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REVIEW

Utility of Mac-2 binding protein glycosylation isomer as an excellent biomarker for the prediction of liver fibrosis, activity, and hepatocellular carcinoma onset: an expert review

Yoshihiro Kamada[1] · Yoshio Sumida[2] · Hirokazu Takahashi[3] · Hideki Fujii[4] · Eiji Miyoshi[5] · Atsushi Nakajima[6] · Japan Study Group of NAFLD (JSG-NAFLD)

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Abstract Mac-2 binding protein glycosylation isomer (M2BPGi) is a liver fibrosis biomarker that originated in Japan and has been covered by health insurance for 10 years. M2BPGi is useful not only for liver fibrosis stage prediction but also for assessment of the degree of liver inflammation and prediction of hepatocellular carcinoma development. The usefulness of M2BPGi for assessing disease progression in patients with various chronic liver diseases has been demonstrated over the past decade in a large number of patients. Recently, there have been many reports from outside Japan, including reports from South Korea, Taiwan, Hong Kong, and China. These studies demonstrated that M2BPGi is an excellent biomarker that can evaluate the progression of liver fibrosis in chronic liver disease. It is also an excellent indicator of liver activity. Recently, a quantitative M2BPGi (M2BPGi-Qt) assay was developed, and future validation in real-world settings is expected. This will enable diagnosis of the progression of liver fibrosis based on more precise test results and is expected to contribute to the early detection and follow-up of diseases caused by chronic hepatitis, as well as post-treatment monitoring. The significance of the M2BPGi-Qt assay will likely become clearer as real-world data accumulate. If new cutoff values for each chronic liver disease stage and activity level using the M2BPGi-Qt assay are set based on real-world data, it is expected that this will become a useful tool to identify cases of liver fibrosis and monitor the progression of chronic liver disease.

Keywords MASLD/MASH · Chronic hepatitis type C · Chronic hepatitis type B · Autoimmune hepatitis · Primary biliary cholangitis

Abbreviations

AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
CHB	Chronic hepatitis type B
CHC	Chronic hepatitis type C
Mac-2bp	Mac-2 binding protein

✉ Yoshio Sumida
sumida19701106@yahoo.co.jp

Yoshihiro Kamada
ykamada@sahs.med.osaka-u.ac.jp

Hirokazu Takahashi
takahas2@cc.saga-u.ac.jp

Hideki Fujii
fujiirola@yahoo.co.jp

Eiji Miyoshi
emiyoshi@sahs.med.osaka-u.ac.jp

Atsushi Nakajima
nakajima-tky@umin.ac.jp

[1] Department of Advanced Metabolic Hepatology, Osaka University Graduate School of Medicine, 1-7, Yamadaoka, Suita, Osaka 565-0871, Japan

[2] Graduate School of Healthcare Management, International University of Healthcare and Welfare, 4-1-26, Akasaka, Minato-ku, Tokyo 107-8402, Japan

[3] Liver Center, Saga University Hospital, 5-1-1 Nabeshima, Saga 849-8501, Japan

[4] Department of Hepatology, Graduate School of Medicine, Osaka Metropolitan University, 1-4-3, Asahimachi, Abeno, Osaka 545-8585, Japan

[5] Department of Molecular Biochemistry and Clinical Investigation, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan

[6] Department of Gastroenterology and Hepatology, Yokohama City University Graduate School of Medicine, 3-9, Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

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M2BPGi	Mac-2 binding protein glycosylation isomer
M2BPGi-Qt	Quantitative M2BPGi assay
MASLD	Metabolic dysfunction-associated steatotic liver disease
HCC	Hepatocellular carcinoma
NIT	Noninvasive test
FIB-4	Fibrosis-4 index
NFS	Nonalcoholic fatty liver disease fibrosis score
ELF test	Enhanced liver fibrosis test
ATX	Autotaxin
T4C7S	Type IV collagen 7S
HA	Hyaluronic acid
CK18-F	Cytokeratin 18 fragment
MRE	Magnetic resonance elastography
PDFF	Proton density fat fraction
VCTE	Vibration-controlled transient elastography
p-SWE	Point shear wave elastography
2D-SWE	2-Dimensional SWE
CAP	Controlled attenuation parameter
WFA lectin	*Wisteria floribunda* Lectin
C.O.I.	Cutoff index
SVR	Sustained virologic response
ETV	Entecavir
TDF	Tenofovir disoproxil fumarate
LSM	Liver stiffness measurement
AIH	Autoimmune hepatitis
PBC	Primary biliary cholangitis
LC	Liver cirrhosis
DAA	Direct-acting antivirals
T2DM	Type 2 diabetes mellitus
IFN	Interferon
HSCs	Hepatic stellate cells
CAFs	Cancer-associated fibroblasts
HCV	Hepatitis C virus
MASH	Metabolic dysfunction-associated steatohepatitis
HR	Hazard ratio

Introduction

In chronic liver disease, the incidence of liver-related events such as hepatocellular carcinoma (HCC), decompensated cirrhosis, and hemorrhagic varices increases with the progression of liver fibrosis [1]. Traditionally, liver tissue diagnosis by biopsy has been used as the gold standard to evaluate the extent of liver disease progression. In recent years, with the evolution of diagnostic imaging methods and blood biomarkers, accurate diagnosis of liver fibrosis using noninvasive tests (NITs) has become possible (Table 1) [2–4]. Among NITs, the fibrosis-4 (FIB-4) index is a very simple and useful scoring system that combines clinical data. Many of the proposed algorithms that combine various NITs use the FIB-4 index as the first stage and other NITs as the second stage [5–7]. We are now able to use many liver fibrosis markers, apoptosis markers, elastography, and liver-fat quantification methods for the evaluation of liver disease progression (Table 1) [2]. By combining these NITs, we have reached the next generation of liver pathophysiological evaluation.

