January 29, 2017

Lian-Sheng Ma,

President and Company Editor-in-Chief

World Journal of Gastroenterology

Dear Lian-Sheng Ma,

Thank you for your careful review of our manuscript entitled, "Rate of local recurrence following radiofrequency ablation of pathologically early hepatocellular carcinoma" **(**ESPS Manuscript No: 32023).

We have read the reviewers' comments carefully and have revised the manuscript accordingly. The revised portions of the manuscript have been written in red. We have also responded to the reviewers' comments in a point-by-point manner, as follows.

Responses to the Reviewers' comments:

Thank you very much for your valuable comments; we have revised the manuscript accordingly.

Responses to Reviewer 1 (No. 01221925)

1 <In the first paragraph of the introduction, in the sentence "although it sometimes includes solitary HCC ≥5 cm as defined according to the Milan criteria" it should be HCC≤5cm>

As you pointed out, we have changed ">5 cm" to "≤5 cm" in the Introduction.

2 <As there were 139 patients with 237 HCCs, were there cases where the same patient would have "early" and "typical" lesions?>

Thank you for your valuable comment. Twenty-five patients with 40 pathologically early HCC lesions had developed typical HCC previously or simultaneously; these lesions were treated using RFA.

3 <Did the number of HCCs in patients with the pathologically "typical" picture have

any effect on recurrence in this study?>

Thank you for your valuable comment. In this study, typical HCC was diagnosed based on contrast-enhanced CT or contrast-enhanced MRI findings. However, in cases with nodules exhibiting hyper-vascularity during the arterial phase and a ***slightly*** hypo-vascular appearance (***slightly*** washout) during the hepatic venous phase or equilibrium phase or during both phases of contrast-enhanced CT or the delayed phase of MRI with Gd-EOB-DTPA, we performed a needle biopsy to confirm the diagnosis of HCC. As a result, 62 nodules were diagnosed as well-differentiated HCC, and 19 were diagnosed as moderately differentiated HCC. If the term "pathologically 'typical' HCCs," as Reviewer 2 described, is used to describe HCCs that have been biopsied, as mentioned above, about 77 percent of the lesions were diagnosed as well-differentiated HCC. In general, most well-differentiated HCCs appear as iso-echoic masses during the post-vascular phase of contrast-enhanced US, and these HCCs are associated with a lower rate of local tumor progression compared with moderately or poorly differentiated HCC, which appear as hypo-echoic regions during the post-vascular phase of contrast-enhanced US. In the present study, however, to focus on the recurrence rate of pathologically early HCC compared with that of typical HCC, we did not evaluate the contrast-enhanced US findings obtained during the post-vascular phase.

4 <Are there any cases of "typical" HCCs where there are biopsies available to confirm the diagnosis? (even at a later time point)? >

Thank you for your valuable comment. The diagnosis of typical HCC was established based on radiologic features. When a lesion was visualized as a hyper-vascular area during arterial phase contrast-enhanced CT or MRI with Gd-EOB-DTPA and as an area of washout during the subsequent phases of contrast-enhanced CT or MRI with Gd-EOB-DTPA, we considered this enhancement pattern to represent a vascular pattern characteristic of typical HCC. Meanwhile, we performed a needle biopsy to confirm the diagnosis in 81/187 lesions with a hyper-vascular appearance during the arterial phase and a slightly hypo-vascular appearance during subsequent phases, and 77 percent of these lesions were diagnosed as well-differentiated HCC based on the results of a needle biopsy

(62 nodules were diagnosed as well-differentiated HCC, 19 were diagnosed as moderately differentiated HCC). To avoid confusion, we have not included these sentences regarding needle biopsies for typical HCC in the text.

5 <Were the recurrences confirmed by biopsy? If yes, is there information on the type of the recurrences, ie "early" or "typical" or another type?>

Thank you for your valuable comment. In this study, one of the 50 pathologically early HCC exhibited local tumor progression. This local tumor progression appeared as a hyper-vascular area during the arterial phase and as a hypo-intense area during the late and hepatobiliary phases of contrast-enhanced MRI with Gd-EOB-DTPA. We suspected that the recurrent nodule after ablation was likely to be a typical HCC, and a biopsy was not performed.

Responses to Reviewer 2 (No. 00070179)

1. < in the introduction change >5cm in <5cm >

   Thank you for pointing out our mistake. We have changed ">5 cm" to "≤5 cm" in the Introduction.

2. <the number of patients with pathologically early HCC is few: I think that it is necessary to outline that this is a pilot study and a multicenter study should be necessary to confirm this results.>

   According to your valuable suggestion, we have included the following sentence in the Discussion: "…the present study was a pilot study, and a multicenter study is needed to confirm the presently reported results" (page 18, paragraph 1, lines 3-5).

