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ORIGINAL ARTICLE

Correlation of serum Mac-2-binding protein glycosylation isomer (M2BPGi) and liver stiffness in chronic hepatitis B infection

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Abstract

Background and aim Mac-2-binding protein glycosylation isomer (M2BPGi) is a novel serum diagnostic marker for liver fibrosis in various liver diseases. We aimed to evaluate its role in assessment of liver fibrosis in chronic hepatitis B infection (CHB) with reference to liver stiffness measurement (LSM).

Methods CHB patients with LSM by transient elastography technology and retrievable serum samples were recruited. Ten-year re-assessments of LSM and M2BPGi were repeated in a patient subgroup.

Results 240 CHB patients (M:F = 116:124; median age 47.5 years) were recruited. The median M2BPGi values for F0/F1/F2, F3 and F4 progressively increased with more advanced stages of liver fibrosis: 0.39, 0.46 and 0.82 COI, respectively ($p < 0.01$). M2BPGi levels correlated well with liver stiffness ($r = 0.611$), FIB-4 ($r = 0.616$), and strongly with APRI ($r = 0.825$) (all $p < 0.001$). Using cut-off values of 0.605 and 0.615 COI, the AUROCs were 0.754 and 0.799 for ≥ F3 and F4, respectively. M2BPGi identified one-quarter patients at risk of advanced fibrosis/cirrhosis otherwise classified into 'grey area' by LSM. In 86 patients with reassessment LSM, 21 (24.4%) showed significant fibrosis regression with corresponding decline in median M2BPGi level (− 0.11 COI) compared with the increase of +0.03 COI in patients without significant fibrosis regression ($p = 0.011$). Male gender, older age, use of potent antiviral therapy and change in serum M2BPGi were independently associated with significant fibrosis regression.

Conclusions Serum M2BPGi can risk-stratify CHB patients whose liver stiffness fell into the 'grey area'. Significant fibrosis regression occurring in one-quarter patients was reflected by a reduction in M2BPGi levels at 10-year interval.

Keywords Cirrhosis · Elastography · Hepatitis B · Liver fibrosis · M2BPGi

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Introduction and aim

Hepatitis B virus (HBV) infects around 257 million people and leads to 1.34 million deaths in year 2015 [1]. Liver-related mortality largely comes from the development of decompensated liver disease including cirrhosis and hepatocellular carcinoma (HCC). Identifying patients who have significant liver fibrosis or cirrhosis is essential for timely treatment with antiviral therapy which may lead to fibrosis or cirrhosis regression, and hence preventing disease progression and decompensation [2]. It is also important for screening of complications as early specific treatment is associated with better survival. Traditionally, liver biopsy is the gold standard for assessing liver fibrosis. However, it is an invasive procedure and there are concerns of sampling error and inter-observer variability [3]. Non-invasive means to assess liver fibrosis are, therefore, advocated, including serum-based and elastography-based tests [4–8]. Many of these tests have been extensively studied in various liver conditions. However, unresolved issues remain including the availability of tests, cost, reproducibility, applicability, validity of the tests in the context of abnormal liver function, and the presence of a "grey area" in transient elastography where severe fibrosis can neither be confidently diagnosed nor excluded [9].

Recently, a novel serum-based marker, Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA^+-M2BP), also known as Mac-2-binding protein glycosylation isomer (M2BPGi), is widely investigated as a potential marker for liver fibrosis in patients with various types of chronic liver diseases including viral hepatitis [10] [11], non-alcoholic fatty liver disease [12], primary biliary cholangitis (PBC) [13], autoimmune hepatitis (AIH) [14] and biliary atresia [15]. For chronic hepatitis B infection (CHB), a recent study showed that M2BPGi is correlated with significant liver fibrosis [16]. Other related studies demonstrate the predictive values of M2BPGi in HBV e antigen (HBeAg) seroconversion (ESC) in treatment-experienced patients [17] and also more importantly, the risk of HCC development in CHB patients [18]. The profile of M2BPGi in CHB patients, therefore, warrants further investigation. Specifically, its performance characteristics with respect to liver stiffness by elastography-based assessment are largely unknown. In addition, changes of this marker in patients with long-term follow-up, as well as the effects of nucleos(t)ide analogue (NA) treatment in CHB patients, should be delineated.