Table 1 NITs used in Japan

Classification of NITs	Name of NITs
Scoring system	FIB-4 index
	NFS
Blood biomarker	
Liver-specific fibrosis makers	ELF test
	T4C7S
	M2BPGi
	ATX
	HA
Apoptosis marker	CK-18F (M30)
Imaging diagnosis	
Liver stiffness measurement	VCTE
	p-/2D-SWE
	MRE
Liver-fat measurement	Attenuation Coefficient (CAP, etc.)
	PDFF

(As of August 2024)

NITs non-invasive tests, *FIB-4* fibrosis-4, *NFS* nonalcoholic fatty liver disease fibrosis score, *ELF test* enhanced liver fibrosis test, *ATX* autotaxin, *T4C7S* type IV collagen 7S, *HA* hyaluronic acid, *CK18-F* cytokeratin 18 fragment, *MRE* magnetic resonance elastography, *PDFF* proton density fat fraction, *VCTE* vibration-controlled transient elastography, *p-SWE* point shear wave elastography, *2D-SWE* 2-dimensional SWE, *CAP* controlled attenuation parameter

Mac-2 binding protein glycosylation isomer (M2BPGi) is a liver fibrosis biomarker that originated in Japan and has been covered by health insurance for 10 years. M2BPGi is a "Mac-2 binding protein (Mac-2bp) glycosylation isomer" that is recognized by *Wisteria floribunda* lectin (also known as WFA + -Mac-2bp) [8]. That is, Mac-2bp having a special glycan structure recognized by WFA is M2BPGi. Initially, there were numerous reports on its use as a liver fibrosis marker with regard to the progression of liver fibrosis. Since then, its usefulness as a biomarker to predict liver cancer has also been reported. M2BPGi also reflects improvements in disease progression during treatment. There have been numerous reports on its usefulness as a biomarker not only for liver fibrosis but also for liver activity. Recently, there have been many reports from outside Japan, including reports from South Korea, Taiwan, Hong Kong, and China, as well as Indonesia and West Africa (Gambia).

This review will outline M2BPGi, its usefulness and future prospects in chronic diseases, and M2BPGi-producing cells and will also touch upon a quantitative M2BPGi (M2BPGi-Qt) assay, which has recently become covered by health insurance. We briefly summarize the papers reporting the usefulness of M2BPGi in chronic liver disease (Table 2).

Mac-2 binding protein (Mac-2bp)

Mac-2bp is a highly glycosylated secreted glycoprotein with seven *N*-glycosylated attachment sites that was first identified in breast cancer tissues and serum in 1986 [9–11]. Koths et al. found that it specifically binds to galectin-3 (Mac-2)

Table 2 M2BPGi and liver fibrosis in chronic liver diseases

Author[a]	Diseases	Number of participants (F/M)	Mean or median[a]	F0	F1	F2	F3	F4	Publication year	Country
Kuno	CHC	160 (88/72)							2013	Japan
Yamasaki	CHC	707 (356/351)	Mean		1.3	2.2	3.3	5.2	2014	Japan
Tamaki	CHC	66 (35/31)	Median		0.81	1.82	2.31	7.5	2015	Japan
Sasaki	CHC	238 (90/148)							2015	Japan
Ura	CHC	159 (genotype 1)	Median	1.2	1.6	3.86	3.53	3.12	2015	Japan
Nishikawa	CHC	386 (205/181)							2016	Japan
Shigefuku	CHC	152 (77/75)							2016	Japan
Huang	CHC	229	Mean	1.68	2.23	3.45	3.48	3.77	2017	Taiwan
Fujita	CHC	122	Median		1.26	1.81	4.03	7.86	2018	Japan
Nakagawa	CHC	947 (510/437)							2020	Japan
Yugawa	CHC	81 (31/50)							2022	Japan
Kawata	CHC	3580 (1905/1675)							2022	Japan
Liu	CHC	1460 (665/795)							2022	Taiwan
Chang	CHC	638 (274/364)	Median (determined by TE)		1.06	1.72	2.18	4.82	2024	Taiwan
Heo	CHB	95 (26/69)							2016	Korea
Nishikawa	CHB	249 (94/155)	Median						2016	Japan
Ishii	CHB	189 (72/117)	Median		0.9	1.4	1.6	3.1	2017	Japan
Ichikawa	CHB	112 (40/72)	Median	0.57	0.75	1.14	1.03	1.64	2017	Japan
Zou	CHB	297 (100/197)	Median	0.54	0,76	1.11	1.16	1.22	2017	China
Jekarl	CHB	151	Mean	0.53	0.68	0.87	1.65		2018	Korea
Mak	CHB	327	Median		0.26	0.34	0.57	1.21	2018	China
Yeh	CHB	160	Median	0.63	0.64	1.36	1.65	2.7	2019	Taiwan
Vincent	CHB	339 (110/229)							2022	Gambia
Pramono	CHB	109 (53/56)							2024	Indonesia
Abe	MASLD	289 (130/159)	Mean	0.57	0.7	1.02	1.57	2.96	2015	Japan
Nishikawa	MASLD	134 (68/66)	Median		0.7	0.7	1.2	1.6	2016	Japan
Shigefuku	MASLD	139 (59/80)							2016	Japan
Ogawa	MASLD	165	Mean	0.43	0.62	0.92	1.12	2.94	2018	Japan
Atsukawa	MASLD	213	Median		0.71	1.17	1.36	1.98	2018	Japan
Park	MASLD	602 (249/353)							2020	Korea
Jang	MASLD	113 (55/58)							2021	Korea
Ito	MASLD	93 (45/48)							2022	Japan
Moon	MASLD	231 (107/124)							2023	Korea
Suzuki	MASLD	38 (15/18)							2023	Japan
Nah	MASLD	108 (46/62)							2024	Korea
Nishikawa	AIH	84 (69/15)	Median		1.5	2.1	3.3	9.8	2016	Japan
Migita	AIH	123 (102/21)							2020	Japan
Umemura	PBC	137 (111/26)	Median	0.4	0.6	1.4	2	3	2015	Japan
Nishikawa	PBC	57 (49/8)	Median		1.4	2.1	2	9.2	2016	Japan

