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M2BPGi Correlated with Immunological Biomarkers and Further Stratified Recurrence Risk in Patients with Hepatocellular Carcinoma

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Hepatocellular carcinoma · M2BPGi · Recurrence · Resection · Immunology · Biomarkers

Abstract

Introduction: Novel biomarkers reflecting liver fibrosis and the immune microenvironment may correlate with the risk of hepatocellular carcinoma (HCC) recurrence. This study aimed to evaluate the prognostic value of serum biomarkers in predicting HCC recurrence. ***Methods:*** Serum biomarkers, including M2BPGi, IL-6, IL-10, CCL5, VEGF-A, soluble PD-1, PD-L1, TIM-3, and LAG-3, were measured in 247 patients with HCC undergoing surgical resection. Factors associated with recurrence-free survival (RFS) and overall survival (OS) were evaluated. The ERASL-post model and IMbrave050 criteria were used to define HCC recurrence risk groups. ***Results:*** Serum M2BPGi levels significantly correlated with FIB-4 score, aspartate transaminase-to-platelet ratio index, ALBI score, alpha-fetoprotein (AFP), alanine transaminase, aspartate transaminase, IL-10, CCL5, VEGF-A, soluble PD-1, PD-L1, TIM-3, and LAG-3 levels. M2BPGi, VEGF-A, soluble PD-1, and TIM-3 levels significantly correlated with RFS. In multivariate analysis, M2BPGi >1.5 COI (hazard ratio [HR] = 2.100, $p < 0.001$), tumor size >5 cm (HR = 1.859, $p = 0.002$), multiple tumors (HR = 2.562, $p < 0.001$), AFP >20 ng/mL (HR = 2.141, $p < 0.001$), and microvascular invasion (HR = 1.954, $p = 0.004$) were independent predictors of RFS. M2BPGi levels significantly stratified the recurrence risk in ERASL-post and IMbrave050 risk groups. An M2BPGi-based model could significantly discriminate RFS in the overall cohort as well as in the IMbrave050 low- and high-risk groups. M2BPGi >1.5 COI was also an independent predictor of OS after resection (HR = 2.707, $p < 0.001$). ***Conclusion:*** Serum M2BPGi levels significantly correlated with surrogate markers of liver fibrosis, liver function, and immunology. M2BPGi is a significant predictor of HCC recurrence and survival after resection and could be incorporated into recurrence-prediction models.

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Introduction

Hepatocellular carcinoma (HCC) stands as the sixth most frequently diagnosed cancer and the third leading cause of cancer-related mortality worldwide [1]. As global population figures continue to rise, a concurrent increase in the incidence of HCC cases and related deaths is anticipated over the next 2 decades [2]. Surgical resection is the curative treatment of choice and leads to a higher chance of long-term cure in well-selected candidates [3]. However, the recurrence rates, particularly within the first 2 years post-resection, remain alarmingly high, ranging from 50% to 70% at the 5-year mark [4–6]. Ongoing clinical trials exploring adjuvant immunotherapy for patients at high risk of recurrence after resection are providing promising insights. The phase III IMbrave050 trial comparing atezolizumab plus bevacizumab versus active surveillance in the adjuvant setting for HCC patients has demonstrated positive results, indicating reduced HCC recurrence at 1 year after curative treatment [7]. In the IMbrave050 trial, high-risk features for resection patients include tumor size exceeding 5 cm, the presence of more than three tumors, microvascular or macrovascular invasion, and poor tumor differentiation. However, these criteria solely focus on tumor-related factors, neglecting the potential impact of tumor markers, host factors including liver fibrosis, and immunological factors that may also contribute to HCC recurrence.

The tumor immune microenvironment of HCC plays a pivotal role in both carcinogenesis and recurrence [8]. Unraveling the mechanisms and identifying predictive biomarkers for HCC recurrence are critical imperatives. Serum cytokines, chemokines, soluble proteins, and the expressions of intrahepatic immune checkpoint proteins could serve as indicators of host antitumor immunity and may offer valuable insights into predicting the risk of HCC recurrence. Previous studies underscore the significant impact of host immune regulators on disease severity and treatment outcomes in chronic viral hepatitis and HCC [9–12]. Serum levels of soluble immune checkpoint targets, including PD-1, PD-L1, CTLA-4, LAG-3, and TIM-3, have demonstrated prognostic value and implications for immunotherapy in HCC patients [13, 14].

Mac 2-Binding protein Gylcan Isomer (M2BPGi) is a recently identified biomarker, which plays an important role in the progression of liver fibrosis, and could be used for assessing liver fibrosis [15]. It has proven useful in assessing liver fibrosis and predicting HCC occurrence during or after antiviral therapy in patients with chronic hepatitis B or C [16]. Although there is suggestive evidence of M2BPGi as a potential biomarker in HCC patients, its prognostic value and correlation with other immunological markers in this context remain unexplored. This study aimed to investigate the prognostic value of M2BPGi and its correlation with various immunological markers in HCC patients receiving curative resection.

Materials and Methods

Patients

Human samples from 247 patients undergoing surgical resection for HCC, including both serum and tissue slides, were obtained from the Biobank, Taipei Veterans General Hospital. Patients with non-curative resection, presence of other malignancy were excluded. The diagnosis of HCC was subsequently confirmed through pathological examination post-surgical resection.

