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MAJOR ARTICLE

Serum Mac-2-Binding Protein Glycosylation Isomer at Virological Remission Predicts Hepatocellular Carcinoma and Death in Chronic Hepatitis B-Related Cirrhosis

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Background. To investigate serum Mac-2-binding protein glycosylation isomer (M2BPGi) levels in predicting hepatocellular carcinoma (HCC) and mortality at virological remission (VR, HBV DNA <20 IU/mL) following antiviral therapy in chronic hepatitis B (CHB) patients with cirrhosis.

Methods. This retrospective cohort study included patients with CHB-related Child-Pugh A cirrhosis undergoing long-term antiviral therapy. Serum M2BPGi levels were quantified and multivariable Cox proportional hazards regression models were used to identify risk predictors for HCC and death.

Results. A total of 126 and 145 patients were included in the derivation and validation cohorts, respectively. The mean age was 56, and the mean M2BPGi level was 1.86 cut-off index (COI) in the derivation cohort. After adjustment for confounders, a higher M2BPGi level at VR significantly predicted HCC (hazard ratio [HR]: 1.58, 95% confidence interval [CI]: 1.19-2.10, P=0.002) and death (HR: 2.17, 95% CI: 1.02-4.62, P=0.044). The M2BPGi ≥3 COI significantly increased the risk of HCC and death in the derivation and validation cohorts. Serial M2BPGi levels declined significantly (P=0.0001) in non-HCC patients only, and remained significantly lower than those who developed HCC afterwards (P=0.039).

Conclusions. Serum M2BPGi levels at antiviral therapy-induced VR predict HCC development and death in patients with CHB-related Child-Pugh A cirrhosis.

Keywords. fibrosis; HBV; liver cancer; M2BPGi; nucleos(t)ide analogs.

Antiviral therapy against hepatitis B virus (HBV) can inhibit viral replication, ameliorate liver inflammation and fibrosis, achieve HBV surface antigen seroclearance, and ultimately reduce disease progression to cirrhosis, hepatic decompensation, hepatocellular carcinoma (HCC), liver-related death, and all-cause mortality [1]. Hepatocellular carcinoma still develops even after long-term viral suppression; therefore, it is crucial to stratify the risk of HCC in patients with undetectable HBV deoxyribonucleic acid (DNA) to intensify the surveillance of HCC [2].

After potent antiviral therapy with virological remission (VR), serum HBV DNA level is no longer predictive for HCC; nevertheless, the severity of liver fibrosis may become a crucial factor for disease progression [3]. Several noninvasive methods have been used to quantify the extent of fibrosis, including serum biomarkers [4], ultrasound-based examinations [5], or FibroScan [6]. In a recent study, a glycoprotein-based serum biomarker, Mac-2-binding protein glycosylation isomer (M2BPGi), has been introduced to correlate with liver fibrosis and risk of HCC in patients with chronic hepatitis C [7], to predict liver reserve and outcome of cirrhosis [8] and liver failure after hepatectomy [9]. In patients with chronic hepatitis B (CHB), M2BPGi levels can also help to estimate liver fibrosis [10, 11] and are associated with an increased risk of HCC [10, 12]. A higher pretreatment M2BPGi level can predict HCC development in patients receiving nucleos(t)ide analog (NUC) therapy [13, 14]. However, previous studies comprised heterogeneous participants including both treated and untreated patients or studies with cirrhotic and noncirrhotic patients.

The M2BPGi levels may decrease after antiviral therapy because of improving liver necroinflammation and fibrosis [11]. For many on-treatment patients, it is also impracticable to check their pretreatment M2BPGi level. In this study, we explored (1) the predictive role of M2BPGi levels for HCC and overall mortality upon antiviral therapy-induced VR in CHB-related

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cirrhotic patients, and (2) the serial changes in M2BPGi levels after VR until the development of HCC.

