

Baseline serum Mac-2 binding protein glycosylation isomer as a predictor of hepatocellular carcinoma in chronic hepatitis B patients: a systematic review and meta-analysis

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Abstract

Background A minimally invasive tool to promptly predict hepatocellular carcinoma (HCC) in chronic hepatitis B (CHB) is currently needed. In this study, we aimed via a meta-analysis to identify the serum Mac-2 binding protein glycosylation isomer (M2BPGi) as a novel glycoprotein-based liver fibrosis marker for predicting HCC in CHB patients.

Methods We conducted a systematic search on PubMed, Scopus, ProQuest, Wiley Online Library, and CINAHL Plus (via EBSCOhost). The articles were screened based on several eligibility criteria and were further assessed for study qualities using the Newcastle-Ottawa Scale. The outcomes were presented as standard mean difference (SMD), hazard ratio (HR), and predictive accuracy parameters of a baseline cutoff index (COI) for serum M2BPGi.

Results Fourteen studies involving 5918 CHB patients were included in this systematic review and meta-analysis. Baseline COI serum M2BPGi was significantly higher in CHB patients who developed HCC than in those who did not (SMD 1.32, 95% confidence interval [CI] 0.91-1.72). A significant HCC risk prediction was also observed (multivariate HR 1.18, 95%CI 1.05-1.32). Baseline COI serum M2BPGi could predict HCC with a pooled sensitivity of 74% (95%CI 50-89%), specificity of 80% (95%CI 65-90%), and area under the summary receiver operating characteristic curve of 0.84 (95%CI 0.81-0.87).

Conclusion High baseline COI serum M2BPGi may predict the development of HCC in CHB patients with moderate-to-high accuracy.

Keywords Biomarker, chronic hepatitis B, hepatocellular carcinoma, Mac-2 binding protein glycosylation isomer, *Wisteria floribunda* agglutinin-positive Mac-2 binding protein

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Conflict of Interest: None

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Introduction

Chronic hepatitis B (CHB) infection is a disease with a global burden. Approximately 20-30% of chronically infected adults with hepatitis B virus (HBV) will develop end-stage clinical consequences, such as cirrhosis and hepatocellular carcinoma (HCC). Furthermore, more than 90-95% of cases of HBV infection in infancy and early childhood will lead to CHB [1]. CHB progression imposes a large mortality burden, as HCC progresses rapidly and, since the treatment options are limited, the outcome is generally poor. In low-income countries, most people with HCC die within a month of detection [2]. Many of them are diagnosed only when they already have advanced liver disease. Therefore, proper clinical management for early detection of HCC is needed to improve the overall survival and quality of life by preventing its progression [2,3].

To achieve effective management of CHB, there should be a thorough assessment. Liver biopsy is recognized as the gold standard to assess the progression of fibrosis;

however, the procedure is limited by its invasiveness, cost, risk of complications, sampling errors and subjective interpretation [4-6]. Additionally, HCC diagnosis and surveillance are commonly based on the detection of tumor markers, such as protein induced by the absence of vitamin K or its antagonist-II (PIVKA-II), α -fetoprotein (AFP), and imaging techniques [7,8]. Hence, there should be more reliable, noninvasive, and inexpensive biomarkers for CHB-related HCC management.

Recently, an extensively glycosylated liver-secreted protein, Mac-2 binding protein glycosylation isomer (M2BPGi), was discovered as a novel biomarker in liver diseases, including liver fibrosis [9]. M2BPGi can accurately distinguish between the stages of liver fibrosis and identify more severe stages of fibrosis. Furthermore, several studies have shown that M2BPGi could also predict the risk of HCC among hepatitis B and C patients [10,11]. In stratifying the risk of HCC, the predictive accuracy of M2BPGi was significantly higher than AFP or HBsAg over a short or intermediate time interval. Hence, the availability of M2BPGi in clinical settings may potentially improve the management of a patient with HBV [3]. The risk prediction accuracy of baseline serum M2BPGi needs to be further established to support its efficacy. Therefore, we aimed to evaluate serum M2BPGi as a novel biomarker for predicting HCC in CHB patients.

Materials and methods

Data search strategy

This systematic review and meta-analysis were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2009 guidelines [12]. A computerized data search of the relevant studies was conducted on PubMed, Scopus, ProQuest, Wiley Online Library, and CINAHL Plus (via EBSCOhost) by 2 independent reviewers, from inception to 2 February 2021. Keywords were constructed based on the Medical Subject Headings (MeSH) terms and other additional terms listed as follows: (“Mac-2 Binding Protein Glycosylation Isomer” OR “Mac-2 Binding Protein Glycan Isomer” OR “M2BPGi” OR “Wisteria floribunda agglutinin-positive human Mac-2 binding protein” OR “Wisteria floribunda agglutinin-positive Mac-2 binding protein” OR “WFA(+)-M2BP”) AND (“Hepatocellular Carcinoma” OR “HCC” OR “Hepatoma” OR “Liver Cell Carcinoma”) AND (“Hepatitis B” OR “Chronic Hepatitis” OR “CHB” OR “Hepatitis B Virus Infection”) AND (“Human”).

Eligibility criteria

The inclusion criteria of this study were as follows: 1) observational study; 2) study population adult patients (>18 years old) with confirmed diagnosis of CHB; and 3) studies that report the relationship between baseline cutoff

index (COI) serum M2BPGi, using either adjusted or unadjusted values of odds ratio (OR), relative risk (RR), hazard ratio (HR), and/or predictive accuracy analysis. The exclusion criteria were as follows: 1) irrelevant titles and abstracts; 2) irretrievable full-text articles; 3) non-English studies; and (4) review articles, case reports, case series, letters to editors and conference abstracts.

Data extraction and quality assessment

Two investigators screened the publications independently. Any disagreements were resolved in a consensus involving an independent third reviewer. The extracted data included author and year of publication, study location, study design, HCC diagnostic method, study population, sample size, age of patients, follow-up period, baseline COI serum M2BPGi levels, and outcome measures (e.g., OR, RR, HR, area under curve [AUC], cutoff, sensitivity, and specificity values). The quality assessment of the selected studies was performed using the Newcastle-Ottawa Scale (NOS). The NOS interpretation in cohort and case-control studies was classified into good-quality (score 7-9), moderate-quality (score 4-6), and poor-quality studies (score 0-3). The quality assessment was conducted by 2 reviewers collaboratively through a group discussion, and the final decision was taken based on the agreement of both reviewers.