F female, *M* male, *CHB* chronic hepatitis type B, *CHC* chronic hepatitis type C, *MASLD* metabolic dysfunction-associated steatotic liver disease, *AIH* autoimmune hepatitis, *PBC* primary biliary cholangitis, *TE* transient elastography

[a]The values of each fibrosis stage are demonstrated as the mean or median

through carbohydrate-specific interactions [12]. Mac-2bp is identical to a previously reported tumor-associated antigen (90 K: named for its molecular weight) that is released into the culture supernatant of human breast cancer cells [13]. Mac-2bp belongs to the scavenger receptor cysteine-rich domain superfamily of proteins that are involved in immune defense and regulation [14]. Mac-2bp is expressed in various tissues, and its expression in macrophages is upregulated by adhesion. In mice, it is upregulated by inflammatory cytokines such as tumor necrosis factor-α and interferon (IFN)-γ [15]. Furthermore, Mac-2bp has been suggested to enhance cell–cell adhesion by binding to extracellular matrix proteins, including β1 integrin, collagen, and fibronectin [16]. Thus, although Mac-2bp is a well-known glycoprotein, its physiological function is not fully understood in detail.

It has been reported that blood Mac-2bp levels are increased in patients with various cancers (e.g., pancreatic cancer, breast cancer, and lung cancer), viral hepatitis, and autoimmune diseases [10]. Mac-2bp is barely detectable in normal liver tissue, but its expression in the livers of patients with chronic hepatitis C (CHC) increases with the progression of fibrosis [17, 18]. We have found that the blood level of Mac-2bp is a biomarker that can predict the histological severity of metabolic dysfunction-associated steatotic liver disease (MASLD) [19, 20]. The name previously known as nonalcoholic fatty liver disease (NAFLD) has been changed to MASLD. In 2023, experts from multiple hepatology societies have recently suggested using the term MASLD instead of NAFLD [21]. MASLD is defined as subjects who have been diagnosed with SLD through imaging or liver biopsy, have one or more of the five cardiometabolic risk factors, and have a low alcohol intake [21]. NAFLD and MASLD are 95–99% identical in diagnoses in the population, and can be considered to be essentially the same disease [22–24]. The NIT thresholds for both diseases are also consistent [23].

Mac-2 binding protein glycosylation isomer

Mac-2bp is a glycoprotein that is rich in *N*-glycosylation sites, including seven potential sites [10, 11]. In addition, the structures of Mac-2bp glycans have been reported to be markedly altered by fibrosis progression in the liver [8]. M2BPGi is a serum liver fibrosis biomarker and a glycosylation isomer that is recognized by WFA lectin [8]. WFA lectin recognizes *N*-acetylgalactosaminide at the terminus of the glycan and specifically binds to the disaccharide LacdiNAc (β-D-GalNAc-[1 → 4]-D-GlcNAc; GalNAc *N*-acetylgalactosamine, GlcNAC N-acetylglucosamine) [25]. Kuno and Narimatsu developed an automated assay system that can detect WFA + -Mac-2bp within 20 min [8]. Initially, M2BPGi was measured as a cutoff index (C.O.I.: negative, positive (1 +), positive (2 +)). Recently, M2BPGi-Qt assays have been developed [26]. In the M2BPGi-Qt test, the accuracy was improved by increasing the number of calibration curve points from two to five and performing analysis using a logistic curve. These assays are expected to assist in diagnosing the progression of liver fibrosis based on more precise test results and to enable early detection, follow-up, and post-treatment monitoring of diseases caused by chronic hepatitis.

M2BPGi has been used clinically in Japan since 2015 as a biomarker for liver fibrosis. For approximately 10 years, real-world data have demonstrated that M2BPGi can differentiate patients at high risk for severe fibrosis from healthy control groups [27], and M2BPGi may be a predictor of hepatocarcinogenesis [28]. Because M2BPGi was identified and developed as a fibrosis marker from the serum of patients with CHC [8], its behavior differs based on the level of fibrosis progression against the background of other etiologies. Therefore, cutoffs for different etiologies should be established [29]. In addition, the pathophysiological mechanism of M2BPGi, which might be different from that of Mac-2bp, is unclear [28]. M2BPGi is a dedicated reagent for the HISCL system (Sysmex Co., Hyogo, Japan) and is currently registered only in Asia.

What types of cells produce M2BPGi?

The glycan structure of a protein varies depending on the cell that produces it, and this structure also changes depending on the state of the cell [30]. These characteristics of glycans make them suitable for medical applications [31]. Several studies have investigated the types of Mac-2bp-producing cells [32–35]. Using immunohistochemical analysis, Bekki and Shirabe found that hepatic stellate cells (HSCs) secrete M2BPGi. They demonstrated that M2BPGi produced from HSCs induces galectin-3 (Mac-2) production in Kupffer cells, and this galectin-3 further activates HSCs, leading to fibrotic changes in the liver. The amount of Mac-2bp produced and its glycan structure change due to the degeneration and activation of HSCs. This is thought to be why M2BPGi can determine the degree of fibrosis. In addition, the produced M2BPGi stimulates Kupffer cells, causing inflammation, which is thought to be one of the reasons why M2BPGi is useful for predicting liver activity. It is reported that Mac-2bp with characteristic glycan structure binds to c-type lectin on macrophage [36] and cleaved Mac-2bp (70K Mac2-bp) attached to cell surface of cancer cells [37]. More interestingly, Gantumur et al. demonstrated that high levels of Mac-2bp mRNA were expressed in HSCs of liver tissue from patients with chronic liver disease, whereas strongly positive staining of Mac-2bp was observed in Kupffer cells but little staining of Mac2-bp in HSC with immunohistological analysis [34]. Since Mac-2bp has the property of

adhering to various proteins, it is thought that Mac-2bp produced in HSCs was adhered to the cell surface of Kupffer cells, which might induce more inflammation signals.