MATERIALS AND METHODS

Patients

This was a retrospective study conducted at the National Taiwan University Hospital ([NTUH] derivation cohort) and the China Medical University Hospital ([CMUH] validation cohort); both were tertiary medical centers in Taiwan. We consecutively screened patients aged ≥20 years with CHB-related cirrhosis who had received long-term NUC therapy (lamivudine, adefovir, entecavir, telbivudine, and tenofovir) with regular follow-up in the liver clinic and available stored sera (in −20°C freezer, every 6 months). The National Health Insurance reimburses lifelong antiviral therapy for patients with CHB-related cirrhosis with evidence of portal hypertension (splenomegaly or esophageal/gastric varices) and HBV DNA ≥2000 IU/mL as per current guidelines [1]. Cirrhosis was diagnosed by histology or from ultrasonographic appearance of nodular liver surface, coarse liver parenchymal texture, and narrowed vessels with irregular intrahepatic vessel contour [5]. Patients with decompensated cirrhosis (Child-Pugh B or C), incomplete clinical data, unavailable stored sera, coinfection with hepatitis C virus, hepatitis D virus (HDV), or human immunodeficiency virus, or past history of or existing HCC were excluded.

The clinical characteristics of patients were collected at the time of VR (HBV DNA level <20 IU/mL), including age, sex, platelet counts, biochemical profiles, alpha-fetoprotein (AFP), HBV DNA, date for HCC, and death. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Boards of NTUH (200909046R) and CMUH (CMUH102-REC1-113). All patients provided written informed consent before enrollment.

Laboratory Tests and Quantification of M2BPGi

The laboratory assays were performed by using commercially standardized automated techniques. Serum HBV DNA levels were measured using the Abbott RealTime HBV assay (Abbott Laboratories, Abbott Park, IL) with a lower detection limit of 20 IU/mL. The sera at the time of VR were retrieved, and the M2BPGi levels were quantified by using an automated chemiluminescence enzyme immunoanalyzer (HISCL-800; Sysmex Co., Kobe, Japan) and expressed as cutoff index (COI), with a range of 0.1–20 COI.

Clinical Evaluation and Endpoints

The patients were observed every 3 months at each hospital. The serum AFP level and abdominal ultrasonography were examined every 3 to 6 months for surveillance of HCC. The data were collected through a standardized clinical record form.

The primary endpoint of this study was the development of HCC, which was diagnosed by either pathology examination or 2 typical dynamic imaging studies (computed tomography, magnetic resonance imaging, or angiography) according to the American Association for the Study of Liver Diseases guidelines [15]. The secondary endpoint was all-cause mortality.

Statistical Analysis

Continuous variables were reported as mean (standard deviation) and categorical data were reported as number (percentage). Differences between groups were evaluated by Student's *t* test or χ^2 statistic as appropriate. Pearson's correlation was used to investigate the association between M2BPGi level and (1) *alanine aminotransferase (*ALT) or (2) AFP. The time at risk was measured from the index date (VR) until the development of HCC and death, loss of follow-up, or December 31, 2016, whichever came first. The risks of HCC and death were modeled using a Cox proportional hazards regression analysis. Those statistical significant parameters ($P < .05$) in univariate analysis were entered in multivariable analysis for adjustment. The clinically relevant cutoff M2BPGi level for HCC risk was calculated by the restricted cubic spline regression with different number of knots. The M2BPGi level was first logarithmically transformed. With the reference M2BPGi level of 1, the best-fitting cubic spline model was determined according to the values of Akaike information criterion (AIC) [4]. Because the average follow-up period was 4 years, we conducted receiver operating characteristic (ROC) curve analysis restricted to patients who were observed for at least 4 years (or until HCC development). The predictability of M2BPGi was expressed as the area under the ROC curve (AUROC), and the optimal cutoff M2BPGi value was obtained by maximizing the Youden's index. The cumulative incidence rates of HCC and death were estimated by Kaplan-Meier analysis and compared using the log-rank test between high and low M2BPGi levels. The linear mixed-effect model with random coefficients was used to compare longitudinal repeated measurements of M2BPGi after VR, by unstructured correlation structure and the time trend. Subject group was included in the model as a fixed effect, and the intercept and slope were treated as random variables. The statistical analysis was performed using STATA (version 13.0; StataCorp, College Station, TX). All tests were 2-sided, and $P < .05$ was considered statistically significant.