Statistical analysis

Meta-analyses were performed for the outcome of pooled standardized mean differences (SMDs) and HRs of baseline COI serum M2BPGi. For the purposes of this meta-analysis, values that were not reported as mean and standard deviation (SD) were transformed before being extracted using methods proposed by Wan *et al* [13] and Luo *et al* [14]. To determine the effect caused by the differences of the population treatment status, the outcome of SMDs was analyzed in subgroups. Publication bias was assessed visually using a funnel plot if a sufficient number of studies were included in the analysis ($n \geq 10$). To determine the overall performance of baseline COI serum M2BPGi for predicting HCC, we further conducted a bivariate meta-analysis for studies that reported sensitivity and specificity values. The pooled values of sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the summary receiver operating characteristic (AUSROC) curve were summarized using the “MIDAS” module in STATA ver. 16.0 (Stata Corporation, College Station, TX, USA). Heterogeneity between studies was assessed using Cochran’s Q statistic and quantified with the Higgins’ I^2 statistic. The level of heterogeneity was determined using I^2 values. Heterogeneity with $I^2 < 25\%$ was considered as negligible, 25-50% as low, 50-75% as moderate and $> 75\%$ as high. A random-effects model was used for the meta-analysis if the I^2 value was greater than 50%. Otherwise, a fixed-effects model was applied.

A P-value of <0.05 was considered as statistically significant. In addition, we performed a sensitivity analysis using the leave-one-out approach, while publication bias was also assessed qualitatively, using funnel plots, and quantitatively, using Egger's regression test [15] to detect potential publication bias. To search for the effect of mean population age and number of females (in %) on the pooled outcome of HR meta-analysis, we performed restricted maximum likelihood random-effects meta-regression analyses. All analyses were performed using RevMan ver 5.4 (The Cochrane Collaboration, The Nordic Cochrane Centre, Copenhagen, Denmark) and STATA ver. 16.0 (Stata Corporation, College Station, TX, USA).

Results

Overview of literature search

The initial search of this study yielded a total of 238 studies obtained from PubMed, Scopus, ProQuest, Wiley Online Library and CINAHL Plus (via EBSCOhost). Of those, 36 studies were screened by titles and abstracts after the removal

of duplicates. Twenty-five were fully reviewed based on the eligibility criteria and 11 of these were excluded because of an inappropriate population (n=3), unmeasured indicator (n=1), not reporting the outcome of interest (n=6), or an incorrect study method (n=1). Fourteen studies were included for both qualitative synthesis and quantitative analysis [3,11,16-27]. The overall study selection process is illustrated in Fig. 1.

Characteristics and results of the selected studies

Table 1 provides a summary of the studies included in this systematic review. The 14 studies included a total of 5918 CHB patients. All studies included men and women (69.55% male), with mean \pm SD age of 51.2 ± 12.9 years. The mean follow-up period was 6.4 years. Of the total studies, 13 had a prospective or retrospective cohort, while one, by Liu *et al* [3], was a nested case-control. The studies were all conducted in Asia, except for one by Jun *et al* [27] that also observed patients in Palo Alto, California. HCC diagnoses in all studies were mainly based on the imaging findings (e.g., computed tomography scan or magnetic resonance imaging). Study populations were divided into 3 subgroups as follows: 1) treated CHB, regardless of the treatment regimen; 2)

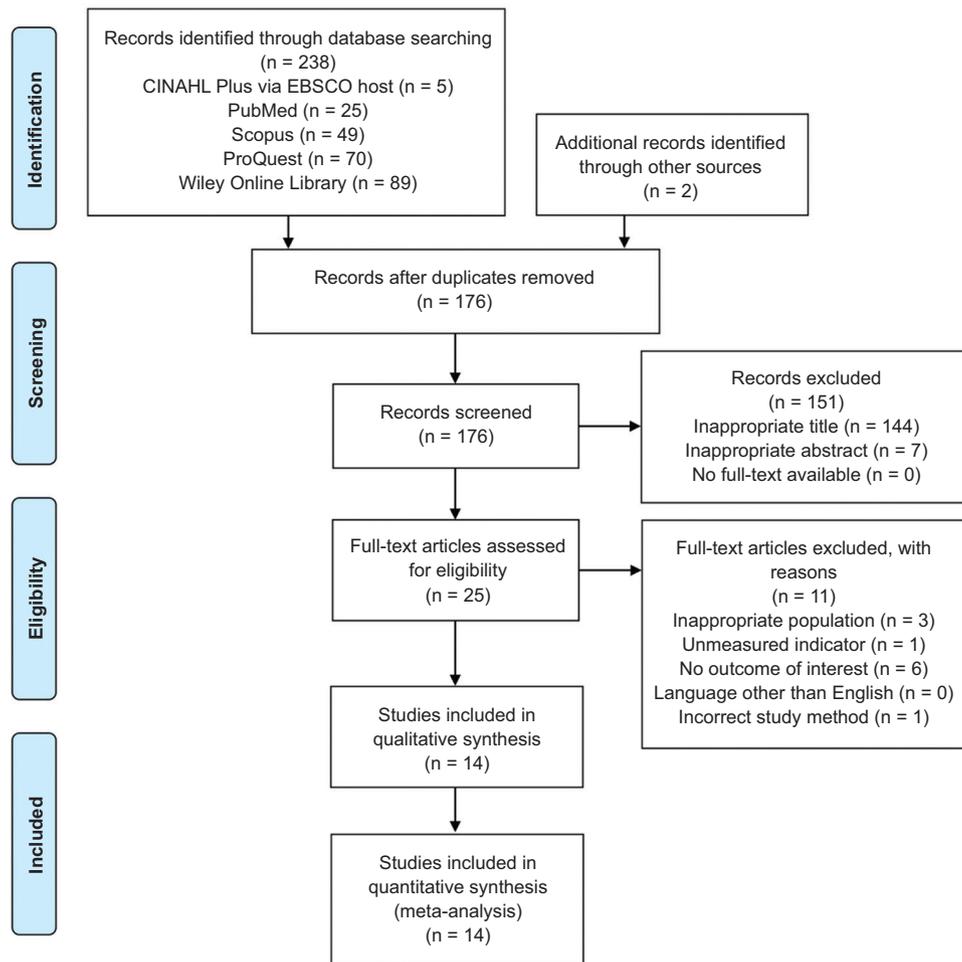


Figure 1 PRISMA flowchart of the study selection process [12]

