

3<sup>rd</sup> June 2014

Re:Manuscript ID 9449

1<sup>st</sup> Revision

” Hepatic clearance measured on <sup>99m</sup>Tc-GSA SPECT for estimation of liver fibrosis”

Dear Editors

We wish to convey our heartfelt thanks in response to your letter dated May 1<sup>st</sup> regarding our paper. We found the reviewers’ kind and detailed suggestions very helpful and have revised the manuscript accordingly.

Please find the revised manuscript (with revisions underlined) as well as point-by-point responses to your and the reviewers’ comments and a description of the corresponding changes made to the manuscript. We are resubmitting this revised manuscript for publication in *World Journal of Gastroenterology*. In point by point response, the number of the pages and lines are taken from the revised manuscripts without underline.

We hope these modifications are sufficient and meet your expectations. We would be pleased to address any further questions that you or your reviewers might have.

Sincerely,

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## Comments to the Reviewer #1

*Major Criticisms: 1) Almost half of the patients had no fibrosis at all, therefore there is significant concern that the data may be underpowered to evaluate whether any of the parameters (HC, LHL15, clinical, etc.) are sufficiently able to discriminate better than other factors the degree of fibrosis. It is suggested that greater patient accrual or cohort expansion, particularly to include greater numbers of patients with fibrosis be included in the study.*

We appreciate your positive comment on the findings of this study.

According to your suggestion, we have enrolled 78 consecutive patients who underwent an initial hepatectomy due to hepatocellular carcinoma. The liver of these patients have showed more severe fibrosis due to hepatitis. According to the change of subject, the results have showed a big change.

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Between January 2011 and March 2014, 78 consecutive patients who underwent an initial hepatectomy due to hepatocellular carcinoma were enrolled in this study.

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## **RESULTS**

### **Patient characteristics**

The clinical characteristics of all participating patients are listed in Table 1. The mean age of the 78 patients was  $66.7 \pm 10.3$  years, and there were 63 men. Of the 78 patients, 71 had chronic liver disease (chronic hepatitis B, n=26; chronic hepatitis C, n=21; non-alcoholic steatohepatitis, n=14; and alcoholic hepatitis, n=10). The remaining patients were diagnosed with normal livers. Concerning the degree of hepatic fibrosis, 10 patients were graded 6, 13 were graded 5, 4 were graded 4, 18 were graded 3, 8 were graded 2, 11 were graded 1, and 14 were graded 0. The mean ICG R15 was  $11.6 \pm 6.0$ .

### **Correlations between the degree of liver fibrosis and quantitative indices of liver functional reserve**

Table 2 shows the correlations between the degree of liver fibrosis and preoperative liver function parameters. The degree of liver fibrosis was positively linearly correlated with ICG R15 and HH15 and negatively linearly correlated with HC.

### **Correlations between quantitative indices for liver functional reserve and conventional liver function tests**

As Table 3 shows, we evaluated the correlations between the preoperative parameters for liver function and conventional liver function tests. LHL15 was correlated with platelet count ( $R=0.235$ ,  $p=0.038$ ) and albumin level ( $R=0.263$ ,  $p=0.020$ ), and HH15 was correlated with total bilirubin level ( $R=0.289$ ,  $p=0.010$ ) and cholinesterase level ( $R=-0.263$ ,  $p=0.020$ ). HC was correlated with all conventional liver function tests after liver resection: platelet count ( $R=0.348$ ,  $p=0.002$ ), prothrombin time ( $R=-0.287$ ,  $p=0.011$ ), albumin level ( $R=0.233$ ,  $p=0.040$ ), total bilirubin level ( $R=-0.345$ ,  $p=0.002$ ), and cholinesterase level ( $R=-0.419$ ,  $p=0.0001$ ).

### **Univariate and multivariate stepwise regression analysis of various factors affecting liver fibrosis**

Univariate analysis showed that platelet count ( $p<0.001$ ), prothrombin time ( $p=0.032$ ), total bilirubin level ( $p=0.001$ ), tumor size ( $p=0.042$ ), MELD score ( $p=0.009$ ), ICG R15 ( $p=0.019$ ), LHL15 ( $p=0.042$ ), HH15 ( $p=0.0004$ ), and HC ( $p<0.0001$ ) were significant predictors of severe cirrhosis. When we entered platelet count, prothrombin time, total bilirubin level, tumor size, MELD score, ICG R15, LHL15, HH15, and HC into a multivariate logistic regression model to identify variables with independent predictive value for severe fibrosis, we found that HC and LHL15 were the significant independent predictors (Table 4).