RESULTS

A total of 156 patients with CHB-related cirrhosis receiving long-term NUC therapy were screened at the NTUH liver clinic during 2007–2016. Among them, 126 patients were included after excluding 2 patients who received NUC therapy after HCC development, 1 with HBV and HDV coinfection, 12 patients with decompensated cirrhosis, and 15 without available sera after VR. At the time of VR, the mean age was 56 years, and 74.6% of them were males. The baseline characteristics are shown in Table 1. The mean M2BPGi level was

Table 1. Baseline Characteristics of Chronic Hepatitis B Patients With Child-Pugh A Cirrhosis Upon Virological Remission From the Derivation and Validation Cohorts[a]

Variables	Derivation N = 126	Validation N = 145
Age, year	56 (11)	54 (10)
Male, n (%)	94 (74.6)	112 (77.2)
ALT, U/L	35 (30)	37 (19)
Platelet, K/μL	145 (65)	131 (56)
Albumin, g/dL	4.4 (0.4)	4.2 (0.4)
T-bil, mg/dL	0.9 (0.4)	1.1 (0.4)
Child-Pugh score	5.1 (0.2)	5.1 (0.3)
AFP, ng/mL	9.4 (41.7)	7.0 (9.7)
M2BPGi, cutoff index	1.86 (2.18)	3.09 (2.89)
Antiviral Therapy		
Entecavir	88	145
Tenofovir	16	0
Combination[b]	11	0
Other[c]	11	0
Follow-up duration, month	50.3 (26.4)	57.9 (27.6)
HCC, n (%)	20 (15.9)	30 (20.7)
Death, n (%)	4 (3.2)	7 (4.8)

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; M2BPGi, Mac-2-binding protein glycosylation isomer; SD, standard deviation; T-bil, total bilirubin.

[a]Data are expressed as mean (SD) or n (%).

[b]Lamivudine/adefovir 5, telbivudine/adefovir 3, entecavir/adefovir 2, entecavir/tenofovir 1.

[c]Lamivudine 2, telbivudine 9.

1.86 COI. After a mean follow-up of 50.3 months, 20 patients developed HCC and 4 died. At the time of VR, there was no correlation between M2BPGi and ALT levels (Pearson's correlation: −0.02, P = .855), but there was a weak correlation between M2BPGi and AFP levels (Pearson's correlation: 0.22, P = .015).

Predictors for Hepatocellular Carcinoma and Death

Univariate analysis showed that age, platelet, albumin, AFP, and M2BPGi levels at the time of VR were predictive of HCC. Multivariable analysis showed that AFP levels (hazard ratio [HR], 1.01; 95% confidence interval [CI], 1.001–1.01; P = .018) and M2BPGi levels (HR, 1.58; 95% CI, 1.19–2.10; P = .002) were significant predictors for HCC development (Table 2).

Moreover, univariate analysis showed that platelet, albumin, total bilirubin, and M2BPGi levels at the time of VR were predictive of death. Multivariable analysis showed that M2BPGi levels (HR, 2.17; 95% CI, 1.02–4.62; P = .044) remained a significant predictor of death (Table 3).

Cutoff Value of M2BPGi for Prediction of Hepatocellular Carcinoma and Death

The best cutoff level of M2BPGi for clinical use was further investigated. Figure 1A showed the best-fitting regression spline, which was based on the smallest AIC value. This model was derived using 2 knots placing on the 33rd and 66th percentiles of M2BPGi distribution. We found that the HCC risks remained similar when ln(M2BPGi) <1.10 (M2BPGi = 2.99),

Table 2. Univariate and Multivariable Analyses for the Risk Predictors of Hepatocellular Carcinoma in the Derivation Cohort

Variables	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Age (1 year increment)	1.07 (1.03–1.11)	.001	1.06 (1.01–1.11)	.024
Male (vs female)	.60 (.24–1.50)	.274		
ALT (1 U/L increment)	1.00 (.99–1.01)	.911		
Platelet (1 K/μL increment)	.98 (.97–.99)	.001	.99 (.98–1.00)	.219
Albumin (1 g/dL increment)	.27 (.11–.71)	.008	4.77 (.90–25.28)	.067
T-bil (1 mg/dL increment)	2.61 (.99–6.86)	.051		
Child score (1 point increment)	2.29 (.53–9.97)	.269		
AFP (1 ng/mL increment)	1.01 (1.004–1.01)	<.001	1.01 (1.001–1.01)	.018
M2BPGi (1 COI increment)	1.46 (1.28–1.68)	<.001	1.58 (1.19–2.10)	.002

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; COI, cutoff index; HR, hazard ratio; M2BPGi, Mac-2-binding protein glycosylation isomer; T-bil, total bilirubin.