Following confirmation of curative resection through contrast-enhanced computed tomography (CECT) or magnetic resonance imaging (MRI) post-surgery, patients underwent regular follow-ups every 3 months. These follow-ups included alpha-fetoprotein (AFP) measurements, ultrasound examinations, and additional CECT or MRI scans. HCC recurrence was suspected in cases of elevated AFP levels or the detection of new hepatic lesions through ultrasound, with confirmation through CECT or MRI.

This study adhered to the prevailing ethical guidelines and principles outlined in the Declaration of Helsinki. Approval for the study protocol was obtained from the Institutional Review Board at Taipei Veterans General Hospital (IRB number: 2023-07-011BC).

Laboratory Tests and Pathology

Clinical data were comprehensively collected from a cohort of patients, encompassing key parameters such as age, sex, Barcelona Clinic Liver Cancer (BCLC) stage, body mass index, Child-Pugh score and class, serum albumin, total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), creatinine, AFP levels, complete blood counts, HBsAg, and anti-HCV. Additional calculated indices included the albumin-bilirubin (ALBI) score, ALBI grade, fibrosis-4 index (FIB-4) score, and aspartate transaminase-to-platelet ratio index (APRI) utilizing established methodologies [17, 18]. Histological features, such as tumor size and number, Edmonson histological grade, microvascular invasion, hepatic steatosis, and Ishak hepatic inflammation and fibrosis scores

[19, 20] were recorded. To further categorize patients based on their recurrence risk profiles, the IMbrave050 criteria [21] and ERASL-post model [22] were employed in defining distinct recurrence risk groups.

Enzyme-Linked Sorbent Assay

The concentrations of serum soluble checkpoint proteins (sPD1, sPD-L1, sTIM3, sLAG3, sCTLA4) and cytokines or chemokines (IL-10, IL-6, CCL5, VEGF) were quantified using commercially available human cytokine ELISA kits (eBioscience, San Diego, CA, USA). The M2BPGi levels were determined using the HISCL M2BPGi reagent (Sysmex Co., Kobe, Japan) on a fully automated immunoanalyzer, HISCL-800 (Sysmex Co., Kobe, Japan). The procedures adhered to the instructions provided by the manufacturers.

Immunohistochemistry

Paraffin-embedded samples were sectioned into 5-μm slices and subsequently subjected to immunohistochemistry. Tumor-cell PD-L1 expression was assessed using the PD-L1 IHC 28-8 pharmDx assay (Agilent Technologies, Santa Clara, CA, USA), with results reported as the percentage of tumor cells exhibiting PD-L1 cell membrane staining at any intensity (% TC). Intra-tumoral CD8, TIM-3, and LAG-3 expressions were assessed using anti-CD8 (4B11, Leica Biosystems, Deer Park, IL, USA), anti-TIM-3 (D5D5R, Cell Signaling, Danvers, MA, USA), and ant-LAG-3 (17B4, Novus Biologicals, Littleton, CO, USA). Post-antibody incubation, adjacent sections underwent staining with diaminobenzidine or amino-ethylcarbazide using the Envision System (DakoCytomation). The immunohistochemistry double staining images were converted to fluorescence using Photoshop. Analysis was conducted utilizing a Leica DM IRB inverted research microscope (Leica Microsystems, Wetzlar, Germany). The expressions of LAG3 and TIM-3 were generally low in this cohort. Therefore, no further correlation analysis of LAG3 and TIM-3 was performed.

Tissue sections were initially screened at low power (×100), and the five most representative fields were carefully selected. To assess immune cell densities, the respective areas of the tumor nest, peritumoral stroma, and normal peritumoral tissue were measured at ×400 magnification. Subsequently, the target immune cells in each area were manually counted and expressed as the number of cells per field. The representative figure of immunohistochemistry staining for PD-L1 expression in HCC tumor tissue are shown in online supplementary Figure 1 (for all online suppl. material, see https://doi.org/10.1159/000540802).

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics V22 (IBM, Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation or as median (ranges) when appropriate. Categorical variables were compared using Pearson χ^2 analysis or the Fisher's exact test, while continuous variables were compared using the Mann-Whitney U test.

The Kaplan-Meier method was employed to estimate survival rates, and the log-rank test was utilized to compare survival curves between different groups. Prognostic factors were analyzed using the Cox proportional-hazards model. Variables showing statistical significance ($p < 0.05$) or those nearing significance ($p < 0.1$) in univariate analysis were included in the multivariate analysis, performed using a forward stepwise Cox proportional-hazards model. A two-tailed $p < 0.05$ was considered statistically significant. For each biomarker, we tested the cutoff value using the mean, median, interquartile range, or the best cutoff for the presence or absence of HCC recurrence based on the receiver-operating characteristic curve (Youden index). The most discriminative value of each biomarker was selected for univariate and multivariate analyses. Consequently, the cutoff values for IL-10 and CCL5 were determined by the upper quartile, while the cutoff values for other factors were determined by the Youden index.

Results

The baseline characteristics of the 247 HCC patients who underwent surgical resection are summarized in Table 1. The primary etiologies of HCC were HBV and HCV infections, accounting for 43.3% and 25.1%, respectively. According to the IMbrave050 criteria, 66.4% of patients were deemed high-risk for recurrence. Based on the ERASL-post criteria, 30% and 8.9% of patients were classified as intermediate- and high-risk for recurrence, respectively.

