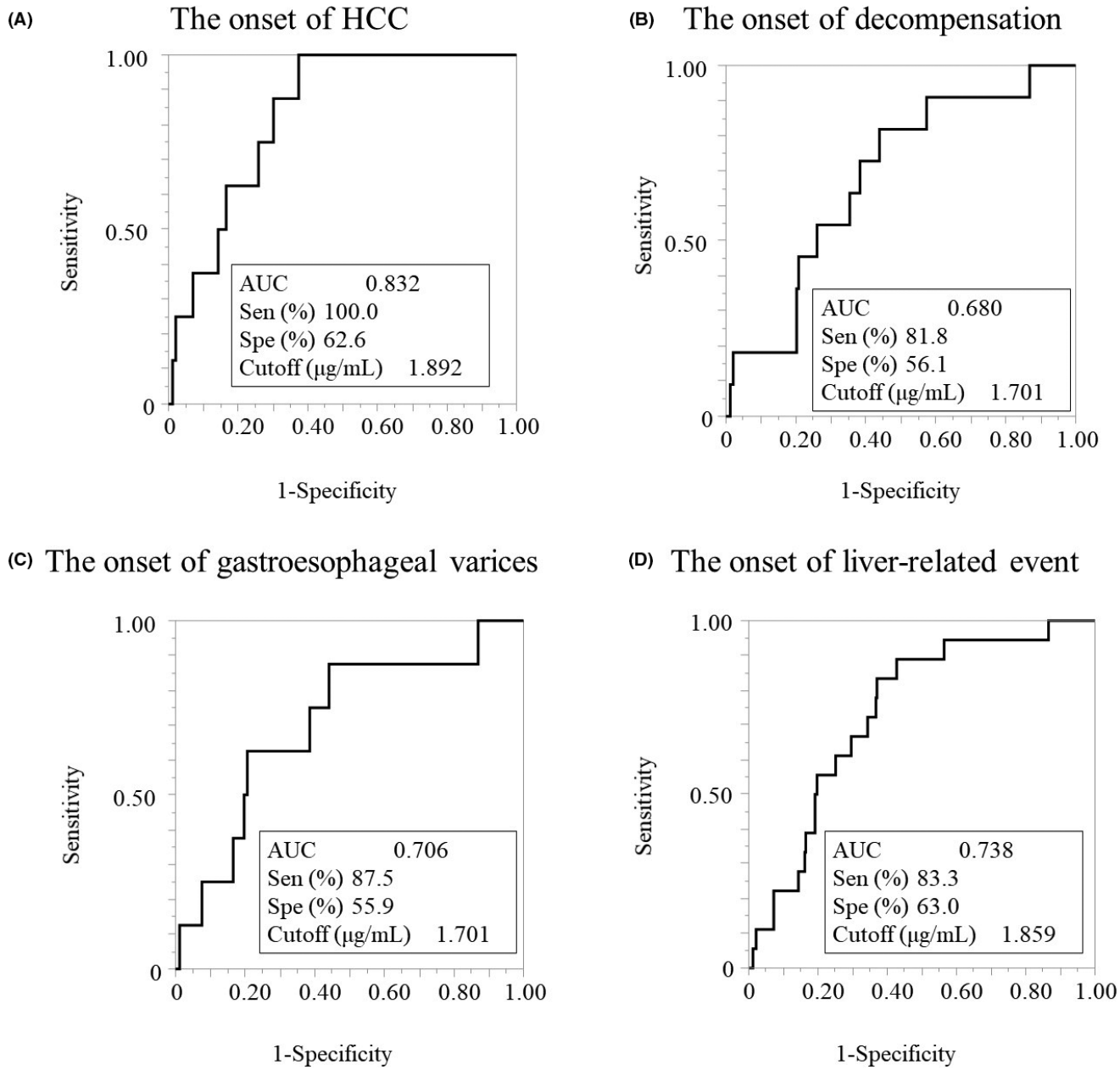

FIGURE 3 Receiver operating characteristic (ROC) analysis of M2BP levels for liver-related events. (A) ROC analysis of M2BP levels for HCC development. (B) ROC analysis of M2BP levels for decompensation development. (C) ROC analysis of M2BP levels for gastroesophageal varices development. (D) ROC analysis of M2BP levels for liver-related event development. Abbreviations: AUC, area under the curve; Sen, sensitivity; Spe, specificity

patients, but the development of HCC in the M2BP Hi group was significantly higher compared with in the M2BP Low group in advanced stage (F3–F4) patients.