Table 3. Univariate and Multivariable Analyses for the Risk Predictors of Death in the Derivation Cohort

Variables	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Age (1 year increment)	1.04 (.95–1.13)	.438		
Male (vs female)	.34 (.05–2.40)	.278		
ALT (1 U/L increment)	.99 (.94–1.04)	.719		
PLT (1 K/μL increment)	.96 (.93–.99)	.020	.99 (.94–1.05)	.814
Albumin (1 g/dL increment)	.05 (.01–.29)	.001	.73 (.02–22.76)	.860
T-bil (1 mg/dL increment)	10.01 (1.43–70.04)	.020	.14 (.00–74.48)	.536
Child score (1 point increment)	6.14 (.63–59.51)	.117		
AFP (1 ng/mL increment)	1.00 (.99–1.02)	.788		
M2BPGi (1 COI increment)	2.12 (1.34–3.35)	.001	2.17 (1.02–4.62)	.044

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; COI, cutoff index; HR, hazard ratio; M2BPGi, Mac-2-binding protein glycosylation isomer; PLT, platelet count; T-bil, total bilirubin.

but it gradually increased while the index was higher than the threshold.

We also performed ROC curve analysis to evaluate M2BPGi's performance to predict HCC in the subgroup of patients with a follow-up >4 years (n = 68). Twenty patients developed HCC, and the AUROC was 0.79 (95% CI, .67–.91) (Figure 1B). The optimal cutoff point of M2BPGi was 3.0 to predict the 4-year risk of HCC, with a sensitivity of 58% and specificity of 90%