Throughout the median follow-up period of 47.4 months (ranging from 1.3 to 128.5 months), 125 patients (50.6%) experienced HCC recurrence after resection, and 67 patients (27.1%) died during the follow-up period.

Correlations between M2BPGi and Other Serum Biomarker Levels and Intra-Tumoral PD-L1 and CD8 Expressions

Serum biomarker levels and intra-tumoral PD-L1 and CD8 expressions in patients with HCC undergoing curative surgical resection are presented in online supplementary

Table 1. Characteristics of the 247 patients with HCC receiving curative surgical resection

Characteristics	
Age, years	65.3±11.1
Sex (male), *n* (%)	174 (70.4)
BMI, kg/m^2	25±3.89
Child-Pugh class A/B, *n* (%)	235/5 (95.1/2)
HCC etiology HBV/HCV/HBV+HCV/nonviral, *n* (%)	107/62/3/75 (43.3/25.1/1.2/30.4)
BCLC stage 0/A/B/C, *n* (%)	26/164/42/15 (10.5/66.4/17/6.1)
IMbrave050 low/high-risk, *n* (%)	83/164 (33.6/66.4)
ERASL-post low/intermediate/high-risk, *n* (%)[a]	145/71/21 (61.2/30/8.9)
Tumor size, cm	5.7±4.4
Multiple tumors, *n* (%)	54 (21.9)
AFP, ng/mL	13.4 (1–1,998,000)
Albumin, g/dL	3.94±0.38
Total bilirubin, mg/dL	0.83±0.43
Albumin-Bilirubin (ALBI) score	−2.621±0.363
ALBI grade 1/2/3, *n* (%)	136/109/2 (55.1/44.1/0.8)
Platelet count, 10^9/L	185±81
ALT, U/L	46.6±45.6
AST, U/L	48.6±42.2
FIB-4	2.99±1.98
APRI	0.68±0.63
Histological features, *n* (%) Edmonson histological grade ≥3 Microvascular invasion Presence of steatosis[a] Ishak hepatic fibrosis score 5-6 (cirrhosis)	 109 (44.1) 148 (59.9) 92 (39) 83 (33.6)
Outcomes, *n* (%) Recurrence Death	 125 (50.6) 67 (27.1)

[a]Available data: ERASL score, *n* = 237 (96%); steatosis, *n* = 236 (95.5%).

Table 1. The majority of patients exhibited quantifiable levels of M2BPGi, CCL5, VEGF-A, soluble PD1, and TIM-3. Less than 10% of patients had quantifiable levels of IL-6, soluble PD-L1, and CTLA-4. Forty-one patients (16.6%) exhibited tumor PD-L1 expression ≥1%, and 64 patients (28.6%) had intra-tumoral CD8 expression >1%.

The correlations between M2BPGi and other serum biomarkers, as well as intra-tumoral PD-L1 expression, are illustrated in Figure 1. The serum M2BPGi level showed significant correlations with the FIB-4 score, APRI, ALBI score, AFP, ALT, AST, IL-10, CCL5, VEGF-A, soluble PD-1, PD-L1, TIM-3, and LAG-3 levels. Furthermore, the M2BPGi level demonstrated a significant correlation with tumor PD-L1 expression ($r = 0.191$, $p = 0.004$). As shown in online supplementary Table 2, there were significant associations between fibrosis markers (FIB-4, APRI) and inflammation markers (ALT, AST) with most serum immunological markers, except for VEGF-A. Additionally, the liver function marker ALBI score was significantly associated with serum soluble CTLA-4, LAG-3, TIM-3, and CCL5. Nevertheless, M2BPGi exhibited the strongest correlations with these