### **ROC curve and interactive dot diagrams of HC and LHL15 for the diagnosis of severe fibrosis**

In Figure 1-A, we present the ROC curves for each of the 2 variables, HC and LHL15, that were identified as the significant independent predictors of severe fibrosis. The AUC of the ROC curves for HC and LHL15 were 0.826 and 0.641, respectively. There was a significant difference between the two values ( $p=0.0146$ ). Based on the analysis employing interactive dot diagrams, the cutoff values for predicting severe cirrhosis with the highest sensitivity and specificity were 298 (sensitivity, 77.8%; specificity, 84.3%) for HC and 0.926 (sensitivity, 74.1%; specificity, 60.8%) for LHL15.

2) Since the main outcome is degree of fibrosis, baseline characteristics should be expressed somehow in relation to degree of fibrosis rather than just the whole cohort at large. This would be helpful for not only the baseline characteristics, but also for the measured aspects of 99mTc-GSA and ICGR15. In addition, noted clinical scoring systems that have been previously validated as being predictive of degree of liver dysfunctions such as MELD or CTP score should be included along with single laboratory values.

We appreciate your comment on our findings. According to your suggestion, we have changed baseline characteristics as follows.

**Table 1 Patient Characteristics**

Variables	n=78
Age	66.7±10.3
Gender (male/female)	63/15
HBs-Ag (+/-)	26/52
HCV-Ab (+/-)	21/57
Alcohol abuse (+/-)	10/68
NASH (+/-)	14/64
Diabetes mellitus (+/-)	25/73
Hyperlipidemia (+/-)	18/60
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )	16.6±7.0
Prothrombin time (INR)	1.05±0.11
Albumin (g/dl)	4.0±0.6
Total bilirubin (mg/dl)	0.8±0.3
Cholinesterase (U/L)	248±70
Tumor size (cm)	49.6±36.9
Tumor number	1.2±0.5
Tumor vascular invasion (+/-)	21/57
Ishak classification 0/1/2/3/4/5/6	14/11/8/18/4/13/10
MELD score	5.3±1.3
CTP score	5.2±0.2
ICG R15 (%)	11.6±6.0

3) The fibrosis scoring system is incompletely defined...how was the scoring system chosen? Does it apply to all etiologies of liver disease? How was it decided? A statement is made that F0-F2 was reflective of non-severe fibrosis, whereas F3 and F4 is considered severe fibrosis? This is very confusing in that fibrosis scores are meant to reflect degree of fibrosis before full-blown cirrhosis has occurred. The Ishak scoring system is suggested as an alternative. 4) While HC appears to be an independent predictor along with other factors, the data do not support the conclusion that HC is superior to other parameters or clinical measurements with respect to predicting fibrosis. The most correct means of assessing these pre-test markers is through the ROC analysis which unfortunately shows no significant differences. This is likely due to many of the reasons listed in comment 1) along with selection of a scoring system for fibrosis that is relatively narrow, though it is ok to select the median value for this range.

As you have rightly pointed out, our classification of fibrosis was not suitable for this study. Accordingly, we have classified the fibrosis based on Ishak classification as follows.

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#### **Histopathological features of liver specimens**

Liver fibrosis was diagnosed using surgical specimens, which were resected at a distance from the tumors. The degree of hepatic fibrosis was assessed and graded 0-6 according to the Ishak classification for chronic hepatitis<sup>[20]</sup>: 0, no fibrosis; 1, fibrous expansion of some portal areas, with or without short fibrous septa; 2, fibrous expansion of most portal areas, with or without short fibrous septa; 3, fibrous expansion of most portal areas with occasional portal-to-portal bridging; 4, fibrous expansion of portal areas with marked bridging (portal to portal as well as portal to central); 5, marked bridging (portal to portal and / or portal to central) with occasional nodules; and 6, cirrhosis, probable or definite. Scores of 0, 1, 2, and 3 were considered to reflect nonsevere fibrosis. Scores of 4, 5, and 6 were recorded as severe fibrosis. Tumor size, tumor number, and tumor vascular invasion (portal vein, hepatic artery, and hepatic vein) were evaluated using surgical specimens.

*Minor Criticisms: 1) The title should include full text for any abbreviations and not simply the acronym itself.*

According to your suggestion, we have changed the title as follows.

#### **Hepatic clearance measured with technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin single-photon emission computed tomography to estimate liver fibrosis**

2) The figure legends and table legends need to be more clear so that the reader can rapidly understand the variables and their definitions along with the basic comparisons made.

According to your suggestion, we have changed the figure legends and table legends as follows.

#### **Figure Legends**

**Figure 1-A. Receiver operating characteristic (ROC) analysis for HC and LHL15.**

There was a significant difference between the two values (p=0.0146).

Abbreviations: AUC: area under the ROC curve; HC: hepatic clearance

**Figure 1-B. Interactive dot diagrams showing HC predicts severe cirrhosis.**

The cutoff value for predicting severe cirrhosis with the highest sensitivity and specificity was 298 (sensitivity, 77.8%; specificity, 84.3%) for HC. The horizontal line indicates the cutoff point with the best separation between the 2 groups (severe fibrosis+, severe fibrosis-).