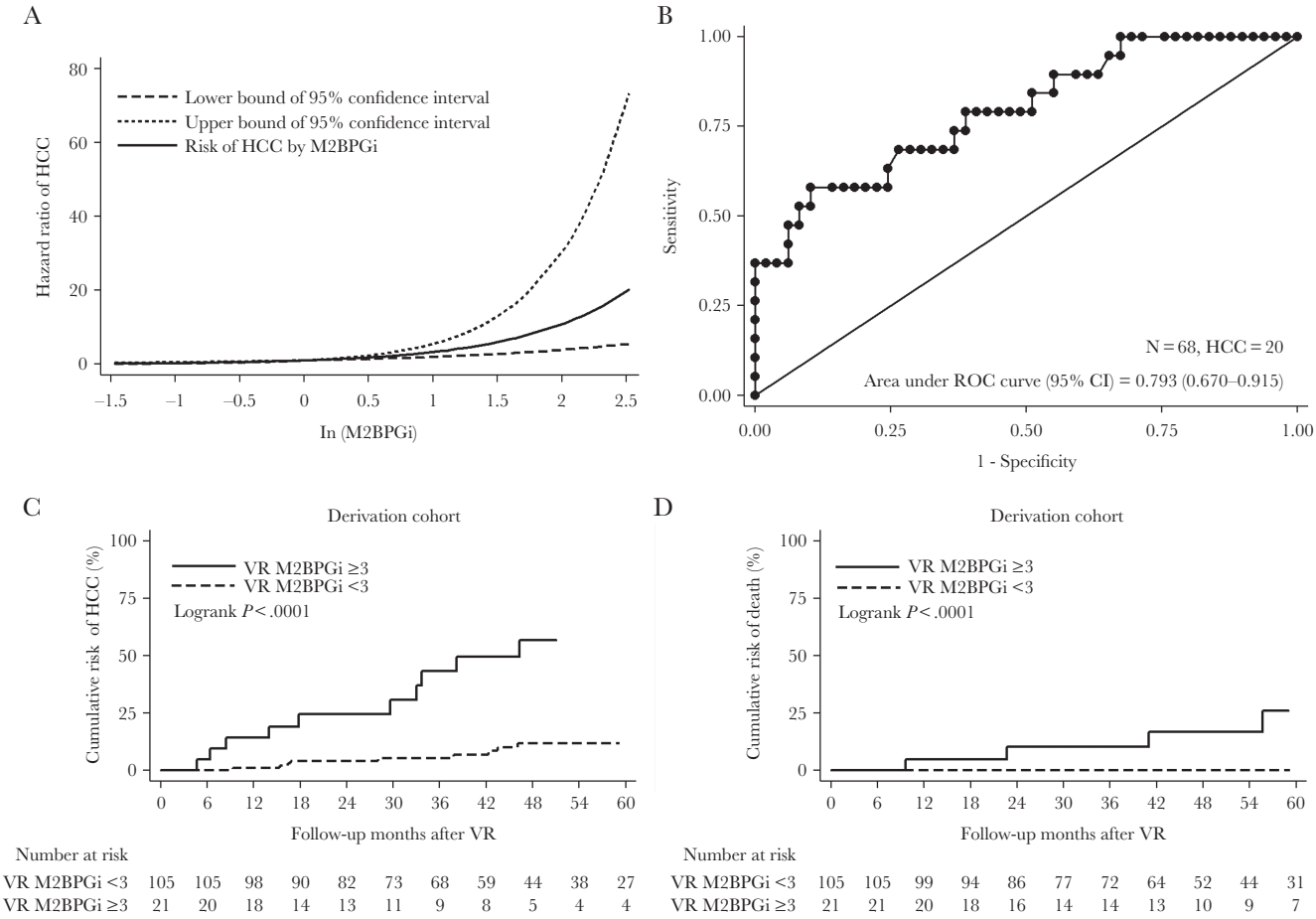

Figure 1. A higher Mac-2-binding protein glycosylation isomer (M2BPGi) level at virological remission (VR) predicts the risk of hepatocellular carcinoma (HCC) and death. (A) The hazard ratio of HCC in relation to M2BPGi level analyzed by the restricted cubic spline regression shows the HCC risk starts to increase when ln(M2BPGi) >1.1 (M2BPGi > 2.99 cutoff index [COI]). (B) Receiver operating characteristic (ROC) curve analysis shows M2BPGi is a modest predictor of HCC development within 4 years of follow-up (area under ROC: 0.793). (C) The M2BPGi ≥3 COI significantly increased the risk of HCC (log-rank *P* < .0001). (D) The M2BPGi ≥3 COI significantly increased the risk of death (log-rank *P* < .0001).

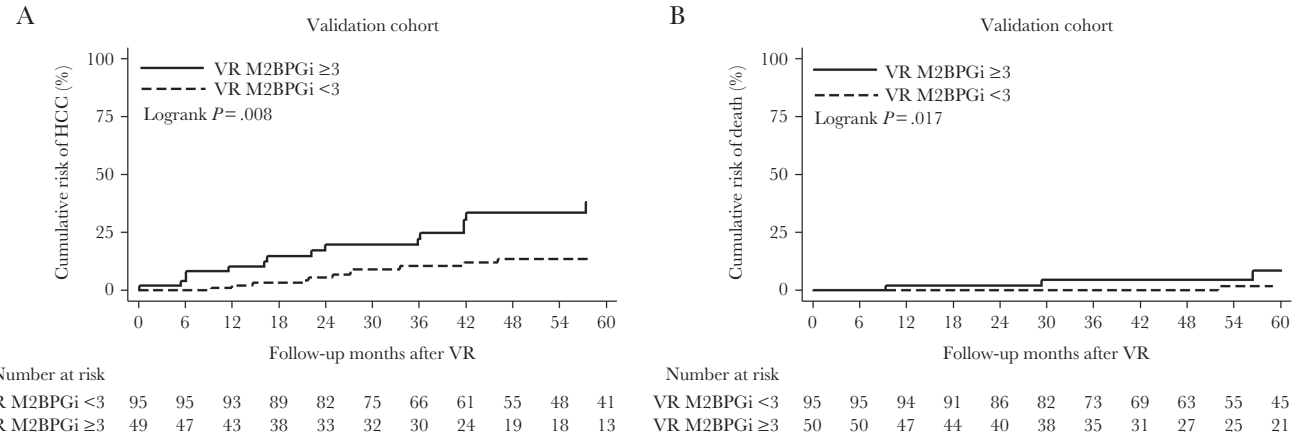

Figure 2. A higher Mac-2-binding protein glycosylation isomer (M2BPGi) level at virological remission (VR) predicts the risk of hepatocellular carcinoma (HCC) and death in the validation cohort. (A) THe M2BPGi ≥3 cutoff index (COI) significantly increased the risk of HCC (log-rank $P = .008$). (D) The M2BPGi ≥3 COI significantly increased the risk of death (log-rank $P = .017$).

(positive predictive value [PPV], 67%; negative predictive value [NPV], 83%) (Supplementary Table 1). Moreover, the AUROC of M2BPGi to predict death was 0.99 (95% CI, .97–1.00), and the M2BPGi level of 3 COI predicted subsequent death with a sensitivity of 100% and specificity of 80% (PPV, 20%; NPV, 100%).

The Kaplan-Meier analysis showed that the cumulative incidence of HCC was significantly higher in patients with M2BPGi level ≥3 COI, compared with those with M2BPGi <3 COI (log-rank $P < .0001$). In addition, the cumulative incidence of death was significantly higher in patients with M2BPGi level ≥3 COI, compared with those with M2BPGi <3 COI (log-rank $P < .0001$) (Figure 1C and 1D).