In the present study, we aimed to investigate the role of M2BPGi in the assessment of liver fibrosis in CHB with reference to liver stiffness measured by transient elastography, and how this marker can complement liver stiffness measurement. We also examined the profile of serum M2BPGi across a long-term follow-up period and the NA-related effects on liver fibrosis.

Materials and methods

Patients

We recruited CHB patients who had valid liver stiffness measurement (LSM) between November 2005 and December 2006 in the Liver Clinics of Queen Mary Hospital, Hong Kong. The inclusion criteria for this study were those with available archived serum and their recruitment process was described previously [19]. Among the 331 (38 HBeAg-positive and 293 HBeAg-negative) patients in the original cohort, 71 patients were excluded because there were no available serum samples taken within 90 days from LSM. In addition, 20 patients were excluded with alanine aminotransferase (ALT) elevated above 5 times the upper limit of normal (ULN, defined as 40 U/L) (see below), leaving 240 patients to be included in the current cohort. Of these, a subgroup of patients ($N=86$) had repeat LSM between November 2015 and December 2016 at 10-year interval from baseline. CHB was defined as persistence of hepatitis B surface antigen (HBsAg) for at least 6 months. None of them achieved HBsAg loss or anti-HBs seroconversion. Patients who had excessive alcohol intake (> 30 g per day for male, > 20 g per day for female) or with co-infection with chronic hepatitis C (CHC), chronic hepatitis D, human immunodeficiency virus, co-existing AIH, PBC, or other major medical illnesses were excluded. Patients with ALT > 5 times ULN [2] were also excluded as such high ALT levels would over-estimate liver fibrosis on transient elastography [20]. Other exclusion criteria include prior liver transplantation, active hepatocellular carcinoma and congestive heart failure which could confound LSM.

Clinical data and laboratory tests

Relevant data including patients' age, gender, HBeAg status, aspartate aminotransferase (AST), ALT levels and platelet (PLT) count were recorded. HBV DNA levels were measured by the COBAS TaqMan HBV Test (Roche Diagnostics, Branchburg, NJ, USA) with detection range of $20–1\times10^8$ IU/mL. Due to logistics of arranging LSM and blood taking with respect to clinic schedules, these blood tests were taken within 90 days of LSM (> 90% of patients had blood taken within 4 weeks from LSM). Serum indices for liver fibrosis including AST–platelet-ratio index (APRI), AST–ALT-ratio (AAR) and Fibrosis-4 index (FIB-4) were estimated using formulae as follows:

APRI = [AST(U/L)/ULN × 100]/PLT (× 10^9/L); AAR = AST/ALT; FIB-4 = [age(years) × AST(U/L)]/[PLT(× 10^9/L) × ALT(U/L)]$^{0.5}$.

Transient elastography

LSM was performed by Fibroscan® (Echosens, Paris, France) using M probe, while XL probe was used for patients whose body mass index was ≥ 30 kg/m^2. Liver stiffness was expressed as the median value of ≥ 10 successful acquisitions in units of kilopascals (kPa). LSM was only considered reliable with a success rate of ≥ 60% and interquartile range (IQR) ≤ 30%. LSM was performed by 3 trained operators who obtained the training certificate from Echosens and had prior experience of performing ≥ 500 transient elastography procedures.

No significant fibrosis (F0/F1) was defined as liver stiffness < 6 kPa. Advanced liver fibrosis (F3) was defined as liver stiffness > 9 kPa (normal ALT) or > 12 kPa (ALT 1–5 × ULN). Cirrhosis (F4) was defined as liver stiffness > 12 kPa (normal ALT) or > 13.5 kPa (ALT 1–5 × ULN). The 'grey area' was defined for liver stiffness measurements between 6 and 9 kPa (normal ALT) and 6–12 kPa (ALT 1–5 × ULN). This classification is in accordance with the European Association for Study of Liver, and Asociación Latinoamericana para el Estudio del Hígado clinical practise guidelines [9]. Since there are no universal definitions for significant fibrosis regression, we define here as fibrosis downstage according to liver stiffness (i.e. F3 or F4 at baseline, becoming F0 or F1 at 10-year reassessment). Similarly, we define significant fibrosis progression as fibrosis upstage according to liver stiffness (i.e. F0 or F1 at baseline, becoming F3 or F4 at 10-year reassessment).