Incidence of cancer in other organs

In our cohort, 24 patients developed new cancers in other organs, and no differences were found in the incidence of new cancers in other organs between M2BP Hi and M2BP Low (Figure 4A). Interestingly, the incidence of new colorectal cancer was higher in M2BP Hi than in M2BP Low. In the M2BP Low group, no new patients with colorectal cancer patients observed (Figure 4B). We compared the development of other organ cancer and colorectal cancer, respectively, by M2BP level and degree of liver fibrosis (Table S1). There were no relationships between the development of other organ cancer and liver fibrosis stage, regardless of the level of M2BP. However, there was a tendency between CRC development and M2BP levels in advanced-stage patients.

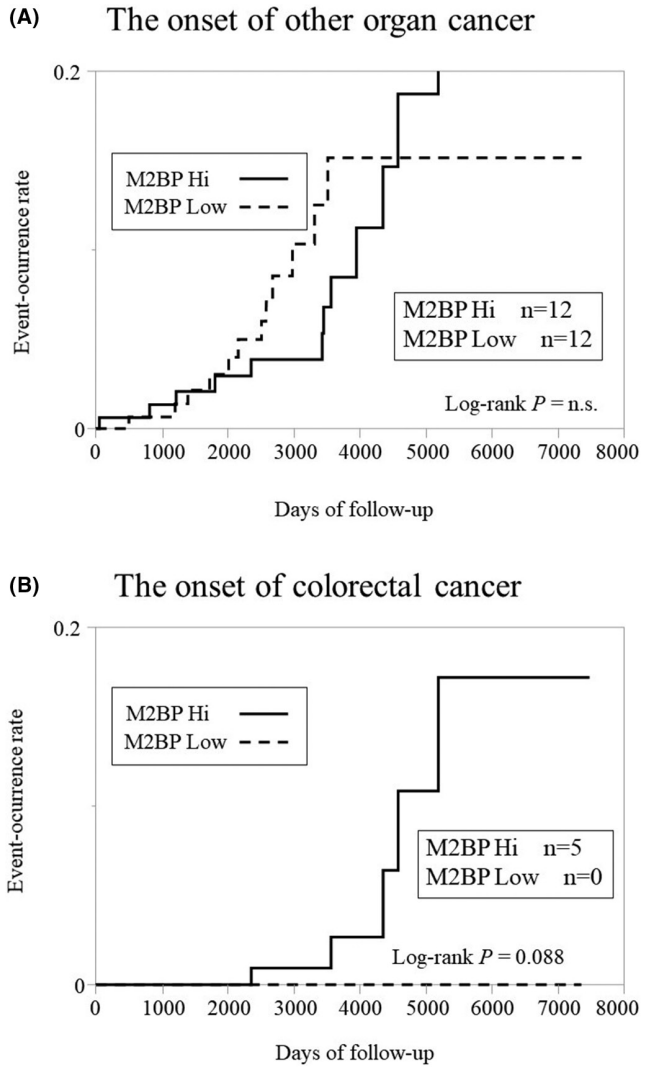

FIGURE 4 Cancer development in other organs according to the serum M2BP levels. (A) Comparison of new cancer development in other organs according to serum M2BP levels. (B) Comparison of new colorectal cancer development according to serum M2BP levels

DISCUSSION

In the present study, serum M2BP levels predicted future liver-related events (HCC, decompensated cirrhosis, and gastroesophageal varices). In particular, M2BP was useful to predict HCC occurrence. None of the patients with NAFLD with low serum M2BP levels (M2BP Low) developed HCC in this study. Additionally, serum M2BP predicted future colorectal cancer occurrence. Interestingly, none of the M2BP Low patients developed colorectal cancer. In contrast, no significant differences were found between M2BP Hi and M2BP Low patients in CVD event development.