Table 1 Basic characteristics and results of baseline serum Mac-2 binding protein glycosylation isomer (M2BPGi) in chronic hepatitis B patients of the included studies

Author [ref.]	Study location	Study Design	HCC diagnostic method	Study population*	Total patients (M/F)	Age (years) [†]	Mean or median follow-up period [‡]	Predictive Accuracy for HCC	
								Cutoff (COI)	AUC
Kozuka <i>et al</i> [20]	Osaka, Japan	Prospective cohort	Presence of arterial hypervascularization and delayed wash-out on dynamic CT and/or MRI	Treated CHB (ETV)	127 (83/44)	43 (23, 79)	N/A	N/A	N/A
Tseng <i>et al</i> [21]	Taipei, Taichung, and New Taipei, Taiwan	Retrospective cohort	Histology/cytology or typical imaging findings (arterial enhancement and venous wash-out by contrast CT or MRI) in hepatic nodules > 1 cm	Treated CHB (ETV/TDF)	899 (669/230)	48 (16)	7.01±3.36 yrs	≥ 1.73	N/A
Murata <i>et al</i> [22]	Shizuoka, Japan	Retrospective cohort	USG/dynamic CT/MRI or FNAB when the hepatic nodule did not show typical imaging features	Treated CHB (NA)	147 (93/54)	55 (20, 82)	6.6 (1.1, 10.9) yrs	> 1.5	0.805
Hsu <i>et al</i> [23]	Kaohsiung, Taiwan	Prospective cohort	Histology/cytology/dynamic images as recommended by the AASLD	Treated CHB (NA)	384 (282/102)	46.6±13.9	72.73 (44.3, 103.75) mos	N/A	N/A
Ichikawa <i>et al</i> [24]	Matsumoto, Japan	Retrospective cohort	N/A	Other (treatment-naive at recruitment, treatment initiated for specific conditions)	112 (72/40)	47 (36, 57)	Mean 173 wks	≥ 0.71	N/A
Kawaguchi <i>et al</i> [25]	Kanazawa, Japan	Prospective cohort	N/A	Treated CHB (NA)	141 (86/55)	50.3±12.1	N/A	N/A	0.660
Heo <i>et al</i> [26]	South Korea	Retrospective cohort	Dynamic CT/dynamic MRI/hepatic angiography or serum AFP as recommended by the KLCSG	Other (sub-populations of treated and untreated CHB)	95 (69/26)	51 (44, 60)	Median 45 mos	N/A	N/A
Kim <i>et al</i> [11]	South Korea	Retrospective cohort	Dynamic CT/dynamic MRI/hepatic angiography or serum AFP as recommended by the KLCSG	Other (Sub-populations of treated and untreated CHB)	1323 (793/530)	51 (41, 59)	60.0 (56.0, 62.1) mos	N/A	N/A
Jun <i>et al</i> [27]	Palo Alto, California and Kaohsiung, Taiwan	Prospective cohort	Histology/dynamic images as recommended by the AASLD	Other (Treatment-naive at recruitment, treatment initiated for specific conditions)	714 (509/205)	45.1±12.1	6.48 (3.21, 11.19) yrs	N/A	N/A

(Contd...)

Table 1 (Continued)

Author [ref.]	Study location	Study Design	HCC diagnostic method	Study population*	Total patients (M/F)	Age (years) [†]	Mean or median follow-up period [‡]	Predictive Accuracy for HCC	
								Cutoff (COI)	AUC
Mak <i>et al</i> [16]	Hong Kong	Prospective cohort	Typical features of arterial phase hyperenhancement and porto-venous wash-out of contrast, with or without histological proof	Other (Treatment-naïve at recruitment, treatment initiated for specific conditions)	207 (118/89)	40 (34, 45)	13.1 (11.8, 15.5) yrs	≥ 0.68	0.883
Mak <i>et al</i> [17]	Hong Kong	Prospective cohort	Typical features of arterial phase hyperenhancement and porto-venous wash-out of contrast, with or without histological proof	Treated CHB (ETV)	285 (225/60)	56.7 (51.5, 62.9)	7.1 (4.4, 8.8) yrs	N/A	0.636
Liu <i>et al</i> [3]	Taiwan	Nested case-control	Chart reviews by gastroenterologists according to the following criteria: Histopathologic confirmation Positive lesions detected by ≥ 2 different imaging techniques (abdominal USG/angiogram/CT) Positive lesions detected by one imaging technique combined with a serum AFP level > 400 ng/mL	Untreated CHB	1070 (794/276)	59.3±9.4	N/A	≥ 2.0	N/A
Cheung <i>et al</i> [18]	Hong Kong	Retrospective cohort	Histology or typical radiological features (arterial enhancement and venous wash-out by triphasic CT/contrast MRI)	Untreated CHB	114 (96/18)	60.6±6.0	N/A	≥ 0.69	0.700
Chuaypen <i>et al</i> [19]	Bangkok, Thailand	Retrospective cohort	Presence of focal hepatic lesions with hyperattenuation in the arterial phase and hypoattenuation in the portal phase in dynamic CT/MRI	Other (Not stated)	300 (227/73)	57.6±8.2	N/A	2.4	0.920

*Other is defined as (1) treatment-naïve at recruitment and treatment is initiated if patients had specific conditions, (2) combined sub-populations of treated and untreated CHB, or (3) no clear statement regarding the treatment status

[†]Data are presented in mean±SD, median (IQR), or median (min, max)

AASLD, American Association for the Study of Liver Disease; AUC, area under curve; CHB, chronic hepatitis B; COI, cut-off index; CT, computed tomography; ETV, entecavir; FNAB, fine needle aspiration biopsy; HCC, hepatocellular carcinoma; KLCSCG, Korean Liver Cancer Study Group; M2BPGi, Mac-2 binding protein glycosylation isomer; MRI, magnetic resonance imaging; N/A, not available or not applicable; NA, nucleot (s) ide analogs; USG, ultrasonography; SD, standard deviation; IQR, interquartile range

untreated CHB; and 3) other. The “other” subgroup was further defined as one of the following: 1) treatment-naïve at recruitment and treatment initiated if patients had a specific condition; 2) combined sub-populations of treated and untreated CHB; or 3) no clear statement regarding the treatment status.