Measurement of M2BPGi

Serum M2BPGi was measured in the samples taken within 90 days of LSM, using the HISCL M2BPGi reagent kit (Sysmex, Hyogo, Japan) on an automatic immunoanalyzer HISCL-800 (Sysmex, Hyogo, Japan). Each serum sample of 10 µL was processed with reaction time of 17 min. M2BPGi levels were expressed as cut-off index (COI) and were calculated based on the following equation:

$COI = ([M2BPGi]_{sample} - [M2BPGi]_{NC})/([M2BPGi]_{PC} - [M2BPGi]_{NC})$, where $[M2BPGi]_{sample}$ represents the M2BPGi count of the serum sample, where PC and NC were positive and negative controls, respectively. The positive control was supplied as a calibration solution. The range of measurement is 0.1–20.0 COI.

Data collection and statistical analysis

Continuous variables were expressed as median (interquartile range). Mann–Whitney *U* test and Kruskal–Wallis test were used for comparison of median between 2 groups and multiple groups, respectively. Categorical variables, expressed as proportions, were compared using χ^2 test and Fisher's Exact test when appropriate. Pearson's correlations were performed to evaluate the serum M2BPGi levels with liver stiffness and other serum indices of liver fibrosis. To evaluate the diagnostic performance of M2BPGi in assessing significant liver fibrosis and cirrhosis, receiver-operating characteristic (ROC) curve analysis was carried out. Diagnostic accuracy was expressed as the specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and area under the ROC curve (AUROC). The optimal cutoff value was obtained by maximising the Youden's index (sensitivity + specificity − 1). Multivariate analysis was performed using binary logistic regression to determine factors that were independently associated with advanced fibrosis and cirrhosis. A two-tailed $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL).

Results

Baseline characteristics

The baseline characteristics of 240 enrolled patients are shown in Table 1. The median age was 47.5 years (interquartile range: 40.9–54.2) and 48.3% were males. The majority was HBeAg negative (90.1%) and treatment naive (85.8%) at the time of initial LSM. There were no significant differences between the median serum M2BPGi values in HBeAg-positive ($N = 23$) patients compared with HBeAg-negative ($N = 210$) patients (0.50 vs. 0.44 COI, respectively, $p = 0.319$). For the treatment-experienced group, the median duration of treatment was 23.5 months. The median ALT level was 26 U/L and the median serum HBV DNA was 3.7 logs IU/mL. The median liver stiffness was 6.9 kPa (IQR: 4.9–11.7 kPa). The median M2BPGi level was 0.45 COI (IQR: 0.31–0.71). There was weak correlation between HBV DNA and liver stiffness ($r = 0.163$, $p = 0.036$), but not between HBV DNA and serum M2BPGi ($r = 0.053$, $p = 0.500$).

Correlation of M2BPGi with liver stiffness

The distribution of fibrosis stage according to liver stiffness was: F0–1: 105 (43.8%); grey area: 55 (22.9%); F3: 27 (11.3%) and F4: 53 (22.1%). The corresponding median M2BPGi values for F0/F1/F2, F3 and F4 progressively

Table 1 Baseline characteristics of patients

N = 240	Median value	IQR
Gender	M:F = 116 (48.3%):124 (51.7%)	–
Age at initial LSM	47.5	40.9–54.2
Number of HBeAg-negative	210 (*n* = 233, 90.1%)[a]	–
Treatment naïve (TN): Treatment experienced (TE)	206 (85.8%): 34 (14.2%)	–
Duration of NA treatment (months)	23.5	10.3–73.7
ALT (U/L)	26	20–41
AST (U/L)	28	23–37
Platelet (x 10^9/L)	217	171–256
HBV DNA (log IU/mL)	3.7	2.3–5.7
Liver stiffness by LSM (kPa)	6.9	4.9–11.7
Proportion in F3/F4	77 (32.1%)	–
Serum M2BPGi level (COI)	0.45	0.31–0.70
AAR	1.00	0.81–1.30
APRI	0.42	0.32–0.65
APRI > 2	4 (1.7%)	
FIB4 score	1.08	0.79–1.62
FIB-4 > 3.25	8 (3.3%)	

AAR AST–ALT ratio, *ALT* alanine aminotransferase, *APRI* AST–platelet-ratio index, *AST* aspartate aminotransferase, *COI* cut-off index, *FIB-4* fibrosis-4 index, *IQR* interquartile range, *LSM* liver stiffness measurement, *M2BPGi* Mac-2-binding protein glycosylation isomer, *NA* nucleos(t)ide analogue