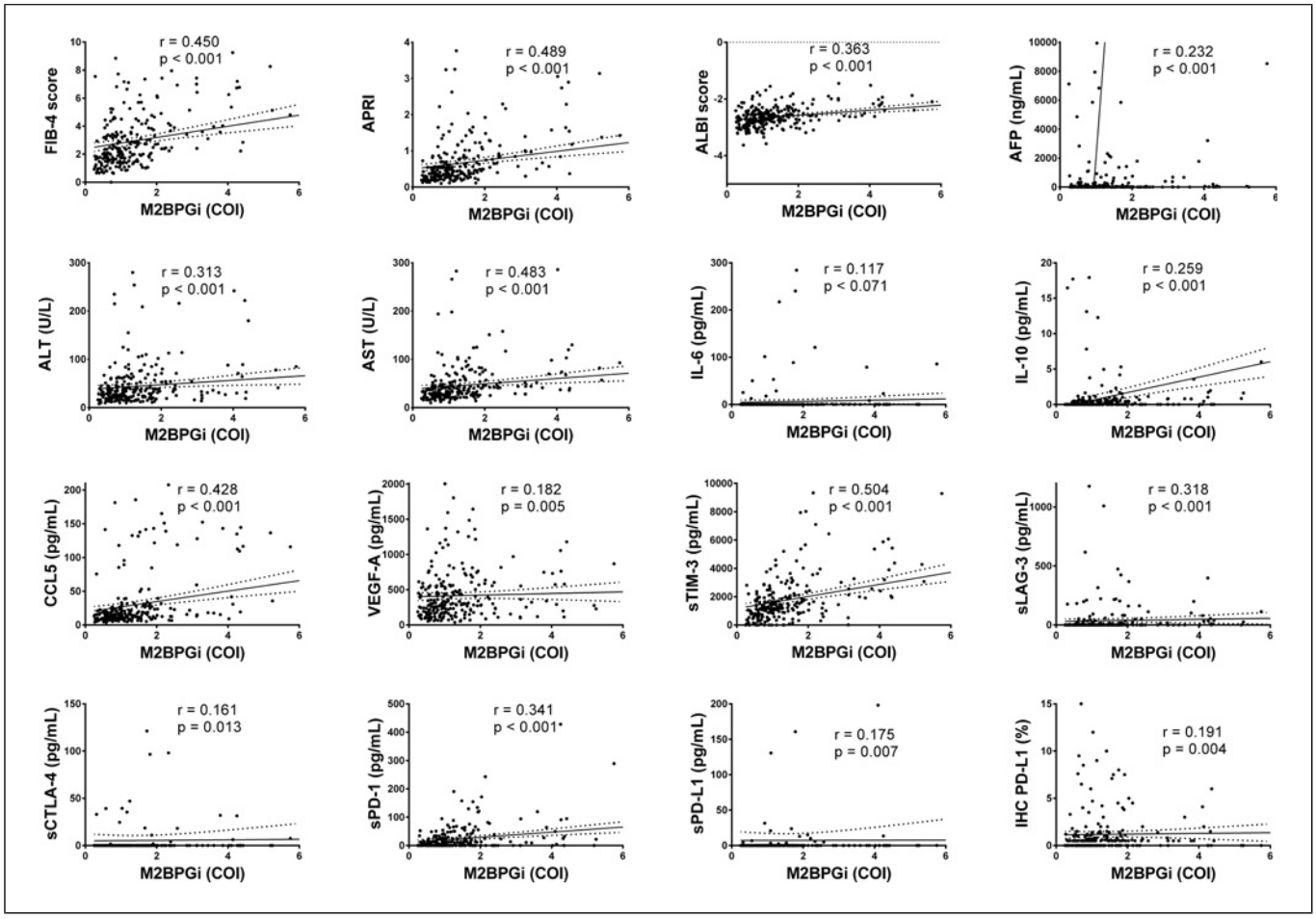

Fig. 1. Correlations between serum M2BPGi level and other serum biomarkers and tumor PD-L1 expression.

surrogate markers of liver fibrosis, inflammation, and liver function compared to other immunological markers.

There was a significant correlation between intratumoral CD8 and PD-L1 expressions (r = 0.156, p = 0.020, online suppl. Fig. 2a). However, no significant correlation was observed between serum M2BPGi and CD8 expression (r = 0.025, p = 0.706, online suppl. Fig. 2b).

There was no significant correlation between M2BPGi and tumor size (r = 0.030, p = 0.643, online suppl. Fig. 3a), tumor number (single vs. multiple tumors: mean M2BPGi 1.51 vs. 1.41 C.O.I., p = 0.473, online suppl. Fig. 3b), or microvascular invasion (absence vs. presence: mean M2BPGi 1.44 vs. 1.51 C.O.I., p = 0.874, online suppl. Fig. 3c).

Factors Associated with Recurrence-Free Survival

Through Kaplan-Meier analysis (Fig. 2a–e), patients with elevated levels of M2BPGi, soluble TIM-3, VEGF-A, soluble PD-1, and IL-10 exhibited significantly poorer recurrence-free survival (RFS), whereas tumor PD-L1 expression showed no association with RFS (Fig. 2f). In univariate analysis, factors associated with RFS included BCLC stage, tumor size, tumor number, AFP, albumin, AST levels, microvascular invasion, VEGF-A, soluble PD-1, TIM-3, and M2BPGi levels (Table 2). In multivariate analysis, independent predictors of RFS were identified as tumor size >5 cm (hazard ratio [HR] = 1.859, p = 0.002), multiple tumors (HR = 2.562, p < 0.001), AFP >20 ng/mL (HR = 2.141, p < 0.001), microvascular invasion (HR = 1.954, p = 0.004), and M2BPGi >1.5 COI (HR = 2.100, p < 0.001). M2BPGi could significantly discriminate RFS in subgroup patients with HBV-related HCC (online suppl. Fig. 4A), HCV-related HCC (online suppl. Fig. 4B), and nonviral HCC (online suppl. Fig. 4C).