We found that hepatocytes are the main source of Mac-2bp by performing immunohistochemical staining of Mac-2bp in MASLD patient livers [35]. We also demonstrated that serum Mac-2bp levels and hepatic gene expression of Mac-2bp significantly correlated in mouse liver disease models [38]. M2BPGi produced by hepatocytes is expected to indicate hepatocyte degeneration. As chronic liver disease progresses, hepatocyte degeneration occurs and function changes. As function changes, the glycan structure of the glycoproteins produced by the hepatocytes changes significantly, and M2BPGi may reflect this. Therefore, it is speculated that M2BPGi may be useful for predicting the onset of liver cancer and decompensated cirrhosis.

Interestingly, when the staining of hepatocytes was scored by immunostaining, we found that there was an inverse correlation between the blood concentration and the degree of staining [35]. We have previously reported that differences in glycan modification, known as fucosylation, change the polar transport of glycoproteins in hepatocytes [39]. We identified Mac-2bp as one of the fucosylation target glycoproteins [19]. Therefore, we believe that the reason for this is that Mac-2bp produced in hepatocytes changes its intracellular polar transport due to changes in its glycan structure, resulting in the production of one that is more likely to accumulate in the cell and one that is more likely to be secreted into the blood.

Yamanaka et al. found that serum M2BPGi levels are upregulated in pancreatic cancer patients [40]. They demonstrated that cancer-associated fibroblasts (CAFs) are sources of M2BPGi, and M2BPGi produced from CAFs promoted the proliferation and invasion of pancreatic cancer cells.

Diagnostic ability of M2BPGi in various diseases

Chronic hepatitis type C

Mac-2bp is weakly detected in the normal liver but strongly detected in hepatocytes from CHC patients as liver fibrosis progresses [17, 18]. Recently, WFA^+- Mac-2bp, also known as M2BPGi, was identified as a novel serum fibrosis biomarker for CHC [8]. WFA recognizes terminal *N*-acetylgalactosaminides and specifically binds with the disaccharide LacdiNAc (β-D-GalNAc-[1 → 4]-D-GlcNAc; GalNAc *N*-acetylgalactosamine, GlcNAc *N*-acetylglucosamine) [25]. This biomarker was developed using a glycan-based immunoassay for the assessment of liver fibrosis severity in CHC patients and could distinguish the glycan structure of WFA-detectable Mac-2bp [8]. Kuno et al. developed an automatic measurement system that can detect WFA^+- Mac-2bp within 20 min. Our meta-analysis suggested that serum M2BPGi is a reliable biomarker to distinguish advanced fibrosis in various chronic liver diseases [41]. It should be noted that blood M2BPGi levels vary depending on the type of chronic liver disease [8, 42–45].

In Japan, M2BPGi has been used clinically as a novel liver fibrosis biomarker since 2015. To date, M2BPGi has been reported as a useful liver fibrosis biomarker not only in Japan, but also in other countries, such as Korea, China, and Indonesia. Liu et al. investigated serum M2BPGi levels in 1460 CHC patients, and they chose the following M2BPGi C.O.I. values: 1.72 (≥ stage 2), 2.65 (≥ stage 3), and 3.93 (stage 4) [46]. M2BPGi is a highly effective biomarker for liver fibrosis in CHC and is highly useful for predicting liver carcinogenesis. Yamazaki et al. measured M2BPGi in 707 CHC patients and found that serum M2BPGi levels were significantly increased with the progression of the liver fibrosis stage, and the risk of HCC development was increased according to M2BPGi elevation [47]. Surprisingly, the time-dependent areas under the receiver operating characteristic curve demonstrated that M2BPGi predicted the development of HCC with higher diagnostic accuracy than that of alpha-fetoprotein. Nagata et al. demonstrated the usefulness of M2BPGi for predicting cancer development after IFN and IFN-free therapy in CHC patients [48]. The utility of M2BPGi for the prediction of HCC occurrence has been reported in many studies, and here we summarize papers reporting the usefulness of M2BPGi in predicting the onset of HCC (Table 3).

After eradication of hepatitis C virus (HCV), serum M2BPGi levels significantly decreased [8, 49–51]. Patients with a high level of M2BPGi even after achieving a sustained virologic response (SVR) had a higher incidence of new-onset HCC and death [49, 52]. Sasaki et al. investigated 238 CHC patients with an SVR and concluded that M2BPGi > 2.0 C.O.I. at 24 weeks after completion of IFN was associated with the development of HCC after SVR. Akuta et al. investigated 1,922 CHC patients with an SVR achieved by direct-acting antiviral (DAA) treatment [53]. They measured M2BPGi before DAA treatment and 24 weeks after achieving an SVR and found an M2BPGi level ≥ 2.5 at baseline and ≥ 1.0 C.O.I. at 24 weeks after achieving an SVR was associated with HCC development after SVR induced by DAA treatment. Interestingly, Kawata et al. investigated the prognosis of 3,580 CHC patients who achieved an SVR and found that M2BPGi levels could predict not only HCC, but also non-HCC malignancies [51]. We also demonstrated serum Mac-2bp levels can predict new HCC and colorectal cancer occurrence in MASLD patients [54]. In addition, our previous report demonstrated that hepatocytes are the main source of Mac-2bp by performing immunohistochemical staining of Mac-2bp in MASLD patient livers [35]. M2BPGi may reflect changes in the

Table 3 M2BPGi utility in the prediction of HCC development/recurrence and prognosis