[a]Missing HBeAg information in 7 patients

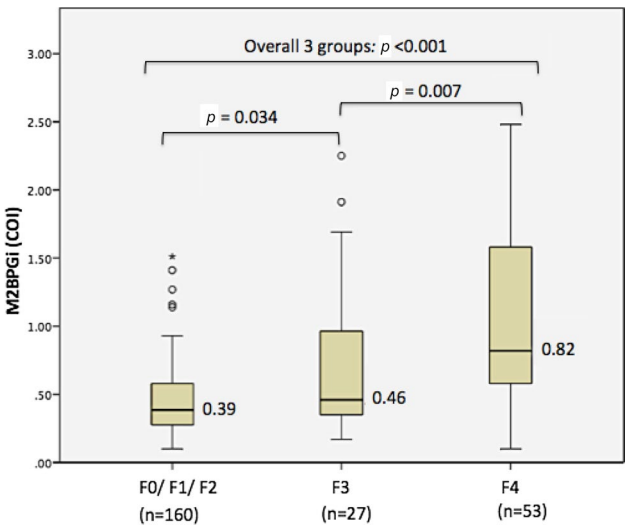

Fig. 1 Serum M2BPGi levels according to fibrosis stage by liver stiffness measurement (*n* = 240)

increased in parallel with more advanced stages of liver fibrosis: 0.39, 0.46 and 0.82 COI, respectively ($p < 0.01$ between 3 groups) (Fig. 1). The correlation of M2BPGi with liver stiffness and other serum-based liver fibrosis indices is shown in Supplementary Table 1. The M2BPGi levels correlated well with liver stiffness (Pearson's correlation coefficient $r = 0.611$, $p < 0.001$), FIB-4 ($r = 0.616$, $p < 0.001$), and strongly correlated with APRI ($r = 0.825$, $p < 0.001$) while AAR did not correlate well ($p = 0.359$).

Diagnostic accuracy of M2BPGi for advanced fibrosis and cirrhosis as classified by transient elastography

Diagnostic accuracies of M2BPGi for advanced liver fibrosis (≥ F3) and cirrhosis (F4) as classified by transient elastography were assessed by constructing AUROC curves. For diagnosing ≥ F3, the AUROC was 0.754 (95% CI 0.686–0.822, $p < 0.001$). By maximising the Youden's index, the optimal cut-off value of serum M2BPGi for diagnosing ≥ F3 was 0.605 COI, with sensitivity, specificity, PPV and NPV of 62.5, 79.4, 60.3 and 80.9%, respectively (supplementary Fig. 1). For diagnosing F4, the AUROC was 0.799 (95% CI 0.729–0.870, $p < 0.001$). By maximising the Youden's index, the optimal cut-off value of serum M2BPGi for diagnosing F4 was 0.615 COI, with sensitivity, specificity, PPV and NPV of 73.6, 77.0, 47 and 91.2%, respectively (supplementary Fig. 2).

Using LSM alone, 55 patients fell into the category of 'grey area' of liver stiffness. The median serum M2BPGi level in this group of patients was 0.37 COI. Among them, 13 (23.6%) had a high serum M2BPGi (≥ 0.615 COI), implying that they are at risk of cirrhosis. Two of them had FIB-4 > 3.25. However, none of them had APRI > 2.

Longitudinal change of M2BPGi and liver stiffness across 10 years

Repeat LSM and serum M2BPGi quantification were performed in 86 CHB patients after 10 years of follow-up. The individual serum M2BPGi levels at both time points are shown in supplementary Fig. 3. Among them, 62 (72.1%) were treatment experienced (44 taking tenofovir disoproxil fumarate (TDF) or entecavir (ETV)). The median serum M2BPGi levels were significantly different between patients with F3/F4 compared to those without advanced fibrosis both at baseline (0.67 COI vs. 0.41 COI, $p<0.001$) and at 10 years (0.62 COI vs. 0.48 COI, $p=0.039$) (Fig. 2). Among these 86 patients, 14 (16.3%) fell into the 'grey area' group at the second LSM. Four out of 14 (28.5%) had a high serum M2BPGi (≥ 0.615 COI) corresponding to F4 fibrosis though elastography classification was in the 'grey area'.