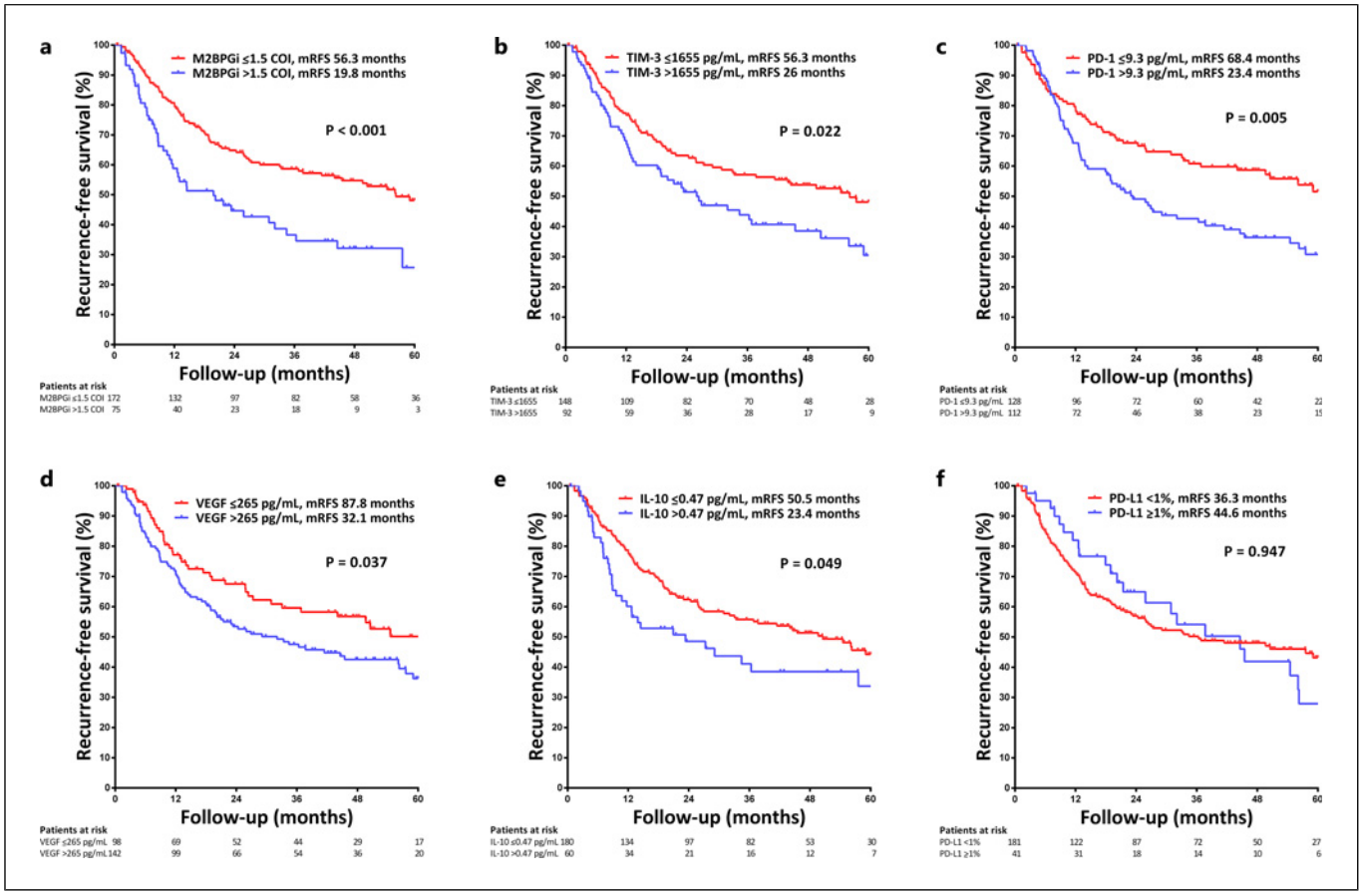

Fig. 2. Recurrence-free survival (RFS) stratified by serum biomarkers. **a** RFS stratified by M2BPGi level. **b** RFS stratified by soluble PD-1 level. **c** RFS stratified by soluble TIM-3 level. **d** RFS stratified by VEGF level. **e** RFS stratified by IL-10 level. **f** RFS stratified by intra-tumoral PD-L1 expression.

M2BPGi further Stratifies Recurrence Risk in ERASL-Post and IMbrave050 Risk Groups

RFS in ERASL-post low-, intermediate-, and high-risk groups were 87.8, 23, and 4.9 months, respectively (online suppl. Fig. 5A). Patients with M2BPGi ≤1.5 COI demonstrated improved survival in ERASL-post low-risk (RFS 87.8 vs. 36.3 months, $p = 0.025$, online suppl. Fig. 5B), intermediate-risk (RFS 26 vs. 12.6 months, $p = 0.014$, online suppl. Fig. 5C), and high-risk groups (RFS 6.1 vs. 3.1 months, $p = 0.124$, online suppl. Fig. 5D).

The RFS in IMbrave050 low- and high-risk groups were 87.8 and 21.8 months, respectively (online suppl. Fig. 6A). Patients with M2BPGi ≤1.5 COI exhibited significantly better survival in both IMbrave050 low-risk (RFS 87.8 vs. 57.6 months, $p = 0.046$, online suppl. Fig. 6B) and high-risk groups (RFS 29.1 vs. 11 months, $p = 0.001$, online suppl. Fig. 6C).

M2BPGi-Based Model further Stratifies Recurrence Risk according to IMbrave050 Criteria

Based on the identified pretreatment predictors of RFS, a new prediction model, consisting of M2BPGi, AFP, tumor size, multiple tumors, and microvascular invasion, was proposed to predict RFS in patients undergoing surgical resection (Table 3). Due to the similar HRs of each factor, patients were assigned one point for each of the five parameters when they fell into the adverse group, as defined by the cut-off. The risk score was defined as the sum of these points, classifying patients into low- (risk score 0–1), intermediate- (risk score 2–3), or high- (risk score 4–5) risk groups (Table 3). Kaplan-Meier analysis demonstrated that the new M2BPGi-based model could significantly discriminate RFS in the overall cohort (Fig. 3a) as well as in the IMbrave050 low- (Fig. 3b) and high-risk groups (Fig. 3c). The M2BPGi-based model could also significantly discriminate RFS in subgroup

