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Reviewer A Comments

First, the purpose of this study was proposed to determine the cut-off values of M2BPGi, however, the appropriate clinical research design should be the diagnostic ability of M2BPGi for hepatic function reserve and nutritional status. In other words, the authors should assess the diagnostic ability of M2BPGi and one step of such works is to determine the cut-off values but its prerequisite is the good diagnostic performance of this new biomarkers. I suggest the authors to revise the title to indicate the clinical research design.
Second, the abstract is not adequate. In the background, the authors need to explain why M2BPGi is potentially useful to replace the conventional biomarkers from a theoretical perspective and indicate the clinical significance of this research topic. The objectives of this study should be describe, not only the determination of cut-off values. In the methods, please describe the inclusion of subjects, the measurements of albumin, bilirubin, AST, PLT, ALT, and M2BPGi. Please briefly describe the ROC analysis. In the results, the diagnostic performance parameters such as AUC, sensitivity, and specificity of M2BPGi should be reported. In the conclusion, the authors should have detailed comments on the clinical implications.
Third, in the introduction of the main text, the authors should explain why M2BPGi is potentially useful to replace these traditional biomarkers and please have comments on the limitations of traditional biomarkers and the strengths of M2BPGi. The clinical significance of this research topic needs to be detailed in this part.
Fourth, the methodology of the main text should first indicate the clinical research design of this study. Please describe the inclusion criteria of subjects and experimental details to measure these biomarkers including M2BPGi. Please clearly indicate the golden diagnosis of hepatic function reserve and nutritional status. In statistics, please describe the threshold values of AUC and other parameters for a good diagnostic test. The authors must explain, if the AUC value is lower than 0.8, does the research work to determine cut-off values, deserve to be done? Please ensure $P<0.05$ is two-sided.

Reply to Reviewer A 's Comments

Thank you for your useful comment.
Firstly, we revised title. Title is "Clinical efficacy of Mac-2-binding protein glycosylation

isomer as a biomarker for albumin–bilirubin grade and the Controlling Nutritional Status score in chronic liver disease: Investigation of cut-off values by the type of chronic liver disease “. Moreover, we indicate the clinical research design as flowchart in Fig.1.
Second, we revised abstract clearly.
Third, we explain why M2BPGi is potentially useful to replace these traditional biomarkers. We have comments on the limitations of traditional biomarkers and the strengths of M2BPGi.
The ALBI score is acknowledged as the gold standard for the assessment of liver function in patients with hepatocellular carcinoma (HCC). Unlike the Child-Pugh score, the ALBI score uses only objective parameters, albumin (Alb) and total bilirubin (T.Bil), enabling a better evaluation. However, the limit of ALBI score is log calculation, so it is a little complicated in clinical use; the limit of CONUT score is leukocyte fraction, so it is affected by infectious diseases.
Liver function largely reflects the prognosis of liver disease. The Child-Turcotte classification published in 1964 as a prognostic criterion for liver cirrhosis was modified by Pugh et al. in 1973 and introduced as the Child-Pugh score, comprising 5 prognostic factors (prothrombin, albumin [Alb], total bilirubin [T.Bil], ascites, and encephalopathy).
Although M2BPGi is a marker of liver fibrosis, it is considered to have a function other than fibrosis because it is increased even in patients at high risk of liver carcinogenesis and inflammatory diseases. This time, we will examine the relationship with ALBI grade and CONUT score, and examine clinical application in hepatic reserve and nutritional status evaluation of patients with chronic liver disease.
Fourth, we indicate flow chart diagram as the research design in Fig.1.
AUC value of 0.8 or higher is a general criterion, but it is meaningful that the AUC value is lower than 0.8.
P <0.05 is a two-sided test because no special settings have been made.
We describe that M2BPGi is useful as a single biomarker for hepatic reserve index and nutritional index in clinical practice.

Reviewer B Comments

1. Keywords: if the authors would like to emphasize “cut-off values”, it should be included in the keywords.

Reply to Reviewer B Comment 1.

We include“cut-off values” in the keywords.

