Dear Professor Ruo-Yu Ma,

Re: 45066, entitled ‘Serum Mac-2 binding protein glycosylation isomer level predicts hepatocellular carcinoma development in E-negative chronic hepatitis B patients’

Thank you for your email concerning the further revision of our manuscript. It is now revised according to the editors’ and reviewers’ comments. The followings are the point-by-point responses to the comments from the reviewers. Our manuscript was prepared and language edited by one of the authors who is a native English speaker.

We have uploaded the powerpoint file containing all figures of the manuscript. Also, the audio core tip file has been modified to mp3 format and uploaded as well.

We hope that the editors and reviewers will find this revised version acceptable for publication in World Journal of Gastroenterology. Thank you again for reviewing our work.

Yours sincerely,
Man-Fung Yuen

Reviewer 1:

The authors investigated the usefulness of Mac-2 Binding Protein Glycosylation Isomer for prediction of development of hepatocellular carcinoma (HCC) in E-negative chronic hepatitis B patients. They stated that HBeAg seroconversion and baseline M2BPGi were significant factors predictive of HCC. They concluded that serum M2BPGi accurately predicted HCC development in treatment-naive CHB patients during long-term follow-up after HBe seroconversion. This study seems to be interesting. However, similar studies have been carried out by Heo (2016), Kim (2017) and Cheung (2017) that cover the same ground. Therefore, I am somewhat concerned about the novelty value of the data presented here.
1) The authors show the indication of the antiviral treatment in CHB patients

Our response: Thank you for your kind comment. In the results section, we stated that “Subsequent antiviral therapy was initiated in 102 (49.3%) patients after a median duration of 5.5 years due to hepatitic flare or diagnosis of liver-related complications including cirrhosis

and HCC." For hepatitis flare, it is defined as ALT > 2 times the upper limit of normal with HBV DNA > 2000 IU/mL according to the EASL guidelines. We also described the indication of treatment, with majority of them being hepatitis flare (83). We have now added this definition in the Methods section, and described the indication of antiviral therapy in supplementary table 1.

2) The authors show the therapy that patients received

Our response: Thank you for your kind comment. The antiviral therapy consisted mainly of nucleos(t)ide analogues, and majority were lamivudine (n=19) or entecavir (n=76). We have now added the details of subsequent antiviral therapy after recruitment in the supplementary table 1.

3) The should explain the reason why cirrhosis is not significant factor for HCC development, because more than 90% of cirrhotic patients developed HCC in this study

Our response: Thank you for your kind comment. We agree that cirrhosis is a well-known risk factor for HCC development. However, we are worried that the numbers in Table 2 may be misinterpreted. The meaning of that row in Table 2 is: Out of the 14 HCC patients, 13 (92.8%) of them had cirrhosis, whereas out of the 193 non-HCC patients, 22 (11.4%) had cirrhosis. Only 13 +22 (i.e. 35 patients, 16.9%) had cirrhosis, as we have stated in the result section (under "Markers of liver fibrosis and cirrhosis"). Therefore, the proportion of patients with cirrhosis in the whole study population is not high.

4) The authors should clearly state the novelty of the current study

Our response: Thank you for your kind comment. We agree with the reviewer that some studies on M2BPGi had been published, which we had already cited in our manuscript (reference number 20 for Kim et al and 21 for Cheung et al). We also cited Heo et al in reference number 23. For all 3 papers mentioned, the patient population was different and the follow-up duration was not long enough. In Kim et al, the incidence of HCC was low - only 3.9% upon a median follow up of about 5 years. Also, the timing of antiviral therapy, which was initiated in about one-quarter patients, was not clear. In Cheung et al, the patients were all treatment-experienced and it was a retrospective case-control study. In Heo et al, the patient population was quite heterogeneous: about half of the patients were HBeAg-positive, and they had a relatively high viral load with two-thirds of them had baseline HBV DNA > 5 logs. Also,

the follow-up duration was relatively short - less than 5 years. The novelty of our study is the predictive value of serum M2BPGi in a homogeneous patient population, i.e. all patients were HBeAg-negative, and were treatment-naive at recruitment. Also, these patients were prospectively followed-up with complete clinical data. Another strength of our study is the long follow-up duration for our patients - median 13.1 years. While we acknowledge and appraise the published studies on this area, we believe the findings of our study are novel in the field.

