May 31, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript No.9664-Review.doc).

Title: Contrast-enhanced Ultrasound: Improving the Preoperative Staging of Hepatocellular Carcinoma and Guiding Individual Treatment

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9664

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

(1) We have summarized the demographic and clinical data of patients and pathologic data of tumors, as shown in table1;

(2) TNM staging of tumors were calculated in table 3;

(3) According to CEUS，changes in operative strategies were shown in table 6.

(4)Advised by the reviewers (Reviewer code: 00070915), we deleted the old table 4(the number of the positive and false positive nodules for CE-CT /MRI, CEUS, IOUS in S-group) and table 5(the number of the positive and false positive nodules for CE-CT /MRI, CEUS, IOUS in L-group). The way results are presented is confusing and requires extra effort from the reader to comprehend them. In addition, we have detailed the results in the "RESULT". Thus those two tables were unnecessary and we followed suggestions of the reviewers.

2 Revision has been made according to the suggestions of the reviewer

(1) According to the suggestions of the reviewers (Reviewer code: 00070915 and 02753135), we have revised our title as "Contrast-enhanced Ultrasound: Improving the Preoperative Staging of Hepatocellular Carcinoma and Guiding Individual Treatment" that may be more accurate reflection of the work presented in the paper.

(2)In the section of the abstract, we put "To investigate the impact of tumor size… in diameter" into the methods section, presented the main result points and deleted the unnecessary numbers, presented the results of changes in surgical strategies by preoperative CEUS and by IOUS finally. Either the P value is stated as being < 0.05 or the exact value is given, not for both.

(3)In the "Materials and Methods": First, our study was a prospective study. We performed CEUS+CT/MRI for consecutive patients who have liver lesions. Thus we added the sentences" A total of 96 consecutive patients were admitted to our center. Of those …… with hemangiomas" to this part followed advice by the reviewer (Reviewer code: 00183029); Secondly, we calculated and compared TNM staging of tumors and analyzed changes in surgical strategies due to CEUS and IOUS. Thirdly, for detailing the methodology, the paragraph, "Ultrasonography was performed by four sonographers with ……was reached", was added.

(4)In the "Result": First, we have summarized the demographic and clinical data of patients and pathologic data of tumors as shown in table1; secondly, the TNM staging of tumors were calculated in table 3.

(5) In the "Operation": Firstly, changes in operative strategies due to the detected lesions by CEUS were shown in table 6; secondly, we clarified the way of 4 patients who had their surgical strategies changed (1 patient with left lobe underwent……required partial-diaphragmatic-resection).

(6) In the "Discussion": we removed the repeated results number and discussed the advantage of CEUS +CE-CT/MRI supported by the result in a descriptive and concise way (as shown in the paragraph" In present study, the overall diagnostic …… who undergoing liver resection.")

3 References and typesetting were corrected

4 Answering reviewers

(1) Answering the first reviewer (Reviewer code: 00503536)

Major points:

1) It is surprising that sensitivity of CE-CT/MRI for the diagnosis of both small and large HCC is too low (72-84%). According to our experience and other reports, HCC over 2cm in diameter is easy to be diagnosed by either CE-CT or CE-MRI, and sensitivity should be >95%. The authors should discuss or explain on that point.

   Thanks for the question. This result may be accounted for the following reasons. Firstly, the etiologies of the HCC are different. To our knowledge, a majority of our patients were HBsAg positive (63/69), while more than half of patients in other studies were HCVAb positive. Thus may be a potential reason for the difference. Secondly, we reported that the mean Ishak score, which were not shown in other studies, was 4.94±1.31. Large regenerative nodules and dysplastic nodules (DN) are very difficult to the differentiate diagnosis from early stage HCC by CT or MRI, which could not detail intratumoral vasculature in real time. These also were the reasons why we conducted this study.

2) It is quite reasonable that combination of the modalities shows higher sensitivity and accuracy than single modality as shown in this study. The authors should discuss why addition of CEUS to CE-CT/MRI improves the accuracy of HCC diagnosis in this study.

   Thanks for your question. As explained above, we wanted to investigate the role of CEUS+CE-CT/MRI in improving the accuracy of HCC diagnosis for patients with HBV related HCCs. In addition, our previous studies have shown that Contrast enhanced intraoperative ultrasound (CEIOUS) is a novel and promising technique that improves specificity and accuracy of IOUS and can influence on surgical strategy and oncological radicality. However, CEIOUS could not overcome the flaws of IOUS which could just be done after laparotomy. Newly identified lesions may require more extensive procedures than initially indicated, such as expanded resection or combined local therapies. In the patients with very extensive disease, surgeons may be obliged to give up the operations. Thus, accurate staging of HCC prior to an operation is extremely important. As mentioned reasons above, we conducted this study.