Validation by the China Medical University Hospital Cohort

To validate the findings, after a similar screening procedure, a total of 145 patients with CHB-related Child-Pugh A cirrhosis who received long-term NUC therapy were included in the CMUH validation cohort. The mean age was 54 years and 77.2% were males. The mean M2BPGi level was 3.09 COI. Thirty patients developed HCC, and 7 patients died after a mean follow-up of 58 months.

Likewise, Kaplan-Meier analysis showed that the cumulative incidence of HCC was significantly higher in patients with M2BPGi level ≥3 COI, compared with those with M2BPGi <3 COI (log-rank $P = .008$). Moreover, the cumulative incidence of mortality was significantly higher in patients with M2BPGi level ≥3 COI, compared with those with M2BPGi < 3 COI (log-rank $P = .017$) (Figure 2).

Serial M2BPGi Levels After Virological Remission

We further investigated serial changes in M2BPGi levels after VR according to the status of HCC development. There were 141 patients with available sera after VR (last sera before the development of HCC in the HCC group [mean: 2 years], and before the end of follow-up for the non-HCC group [mean: 5 years]), including 28 patients with sera at the time of HCC diagnosis (mean: 3 years). The M2BPGi levels remained unchanged during antiviral treatment in patients who developed HCC afterwards ($P = .691$), but they declined significantly after VR in those without HCC ($P = .0001$). The M2BPGi levels were significantly higher in the HCC group by repeated measurements ($P = .039$) (Figure 3).

DISCUSSION

In this study, we demonstrated that serum M2BPGi level at the time of VR was a significant and independent risk predictor of subsequent HCC development and all-cause mortality in patients with CHB-related Child-Pugh A cirrhosis undergoing long-term antiviral therapy. An M2BPGi level ≥3 (vs <3) COI at VR significantly increased the risk of HCC or death, which was validated by another independent cohort. Furthermore, serum M2BPGi levels decreased significantly after antiviral therapy only in the non-HCC subgroup, but they remained unchanged in patients who developed HCC afterwards. Our results suggested serum M2BPGi levels may be useful for HCC surveillance in NUC-treated patients with CHB-related Child-Pugh A cirrhosis.

The Mac-2 binding protein (M2BP) is a secreted glycoprotein (~90 kDa) that presents 7 N-glycans per monomer [16]. The M2BP is highly polymerized in serum to form a large, sweet doughnut-like structure [17], which is secreted by hepatic stellate cells (HSCs) in the liver and macrophages from other organs. The M2BP interacts with collagens IV, V, and VI, fibronectin, and nidogen [18]. The glycan structure of M2BP may change and reflect the degree of cell differentiation and canceration [16]; for example, the N-glycosylation of M2BP changes during the progression of liver disease, and the amount of altered M2BP increases in advanced fibrosis. The *Wisteria floribunda* agglutinin (WFA) recognizes the altered M2BP specifically produced by HSCs (termed