Table 2. Univariate and multivariate analyses of factors associated with RFS

	Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age, years >60 versus ≤60	0.773	(0.530–1,127)	0.181			
Sex Female versus male	1.206	(0.813–1.790)	0.352			
BMI, kg/m^2 >25 versus ≤25	1.142	(0.802–1.627)	0.461			
BCLC stage B-C versus 0-A	3.382	(2.338–4.892)	<0.001			NS
ALBI grade 2-3 versus 1	1.344	(0.939–1.924)	0.106			
Tumor size, cm >5 versus ≤5	2.321	(1.623–3.320)	<0.001	1.895	(1.275–2.818)	0.002
Tumor number Multiple versus single	2.343	(1.600–3.430)	<0.001	2.562	(1.733–3.789)	<0.001
AFP, ng/mL >20 versus ≤20	2.254	(1.576–3.225)	<0.001	2.141	(1.476–3.105)	<0.001
Bilirubin, mg/dL >1.2 versus ≤1.2	1.527	(0.952–2.449)	0.079			NS
Albumin, g/dL >3.5 versus ≤3.5	0.574	(0.334–0.986)	0.044			NS
Platelet count, 10^9/L >150 versus ≤150	0.956	(0.664–1.377)	0.808			
ALT, U/L >40 versus ≤40	1.403	(0.979–2.010)	0.065			NS
AST, U/L >40 versus ≤40	2.083	(1.459–2.974)	<0.001			NS
FIB-4 >3.25 versus ≤3.25	1.329	(0.918–1.924)	0.131			
APRI >1.0 versus ≤1.0	1.291	(0.819–2.034)	0.272			
Histological grade ≥3 versus <3	1.601	(1.126–2.277)	0.009			NS
Microvascular invasion Yes/No	2.854	(1.890–4.309)	<0.001	1.954	(1.240–3.079)	0.004
Presence of steatosis Yes/No	1.173	(0.816–1.687)	0.389			
Presence of cirrhosis Yes/No	1.266	(0.876–1.830)	0.209			
IL-6 >LLOQ Yes/No	1.412	(0.759–2.626)	0.276			
IL-10, pg/mL >0.47 versus ≤0.47	1.486	(0.999–2.212)	0.051			NS

Table 2 (continued)

	Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value
CCL5, pg/mL >25 versus ≤25	1.466	(0.993–2.163)	0.054			NS
VEGF-A, pg/mL >265 versus ≤265	1.494	(1.023–2.188)	0.038			NS
sPD-L1 >LLOQ Yes/No	1.457	(0.801–2.650)	0.218			
sPD1, pg/mL >9.3 versus ≤9.3	1.673	(1.167–2.398)	0.005			NS
sTIM-3, pg/mL >1,665 versus ≤1,665	1.520	(1.060–2.180)	0.023			NS
sLAG-3 >LLOQ Yes/No	1.092	(0.732–1.630)	0.665			
sCTLA-4 >LLOQ Yes/No	1.456	(0.801–2.648)	0.218			
M2BPGi (COI) >1.5 versus ≤1.5	1.979	(1.369–2.862)	<0.001	2.100	(1.435–3.074)	<0.001
PD-L1 expression >1% versus ≤1%	0.750	(0.489–1.151)	0.188			
CD8 expression >1% versus ≤1%	0.922	(0.607–1.400)	0.702			

HR, hazard ratio; CI, confidence interval; NS, not significant; LLOQ, lower limit of quantification; BMI, body mass index.

Table 3. Proposed new M2BPGi-based model for HCC patients receiving surgical resection

Points	0	1
M2BPGi (COI)	≤1.5	>1.5
AFP, ng/mL	≤20	>20
Tumor size, cm	≤5	>5
Tumor number	Single	Multiple
Microvascular invasion	Absence	Presence
Risk score	**Risk group**	
0–1	Low	
2–3	Intermediate	
4–5	High	

patients with HBV-related HCC (online suppl. Fig. 4D), HCV-related HCC (online suppl. Fig. 4E), and nonviral HCC (online suppl. Fig. 4F).

Factors Associated with Overall Survival

In univariate analysis, factors associated with overall survival (OS) included BCLC stage, tumor size, AFP, albumin, ALT, AST levels, microvascular invasion, soluble TIM-3, and M2BPGi levels (online suppl. Table 3). The 5-year survival rate for patients with M2BPGi ≤1.5 and >1.5 COI was 77.5% and 50.7%, respectively ($p < 0.001$, online suppl. Fig. 7). In multivariate analysis, independent predictors of OS were identified as tumor size >5 cm (HR = 1.931, $p = 0.021$), AFP >20 ng/mL (HR = 1.797, $p = 0.034$), microvascular invasion (HR = 2.143, $p = 0.049$), and M2BPGi >1.5 COI (HR = 2.707, $p < 0.001$).