Reviewer B Comments

2. Page 5, line 105: “Thus, in this study, we aimed to evaluate the use of M2BPGi as a surrogate marker in patients with CLD.” This sentence for the study purpose is quite unclear. Surrogate marker for what? Please describe the study purpose clearly.

Reply to Reviewer B Comment 2.

Thank you for your useful comment.

Although M2BP was expected as hepatic fibrosis, its involvement in hepatic carcinogenesis was also reported in clinical practice, and this study aims to clarify that M2BPGi can be clinically used as a hepatic reserve marker and nutritional index without pushing the search for alternative markers to the forefront in clinical practice as an important biomarker.

Reviewer B Comments

3. Page 5, line 112: “…. M2BP level was measured at their first visit to Saiseikai Niigata Hospital for CLD between January 2012 and April 2020.” It is unclear whether this study is a retrospective analysis of already collected, routine clinical and laboratory data or intentionally measured M2BPGi level for this study. In Introduction Section, the authors described that “In 2015, Mac-2-binding protein glycosylation isomer (M2BPGi), a carbohydrate antigen marker associated with fibrosis, was identified as a novel marker for liver fibrosis.”. However, this study enrolled the patients from Jan 2012 with M2BPGi level that was measured at their first visit. Unless the authors archived serum samples for the later M2BPGi level measurement, this time frame cannot be understood. Moreover, there is no description on the M2BPGi level measurement assays.

Reply to Reviewer B Comment 3.

Thank you for your important point. We mistake enrolled the study period.

We revised that this study enrolled the patients from January 2015 with M2BPGi level that was measured at their first visit after M2BPGi measurement covered by insurance. We added flowchart in Methods as Fig.1.

Reviewer B Comments

4. In Methods Section, there is no description on the mALBI grade.

Reply to Reviewer B Comment 4.

The description about mALBI grade has been added in the text line 133-135.

Reviewer B Comments

5. The first paragraph of Results Section corresponds to the basic characteristics of the study population, which is not the "results" but the "methods". This part should be moved to the Methods Section.

Reply to Reviewer B Comment 5.

Thank you for your comment. We move to the Methods Section according to your comment.

Reviewer B Comments

6. The Results Section spans more than 4 pages and mostly duplicates the contents of Table 2 and Table 3. Such kinds of duplicated presentation should be avoided, and only key points should be described in the main text.

Reply to Reviewer B Comment 6.

Thank you for your comment, we describe only key points clearly.

Reviewer B Comments

7. Page 7, line 164: "The pairwise Wilcoxon test revealed significant differences among some groups." Regarding this sentence, I cannot find any supporting data (or presentation in figures). In Methods Section (page 6, line 142), it is described that "Pairwise comparisons were made using Wilcoxon's rank-sum test for between-group comparisons." Which groups were compared and what data were obtained?

Reply to Reviewer B Comment 7.

We revised to present in Fig 2, 3 and 4.

Reviewer B Comments

8. Page 7, line 167 – page 8, line 173: Regarding "correlations", there is no description in the "statistical analysis" section. Instead of "positive correlation", the authors have to provide more objective interpretation criteria on the r values in the "statistical analysis" section and interpret the correlation in the "Results" Section.

Reply to Reviewer B Comment 8.

We describe r values and correlation in Methods Section clearly.

Reviewer B Comments

9. Page 8, line 169: Please check the typo error (A2BPGi).

Reply to Reviewer B Comment 9.

Thank you for your comment. We corrected checked the typo error in the text.

Reviewer B Comments

10. Page 8, line 169 – 173: Regarding “FIB-4 index”, investigating its correlation with CONUT score or ALBI grade is not included in the study purpose. If the authors would like to include this portion in the Results Section, the study purpose should be changed.

Reply to Reviewer B Comment 10.