M2BPGi and HCC development

Author*	Diseases	Number of participants (F/M)	Number of HCC patients	Median study period (wks)	M2BPGi threshold value for HCC	Publication year	Country
Sasaki	CHC	238 (90/148)	16	474	> 2.0 (post SVR)	2015	Japan
Hasegawa	CHC-LC	165 (72/93)	68	201	≥ 6.15	2016	Japan
Sato	CHC SVR	355 (139/216)	12	151	≥ 2.80	2016	Japan
Yamasaki	CHC	707 (356/351)	110	428	≥ 4.0	2014	Japan
Nagata	IFN or IFN-free therapy CHC HCC	119 (59/70)	8	370	≥ 2.2	2016	Japan
Akuta	CHC SVR by DAA	1922 (1114/808)	43	52	≥ 2.5 (pre-DAA treatment) ≥ 1.0 (SVR 24)	2019	Japan
Ichikawa	CHB	112 (40/72)	15	173	≥ 0.71	2017	Japan
Kim	CHB	1323 (530/793)	52	258	≥ 1.8	2017	Korea
Mak	CHB	225 (48/177)	100	370	≥ 1.15 (before NA treatment)	2019	China
Chen	CHB	899 (242/657)	115	313	≥ 1.0 (at 5 years of NA treatment)	2024	Taiwan
Tseng	CHB	Discovery cohort 899 (230/669) Validation cohort 384 (102/282)	Discovery cohort 64 Validation cohort 36	Discovery cohort 366 Validation cohort 351	≥ 1.73 (during ETV treatment)	2020	Taiwan
Suzuki	CHB	466 (202/264)	33	206	N.A	2021	Japan
Heo	CHB	95 (26/69)	7	193	1.8	2016	Korea
Cheung	Antiviral therapy CHB	57 (9/48) (HCC) 57 (9/48) (non-HCC)	57	167 (HCC) 188 (non-HCC)	≥ 0.69	2017	China
Kawanaka	MASLD	331 (161/170)	51	419	≥ 1.255	2018	Japan
Kanno	MASLD	85 (45/40)	36	417	≥ 3.11 (stage 4 patients)	2019	Japan

M2BPGi utility in the prediction of HCC recurrence and prognosis

Author*	Diseases	Number of participants (F/M)	Number of HCC recurrences	Median study period after therapy (wks)	M2BPGi threshold value for HCC recurrence	Publication year	Country
Hanai	LC	59 (18/41)	3	48	≥ 5.0 (for mortality)	2015	Japan
Toyoda	HCC curative resection	240 (57/183)	117	276	≥ 3.00	2016	Japan
Yugawa	After resection of HCC	150 (41/109)	34	257	≥ 1.58	2015	Japan
Fujiyoshi	After resection of HCC	376 (70/306)	80 (CHC patients) 20 (non-CHC patients)	172	> 4.615 (CHC patients) > 1.435 (non-CHC patients)	2015	Japan

HCC hepatocellular carcinoma, *DAA* direct-acting antivirals, *LC* liver cirrhosis, *wks* weeks, *SVR* sustained virologic response, *IFN* interferon, *ETV* entecavir

glycan structure of hepatocytes accompanying the progression of chronic liver disease. Therefore, because the change in the glycan structure of Mac-2bp produced by degenerated hepatocytes that leads to the development of HCC can be detected as M2BPGi, it is thought that M2BPGi may be useful for predicting liver carcinogenesis in various chronic

liver diseases. Degenerated hepatocytes might produce any hepatokines that would cause progression of non-HCC malignancies.

Chronic hepatitis type B (CHB)

Our meta-analysis suggested that serum M2BPGi is a reliable biomarker for distinguishing advanced fibrosis in various chronic liver diseases other than CHC, including CHB [41]. In CHB patients, serum levels of M2BPGi demonstrated stepwise increases with the liver fibrosis stage progression [55–57]. Nishikawa et al. investigated serum levels of M2BPGi in 386 CHC patients and 249 CHB patients and demonstrated its value was higher in CHC patients than in CHB patients, even for the same degree of fibrosis [55]. Ishii et al. investigated 189 treatment-naïve CHB patients and demonstrated that M2BPGi is a useful biomarker not only for liver fibrosis stage prediction, but also for liver inflammation activity [56]. Ichikawa et al. investigated 112 treatment-naïve CHB patients and found M2BPGi values were useful for assessing the liver fibrosis stage and were independently associated with HCC development [57]. The predictive ability of M2BPGi for HCC development in CHB patients has been reported in many studies [57–62]. Mak et al. investigated 100 HCC patients and 185 matched CHB patients without HCC during entecavir (ETV) treatment [63]. They found that patients with high M2BPGi values (≥ 1.15 C.O.I.) had higher risk for subsequent HCC development during ETV treatment. Tseng et al. investigated 899 CHB patients receiving long-term ETV therapy [59]. Among the 899 patients, 64 patients developed HCC, and they found patients with higher serum M2BPGi levels (≥ 1.73 C.O.I.) had an increased HCC risk compared with the low M2BPGi group, with a hazard ratio (HR) of 5.80. Chen et al. investigated 899 CHB cirrhosis patients who did not develop HCC within the first 5 years of treatment (ETV or tenofovir disoproxil fumarate (TDF)). Among the 899 patients, 115, 57, and 37 experienced new HCC development, all-cause mortality, and liver-related mortality, respectively. They found that an increased M2BPGi level at 5 years of treatment was an independent predictor of HCC occurrence beyond year 5. They also found that an M2BPGi level of 1.0 C.O.I. at 5 years of treatment (EVT, TDF) could stratify the risk of all-cause and liver-related mortality in CHB patients with cirrhosis. M2BPGi was useful for predicting the development of HCC in CHB patients undergoing antiviral therapy in an age- and sex-matched study [64].

Reports of M2BPGi in CHB patients are increasingly originating from countries other than Japan, including Korea, China, Indonesia, and West Africa (Gambia) [43, 62, 65, 66]. Zou et al. investigated serum M2BPGi values and liver stiffness measurements assessed by FibroScan in 89 Chinese CHB patients during nucleot(s)ide analog therapy and found that serum M2BPGi can predict the liver fibrosis stage during both progression and regression.