Discussion

In this study, we assessed the prognostic value of various novel serum biomarkers in patients undergoing surgical resection for HCC. Our findings revealed a significant correlation between serum M2BPGi and other surrogate markers of liver fibrosis, liver function reserve,

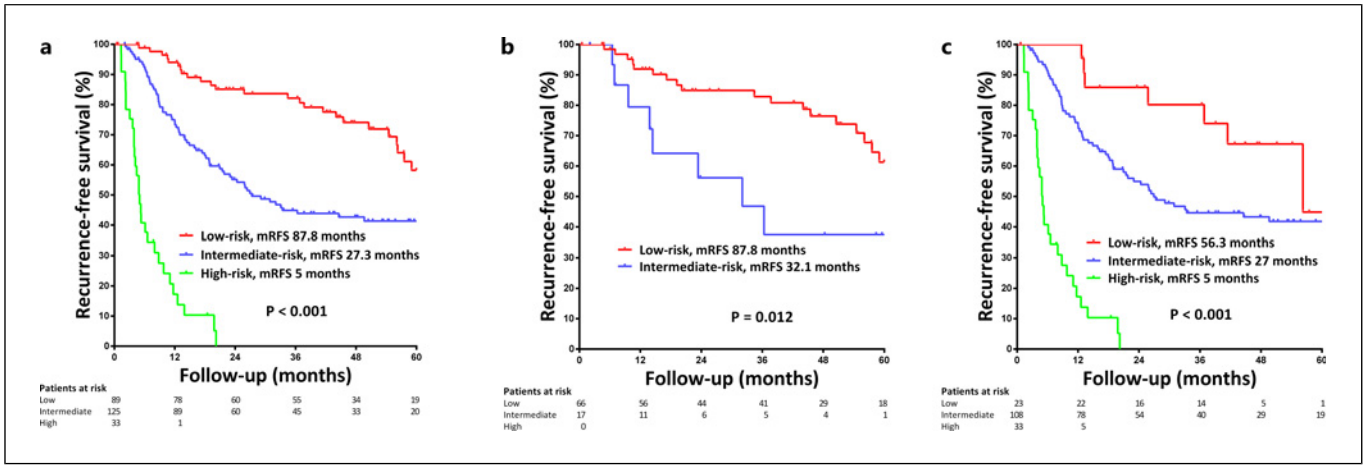

Fig. 3. Recurrence-free survival (RFS) stratified by the M2BPGi-based model. **a** RFS stratified by the M2BPGi-based model in the overall cohort. **b** RFS stratified by the M2BPGi-based model in the IMbrave050 low-risk group. **c** RFS stratified by the M2BPGi-based model in the IMbrave050 high-risk group.

and immunology. Moreover, M2BPGi emerged as a significant predictor of both RFS and OS following surgical resection.

The recurrence rate of HCC remains high following surgical resection, with multiple factors contributing to recurrence. Tumor-related factors, including tumor size, number, microvascular invasion, poor differentiation, AFP, liver function, and fibrosis, are well-established clinical predictors of HCC recurrence [23]. Identifying patients at high risk of recurrence can inform a more vigilant post-resection monitoring strategy, and adjuvant immunotherapy may be beneficial for such individuals. The IMbrave050 trial has demonstrated positive outcomes, indicating a reduced risk of HCC recurrence in high-risk patients receiving adjuvant atezolizumab plus bevacizumab after curative treatment. However, the IMbrave050 criteria for high recurrence risk only incorporate tumor size, number, microvascular invasion, and poor differentiation, neglecting AFP and other host factors [21]. Consequently, the IMbrave050 criteria may be inadequate in identifying high-risk patients. The ERASL-post model, incorporating tumor size, number, microvascular invasion, AFP, gender, and ALBI grade, could potentially offer improved accuracy compared to the IMbrave050 criteria, as it considers both tumor- and host-related factors [22]. Nonetheless, these clinical criteria remain imperfect, necessitating the exploration of novel biomarkers to enhance the precision of clinical prediction models.

The tumor immune microenvironment has been recognized as a pivotal factor in HCC carcinogenesis and recurrence. Immunological biomarkers may provide insights into the HCC immune microenvironment, offering prognostic value for predicting HCC recurrence. In this study, we selected several serum biomarkers implicated in HCC carcinogenesis or associated with therapeutic potential in HCC. IL-6 [24], IL-10 [25], and CCL5 [26] have demonstrated involvement in HCC progression. Soluble PD-1, PD-L1, CTLA-4, and VEGF-A are relevant to currently approved systemic therapies for HCC, such as atezolizumab plus bevacizumab, durvalumab plus tremelizumab, nivolumab plus ipilimumab, and pembrolizumab [6]. TIM-3 and LAG-3 are also novel targets of immune-checkpoint inhibitors under investigation in HCC [27]. Tumor PD-L1 expression is a crucial marker in the era of immunotherapy, demonstrating correlation with HCC aggressiveness [28]. M2BPGi has proven useful as a marker for predicting liver fibrosis and HCC risk in patients with chronic liver disease across various etiologies [29–33].

In this study, we assessed the correlation between M2BPGi and other serum biomarkers, as well as tumor PD-L1 expression. Intriguingly, we observed a significant correlation between M2BPGi and surrogate markers of liver fibrosis (FIB-4 score, APRI), liver function reserve (ALBI score), liver inflammation (ALT, AST), tumor marker (AFP), angiogenesis (VEGF-A), and nearly all measured immunological biomarkers (IL-10, CCL5, soluble PD-1, PD-L1, TIM-3, and LAG-3). AFP represents not only a marker of tumor burden but also correlates with liver inflammation, fibrosis, and tumor biology. In contrast, there was no significant

correlation between M2BPGi and tumor factors such as tumor size, tumor number, and microvascular invasion [20, 34].