5) It would be of great interest to analyze relationship between M2BPGi levels and lens culinaris-agglutinin-reactive fraction of AFP (AFP-L3) levels.

Our response: Thank you for your kind comment. We agree that AFP-L3 is an alternative tumour marker for HCC. This marker is however not yet widely available, and is also not available in our laboratory. In our manuscript, we analyzed baseline AFP, which did not show statistical difference between patients with or without subsequent HCC development.

Reviewer 2:

The authors include a specific group of patients with spontaneous e seroconversion. I think baseline M2BPGi is the most important. In table 3, comparing the level of M2BPGi is meaningless because you treated the most HCC patients with antiviral agent. It may have same implication in Fig 2 perhaps you also had treatment for those with cirrhosis when you found they had activity of hepatitis, of which elevated M2BPGi is expected.

Our response: Thank you for your comment. We believe the reviewer is referring to Table 2 (as we only have 2 tables in the original manuscript), which listed the median serum M2BPGi values in HCC and no HCC patients at baseline, 5-year and 10-year. We agree with the reviewer that antiviral treatment may confound the M2BPGi level, as reported in Mak et al (reference number 18) that long term antiviral treatment led to decline in serum M2BPGi level and also reduction in histological fibrosis. We have now performed additional analysis and reported in the results section under 'Markers of liver fibrosis and cirrhosis': " Since almost half patients were eventually started on antiviral therapy, it would be impractical to exclude these patients from subsequent analysis. Previous report stated that antiviral therapy would lead to decline in serum M2BPGi level and reduction in histological fibrosis (reference no. 18). To address this issue, we performed additional analysis to compare the serum M2BPGi levels at 5-year and 10-year between those who were subsequently initiated with antiviral therapy. The

median serum M2BPGi levels in patients needing treatment compared to patients not needing treatment were 0.50 (IQR 0.37 - 0.71) COI vs. 0.50 (IQR 0.28 - 0.64) COI and 0.66 (IQR 0.43 - 0.95) COI vs. 0.52 (0.34 - 0.62) at 5-year (p=0.167) and 10-year (p<0.001), respectively." and we elaborated this in the discussion section on this issue: " Antiviral treatment cannot be withheld if treatment indications were reached, including not only hepatitic flare but also development of cirrhosis or HCC. Therefore, 49.3% of patients in this study eventually received treatment at a median duration of 5.5 years. We showed that subsequent antiviral therapy did not significantly change the serum M2BPGi levels at 5-year, probably related to the short duration of treatment. Paradoxically, the median M2BPGi level was significantly higher at 10-year in those needing antiviral treatment compared to those who did not (0.66 vs. 0.52, p<0.001). This reflects the intrinsically more advanced liver disease in those needing antiviral treatment. As the timing of antiviral therapy initiation was heterogenous (IQR 2.7 - 8.6 years after recruitment), it is difficult to assess the effect of antiviral therapy on fibrosis regression, taking into consideration the differences in the severity of liver disease accumulated at the timing of therapy. Therefore, the differences in serum M2BPGi levels between HCC and non HCC group at 5-year and 10-year could not be explained by antiviral therapy alone."

Moreover, the M2BPGi level in patients without cirrhosis (Figure 2) or without HCC (Figure 3) did not show significant decline across the 3 time points despite the initiation of antiviral therapy in some of these patients. Therefore, we would like to keep the original presentation of the median serum M2BPGi levels at all 3 time points.