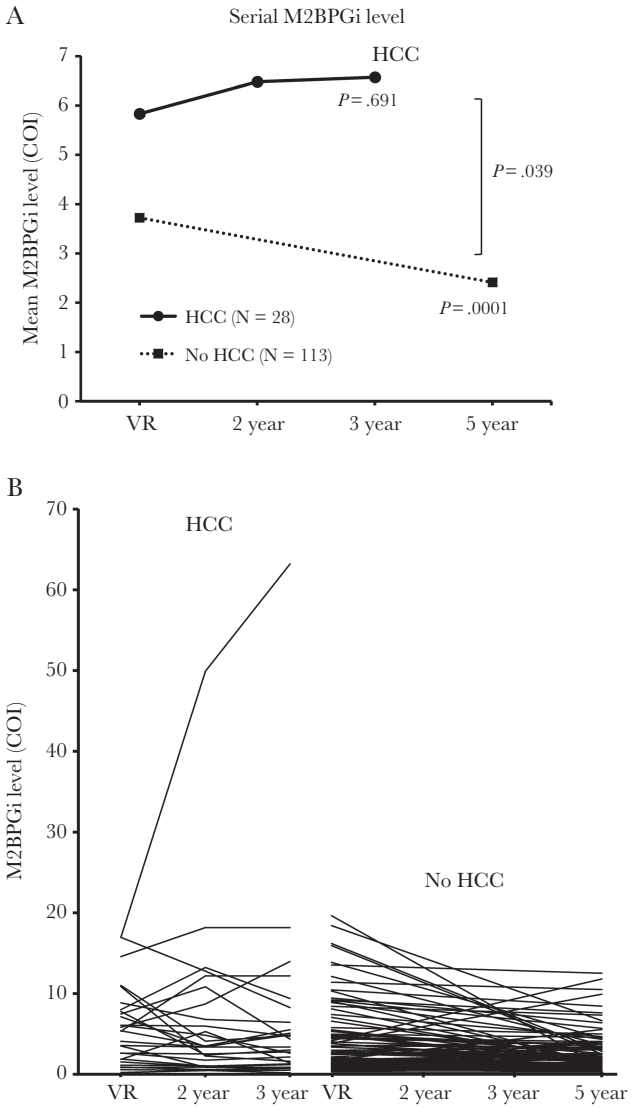

Figure 3. Serial changes in serum Mac-2-binding protein glycosylation isomer (M2BPGi) levels after virological remission (VR) stratified by the development of hepatocellular carcinoma (HCC). (A) The mean M2BPGi levels remained stationary (P = .691) during antiviral treatment in patients who developed HCC afterwards (at a mean of 3 years after VR), but they declined significantly (P = .0001) in those without HCC at a mean of 5 years after VR. The M2BPGi levels after VR remained significantly higher in the HCC group compared with the non-HCC group (P = .039). (B) The M2BPGi levels of individual patients stratified by the development of HCC. COI, cutoff index.

WFA$^+$-M2BP, or M2BPGi) [17]. Hepatic stellate cells are the major producers of M2BPGi, which acts like a juxtacrine messenger to enhance Mac-2 protein (galectin-3) expressed in Kupffer cells, and the Mac-2 protein in turn activates HSCs to become fibrogenic [19]; therefore, M2BPGi reflects the activation of HSCs during fibrogenesis [20]. In a clinical setting, M2BPGi was found to be a liver fibrosis marker in a variety of liver diseases, such as primary biliary cholangitis [21], autoimmune hepatitis [22], and nonalcoholic fatty liver disease [23].

Several risk predictors of HCC have been proposed for clinical use, including the REACH-B, CU-HCC, GAG-HCC, PAGE-B, and APA-B scores [24, 25]. The CU-HCC and GAG-HCC scores highlight that cirrhosis is an important risk factor of HCC in treatment-naive Asian patients [26, 27]. An important clinical question is whether these HCC risk predictors also work for patients undergoing long-term antiviral therapy, because HBV DNA is suppressed and cirrhosis may regress [28]. The pretreatment or on-treatment platelet count [24, 25] or the AFP levels have been demonstrated to be independent predictors of HCC in patients undergoing antiviral therapy [1, 25, 29]. Liver stiffness measurement (LSM) by FibroScan could be another useful predictor of HCC development in patients with CHB, regardless of the use of antiviral therapy [30]. The REACH-B, CU-HCC, and GAG-HCC scores (including baseline HBV DNA level) can accurately predict subsequent development of HCC in patients even during antiviral therapy [31]; however, the modified REACH-B score (LSM value replaces HBV DNA) seemed better when compared to conventional models [32], indicating that the underlying liver disease (fibrotic burden) may be more important than the virus itself for HCC development in the treated populations. Moreover, in patients already undergoing antiviral therapy, HBV DNA level was usually undetectable, and therefore risk predictors without HBV DNA is more clinically relevant at this scenario.

In addition to being a fibrosis marker, Mac-2 protein as a predictor for HCC was further explored. It has been associated with cell adhesion, growth regulation, cytokine production, T-cell apoptosis, and immune responses [20], and it may stimulate cancer progression [33]. The combination of M2BPGi and AFP could improve the sensitivity of a single test in the diagnosis of HCC [34]. Activated HSCs promote tumorigenicity of HCC [35], and they act as key modulators of fibrosis and tumor cell microenvironment [36]. Therefore, M2BPGi (reflecting activated HSCs) may be associated with HCC development in patients with liver fibrosis. A recent study using the REVEAL-HBV cohort found that an M2BPGi level of ≥2 COI (compared with <1 COI) was a strong and independent short-term predictor of HCC within 1–2 or 2–5 years in CHB patients with or without cirrhosis, respectively [37]. In that study, HBV viral load played no role on HCC risk in the years closer to HCC diagnosis, and the researchers also found that M2BPGi levels significantly increased before HCC diagnosis. It is interesting to note that M2BPGi levels remained a significant predictor of noncirrhotic HCC within 2–5 years, suggesting that M2BPGi might serve as an HCC predictor regardless of liver fibrosis. Our data further validated their findings in another clinical scenario of CHB patients with viral suppression by NUCs. Achieving VR by antiviral therapy is insufficient to eliminate HCC risk completely. Thus, risk-stratified HCC surveillance among patients who received long-term NUC therapy is crucial [2]. In our study, we included a homogenous population of CHB-related cirrhotic patients (Child-Pugh A)