M2BPGi has been clinically applied as a diagnostic marker for liver fibrosis over the past decade. However, its role in patients with chronic liver disease and HCC is not completely elucidated. In patients with chronic hepatitis, including hepatitis B, hepatitis C, and metabolic dysfunction-associated steatotic liver disease, several pro-inflammatory molecules (such as CCL5) and immunosuppressive molecules (such as IL-10, PD-1, TIM-3, and LAG-3) contribute to the pathogenesis of chronic hepatic inflammation [35]. Chronic hepatic inflammation further induces the secretion of M2BPGi from hepatic stellate cells, driving the progression of liver fibrosis [36].

In this study, M2BPGi showed significant correlations with several immunological markers, as well as markers of liver fibrosis, inflammation, and liver function. Although these immunological markers also had significant correlations with liver function markers, M2BPGi showed stronger correlations with these liver function markers than other immunological markers, suggesting that M2BPGi may play a significant role in representing the tumor microenvironment of HCC. Additionally, M2BPGi shows the highest correlation with most serum immunological biomarkers compared to FIB-4 and APRI. These findings suggest that M2BPGi serves not only as a marker of hepatic fibrosis but also as an indicator of hepatic inflammation. M2BPGi may be associated with multiple aspects of the tumor immune microenvironment in HCC, independent of tumor factors.

Tumor-infiltrating $CD8^+$ T cells are crucial in modulating antitumor responses. Our study revealed a significant correlation between CD8 and PD-L1 expression. However, no significant correlation was found between tumor CD8 expression and M2BPGi, RFS, or OS. This indicates the need for a more in-depth analysis of the functional role of $CD8^+$ T cells to determine their prognostic value in HCC.

Our study demonstrated significant correlations between M2BPGi, VEGF-A, soluble PD-1, and TIM-3 levels with RFS, indicating their prognostic potential in HCC patients. In multivariate analysis, M2BPGi emerged as a robust predictor of RFS, independent of other well-known clinical factors associated with RFS. M2BPGi levels were effective in further stratifying the recurrence risk within each risk group defined by the ERASL-post model or IMbrave050 criteria. We proposed an M2BPGi-based model by incorporating five independent predictors of RFS identified through multivariate analysis. This novel model demonstrated enhanced discrimination of recurrence risk in both IMbrave050 low- and high-risk groups. M2BPGi and the M2BPGi-based model may also discriminate RFS in patients with HBV-related HCC, HCV-related HCC, and nonviral HCC using the same cutoff value of M2BPGi, suggesting that M2BPGi can be broadly applied across various HCC etiologies. Improved accuracy in predicting recurrence risk could facilitate the selection of patients who would benefit from adjuvant immunotherapy following surgical resection.

This study has some limitations. First, it is a single-center study, and further external validation is required to confirm the prognostic role of M2BPGi. Second, while M2BPGi can identify patients at high risk of recurrence, its role in predicting the response to adjuvant immunotherapy remains unclear. Additional studies are warranted to explore the correlation between M2BPGi and the efficacy of immunotherapy.

In conclusion, serum M2BPGi levels were significantly correlated with surrogate markers of liver fibrosis, liver function, and immunology. M2BPGi emerged as a significant predictor of HCC recurrence and survival after resection, suggesting its potential incorporation into recurrence-prediction models.

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Statement of Ethics

This study adhered to the prevailing ethical guidelines and principles outlined in the Declaration of Helsinki. Approval for the study protocol was obtained from the Institutional Review Board at Taipei Veterans General Hospital (IRB number: 2023-07-011BC). Written informed consents from sample donors were obtained at time of their donation to the Biobank, Taipei Veterans General Hospital.

Conflict of Interest Statement

ICL has received honoraria from Gilead Sciences, Bristol-Meyers Squibb, Abbvie, Merck Sharp and Dohme, Bayer, Eisai, Ipsen and Roche, and has served in an advisory role for Gilead Sciences. YHH has received research grants from Gilead Sciences and Bristol-Meyers Squibb, and honoraria from Abbvie, Gilead Sciences, Bristol-Meyers Squibb, Ono Pharmaceutical, Merck Sharp and Dohme, Eisai, Eli Lilly, Ipsen, and Roche, and has served in an advisory role for Abbvie, Gilead Sciences, Bristol-Meyers Squibb, Ono Pharmaceuticals, Eisai, Eli Lilly, Ipsen, Merck Sharp and Dohme and Roche. Other authors declare no conflicts of interest.

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Author Contributions

I.-C.L.: study concept and design, obtained funding, data acquisition, analysis and interpretation of data, drafting of the manuscript, statistical analysis, and study supervision. H.-J.L.: preparation of biobank samples. L.-C.W. and Y.-C.Y.: preparation of tissue array, immunohistochemistry staining, and pathology analysis. H.-J.L., G.-Y.C., C.-Y.H., S.-C.C., J.-C.L., M.-C.H, and Y.-H.H.: data acquisition.

Data Availability Statement

The data that support the findings of this study are not publicly available due to Taipei Veterans General Hospital retains the ownership of biobank data related to this study, but are available from the corresponding author [I.-C.L.] upon reasonable request and approved by the Institutional Review Board at Taipei Veterans General Hospital. Further inquiries can be directed to the corresponding author.

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