Metabolic dysfunction-associated steatotic liver disease

M2BPGi is a useful predictor of MASLD at fibrosis stages ≥ 2 and ≥ 3 [67]. It is not affected by age and can be judged by a single cutoff point [68]. As with other chronic liver diseases, M2BPGi is also a useful liver fibrosis biomarker for MASLD. In addition, many studies demonstrated that M2BPGi is useful for the discrimination of metabolic dysfunction-associated steatohepatitis (MASH) from MASLD [42, 67, 69–72]. Abe et al. first demonstrated the usefulness of M2BPGi in 289 biopsy-proven MASLD patients [42]. In their study, the optimal cutoff values were 0.59 ≥ stage 1, 0.90 ≥ stage 2, 0.94 ≥ stage 3, and 1.46 for stage 4. Other reports also demonstrated approximately the same cutoff values of M2BPGi for the MASLD fibrosis stage. Shigefuku et al. compared serum M2BPGi levels in 72 CHC patients and 58 MASLD patients [73]. The cutoff values of advanced liver fibrosis (stage 3–4) were 3.28 C.O.I. in CHC patients and 1.06 C.O.I. in MASLD patients. Considering these findings in MASLD patients, M2BPGi ≥ 1.0 C.O.I. would be a suitable indicator of advanced liver fibrosis in MASLD patients.

Kim et al. investigated chronic liver disease patients and healthy subjects in a propensity score-matched study by age and sex [71]. They used the FIB-4 index and M2BPGi to generate a diagnostic algorithm for advanced liver fibrosis. The FIB-4 index is a well-known, simple scoring system calculated using four factors (aspartate aminotransferase, alanine aminotransferase (ALT), platelet count, and age) [74, 75]. The FIB-4 index can be easily calculated, is widely available in clinical settings, has a low cost, and is the most validated measure for the prediction of MASLD with severe liver fibrosis. In addition, some clinical practice guidelines have recommended it as an initial triage tool in clinical practice [2, 76–78]. M2BPGi is a useful predictor of advanced fibrosis in MASLD patients [67] and is not affected by age. The combination of the FIB-4 index and M2BPGi would be an excellent method for the discrimination of advanced liver fibrosis in MASLD patients. Based on a proposal by Kim et al. [71], we aim to propose an algorithm to determine advanced liver fibrosis in MASLD patients (Fig. 1).

At the same time as Kuno et al. reported the development of M2BPGi measurement, we reported that Mac-2bp is a useful biomarker for MASLD [19]. Mac-2bp is a useful measure for differentiating MASH, and it increases with liver fibrosis progression [20, 35]. In addition, we demonstrated that serum Mac-2bp levels can predict new HCC and colorectal cancer occurrence in MASLD patients [54]. Serum Mac-2bp levels would be useful not only for the prediction of HCC but also for the prediction of non-HCC

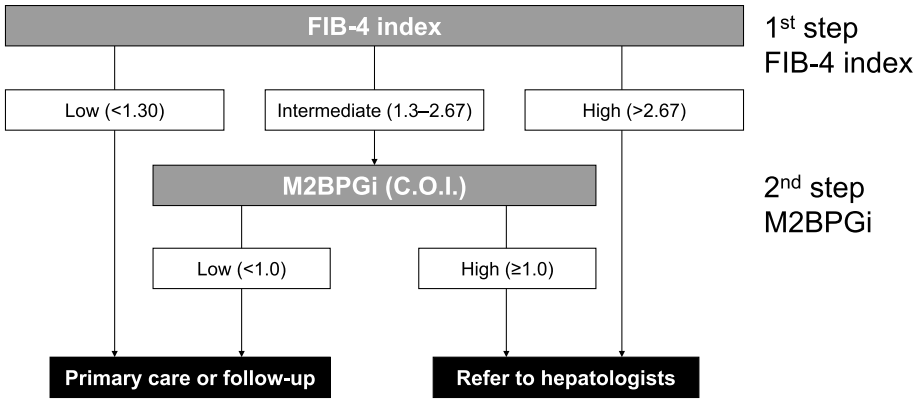

Fig. 1 Proposal of an algorithm to determine advanced liver fibrosis in MASLD patients. As a first step, MASLD patients are divided by their FIB-4 index. In the second step, patients who are considered to be in the intermediate group according to the FIB-4 index are divided according to the M2BPGi value (≥ 1.0 C.O.I.)

malignancies. M2BPGi also can predict HCC development and gastroesophageal varix formation in MASLD patients [79, 80]. Kawanaka et al. investigated 331 biopsy-proven MASLD patients with a 419-week mean study period. Among 331 patients, 51 developed HCC. MASLD patients with M2BPGi ≥ 1.255 C.O.I. demonstrated a significantly higher rate of HCC development than those with M2BPGi < 1.255.

Autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC)

Serum levels of M2BPGi in AIH and PBC patients are higher than those in patients with other chronic liver diseases, except for CHC. This is because liver inflammation has significant effects on M2BPGi values in these diseases. Nishikawa et al. investigated 84 biopsy-proven AIH patients [45]. They examined the serum M2BPGi values with regard to liver fibrosis stage and liver inflammation degree. The median values of M2BPGi in AIH patients with liver fibrosis stages *F*1, *F*2, *F*3, and *F*4 were 1.5, 2.1, 3.3, and 9.8 C.O.I., respectively. The median values of M2BPGi in AIH patients with liver inflammation stages *A*1, *A*2, and *A*3 were 1.6, 2.5, and 5.4, respectively. The authors also investigated M2BPGi values in 57 biopsy-proven PBC patients [44]. The median values of M2BPGi in PBC patients with liver fibrosis stages *F*1, *F*2, *F*3, and *F*4 were 1.4, 2.1, 2.0, and 9.2, respectively. The median values of M2BPGi in PBC patients with liver inflammation stages *A*1, *A*2, and *A*3 were 1.6, 1.9, and 3.7, respectively. These results indicate that serum levels of M2BPGi increase not only with liver fibrosis progression, but also with liver inflammation degree. Umemura et al. observed 137 PBC patients for an average of 7.7 years and investigated the relationships between initial M2BPGi values and prognosis in these patients [81]. The median values of M2BPGi in PBC patients with liver fibrosis stages *F*0, *F*1, *F*2, *F*3, and *F*4 were 0.4, 0.6, 1.4, 2.0, and 3.0 (C.O.I.), respectively. The cutoff values of M2BPGi in PBC patients with liver fibrosis stages ≥ *F*1, ≥ *F*2, ≥ *F*3, and F4 were 0.7, 1.0, 1.4, and 2.0 (C.O.I.), respectively. Interestingly, the serum M2BPGi level was useful as a marker to predict not only liver fibrosis but also the prognosis in patients with PBC. Patients with a serum M2BPGi level of 2.0 (C.O.I.) or higher had a lower survival rate (HR 18.59) and a higher rate of progression to decompensated cirrhosis than those with a serum M2BPGi level of less than 2.0 (C.O.I.) (HR 11.55).