In these 86 patients, the median change in M2BPGi weakly correlated with change in liver stiffness ($r=0.231$, $p=0.033$). The proportion of patients with $\geq$ F3 reduced from 40.7% to 16.3% ($p<0.001$) (Fig. 3). This phenomenon was observed in both treatment-naïve and treatment-experienced patients (supplementary Fig. 4). NA treatment, taken as a whole, was not associated with significant change in serum M2BPGi level (− 0.01 COI vs. +0.08 COI, $p=0.402$) nor liver stiffness (− 2.8 kPa vs. − 1.7 kPa, $p=0.693$) when compared to untreated patients. When comparing the subgroup of 44 patients on TDF/ETV with 18 patients taking other NAs (lamivudine, adefovir or telbivudine), liver stiffness reduction was significantly greater in the former group (− 3.4 kPa vs. − 1.0 kPa, $p=0.013$). The change in serum M2BPGi was not significantly different between the 2 groups (+0.015 COI vs. − 0.09 COI, $p=0.368$).

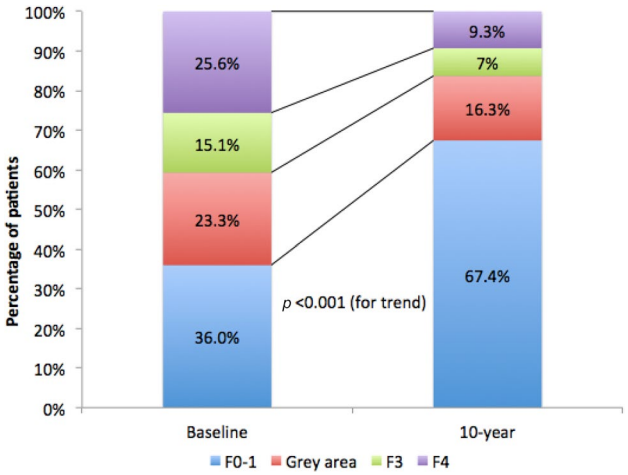

Fig. 3 Distribution of fibrosis stage according to liver stiffness across 10 years ($n=86$)

Factors associated with significant fibrosis regression

Among the 86 patients with 10-year follow-up data, 21 (24.4%) showed significant fibrosis regression as defined by fibrosis downstage according to liver stiffness (i.e. F3 or F4 at baseline, becoming F0 or F1 at 10-year reassessment). In contrast, none of the patients showed significant fibrosis progression according to liver stiffness (i.e. F0 or F1 at baseline, becoming F3 or F4 at 10-year reassessment). The median change in serum M2BPGi level was − 0.11 COI in those with significant fibrosis regression compared to +0.03 COI in the other patients who did not show significant fibrosis regression ($p=0.011$) (Fig. 4). Apart from

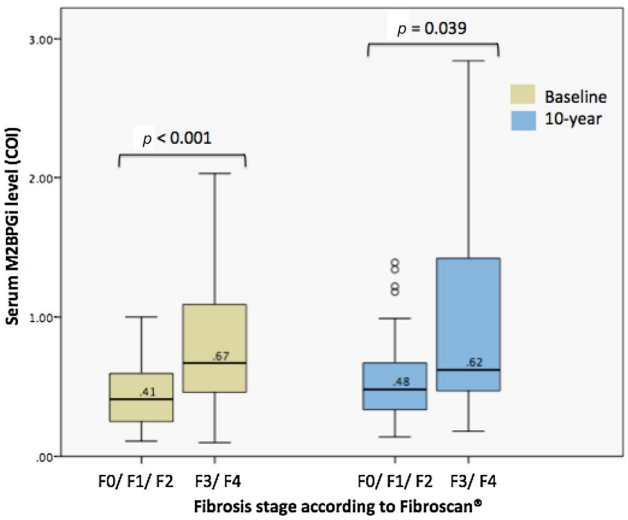

Fig. 2 Serum M2BPGi levels according to fibrosis stage by liver stiffness measurement across 10 years ($n=86$)