3) The authors should discuss on the cost-effectiveness of the examination (single or in combination). Do the author recommend the combination of CEUS with CE-CT/MRI as a routine surveillance in high risk patients?

   Thanks for your suggestion. As reported by Tanaka et al. that contrast-enhanced ultrasonography surveillance for HCC was a cost-effective strategy for liver cirrhosis patients and gains their longest additional life years (title: Cost-effectiveness analysis on the surveillance for hepatocellular carcinoma in liver cirrhosis patients using contrast-enhanced ultrasonography), CEUS could be a cost-effective examination. In this study we aimed to investigate the clinical role of CEUS in improving the diagnostic accuracy and staging of HCCs. According to our research, we suggest that the preoperative contrast enhanced ultrasound (CEUS) examination should be a standard for patients undergoing liver resection.

Minor points:

1) Tables 4 and 5 are not organized ones and need to be revised.

   Thanks for your suggestion. We have detailed the results what was shown in the table 4(the number of the positive and false positive nodules for CE-CT /MRI, CEUS, IOUS in S-group)

and table 5(the number of the positive and false positive nodules for CE-CT /MRI, CEUS, IOUS in L-group) in the "RESULT". Another reviewer thought the way results were presented was confusing and required extra effort from the reader to comprehend them. Following your and another reviewer´ s advices, we deleted those two tables.

2) There are some spelling mistakes.

Thanks for your suggestion. We have sought for a copyediting service provided by professional English language editing company (American Journal Experts) and earned an editing certificate.

(2) Answering the second reviewer (Reviewer code: 00503536)

1) In their study, CEUS was performed in 69 patients after CT or MRI evidence of focal liver lesions and under the diagnosis of HCC. To differentiate between malignant and benign liver lesions, their CEUS diagnostic efficacy was excellent, with 89.4% sensitivity, 97.0% specificity, 98.8% positive predictive value, 76.2% negative predictive value and 91.3% accuracy. The CEUS + CE-CT/MRI imaging modality had 100% positive predictive value in both tumor size < 5 cm and in tumor size > 5 cm, the diagnostic performance appeared much better than might be expected in clinical practice. Given the fact that the diagnostic accuracy of CEUS for HCC may be compromised by a high rate of operator- and machine-dependent variability, which calls for authors to explain how they standardized the procedures to achieve good results. There is no clear description on the techniques and the procedures how to obtain such a good diagnostic efficacy. In addition, authors should explain why their CEUS accuracy, specificity, positive predictive value and negative predictive value for differentiation between malignant and benign liver lesions were better in tumors with diameter ≤5cm than those with diameter >5cm.

Thanks for the question. Ultrasonography was performed by four sonographers with approximately 6, 8, 10, and 30 years of experience in abdominal US. In the case of disagreement, the sonographers engaged in joint discussions until a consensus was reached.

An iU22 ultrasound system (Philips Royal Electronic Corporation, The Netherlands) with a C5-1 transducer was used during the preoperative CEUS examinations, to start the mode of CEUS, to display clear of the lesions and to quickly push SonoVue (Bracco of Italy) 2.4 to 4.8 ml through the antecubital vein. The pipe was washed with 5ml of 0.9 % sodium chloride solution. A timer began at the same time the contrast agent was injected, and continuous observations occurred for at least five minutes. All phases of contrast enhancement, including the arterial phase (10-20s to 25-35s after injection), the portal phase (30-45 s to 120 s) and the delay phase (> 120 s) were recorded and analyzed. HCCs were characterized by the mode of enhancement, which showed hyper-enhancement in the arterial phase and wash-out of microbubbles during the portal or late phase. If the lesion did not exhibit wash-out during the portal and late phases, the lesions were defined as benign solid lesions.

As discussed in the Limitation, sample of subjects in our study was relatively small that may result in the difference between S-group and L-group. More studies are needed to confirm the influence of tumor size on the results of these imaging studies.

2) This is a prospective study. Please describe methods of patient selection. Was a consecutive or random sample of patients enrolled? Is there inclusion or exclusion criteria? Were all patients included in the final analysis?

Thanks for your advice. Our study was a prospective study. We performed CEUS+CT/MRI for consecutive patients who have liver lesions. A total of 96 consecutive patients were admitted to our center. Of those, 69 HCC patients and 8 ICC patients underwent liver resection; 11 patients underwent radio frequency ablation; 4 patients had liver metastases, 2 patients had focal nodular hyperplasia and 2 patients had hemangiomas. We enrolled the 69 patients who underwent hepatic resection for HCC in our study.