Application of M2BPGi to predict the prognosis of patients with liver cirrhosis and HCC treatment

Many researchers have investigated the usefulness of M2BPGi for the prognosis prediction of liver cirrhosis patients or HCC patients who received any therapies (Table 3). Hanai et al. demonstrated the usefulness of M2BPGi for predicting the prognosis of patients with liver cirrhosis [82]. They found that liver cirrhosis patients with elevated M2BPGi levels (≥ 5.0 C.O.I.) had increased mortality. M2BPGi can predict cancer occurrence after curative treatment of HCC [64, 83–85]. M2BPGi is also useful for the prediction of complications, including liver failure after transcatheter arterial chemo-embolization, systemic chemotherapy, HCC surgery, and liver transplantation [86–92]. These study results demonstrate the usefulness of M2BPGi for prognostic prediction of chronic liver diseases and are expected to be widely used in clinical practice.

Other organ diseases

There have been reports on the significance of measuring blood M2BPGi levels for several diseases other than chronic liver disease. The effectiveness of this method has been reported for pulmonary fibrosis [93], biliary atresia [94], pancreatic cancer [40], and adult-onset Still's disease [95]. Interestingly, this method has also been reported to be useful in predicting the onset of type 2 diabetes mellitus (T2DM) [96]. Higher serum M2BPGi levels were significantly associated with newly T2DM onset. A high proportion of MASLD patients with T2DM have advanced liver fibrosis [97, 98].

Strikingly, when subjects with M2BPGi > 1.0 C.O.I. were diagnosed with T2DM at health checkup, the prevalence of advanced fibrosis increased to 50% [99]. Interestingly, serum M2BPGi levels also are associated with micro- and macro-angiopathy in people with T2DM [100]. Serum M2BPGi levels could be used to assess T2DM complications. As mentioned previously, serum Mac-2bp levels can predict not only newly developed HCC, but also colorectal cancer occurrence in MASLD patients [54]. These findings indicate that serum M2BPGi levels can predict chronic organ inflammation.

M2BPGi in health check-ups

There have been several attempts to use M2BPGi as a screening test in health check-ups. Of course, the diagnosis of liver fibrosis by measuring M2BPGi is appropriate [101]. Other than liver fibrosis prediction, M2BPGi measurement is useful for screening for carotid artery sclerosis [102]. Nah et al. reported that M2BPGi is useful for evaluating lifestyle interventions [101]. In addition, T2DM is closely associated with MASLD pathophysiology [97, 98]. Castera investigated 330 T2DM patients with persistently high ALT values and found that the prevalence rates of MASH, advanced liver fibrosis ($\geq F3$), and cirrhosis were 58%, 38%, and 10%, respectively [98]. Park et al. investigated 952 subjects who received health check-ups regarding the risk for advanced liver fibrosis as assessed by the FIB-4 index and nonalcoholic fatty liver disease fibrosis score [99]. They concluded that patients with T2DM with M2BPGi > 1.0 C.O.I. had a high probability of advanced liver fibrosis. Interestingly, Hashimoto et al. demonstrated serum levels of M2BPGi could predict both micro- and macro-angiopathy in patients with T2DM [100]. Serum M2BPGi levels are decreased by lifestyle interventions and drug treatment in MASLD patients [101, 103]. Tamai et al. demonstrated that M2BPGi is useful for MASH screening in health check-ups [104].

