26th November 2014

Professor Ya-Juan Ma
Science Editor, Editorial Office
Baishideng Publishing Group Inc

Dear Professor Ma,

On behalf of my colleagues, I would like to resubmit the revised manuscript entitled **"*Factors predicting aggressiveness of non-hypervascular hepatic nodules detected on the hepatobiliary phase of gadolinium ethoxybenzyl diethylene-triamine-pentaacetic-acid enhanced MRI studies in cirrhotic patients*"** (MS#13915) for your consideration of the publication in *World J Gastroenterology*. I would like to thank you and the reviewers for a thorough review of our work and the thoughtful comments and suggestions. The revision was made in accordance with the reviewer's comments and suggestions, especially the statistical methods for formulation and validation were replaced with a generalized estimating equation model and bootstrap resampling, respectively, and changes are highlighted in red in the revised text. A point-by-point response to the comments and suggestions is provided below. It would bring great pleasure to us to have the revised version accepted for publication in your prestigious journal.

Sincerely,

Takeshi Suda
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Reply to the Reviewer

Reviewer a:

Q1. Basically, is this really a randomized controlled study? There is no description about calculation of sample size. Moreover, the authors stated in the paragraph of Patients and nodules that this study was a retrospective study. Which is true? The authors should clearly state calculation of sample size in case of a randomized controlled study. There seems to be no clear description of sample number in each group in Materials and Methods section or Results section. Moreover, supplement Tables show that all nodules from case 21 to 29 belong to validation group. It seems funny, if this study is a randomized controlled study.

A1. We never described this study is a randomized controlled study. As we stated, this study is a retrospective study. We apologize if our strategy to divide cases into two groups with block randomization makes the reviewer confused. In the revised version, we analyze all cases in one group for formulation and employed a resampling method for validation. I hope the revision makes the reviewer clear in this issue.

Q2. As the authors mentioned in the Discussion, these findings obtained from such small number of cases cannot draw definite conclusions, except for a randomized controlled study with a definite study design.

A2. We completely agree with the reviewer. However, based on the nature of MRI in terms of study cost, equipment availability, and a time consuming study protocol, it is quite hard to conduct a randomized controlled study with a large population. Instead, we alleviated our shortcomings in sample number by using a resampling method in the revised version. We performed 1,000 times iteration to mimic a cohort consisting of larger number of cases. We know that this is not enough to draw definite conclusions, but we hope our conclusions can encourage hepatologists to design a true randomized controlled study in a near future and reach final conclusions, which contribute to decision making in the early diagnosis and treatment of hepatocellular carcinoma.

Reviewer d:

Q1. One of the main limitations of the study is that multivariate logistic regression was used to model pseudoreplicate data (73 NHNs in 29 patients). This fact, contravenes one of the fundamental assumptions of logistic regression, which is the independence of errors. Therefore, I would recommend the use of other statistical strategies such as mixed-effects models to overcome this limitation.

A1. Thank you for your productive comments and suggestions. According to the reviewer's comment, we reanalyzed the data using a generalized estimating equation model instead of multivariate logistic regression in the revised version. In the result, the five factors different from those chosen in the original analysis were selected as significant explanatories for the disease progression. We agree that the new selection is much reasonable from the clinical point of view.

Q2. The relatively small number of cases and the retrospective experimental design are major weakness of this study. These methodological shortcomings compromise the robustness of the conclusions. A prospective study or a large number of cases would support more robustly the author's conclusion.

A2. I completely agree with the reviewer. However, based on the nature of MRI in terms of study cost, equipment availability, and a time consuming study protocol, it is quite hard to conduct a randomized controlled prospective study with a large population. Instead, according to the reviewer's first comment, we analyzed all cases at once in the revised version instead of dividing them into two groups using a generalized estimating equation method. Furthermore, we used a resampling method to validate the formula in the revised version. We performed 1,000 times iteration to mimic a cohort consisting of larger number of cases. I know that this is not enough to draw definite conclusions, but I hope our conclusions can encourage hepatologists to design a true randomized controlled study in a near future and reach final conclusions, which contribute to decision making in the early diagnosis and treatment of hepatocellular carcinoma.

Q3. In Table 2, can the authors provide confidence intervals? This would help the readers assess the precision of the model and evaluate for any overlap.

A3. We did so in the revised version.

Q4. Could the authors provide more information on goodness-of-fit summary statistic for the model (e.g.: Lemeshow-Hosmer) and the goodness-of-fit statistics for the independent variables? In addition, and due to the small number of cases, I would recommend performing ten-fold cross validation to assess how well the model predicts based on new information. The authors assessed the accuracy of the model by dividing the 73 NHNs cases in one training and one test group. This fit measure may be insufficient and biased by over-fitting, considering the proportion of cases and variables.

A4. Thank you for your expertise. Along with the reviewer's suggestions, we used Bootstrap resampling samples for the validation study in the revised version as described above. We believe that this revision enables to increase the sample size and alleviate over-fitting effects using 13 variables.

Q5. The author should consider asking a native English speaker and writer to edit the manuscript for grammar and readability.

A5. The original version of this manuscript was edited by one or more native English-speaking editors at Nature Publishing Group Language Editing Service. If there are grammatical error and/or unclear meaning in the revised version, could you specifically indicate each point? We are very happy to learn a clearer and more understandable expressions and word usage.

<u>Reviewer f:</u>

Q1. This appears to be significant on a regression analyze but has limited applicability as well as no formal pathologic confirmation as of yet. Obviously pathologic confirmation would be desirable.

A1. Thank you for your important comments. We agree that the pathological confirmation is inevitable if a formula aims at the diagnosis of hepatocellular carcinoma. However, our focus is not the definite diagnosis, but rather recommendation to investigate further the nodules, which do not show typical image findings. Although pathologic confirmation would be desirable, we believe that either growth

in size or gaining vascularity in an arterial phase or both is meaningful surrogates for histological confirmation in this setting. Anyway, this manuscript just provides one of ways to distinguish nodules showing progressive characters from indolent one. We hope the publication of this type of paper makes more hepatologists interest and commit in this issue.

Reviewer l:

Q1. Very good job. I am personally waiting a future paper of yours evaluating the prognostic value of your formula in a larger group of patients. Keep up the good work.

A1. Thank you for your encouraging comments.