Abbreviations: HC: hepatic clearance.

**Figure 1-C. Interactive dot diagrams showing LHL15 predicts severe cirrhosis.**

The cutoff value for predicting severe cirrhosis with the highest sensitivity and specificity was 0.926 (sensitivity, 74.1%; specificity, 60.8%) for LHL15. The horizontal line indicates the cutoff point with the best separation between the 2 groups (severe fibrosis+, severe fibrosis-).

**Table Legends**

**Table 1: Patient Characteristics**

Abbreviations: HBs-Ag: hepatitis B surface antigen; HCV-Ab: hepatitis C virus antibody; NASH: nonalcoholic steatohepatitis; MELD score: model for end-stage liver disease score; CTP score: Child-Turcotte-Pugh score; ICG R15: indocyanine green dye retention at 15 min.

**Table 2: Correlation between the degree of liver fibrosis and quantitative indices of liver functional reserve**

The degree of liver fibrosis was correlated with ICG R15, HH15, and HC.

Abbreviations: ICG R15: indocyanine green dye retention at 15 min; HC: hepatic clearance

**Table 3: Correlations between quantitative indices for liver functional reserve and conventional liver function tests**

LHL15 was correlated with platelet count and albumin level. HH15 was correlated with total bilirubin level and cholinesterase level. HC was correlated with all conventional liver function tests.

Abbreviations: ICG R15: indocyanine green dye retention at 15 min; HC: hepatic clearance.

**Table 4: Univariate and multivariate analysis of variables predictive of severe fibrosis**

Platelet count, prothrombin time, total bilirubin level, tumor size, MELD score, ICG R15, LHL15, HH15, and HC were significant predictors of severe cirrhosis in the univariate analysis. In the multivariate analysis, HC and LHL15 were the significant independent predictors.

Abbreviations: HBs-Ag: hepatitis B surface antigen; HCV-Ab: hepatitis C virus antibody; NASH: nonalcoholic steatohepatitis; MELD score: model for end-stage liver

disease score; CTP score: Child-Turcotte-Pugh score; ICG R15: indocyanine green dye retention at 15 min; HC: hepatic clearance.

*3) Are there any technical concerns with the use of this technology with respect to other co-morbidities?*

We appreciate your comment on our findings. According to your suggestion, we have checked technical concerns with respect to other co-morbidities, such as diabetes mellitus, hyperlipidemia. Please see Table 1 and Table 4. As a result, there were no concerns. We have changed baseline characteristics and analyses of variables predictive of severe fibrosis.

## Comments to the Reviewer #2

1) The author should clarify the indications for hepatectomy (as regard the HCC patients, the author should clarify number and size of tumours).

2) what about the condition of the hepatic vasculature in those patients (portal vein, hepatic veins and hepatic artery) whether they have patent, thrombosed or attenuated vessels or impaired blood flow as a complication of HCC.

3) As regard patients with NASH, the author should clarify whether those patients had DM, dyslipidemia or unknown aetiology.

We appreciate your comment on our findings. According to your suggestion, we have enrolled 78 consecutive patients who underwent an initial hepatectomy due to hepatocellular carcinoma. And we have changed baseline characteristics as follows.

**Table 1 Patient Characteristics**

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CTP score	5.2±0.2
ICG R15 (%)	11.6±6.0



4) Why the author did not use Metavir or Ishak classification of liver fibrosis ???(both are more commonly used as a universal classification of liver fibrosis).

As you have rightly pointed out, our classification of fibrosis was not suitable for this study. Accordingly, we have classified the fibrosis based on Ishak classification as follows.

Page 10 line 11

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According to the change of methods, the results have showed a big change.

Page 11 line 10

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#### **Univariate and multivariate stepwise regression analysis of various factors affecting liver fibrosis**

Univariate analysis showed that platelet count (p<0.001), prothrombin time (p=0.032), total bilirubin level (p=0.001), tumor size (p=0.042), MELD score (p=0.009), ICG R15 (p=0.019), LHL15 (p=0.042), HH15 (p=0.0004), and HC (p<0.0001) were significant predictors of severe cirrhosis. When we entered platelet count, prothrombin

time, total bilirubin level, tumor size, MELD score, ICG R15, LHL15, HH15, and HC into a multivariate logistic regression model to identify variables with independent predictive value for severe fibrosis, we found that HC and LHL15 were the significant independent predictors (Table 4).

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We highly appreciate your valuable comments.

We have revised the manuscript according to the suggestions of the reviewers, and the references and typesetting were corrected.

The changes suggested by the reviewers have greatly improved the quality of our manuscript, and we hope that you will find that we have satisfactorily addressed all the pertinent issues in the revised version. We are grateful for the opportunity to submit our revised manuscript, and we look forward to your positive reply.

Thank you again for considering our paper.

Sincerely yours,

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