3) Authors should comment whether the conduct or interpretation of the CEUS findings have

introduced bias? Were the CEUS and CE-CT/MRI imaging results interpreted and documented by experts before operation, most importantly, without knowledge of the results of the operative findings?

Thanks for your question. Ultrasonography was performed by four sonographers with well experience in abdominal US. They were aware that the patients were at risk of developing HCC but they did not know any further information, e.g., AFP levels. In the case of disagreement, they engaged in joint discussions until a consensus was reached. Thus may avoid the bias of the conduct or interpretation of the CEUS findings. Lesions depicted by each imaging modality were counted and mapped. The arterial, portal and late phases of contrast enhancement were recorded and analyzed. All of the imaging results were interpreted and documented by experts before operation. In addition, the results of the operative findings were shown in table 6 and only 4 surgical strategies were changed because of newly detected lesions which were not in the same segments as old ones (1 patient with left lobe underwent a additional partial resection, two patients had lesions in other segments and underwent expanded resection, and, in the last patient, a lesion invaded the diaphragm and required partial-diaphragmatic-resection).

4) It is helpful if authors could present the clinicopathological features and preoperative liver function status of patients, including sex, age, interval between two images, liver function, Child-Pugh score, MELD score, tumor characteristics, alpha fetoprotein level, tumor size, tumor number, frequency of vascular invasion, and liver cirrhosis.

Thanks for your suggestion. We have summarized the demographic and clinical data of patients and pathologic data of tumors, as shown in table 1.

5) Intrahepatic peripheral cholangiocarcinoma (ICC) in cirrhosis patients may display a vascular pattern similar to HCC on CEUS, it has been reported that CEUS misdiagnosed ICC as HCC in 52% of cases, compared to 4.2 and 9.1% of CT and MRI respectively. Had any patients with ICC misdiagnosed as HCC preoperatively in the series?

A total of 8 patients for ICC was underwent liver resection. CEUS misdiagnosed ICC as HCC in 37.5% (3/8) of patients, compared to 12.5% (1/8) of CT/MRI. But the sample size was small, thus we didn't do statistic analysis.

6) Please illustrate the change of therapeutic decision in the decision-making process before and after adding CEUS in the imaging modalities for the patients.

Thanks for your suggestion. We have illustrated the changes of therapeutic decision in the decision-making process before and after adding CEUS in the imaging modalities in table 6. Surgical strategies were changed in 15.9% (11/69) of patients due to preoperative CEUS. Finally, only 4 patients' surgical strategies were changed because of newly detected lesions by IOUS.

7) There are many typographic and grammar errors in the manuscript.

Thanks for your review. We have sought for a copyediting service provided by professional English language editing company (American Journal Experts) and earned an editing certificate.

(3) Answering the third reviewer (Reviewer code: 02753135)

1) Title: - seeing that all patients had a pre-op histological diagnosis, a more accurate reflection of the work presented in the paper would be to refer to staging, i.e.: improving staging of HCC…..

Our heartfelt thanks for your suggestion. Following your and another reviewer' s advices, We have revised our title as "Contrast-enhanced Ultrasound: Improving the Preoperative Staging of Hepatocellular Carcinoma and Guiding Individual Treatment".

2) Abstract: - again review the use of the word "diagnosis"- the paper basically deals with the value of CEUS prior to surgery. IOUS is done during all operations, so the surgical strategy changes due to IOUS are not really new. The basic question is; what is the potential for CEUS to change surgical strategies pre-op

Thanks for your suggestion. The results of the operative findings potentially changed by CEUS were shown in table 6 in revised manuscript.

3) - Fellow-up should read Follow-up- Either the P value is stated as being < 0.05 or the exact value is given, no need for both General

Thanks for your suggestion, we have revised them.

4) - It would be considered standard to do both CT and MRI prior to surgery. In this study only 2 patients had both which is a bit surprising. Can the authors comment on this.

   Thanks for your question. HBV infections account for the majority of cirrhosis and primary liver cancer in China. Elevated AFP level is a good maker for diagnosing HCC related with HBV infection, as shown in our study. Based on the Chinese guideline of the primary liver cancer, combining with AFP level, conventional ultrasound, CT and or MRI is the effective way to diagnose the primary liver cancer. Thus, in this study only 2 patients had both CT and MRI.

5) - It would enhance the value of the paper if the authors can say which of the tests (CT or MRI independently) correlates best with the CEUS, and if a conclusion can be drawn for when both are not feasible.