The baseline was defined as the measurement of serum M2BPGi level at the time of enrollment or the start of treatment. Baseline COI serum M2BPGi was higher in CHB patients with HCC development compared to those without HCC development and the difference was found to be statistically significant in all 10 studies ($P < 0.05$). Seven studies reported outcome measures in the form of HR, whereas 2 studies [16,17] reported outcome measures as OR. Among the former, 2 studies [20,22] did not observe statistically significant findings for HR between baseline serum M2BPGi with the development of HCC. The cutoff for baseline COI serum M2BPGi in predicting HCC varied across studies, ranging from 0.68-2.40. The AUC of baseline serum M2BPGi ranged from 64-92%. The quality assessment of each study using the NOS score is listed in Table 2. All included studies were considered good-quality studies, except for one study by Chuaypen *et al* [19] which was considered as moderate-quality.

Meta-analysis of baseline COI serum M2BPGi levels in HCC vs. non-HCC CHB patients

A total of 4081 CHB patients, 826 with HCC and 3255 without HCC, from 10 studies were included in this subgroup meta-analysis (Fig. 2A) to compare the SMDs of baseline COI serum M2BPGi levels between CHB patients who developed HCC and those who did not. A random-effects model was used for the analysis, since heterogeneity was detected in the data ($I^2=94%$). The overall pooled analysis showed that baseline COI serum M2BPGi levels in HCC patients were significantly higher compared to those without HCC (SMD 1.32, 95% confidence interval [CI] 0.91-1.72). The test for subgroup differences suggests that there was a statistically significant subgroup effect ($P=0.003$), meaning that treatment status significantly modified the differences in means of baseline COI serum M2BPGi levels of CHB patients who developed HCC in comparison to those who did not. A funnel plot of the included studies showed an asymmetrical distribution (Fig. 2B).

Subgroup analysis of baseline COI serum M2BPGi levels in HCC vs. non-HCC CHB patients

Treated CHB

Four studies with a total of 972 CHB patients who received treatments were included in this subgroup analysis (Fig. 2A). Evidence of heterogeneity was detected ($I^2=93%$); therefore, a random-effects model was used for the analysis. The pooled SMD was 1.32 (95%CI 0.63-2.01), which indicated that CHB patients who developed HCC had higher baseline COI serum M2BPGi levels compared to those who did not develop HCC, with a statistically significant difference.

Untreated CHB

A total of 1184 CHB patients from 2 studies who did not receive treatment were included in this subgroup analysis (Fig. 2A). A random-effects model was used for the analysis, since substantial heterogeneity was detected in the data ($I^2=66%$). The pooled analysis data showed a significantly higher mean value of baseline COI serum M2BPGi levels in CHB patients who developed HCC compared to those who did not (SMD 0.54, 95%CI 0.21-0.87).

Others

A total of 1925 CHB patients from 4 studies, with the subgroup defined as above, were evaluated in this subgroup analysis (Fig. 2A). Heterogeneity across studies was detected ($I^2=91%$); therefore, a random-effect model was used for the analysis. The pooled analysis showed a statistically significant result, with higher baseline COI serum M2BPGi levels in CHB patients who developed HCC compared to those who did not (SMD 1.73, 95%CI 1.07-2.38).

Meta-analysis of HR of baseline COI serum M2BPGi levels with the risk of HCC development

Univariate HR analysis

Univariate analysis results from 5 studies, using a Cox proportional hazard model to assess the association of baseline serum M2BPGi level with HCC development, were included in this meta-analysis (Fig. 3A). A random-effects model was used because of the heterogeneity of the data ($I^2=91%$). The pooled analysis showed that baseline serum M2BPGi levels were significantly associated with HCC development in CHB patients (HR 1.28, 95%CI 1.11-1.48).

Multivariate HR analysis

Five studies that tested the association between baseline serum M2BPGi level and HCC development using a multivariable Cox proportional hazard model were included in this meta-analysis (Fig. 3B). Heterogeneity was detected across studies ($I^2=72%$); thus, a random-effects model was used for the analysis. The pooled multivariate HR was 1.18 (95%CI 1.05-1.32), indicating that baseline serum M2BPGi levels were significantly associated with the development of HCC in CHB patients.

Predictive accuracy of baseline COI serum M2BPGi for HCC

Seven studies that reported the sensitivity and specificity values of baseline COI serum M2BPGi in the prediction of HCC were included in the meta-analysis (Fig. 4). Results of the pooled sensitivity, specificity, PLR, NLR, DOR, and AUSROC

Table 2 Quality study assessments of cohort and case-control studies using Newcastle-Ottawa Scale (NOS) score

NOS score of cohort studies										NOS score of case-control study					
Components	Kozuka <i>et al</i> [20]	Tseng <i>et al</i> [21]	Murata <i>et al</i> [22]	Hsu <i>et al</i> [23]	Ichikawa <i>et al</i> [24]	Kawaguchi <i>et al</i> [25]	Heo <i>et al</i> [26]	Kim <i>et al</i> [11]	Jun <i>et al</i> [27]	Mak <i>et al</i> [16]	Mak <i>et al</i> [17]	Cheung <i>et al</i> [18]	Chuaypen <i>et al</i> [19]	Components	Liu <i>et al</i> [3]
Selection															
Representativeness of the exposed cohort	*	*	*	*	*	*	*	*	*	*	*	*	*	Adequateness of the case definition	*
Selection of the non-exposed cohort	*	*	*	*	*	*	*	*	*	*	*	0	0	Representativeness of the cases	*
Ascertainment of exposure	*	*	*	*	*	*	*	*	*	*	*	*	*	Selection of controls	*
Demonstration that outcome of interest was not present at start of the study	*	*	*	*	0	0	*	*	*	*	*	*	0	Definition of controls	0
Comparability															
Comparability of cohorts on the basis of design or analysis	**	**	**	**	**	**	**	**	0	**	**	**	**	Comparability of cases and controls on the basis of the design or analysis	**
Exposure															
Assessment of outcome	*	*	*	*	*	*	*	*	*	*	*	*	*	Ascertainment of exposure	*
Enough follow-up time length for outcome to occur	*	*	*	*	*	*	*	*	*	*	*	*	0	Same method of ascertainment for cases and controls	*
Adequacy of follow up of cohorts	*	*	*	*	*	*	*	*	*	*	*	*	0	Non-response rate	*
Study quality															
Total score	9	9	9	9	8	8	9	9	7	9	9	8	5	Total score	8
Interpretation	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Moderate	Interpretation	Good