Quantitative M2BPGi measurement

Initially, M2BPGi was measured as a C.O.I. (negative, positive (1 +), positive (2 +)). Recently, methods for measuring M2BPGi have shifted to quantitative approaches (M2BPGi-Qt, AU/mL), and this measurement is already covered by insurance in Japan [26]. M2BPGi-Qt improves accuracy by increasing the number of points in the calibration curve from 2 to 5 and performing the analysis using a logistic curve. M2BPGi-Qt uses AU/mL as the unit and adjusts 1 C.O.I. in the current qualitative measurement method of M2BPGi to 1 AU/mL. These advances will help minimize the variability in cut-off values among different etiologies of chronic liver disease [26]. These will enable diagnosis of the progression of liver fibrosis based on more precise test results and is expected to contribute to the early detection and follow-up of diseases caused by chronic hepatitis, as well as post-treatment monitoring. Uojima et al. demonstrated that M2BPGi-Qt assays can predict the liver fibrosis stage and inflammation degree, regardless of etiology [105]. In CHC patients ($n = 347$), the median M2BPGi-Qt values for liver fibrosis stages *F*0, *F*1, *F*2, *F*3, and *F*4 were 0.95, 1.32, 1.63, 2.10, and 3.07 (AU/mL), respectively. In CHB patients ($n = 163$), the median M2BPGi-Qt values for liver fibrosis stages *F*0, *F*1, *F*2, *F*3, and *F*4 were 0.87, 0.99, 1.07, 2.27, and 2.35 (AU/mL), respectively. In MASLD patients ($n = 158$), the median M2BPGi-Qt values for liver fibrosis stages *F*0, *F*1, *F*2, *F*3, and *F*4 were 0.73, 0.79, 1.34, 1.81, and 2.37 (AU/mL), respectively. In AIH patients ($n = 153$), the median M2BPGi-Qt values for liver fibrosis stages *F*0, *F*1, *F*2, *F*3, and *F*4 were 2.23, 1.76, 3.33, 4.61, and 3.31 (AU/mL), respectively. The M2BPGi-Qt values were higher in AIH patients than in patients with other chronic liver diseases, and the M2BPGi-Qt levels did not increase with progression of fibrosis. In PBC patients ($n = 111$), the median M2BPGi-Qt values for liver fibrosis stages *F*0, *F*1, *F*2, and *F*3 were 0.83, 0.95, 1.19, and 1.68 (AU/mL), respectively. The M2BPGi-Qt values increased with progression of the liver fibrosis stage in chronic liver diseases, but this trend was not typically observed in AIH patients. The authors also investigated the correlations between M2BPGi-Qt levels and activity stages in each liver disease. In CHC patients, the median M2BPGi-Qt values for liver-activity stages *A*0, *A*1, *A*2, and *A*3 were 0.74, 1.41, 2.41, and 5.10 (AU/mL), respectively. In CHB patients, the median M2BPGi-Qt values for liver-activity stages A0, A1, A2, and A3 were 0.87, 1.01, 1.91, and 4.02 (AU/mL), respectively. In MASLD patients, the median M2BPGi-Qt values for liver-activity stages *A*0, *A*1, *A*2, *A*3, and *A*4 were 0.83, 0.77, 1.25, 1.57, and 1.98 (AU/mL), respectively. In AIH patients, the median M2BPGi-Qt values for liver activity stages *A*0, *A*1, *A*2, and *A*3 were 0.81, 1.46, 3,17, and 6.83 (AU/mL), respectively. In AIH patients, the M2BPGi-Qt value was more dependent on liver inflammation activity than on liver fibrosis stage. In all chronic liver diseases, the M2BPGi-Qt values increased with the progression of liver activity. In their study [105], the cutoff values for estimating liver cirrhosis for HCV, HBV, NAFLD, AIH, and PBC were 2.80, 2.35, 2.36, 2.50, and 1.54 (AU/mL), respectively. These results indicated that M2BPGi-Qt assays can predict not only the liver fibrosis stage, but also the degree of inflammatory activity in chronic liver diseases. M2BPGi-Qt assays would be useful for clinical monitoring of the severity of chronic liver disease. No other real-world data regarding M2BPGi-Qt assays have been reported, and the significance of M2BPGi-Qt assays will likely become clearer as data accumulate.

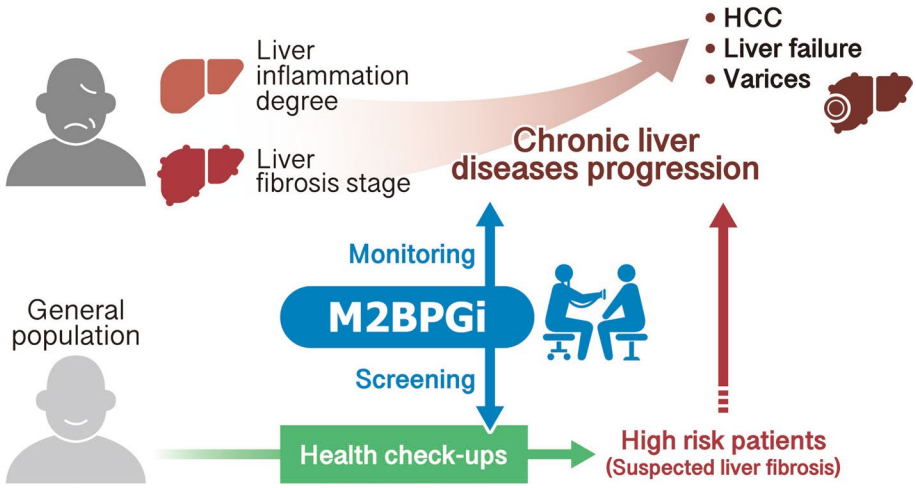

Fig. 2 Schematic image for the clinical use of M2BPGi. In health check-ups, M2BPGi can help identify high-risk patients (suspected liver fibrosis). For hospitalized patients, M2BPGi can be used to monitor the progression of chronic liver disease (evaluating inflammation and fibrosis and predicting HCC, liver failure, and varices)

Issues and future prospective of M2BPGi

M2BPGi was originally developed as a glycan biomarker for CHC patients [8]. Therefore, the liver fibrosis prediction abilities for the other chronic liver diseases (MASLD, CHB, PBC, and AIH) are relatively lower than that for CHC [8, 42–45]. This is the first problem to be pointed out. The new quantitative method for M2BPGi (M2BPGi-Qt) was established as more accurate method than qualitative measurements (M2BPGi (C.O.I.)), and M2BPGi-Qt is more precise at high M2BPGi levels than qualitative method [26]. The difference in the usefulness of M2BPGi (C.O.I.) depending on the disease may be improved by M2BPGi-Qt. However, the glycan structures of various glycoprotein are known to be different in different cells and disease conditions [30]. Ideally, I would like to see more development of M2BPGi for each liver disease in the future. Another current issue is that it is still unknown whether M2BPGi can accurately detect changes in disease pathology. We hope that future longitudinal studies, especially detailed disease investigations using M2BPGi-Qt, will clarify the relationship between disease pathology and M2BPGi.

Conclusion

M2BPGi is an excellent biomarker that can evaluate the progression of liver fibrosis in chronic liver disease. It is also an excellent indicator of liver activity. Recently, a quantitative method (M2BPGi-Qt) was developed, and future validation in real-world settings is expected. If new cutoff values for each chronic liver disease stage and activity using M2BPGi-Qt assays are set based on real-world data, it is expected that this method will become a useful tool for identifying cases of liver fibrosis and monitoring the progression of chronic liver disease (Fig. 2).

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