   Thanks for your suggestion. When divided the patients into two groups (CT group and MRI group), we found that there was no significant difference between two groups ($P>0.05$). In addition, the sample size of subjects was relatively small. So we didn't report the relative result.

6) - Although all patients had surgery, how many would have had their management changed based on CEUS findings?

   Thanks for your suggestion. We have illustrated the changes of therapeutic decision in the decision-making process before and after adding CEUS in the imaging modalities in table 6. Surgical strategies were changed in 15.9% (11/69) of patients due to preoperative CEUS. Finally, only 4 patients´ surgical strategies were changed because of newly detected lesions by IOUS.

7) - The study ended in October 2013, so only a very short follow up period is available. Are there any findings during the follow up period that confirm the benign nature of some of the lesions?

   The benign lesions were proven by histopathology and follow-up. During the follow-up, those lesions didn't transform to carcinoma. Otherwise, if the patient with micro-vascular invasion, continuous elevated AFP level and other risks of tumorigenesis and recurrence, TACE, Sorafenib and immuno-enhancement were advised. All of these may lead to a good outcome.

8) - 4 patients had their surgical strategy changed; can the authors perhaps clarify in what way this was?

   Thanks for your question. We have clarified it in the revised manuscript. Finally, only 4 patients´ surgical strategies were changed because of newly detected lesions which were not in the same segments as old ones (1 patient with left lobe underwent a additional partial resection, two patients had lesions in other segments and underwent expanded resection, and, in the last patient, a lesion invaded the diaphragm and required partial-diaphragmatic-resection).

9) Please refer also to the manuscript in which poor grammar is highlighted in green and paper content comments in yellow.

   Thanks for your suggestion. We have sought for a copyediting service provided by professional English language editing company (American Journal Experts) and earned an editing certificate.

(4) Answering the fourth reviewer (Reviewer code: 00070915)

1) Title: the word "preoperative" should be added to the title before diagnosis of hepatocellular carcinoma… in order to differentiate from the intraoperative diagnosis provided by the IOUS which is analyzed in the paper?

   Thanks for your suggestion. According to your and another reviewer´s advices, we have revised our title as "Contrast-enhanced Ultrasound: Improving the Preoperative Staging of Hepatocellular Carcinoma and Guiding Individual Treatment" that may be more accurate reflection of the work presented in the paper.

2) The results section of the abstract is too long. There is no need to present so many numbers in that section, just the main result points. In the same section the phrase: "To investigate the impact of tumor size… in diameter." Should be in the methods section and not in the results. ?

Thanks for your suggestion. We have put "To investigate the impact of tumor size… in diameter" into the methods section and presented the main result points and deleted the unnecessary numbers.

3) Several grammatical and spelling errors are found within the manuscript; the quality of English is marginally acceptable so the text requires further language editing?
   Thanks for your suggestion. We have sought for a copyediting service provided by professional English language editing company (American Journal Experts) and earned an editing certificate.
4) Table 5 presents nodules in L-group while in the text it is used to link to a table from the S-group paragraph. ? Tables 4 and 5 are unnecessary; they do not help in presenting the results clearly and descriptively. ? The way results are presented is confusing and requires extra effort from the reader to comprehend them. Tables (especially 4 & 5) do not contribute to that cause. ?
   Following your advice, we deleted those two tables. Additionally we have described the results in the "RESULT" instead of the two tables.
5) Specific results numbers should not be repeated in Discussion section. Results needed to support key points in Discussion should be presented in a descriptive and concise way?
   Thanks for your suggestion. In the "Discussion" of revised manuscript, we removed the repeated results number and discussed the advantage of CEUS +CE-CT/MRI supported by the result in a descriptive and concise way (as shown in the paragraph" In the present study, the overall diagnostic …… undergoing liver resection.").
6) The major advantage of the combination of CEUS+CE CT/MRI that is to improve the accuracy of diagnosis preoperatively was not thoroughly investigated. Instead of detecting the patients in whom treatment strategy was altered because of the use of the CEUS+CE-CT/MRI, just the surgical strategy change because of the use of IOUS was investigated. The point stated by the authors that in only 5.7% surgical strategy was changed is just an indirect finding.
   Thanks for your advice. We have illustrated the changes of therapeutic decision in the decision-making process before and after adding CEUS in the imaging modalities in table 6. Surgical strategies were changed in 15.9% (11/69) of patients due to preoperative CEUS. Finally, only 4 patients´ surgical strategies were changed because of newly detected lesions by IOUS. It may support the major advantage of the combination of CEUS+CE CT/MRI better.

Thank you again for publishing our manuscript in the World Journal of Gastroenterology.

Sincerely yours,

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