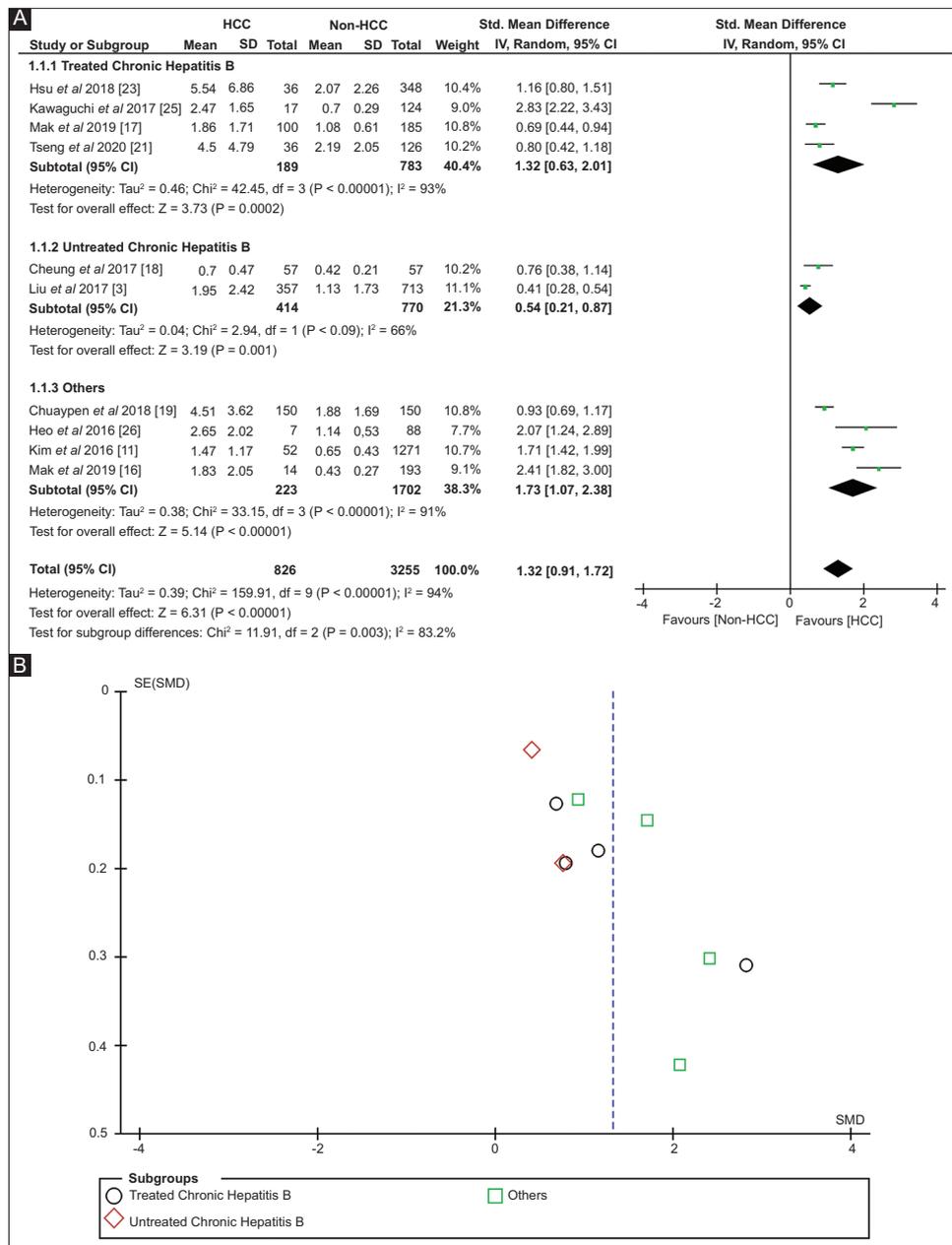


Figure 2 SMD of baseline serum Mac-2 binding protein glycosylation isomer levels in patients with chronic hepatitis B, according to the development of hepatocellular carcinoma. (A) forest plot, (B) funnel plot
HCC, hepatocellular carcinoma; SD, standard deviation; SE, standard error; SMD, standardized mean difference; CI, confidence interval

curve were 74% (95%CI 50-89%), 80% (95%CI 65-90%), 3.73 (95%CI 2.31-6.03), 0.33 (95%CI 0.17-0.64), 11.35 (95%CI 5.46-23.60), and 0.84 (95%CI 0.81-0.87), respectively.

Sensitivity and publication bias analyses

Sensitivity analysis was performed to assess the influence of each individual study on the pooled subgroup and overall results. The sensitivity analyses suggested that the statistical significance of pooled subgroups and overall point estimates

in all meta-analyses were unaffected by any study. For the SMD of M2BPGi (Fig. 2B), an asymmetrical distribution of studies was observed in the funnel plot, indicating that there was a potential publication bias. This finding was further confirmed by a significant Egger’s test result (Z=4.25; P<0.001).