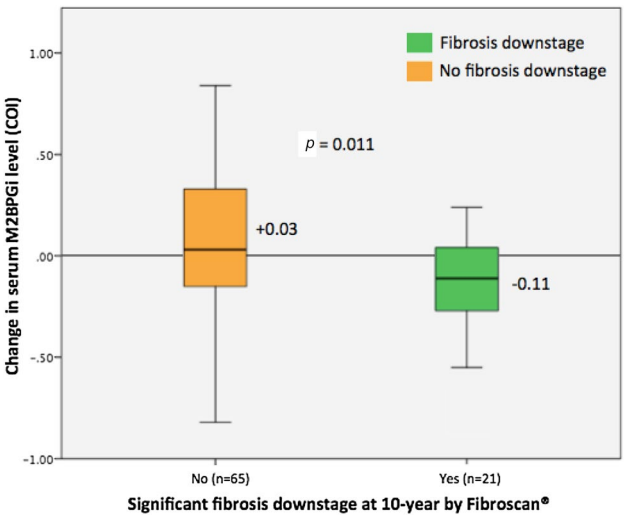

Fig. 4 Change in serum M2BPGi level in patients with or without fibrosis regression

change in serum M2BPGi, older age and male gender were also associated with significant fibrosis regression (Supplementary Table 2). Use of NA *as a whole* was not associated with significant fibrosis regression ($p=0.303$). However, when *specific types of NA* were used (TDF or ETV), it was associated with significant fibrosis regression (OR 9.714, 95% CI 1.180–79.986, $p=0.035$) compared to other NAs. Multivariate logistic regression showed that change in serum M2BPGi (OR 0.189, 95% CI 0.038–0.943, $p=0.042$) and use of TDF/ETV (OR 29.65, 95% CI 1.55–565.5, $p=0.024$) were independently associated with significant fibrosis regression (Table 2).

Discussion

Early diagnosis of advanced liver fibrosis in CHB is crucial for patient management. First, patients with advanced fibrosis carry a higher risk of development of cirrhosis-related complications and HCC, requiring more intensive screening and monitoring strategy. Second, these patients need timely antiviral treatment to reduce the long-term risk of liver-related complications. The use of liver biopsies to assess liver fibrosis has been decreasing due to its inherent procedure-related risks, sampling bias and inter-observer variability. Also, histological fibrosis stage is in fact a categorical variable, where every stage of histological fibrosis contains a wide range of spectrum in terms of fibrous load. Recent advances in non-invasive modalities of liver fibrosis assessment aim to find an ideal test which is convenient, accessible, accurate, reproducible and safe for identification of patients with significant liver disease. Transient elastography or liver stiffness measurement is the most widely investigated and extensively validated among the many, and is now a recognised surrogate assessment tool for liver fibrosis. In this study, we find that serum M2BPGi level, a novel marker of liver fibrosis in CHB, correlated well with liver stiffness ($r=0.611$, $p<0.001$). This finding is consistent with the findings from Zou et al. which reported good correlation of serum M2BPGi with liver stiffness ($r=0.614$, $p<0.0001$) and histological fibrosis stage ($r=0.451$, $p<0.001$) [16].

Table 2 Multivariate analysis of factors associated with significant fibrosis regression at 10-years

	Odds ratio	95% CI	*p* value
Age	1.026	0.961–1.095	0.443
Gender (male)	2.443	0.595–10.027	0.215
Use of TDF/ETV	*29.65*	*1.555–565.5*	*0.024*
Change in M2BPGi	*0.189*	*0.038–0.943*	*0.042*

CI confidence interval, *ETV* entecavir, *M2BPGi* Mac-2-binding protein glycosylation isomer, *TDF* tenofovir disoproxil fumarate

It demonstrated good diagnostic accuracies for advanced fibrosis (AUROC: 0.754) and cirrhosis (AUROC: 0.799) using cut-off levels of 0.605 COI and 0.615 COI, respectively, with corresponding NPVs being 80.9% and 91.2%, respectively. This marker is, thus, particularly helpful for excluding those at high risk of advanced fibrosis and cirrhosis in CHB patients whose LSM fell into the 'grey area', so that additional non-invasive tests or liver biopsy might only be considered for 'grey area' patients with high serum M2BPGi.