M2BPGi has a liver fibrosis marker as an origin, but it has been reported that it also correlates well with the FIB-4 index, which is also an internationally standard index for liver fibrosis. In addition, M2BPGi has penetrated in clinical practice as a marker for predicting liver carcinogenesis, and its usefulness as a marker for hepatic reserve has been reported (function of ＋α). This time, we added FIB-4 to explain the base of M2BPGi, and we didn't describe it meaninglessly (it may not be well communicated to the reviewers). In addition, the FIB-4 index is a scoring system like ALBI and CONUT, and it is undeniable that the calculation is troublesome.

M2BPGi is a single marker I think that the strength may be described somewhere in the text.

Reviewer B Comments

11. Page 12, line 268 – 271: “Various studies have evaluated … Although M2BPGi has the potential for use … prognosis in clinical practice, clinical data are insufficient.” References are needed for these sentences.

Reply to Reviewer B Comment 11.

We added references.

Reviewer B Comments

12. Page 12, line 276: “M2BPGi, …. the changes in N-glycan during fibrosis can be identified using lectin WFA.” These two sentences should be described in Introduction Section.

Reply to Reviewer B Comment 12.

Thank you for your comments. We describe in Introduction section as your comments.

Reviewer B Comments

13. Page 12, line 281: “This can be evaluated using Sysmex's automated immunoassay system (HISCL), … on the date of blood sampling.” This sentence should be described in the Methods Section.

Reply to Reviewer B Comment 13.

We described in the Methods Section.

Reviewer B Comments

14. Page 13, line 290: “To date, only a few studies have investigated the relationship between M2BPGi and ALBI grade or CONUT score in liver disease.” References are needed for this sentence.

Reply to Reviewer B Comment 14.

We added references.

Reviewer B Comments

15. Page 13, line 294 – 303: Do the authors mean that M2BPGi level can replace the CONUT score? What is the clear application of the M2BPGi level in the clinical practice? Please clarify it.

Reply to Reviewer B Comment 15.

The limit of the CONUT score includes lymphocytes and may be affected in the case of coinfection. As a result of our examination this time, it was shown that it will be replaced by the M2BPGi single biomarker, so I think it may be explained that it is planned.

Reviewer B Comments

16. Page 14, line 313: Please check the typo error (M2BP). It is not confined to this sentence. Throughout the manuscript, there are many typo errors.

Reply to Reviewer B Comment 16.

We corrected many typo errors in the text.

Reviewer B Comments

17. Table 2 and Table 3: What do the authors mean by “isolation performance” in the Table headings? In the statistical analysis section, the authors said the ROC curve analysis was performed to evaluate the diagnostic performance of M2BPGi in assessing hepatic function reserve and nutritional status (page 6, line 143). To explore “diagnostic performance”, diagnostic gold standard should be designated. Can CONUT score or ALBI grade (or mALBI grade) be considered diagnostic gold standard for assessing hepatic function reserve and nutritional status? If so, this portion should be described clearly in Methods section.

Reply to Reviewer B Comment 17.

We revised Table headings.

The major limitation of the Child-Pugh scoring system is that it includes several

subjective parameters (hepatic encephalopathy and ascites) and interrelated parameters (ascites and serum albumin). Ascites can be easily influenced by diuretic use or dehydration state. Diagnosing minimal or covert hepatic encephalopathy involves difficulties. Hence, the ALBI score uses only objective parameters, albumin (Alb) and total bilirubin (T.Bil), enabling a better evaluation. Hence, due to the accessibility of obtaining these laboratory parameters, the CONUT score is convenient and easy to use for determining the nutritional status of CLD patient. In this scoring system, the level of malnutrition is classified into four levels: normal, mild, moderate, and severe; the higher the score, the more severe the malnutrition is. Especially, the value of M2BPGi can be determined moderately by CONUT, which is useful for early clinical intervention of nutritional treatment in this study.

Reviewer B Comments

18. Throughout the text, there are lots of typo errors and random use of terminology. The manuscript writing should be trimmed a lot (for example, M2BPGi vs. M2BP, level vs. value, grade vs. score, etc.).

Reply to Reviewer B Comment 18.

We rechecked many typo errors. Moreover, we stratified (for example, M2BPGi vs. M2BP, level vs. value, grade vs. score, etc.).