Meta-regression analysis

Meta-regression analysis in the univariate HR meta-analysis showed that mean population age (Z=0.44; P=0.66)

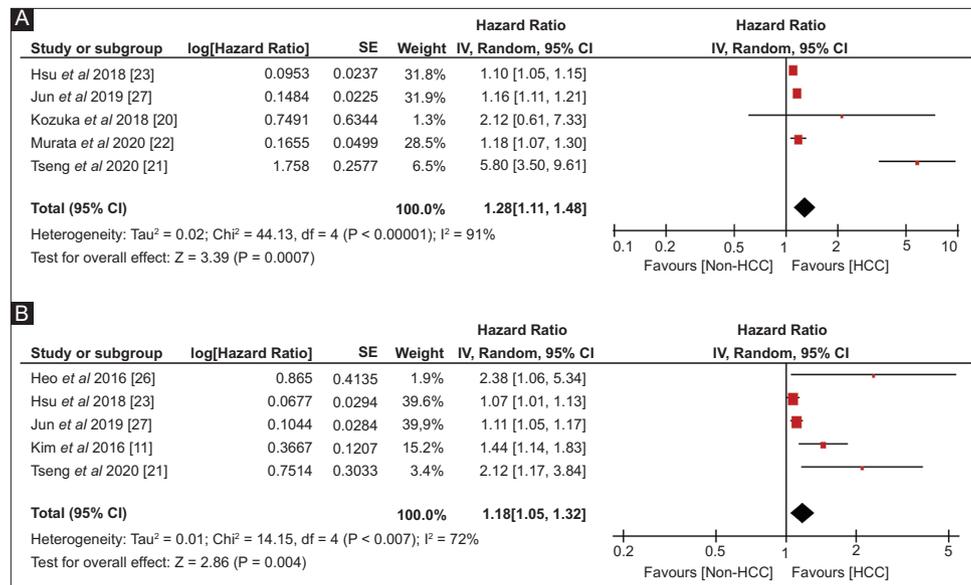


Figure 3 Hazard ratio of baseline serum Mac-2 binding protein glycosylation isomer levels in patients with chronic hepatitis B, according to the development of hepatocellular carcinoma. (A) univariate analysis, (B) multivariate analysis
HCC, hepatocellular carcinoma; SE, standard error; CI, confidence interval

and the percentage of females ($Z=-0.67$; $P=0.50$) had no effect on the M2BPGi in predicting HCC risk in CHB patients. Furthermore, meta-regression analysis in the multivariate HR meta-analysis of M2BPGi also showed no significant influence of mean population age ($Z=1.58$; $P=0.11$) on the HR of HCC risk. However, we found a significant positive effect of female percentage on the M2BPGi in predicting HCC risk in CHB patients ($Z=2.08$; $P=0.04$), meaning that a greater proportion of female patients would increase the capability of M2BPGi to predict future HCC risk.

Discussion

To the best of our knowledge, the current study was the first meta-analysis to investigate the value of baseline serum M2BPGi in predicting the HCC progression of CHB patients. Our study showed that baseline serum M2BPGi was a useful biomarker for predicting the progression of CHB patients to HCC, as indicated by its hazard ratios. We further analyzed the pooled accuracy of baseline serum M2BPGi, which showed moderately high sensitivity and specificity accompanied by moderate PLR and NLR. In such a scenario, based on Bayes' theorem [28], baseline serum M2BPGi can be a valuable test for predicting the need for aggressive therapy when the pre-test probability of HCC is uncertain (34-66%) or unlikely (10-33%). The latter findings indicated that baseline serum M2BPGi in HCC-related CHB patients was a reliable and helpful biomarker in confirming the practitioners' treatment decision.

In recent years, glycoprotein-based biomarkers have been introduced as novel biomarkers for several diseases. One of the examples is the detection of liver cell activities,

such as cell adhesion mediation and fibrosis promotion, using M2BP [10,29,30]. As the ligand of galectin-3, M2BPGi will communicate with Mac-2-positive cells to induce several biological activities, including cell adhesion, growth regulation, cytokine production, T-cell apoptosis and immune response. Therefore, M2BPGi would act as the juxtacrine-acting messenger for the activation of hepatic stellate cells (HSCs) during the progression of liver fibrosis [30]. M2BP, or Mac-2 binding protein, is a highly glycosylated, secreted protein that consists of 90 kDa subunits, containing sialylated multibranched N-glycans, and also acts as a ligand to galectin-3 (Mac-2). When extensively glycosylated, the M2BP becomes M2BPGi, or also known as hyperglycosylated *Wisteria floribunda* agglutinin (WFA)-positive Mac-2 binding protein (WFA⁺-M2BP) [9,17]. WFA is an optimal lectin substance used to detect a specific fibrosis-related glycoalteration [9,29]. Therefore, both M2BPGi and WFA⁺-M2BP were considered the same. However, recent studies favor using the name M2BPGi, rather than WFA⁺-M2BP [9,16,29].

This substance presents in small amounts throughout many tissues of the human liver, including the extracellular matrix (ECM) [9,17]. As it is produced by HSCs, it will act as a messenger from them to Kupffer cells, promoting fibrogenesis [16]. Any injuries, such as infections, may cause inflammation and further activate the Kupffer cells and hepatocytes to release cytokines. Activated HSCs may release M2BPGi along with other ECM, including TIMP-1, PIINP and hyaluronic acid, which will cause dysfunction of hepatic sinusoidal epithelial cells, leading to the progression of liver fibrosis [31]. The sugar chain structure of M2BP would change in response to the progression of hepatic fibrosis [16,29,32]. In chronic liver diseases, such as liver fibrosis, the WFA will recognize the N-acetylgalactosamine residue of N- and O-glycans on the M2BP, only modified by a fibrosis-specific