Apart from LSM, serum M2BPGi demonstrated strong linear correlation with APRI ($r=0.825$, $p<0.001$) and good linear correlation with FIB4 ($r=0.616$, $p<0.001$). There are several major disadvantages of using LSM to assess liver fibrosis. First, patients with ALT > 5 times ULN should be excluded from LSM [9]. Second, unreliable results were reported in up to 11.6–15.8%, mostly due to patient obesity [21, 22]. Third, technical failure of LSM is inevitable in certain patients, and the reported failure rate was 2.7–3.1% [21, 22]. In contrast, there is virtually no technical failure in measurement of serum M2BPGi. Other advantages of serum M2BPGi measurement include its convenience without the need of fasting, and that it is an independent one-marker test. More importantly, serum M2BPGi identified 23.6% and 28.5% of patients at risk of cirrhosis whose fibrosis stage was otherwise classified as 'grey area' according to LSM at baseline and 10-year interval, respectively. This finding is clinically important because one-quarter of patients in the 'grey area' group may be at a higher risk of advanced fibrosis and cirrhosis, while the remaining three-quarters with low serum M2BPGi were at low risk. It is suggested that liver biopsy should be performed in all patients in the 'grey area' group if results influence management, according to the European Association for the Study of the Liver/AsociaciÓn Latinoamericana para el Estudio del HÍgado (EASL-ALEH) guidelines [9]. From the findings of our study, serum M2BPGi can potentially risk-stratify 'grey area' patients in identifying those who will benefit from a liver biopsy to exclude advanced fibrosis or cirrhosis, to allow a more aggressive treatment and monitoring approach instead of watch-and-wait. We propose an algorithm for complementary use of combination tests for non-invasive assessment of liver fibrosis as shown in supplementary Fig. 5. When 'grey area' is encountered after liver stiffness measurement, serum M2BPGi level can provide additional diagnostic information. A low serum M2BPGi < 0.605 COI in a patient who belongs to the 'grey area' confidently excludes the diagnosis of advanced fibrosis or cirrhosis. In contrast, if patients belonging to 'grey area' have serum M2BPGi levels higher than the cut-off level of 0.605 COI, it remains inconclusive whether the patient has advanced liver fibrosis or cirrhosis, and thus, further testing with other assessment tools for liver fibrosis including liver biopsy should be considered.

The derived cut-off level of 0.615 COI to exclude cirrhosis is lower than that reported in Mak et al. which compared serum M2BPGi with liver biopsy and the cut-off level for cirrhosis in that paper was found to be 0.96 COI [23]. This may be related to the inclusion of more patients requiring treatment in that study (285 baseline-treated samples out of 554, i.e. 51.4%) compared to the current study (14.2%). Patients requiring antiviral therapy at inclusion indicated a state of more active and advanced liver disease with more hepatic necroinflammation as reflected by higher ALT in patients in Mak et al. (median ALT: 74 U/L) compared to patients in the current study (median ALT: 26 U/L). From unpublished data, there was weak correlation between serum M2BPGi and ALT ($r=0.131$, $p=0.002$). Thus, ALT likely confounds the serum M2BPGi levels as in transient elastography, and the derived cut-off for advanced fibrosis or cirrhosis would be different in patients with normal ALT compared to those with elevated ALT. Ideally, cut-off levels for advanced fibrosis and cirrhosis should be separately evaluated for treatment-naive and treatment-experienced patients, and should be studied using a large sample size.