Previously, we found that M2BP is a main target protein of fucosylation, a glycosylation modification.[13] Serum M2BP levels can distinguish NASH from NAFLD.[13,16] Additionally, serum M2BP levels can predict liver fibrosis progression in patients with NAFLD.[17] *Wisteria floribunda agglutinin* (WFA)–positive M2BP (WFA$^+$-M2BP) was identified as a serum fibrosis biomarker for CHC.[21] This biomarker was developed using a glycan-based immunoassay to assess liver fibrosis severity in patients with CHC and could distinguish the glycan structure of WFA-detectable M2BP.[21] In Japan, WFA$^+$-M2BP has been used clinically as a liver fibrosis biomarker (Mac-2 binding protein glycosylation isomer [M2BPGi]) since 2015. M2BPGi is very useful as a liver fibrosis biomarker for CHC and to predict HCC development in patients with CHC.[22] Our previous study revealed that compared with M2BPGi, M2BP showed a greater ability to predict NAFLD fibrosis stage.[23] Our study demonstrated that M2BP is a useful predictive biomarker for HCC development in patients with NAFLD. Additionally, M2BP predicts gastroesophageal varices development. These findings indicated that M2BP is a useful biomarker for liver-related event occurrence.

Colorectal cancer is a metabolic syndrome–related cancer.[24] Additionally, NAFLD is closely related to colorectal cancer development.[10] Kim et al. investigated 25,947 patients with NAFLD with a median 7.5 years of follow-up and found that male patients with NAFLD show a higher association with the development of colorectal cancer. The data of many epidemiological studies have revealed a significant association of NAFLD with the risk of colorectal adenomas and cancer.[25] Our study demonstrated that high levels of serum M2BP are a useful biomarker to predict colorectal cancer development in patients with NAFLD. Our preliminary data using M2BP knockout mice showed an abnormality in hepatic metabolism, although no significant change was observed in the mouse body weight. Thus, M2BP is not only an NAFLD biomarker but is also a predictive biomarker for colorectal cancer.

In this study, the CVD event incidence in patients with NAFLD was 3.3/1000 PY (NAFL, 1.54/1000 PY; NASH, 6.07/1000 PY). This incidence was very low compared with the data on Caucasian NAFLD and comparable with the data on the Caucasian general population.[9] A study from China demonstrated that 6 of 307 (2%) patients with NAFLD died from CVD events.[26] In this study, 72 among 307 patients with NAFLD (23.5%) were nonobese (BMI < 25 kg/m^2). In our study, 107 among 353 patients (30.3%) were nonobese. Lean NAFLD was first described in Asia and has been recognized globally.[27] In Japan, the ratio of lean NAFLD is higher than that in Western countries.[28,29] Lean patients with NAFLD have fewer complications of metabolic syndrome, and this finding may contribute to the low complication rate of CVD in Asians. Asian patients with NAFLD would have a different prognosis from Caucasian patients with NAFLD; therefore, the prediction of CVD event in our cohort would be difficult compared with other Caucasian NAFLD cohorts.

Our study has several limitations. First, the follow-up period was relatively short to monitor the survival of patients with NAFLD. The relatively small number of patients may explain the lack of significant differences in the statistical analysis of follow-up data. Second, we did not measure the *PNPLA3* (patatin-like phospholipase domain-containing protein 3) gene polymorphism, which is more common in Asian than in Western populations.[30] This gene polymorphism has homozygous mutations in approximately 20% of the general Japanese population[31] and is associated with NAFLD onset and progression.[32,33]

In conclusion, serum M2BP levels are a useful biomarker to predict liver-related events, particularly HCC. Additionally, M2BP is a potential biomarker of colorectal cancer development. Longer-term follow-up studies are warranted to examine the long-term predictive ability of M2BP for complications associated with NAFLD.

ETHICAL COMMITTEE APPROVAL

The protocol and informed consent were approved as a multicenter study by each institutional review board at Osaka University Hospital, Kochi Medical School Hospital, Hiroshima University Hospital, Ikeda Municipal Hospital, Otemae Hospital, and Osaka City University Hospital. Written informed consent was obtained from all the subjects at the time of liver biopsy or enrollment at each institute, and the study was conducted in accordance with the Helsinki Declaration.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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