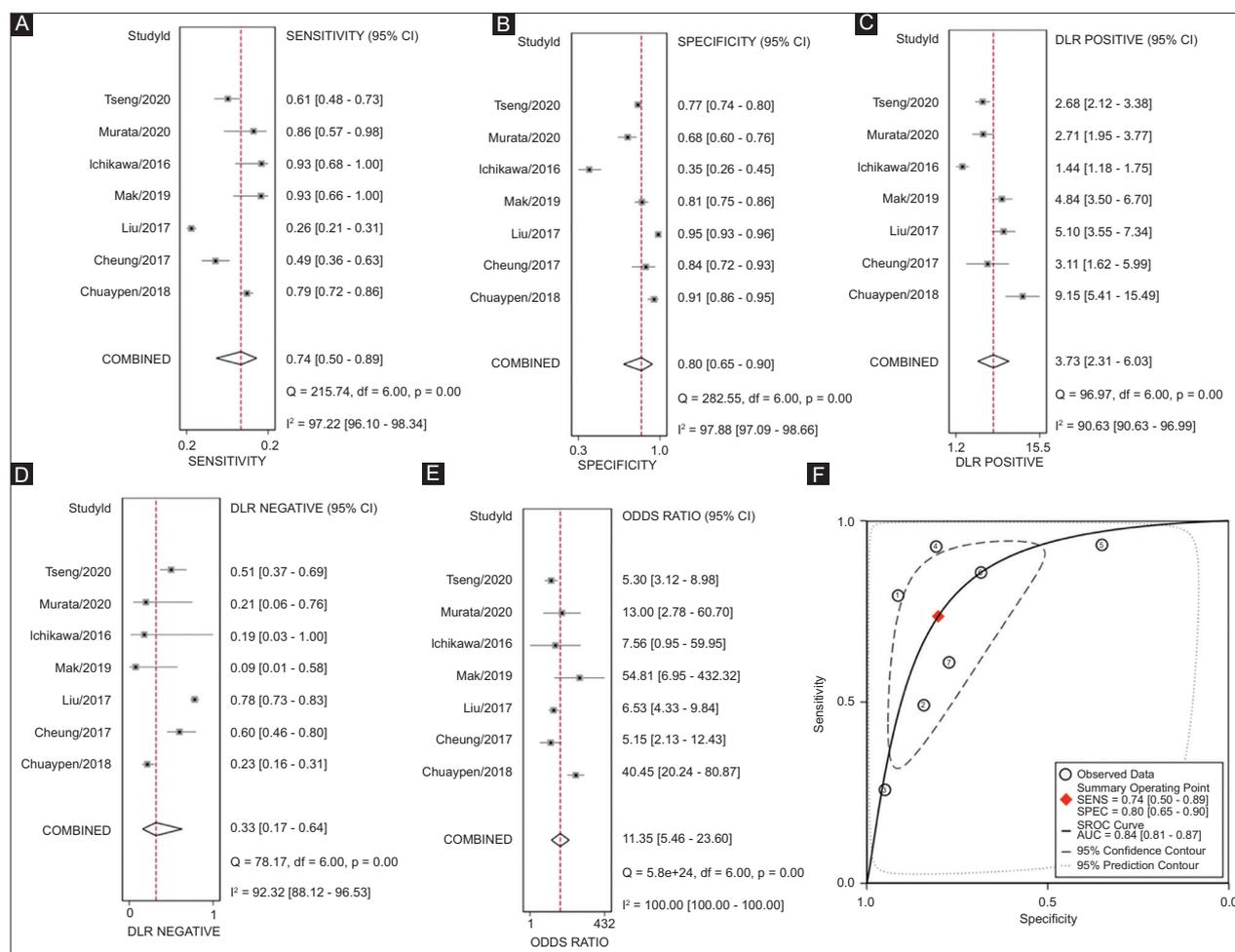


Figure 4 Pooled accuracy analysis of baseline serum Mac-2 binding protein glycosylation isomer: (A) sensitivity, (B) specificity, (C) positive likelihood ratio, (D) negative likelihood ratio, (E) diagnostic odds ratio, and (F) area under the summary receiver operating characteristic curve in predicting the development of hepatocellular carcinoma in patients with chronic hepatitis B
CI, confidence interval; AUC, area under curve; DLR, diagnostic likelihood ratio

sugar chain. Thus, the larger the fibrosis area, the higher the M2BPGi levels [31]. In addition, M2BPGi levels would rise as part of the host's response to infection and cancer cells [9]. M2BPGi can also be a useful tool for diagnosing several diseases, such as acute and chronic viral hepatitis, mortality in liver cirrhosis, biliary atresia, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, primary biliary sclerosis, autoimmune hepatitis, primary sclerosing cholangitis, and even interstitial pulmonary fibrosis [9,33].

Recent findings showed that serum M2BPGi was highly correlated with the progression of liver fibrosis. The serum level of M2BPGi increased significantly along with liver fibrosis progression. Moreover, this serum level may reflect the activation of HBV-related oncogenic factors, given its properties as the ligand of galectin-3 [17,22,24]. M2BPGi may bind and express galectin-3, thus activating the mTOR signaling that promotes HCC malignancy. The cancer also progresses further because of mitogen-activated protein kinase (MAPK) signaling, which enhances the mTOR signaling pathway from M2BPGi-induced galectin-3 [34]. Hence, serum M2BPGi

levels had been associated with the risk of HCC [17,22,24]. Serum M2BPGi not only acts as a serum surrogate marker for hepatic fibrosis, but could eventually serve as a useful predictive biomarker for the development of HCC during routine follow up [9,22,29].

Only one study specifically addressed HSCs as producers of M2BPGi, while one other addressed cirrhotic liver stromal cells in this role [34,35]. It is known that HSCs are sensitive to stimuli generated during hepatitis, inflammation or the tissue injury process [36]. M2BPGi itself could also be a stimulus for activating and inducing HSCs to change from their dormant form into a myofibrillar form that expresses proliferative, migratory and invasive properties [35]. The level of M2BPGi will be expected to increase, as one study showed that the increase of M2BPGi level reflects the activation of HSCs [37]. The latter statements suggest that the ongoing process during HBV infection would affect the production of M2BPGi through HSCs activation, further affecting the probability of HCC development. Several studies also highlighted that the patients treated for hepatitis B had significantly lower

serum levels of M2BPGi after the treatment, indicating that suppressing the infection process will decrease M2BPGi production [17,22,23,25,32,38]. HBV may also contribute to the M2BPGi level by having a direct effect on HSCs [39-41]. Some studies showed that hepatitis B protein-X activates the HSCs to express fibrotic properties [39,40]. Another study showed that HBe-antigen may prevent the apoptosis of HSCs [41]. On the other hand, an existing HCC can in turn signal dormant HSC with transforming growth factor (TGF)- β or connective tissue growth factor, activating the HSCs, and thus may increase M2BPGi production [36,42]. A study showed that M2BPGi could activate dormant HSC via M2BP/galectin-3 and Kupffer cells [35]. Activated HSC also may promote HCC tumorigenesis through the production of growth factors, cytokines, angiogenesis signals, and immune suppression [36,42].