Longitudinal assessment of liver fibrosis by transient elastography has been increasingly utilized and reported in both treated and untreated patients [19, 24–26], although the best cut-off values for advanced liver disease, or thresholds to define fibrosis progression/regression, are yet to be defined. This study is the first to demonstrate the paired assessment of serum M2BPGi level and liver stiffness across long-term follow-up of 10 years in CHB patients. Serum M2BPGi can accurately differentiate F3/F4 from F0/F1/F2 even with a 10-year interval. Although some other studies showed that long-term NAs is associated with fibrosis regression [27, 28], there is still a significant proportion of patients suffering the opposite with ongoing liver fibrosis and decompensation. From this study, fibrosis regression happened in 24.4% of patients and may be reflected by a change in serum M2BPGi. A reduction in serum M2BPGi level was independently associated with significant fibrosis regression after 10 years (OR 0.189, 95% CI 0.038–0.943, $p=0.042$). Reduction in serum M2BPGi associated with histological fibrosis regression upon long-term NA has been described in patients with paired liver biopsies [23]. While use of NAs *as a whole* was not a significant factor for fibrosis regression, analysing the subgroup of patients using more potent NAs (TDF or ETV) showed significant fibrosis regression (OR 29.65, 95% CI 1.55–565.5, $p=0.024$) compared to non-TDF/ETV NAs. Liver stiffness reduction was also significantly greater for those taking potent NAs compared to taking non-potent NAs (− 3.4 kPa vs. − 1.0 kPa, $p=0.013$). Compared to other NAs (lamivudine, telbivudine and adefovir), TDF and ETV achieve higher rate of ALT normalisation and serum HBV DNA undetectability at 1 year [2]. Although there is no direct comparison of their efficacies in fibrosis regression following long-term treatment by different NAs, the efficacy in fibrosis regression is expected to be superior for more potent NAs when hepatocyte necroinflammation is better controlled, leading to faster quiescence of fibrogenesis process led by activated hepatic stellate cells and allow time for fibrinolysis [29]. On the other hand, the change in serum M2BPGi was not significantly different between patients taking potent NAs compared to those taking non-potent NAs (+0.015 COI vs. − 0.09 COI, $p=0.368$). Since transient elastography is known to be significantly confounded by ALT, the improvement in liver stiffness after potent NA may partially represent normalisation in ALT rather than solely fibrosis regression. In comparison, the effect of ALT on serum M2BPGi is less well known, although it remains possible that ALT might modestly affect serum M2BPGi levels given the very weak correlation between the two variables as mentioned above. Moreover, the insignificant change in M2BPGi after long-term NA, regardless of potent NAs or not, was likely attributed by a small number of patients in the current study. Larger-scale studies are, therefore, needed to demonstrate the effect of long-term NA on serum M2BPGi levels. Male gender and older age are known risk factors for cirrhosis and HCC. The baseline liver stiffness for male and female patients was 10.2 and 6.1 kPa, respectively ($p<0.001$, data not shown). Similarly, the baseline liver stiffness for patients <50 and ≥ 50 was 6.7 and 11.5 kPa, respectively ($p=0.001$, data not shown). Therefore, male patients and older patients had higher disease load to begin with, allowing a bigger room for improvement following antiviral therapy, and are, therefore, associated with significant fibrosis regression (OR = 1.026 and 2.443, respectively). The insignificant change in M2BPGi in the TDF/ETV-treated group compared to other NAs could be related to the small number of patients, and thus should be further assessed with larger population in longitudinal studies. Also, the minimum decline in serum M2BPGi which can be regarded as significant fibrosis regression will need further studies to delineate.

There were three limitations in this study. First, no liver biopsies were performed to validate the fibrosis stage estimated by LSM and serum M2BPGi. This would be of particular interest in the 'grey area' group of patients and should be further addressed in future studies. Second, the specificity of serum M2BPGi remains an issue, as its levels might also be influenced by acute liver injury [30] and other medical illnesses, e.g. lung fibrosis [31], heart failure [32], acute coronary syndrome [33], and pancreatic ductal adenocarcinoma [34]. We had excluded patients with significant liver necroinflammation by ALT and other major medical illnesses in this study to minimise this limitation. Third, while significant fibrosis (F2) is also an important treatment indication, it is not evaluated in this study due to the fact that liver fibrosis assessment was referenced to

transient elastography which does not give a diagnosis of F2 but rather, a grey zone for those who fall into neither the F3/F4 range nor the F0/F1 range. The role of serum M2BPGi in this setting, or other non-invasive tests, would, therefore, be to further stratify whether these patients would be at high risk of F3/F4 or not. For the use of M2BPGi in diagnosing F2, Zou et al. reported good performance characteristics of this marker with reference to liver biopsy in CHB patients (AUROC 0.753) [16]. More data in the role of M2BPGi in diagnosis of F2 are also warranted.

In summary, serum M2BPGi correlated well with liver stiffness and was an accurate marker to diagnose ≥ F3 and F4 as classified by liver stiffness in patients with CHB. Significant fibrosis regression occurred in one-quarter patients and this was reflected by a reduction in serum M2BPGi levels across a 10-year interval of follow-up. One-quarter of patients belonging to the 'grey area' group by LSM had high serum M2BPGi levels, which should prompt further studies to investigate the clinical implication of this finding. Serum M2BPGi can potentially play a complementary role with LSM in non-invasive assessment of liver fibrosis.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest to disclose for the authors Lung-Yi Mak, Danny Ka-Ho Wong, Wai-Kay Seto, Qin Ning, Ka-Shing Cheung, James Fung, Ching-Lung Lai, and Man-Fung Yuen.

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