under long-term oral antiviral therapy. After adjustment for relevant confounding factors, higher M2BPGi levels at VR independently predicted subsequent development of HCC (HR, 1.58) and all-cause mortality (HR, 2.17). The higher cutoff value of M2BPGi (≥3 COI) reflected higher severity of liver disease (cirrhosis with portal hypertension) in our patients. The NPVs were very high (HCC, 90% and death, 100%), which made serum M2BPGi levels a crucial marker for excluding subsequent poor outcomes. The M2BPGi level was also a better predictor for all-cause mortality (HR, 2.17) than HCC (HR, 1.58), and, consistently, the AUROC for predicting 4-year risk of death (0.99) was higher than HCC (0.79), indicating that M2BPGi also predicted cirrhotic complications and cirrhosis related death other than HCC. The serum M2BPGi levels significantly decreased after NUC treatment in patients with CHB [14]. However, we found that the serial M2BPGi levels during antiviral therapy only declined in non-HCC patients but remained unchanged in those with subsequent HCC, indicating that M2BPGi might serve as a tumor marker for HCC in CHB-related cirrhosis. The persistently elevated M2BPGi level leading to HCC can be explained by a high fibrotic burden passing the point of no return, and leads to the development of HCC. Alternatively, the occurrence of cell atypia would alter the surrounding microenvironment and caused the activated HSCs to overproduce M2BPGi.

Compared with other noninvasive examinations, there are several advantages to measuring M2BPGi. The patients do not need to visit large hospitals physically for the test. The M2BPGi levels are not influenced by fluctuations in ALT value or inflammation, which can cause falsely high estimates in other fibrosis tests (eg, fibrosis-4 [FIB-4] or FibroScan) [17]. The M2BPGi has a superior diagnostic accuracy for advanced fibrosis (F3) and cirrhosis (F4) compared with FIB-4 [17], and FIB-4 has been shown unsuitable to evaluate fibrosis improvement in CHB patients receiving antiviral therapy [38]. However, there are several limitations in this study. The patient characteristics were not matched completely in the derivation and validation cohorts; therefore, the mean M2BPGi levels were different, and selection bias was unavoidable from a retrospective design. Whether the findings in Asian cohorts could be extrapolated to whites remains to be examined. The timing for M2BPGi measurement for outcome prediction is still controversial. The M2BPGi level reflects both necroinflammation and fibrosis before treatment, whereas it reflects predominantly fibrotic burden after treatment. A recent study by Hsu et al [14] suggested that pretreatment M2BPGi level is more predictive than the level at year 1 or 2, whereas another study by Shinkai et al [39] suggested that 48-week M2BPGi level is superior to baseline. Because we did not have the pretreatment M2BPGi levels for investigation, this study could not validate or define the best timing to check M2BPGi level. The proposed cutoff value by Shinkai et al [39] was 1.215 COI, which is lower than our suggested value (3 COI); however, their target population had less fibrotic burden (84% noncirrhotic), and some patients still had HBV viremia at 48 weeks of therapy. Our study suggested that M2BPGi at VR is predictive for HCC and death, which is more practical in clinical settings. More investigations should be conducted to clarify the role of M2BPGi at different time points during the natural course of CHB.

CONCLUSIONS

In conclusion, from 2 cohorts of patients with CHB-related Child-Pugh A cirrhosis undergoing long-term antiviral therapy, higher M2BPGi levels (≥3 COI) at the time of VR predict subsequent development of HCC and all-cause mortality. Serum M2BPGi levels decrease significantly after antiviral therapy only in non-HCC patients, but they remain unchanged in patients who develop HCC afterwards. Serum M2BPGi levels at VR may help stratify the intensity of HCC surveillance in NUC-treated patients with CHB-related Child-Pugh A cirrhosis.

Supplementary Data

Supplementary materials are available at The *Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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