From a molecular perspective, carcinogenesis caused by HBV is promoted by several mechanisms. Signaling pathways modified during HBV infections are Wnt signaling, PI3K/Akt/mTOR and Ras/ERK1/2. This will result in altered expression of C-myc, cyclin D1, P21^{cip1}, NF κ B, JAK/STAT, which regulate cell cycle, proliferation, differentiation, survival, growth, and hepatocyte mobility, and promote HCC [43]. HBV may directly promote carcinogenesis through host cell gene mutations by the insertion of viral genetic material, or through hepatitis B protein-X protein. Chronic inflammation resulting from infection causes chronic hepatitis, fibrosis and cirrhosis, and eventually leads to HCC [44].

It is widely known that liver fibrosis is the most recognized risk factor for HCC [36]. Several studies show that M2BPGi level could reflect fibrosis severity in patients with hepatitis B [24,30,35,38]. M2BPGi could also further promote liver fibrosis by activating HSCs and making them fibrogenic [35]. HSCs are the liver's collagen, ECM and tissue metalloproteinase producers, and make a large contribution to the fibrosis process in liver diseases [45]. During the fibrosis process, HSCs, along with portal fibrocytes, transform into a fibrosis-associated fibroblast and send multiple signals, such as ECM, growth factors, inflammatory cytokines, TGF- β and immunomodulatory receptor ligands that promote a suitable microenvironment for HCC growth and survival [46].

Some studies showed conflicting evidence, as high levels of M2BPGi could be detected in HBV patients with HCC regardless of liver fibrosis severity, suggesting that M2BPGi may have a role in hepatocarcinogenesis that does not involve the fibrosis process [11,19]. M2BPGi can further enhance HCC malignancy through inducing M2BP/galectin-3 expression on Kupffer cells and HCC, activating the mTOR pathway through galectin-3 signaling, as one study confirmed that galectin-3 knockdown reduced the effect of M2BPGi on HCC [34]. It is known that HBV infection activates the mTOR pathway, but through the PI3K/Akt/mTOR signaling mechanism, which is used to regulate the viral life cycle [47]. mTOR signaling normally serves in the regulation of cellular lipid metabolism, growth, motility and survival, and after HBV infection the pathway is associated with more aggressive HCC tumor progression and survival [48]. Thus, during HBV infection, M2BPGi may promote HCC through activation of HSC or

through enhancement of mTOR signaling. However, high levels of M2BPGi may not always reflect liver fibrosis, as elevated levels of M2BPGi are also found during acute liver injury [49].

Our current study also stated 2 important findings: 1) baseline serum M2BPGi in CHB patients who developed HCC was higher than in those who did not, regardless of the treatment status in the subgroup analysis; and 2) treatment status significantly modified the baseline COI serum M2BPGi levels. A previous study reported that treated and untreated CHB patients remained at risk for HCC [23]. Pharmacological therapies in CHB patients are certainly beneficial in reducing the inflammatory response and serum HBV-DNA in hepatitis, but they may not reduce the probability of having HCC in the future [25]. Other factors, aside from treatment status, may affect the risk of HCC, such as age, cirrhosis, liver stiffness, severity of hepatic dysfunction, AFP levels, being male, and having diabetes mellitus [23]. Those factors, apart from treatment status, might have affected our second finding, and there is still a lack of studies discussing the effect of hepatitis B treatment on baseline serum M2BPGi levels. However, taken together, our findings may still indicate that M2BPGi is of significant clinical value for predicting the risk of HCC in CHB patients, since it is not affected by treatment status, regardless of other possible factors.

Although some studies showed M2BPGi to be superior in detecting HCC, M2BPGi does not reflect HCC severity or disease stage, implying that its use may be limited to serving as an early HCC detection tool [3,19]. M2BPGi levels may also be elevated during chronic heart failure and idiopathic pulmonary fibrosis, and may thus misdirect the diagnosis [9,50]. These observations indicate that M2BPGi levels could not be used alone in predicting HCC, as different parameters may be required to complement the HCC diagnosis, depending on the disease stage and progression.

Our study had several limitations. First, our results showed considerable heterogeneity between studies. Thus, they should be interpreted more cautiously. Second, the potential of publication bias was present, as indicated by the funnel plot and Egger's test. This could be explained by the fact that our study only included English articles. Studies with non-significant findings are more likely to be published in a non-English journal [51]. Furthermore, publication bias could not be determined in several outcomes, since only a small number of studies were included in the analyses. Despite the limitations, to the best of our knowledge, our work is the first study to discuss the role of serum M2BPGi for predicting HCC specifically in CHB patients, through a sufficiently comprehensive systematic review and meta-analysis. Therefore, our study provides further evidence regarding the utility of serum M2BPGi in clinical practice, apart from the currently available and established biomarkers.

In conclusion, our study demonstrated that baseline serum M2BPGi was higher in CHB patients who developed HCC as compared to those who did not, regardless of the treatment status. Baseline serum M2BPGi could serve as a novel predictor of HCC development in CHB patients, given its moderate-to-high accuracy. Further larger-sized and well-designed studies

directly comparing the properties of M2BPGi with those of other traditional HCC biomarkers are warranted to confirm the current findings.

Summary Box

What is already known:

- Mac-2 binding protein glycosylation isomer (M2BPGi) is an extensively glycosylated liver-secreted protein, also known as a biomarker in liver fibrosis, including distinguishing each of its stages and representing more severe stages of fibrosis
- The exact risk predictive accuracy of baseline serum M2BPGi for the development of hepatocellular carcinoma (HCC) in patients with chronic hepatitis B (CHB) remains unclear and needs further establishment to support its efficacy

What the new findings are:

- The pooled analysis of the current study demonstrated that baseline serum M2BPGi was significantly higher in CHB patients who developed HCC compared to those who did not, regardless of the treatment status
- High baseline serum M2BPGi, with a cutoff value of 0.68-2.40 or higher, showed a significant capability for predicting HCC development in CHB patients
- Baseline serum M2BPGi possessed moderate-to-high accuracy for predicting HCC development in CHB patients, with a sensitivity of 74% and a specificity of 80%

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