3. <in this setting it should be interesting to evaluate also the intra-hepatic recurrences and survival rates.>

   Thank you for your valuable comment. In this study, patients who had both pathologically early HCC and typical HCC simultaneously were not excluded from this study, but only 10 of the 50 pathologically early HCC cases had primary pathologically early HCC. Patients with primary pathologically early HCC were

compared with those with primary typical HCC (30/187) using a long-rank test to determine whether such factors influenced the rate of intra-hepatic recurrence. However, no significant difference was observed (P = 0.418). In addition, patients with primary pathologically early HCC were also compared with those with primary typical HCC to determine whether such factors influenced the overall survival rate when examined using a log-rank test. However, no significant difference was observed (P = 0.122), possibly because of the relatively small number of patients with primary pathologically early HCC or primary typical HCC in this study. The rates of intra-hepatic recurrence and overall survival for patients with primary pathologically early HCC will need to be studied in the future. In the Discussion section, we have revised the sentences as follows: "…only 10 of the 50 pathologically early HCC cases had primary pathologically early HCC; therefore, the rates of intra-hepatic recurrence-free survival and of overall survival after ablation were not evaluated for cases with primary pathologically early HCC or for those with primary typical HCC" (page 21, paragraph 2, lines 5-9).

4. <I prefer to use "local tumor progression" as suggested by Ahmed M. Image-guided tumor ablation: standardization of terminology and reporting criteria—a 10-year update. Radiology. 2014;273:241–260.>

   According to your suggestion, we have changed "local recurrence" to "local tumor progression" throughout the manuscript, including the title.

Responses to reviewer 3 (NO 03647831)

Major Comments

1 <Did all of the 50 pathologically early HCC appear as hypo-intense mass during the hepatobiliary phase of contrast-enhanced MRI with Gd-EOB-DTPA? Were there any exceptions?>

Thank you for your valuable comment. One of the 50 pathologically early HCCs had appeared as an almost iso-intense area during the hepatobiliary phase of contrast-enhanced MRI with Gd-EOB-DTPA. The remaining 49 lesions appeared as hypo-intense areas during the hepatobiliary phase of contrast-enhanced MRI with

Gd-EOB-DTPA. In this study, however, we focused on the recurrence rate of pathologically early HCC, compared with that of typical HCC; thus, we did not include these sentences regarding the findings for the hepatobiliary phase in the text.

2 <How many early HCCs were examined by CTHA and /or CTAP? >

Thank you for your valuable comment. We did not evaluate the images of pathologically early HCCs using CTHA or CTAP.

3 <How many pathologically early HCC showed defect of portal blood flow?>

Thank you for your valuable comment. Two of the 50 pathologically early HCCs appeared as hypo-vascular nodules during the portal phase of contrast-enhanced US. These lesions had a hypo-vascular appearance during three phases (arterial, portal, and post-vascular phases), indicating reductions in both hepatic arterial and portal venous flows. In the present study, however, we focused on the recurrence rate of pathologically early HCC, compared with that of typical HCC; thus, we did not include these sentences regarding the portal phase findings for contrast-enhanced US in the text.

4 <Among 50 pathologically early HCCs, only 1 nodule was associated with a local recurrence. It is desirable that the authors describe about the details of this case as figure 1-3, especially about the tumor size, imaging characteristic, pathological characteristic, and tumor volume doubling time. Imaging and pathological features of the recurrent HCC should be also described. >

According to your valuable suggestion, we have changed Figure 3 from a case with no local tumor progression to a case showing the local tumor progression of pathologically early HCC; we have also included a detailed description of Figure 3 in the figure legends.

5 <If possible, it is desirable that the overall survival and recurrence free survival are examined after excluding patients who had both pathologically early HCC and typical HCC simultaneously. The relationship between local recurrence and tumor markers (AFP, AFP -L3%, DCP) can be also determined in this setting. >

Thank you so much valuable suggestion. In this study, only 10 of the 50 pathologically early HCC cases were primary pathologically early HCC. Patients with primary pathologically early HCC were compared with those with primary typical HCC (30/187) using a long-rank test to determine whether such factors influenced the rate of intra-hepatic recurrence. However, no significant difference was observed (P = 0.418). In addition, patients with primary pathologically early HCC were also compared with those with primary typical HCC to determine whether such factors influenced the overall survival rate when examined using a log-rank test. However, no significant difference was observed (P = 0.122). Because the number of primary pathologically early HCCs that were treated with RFA was relatively small in this study, the rates of intra-hepatic recurrence and overall survival among patients with primary early HCC should be further studied in the future. We have revised the Discussion section as follows: "…only 10 of the 50 pathologically early HCC cases were primary pathologically early HCC; therefore, the intra-hepatic recurrence-free survival and overall survival rates after ablation were not evaluated for cases with primary pathologically early HCC or for those with primary typical HCC" (page 21, paragraph 2, lines 5-9). The relationships between local tumor progression and tumor markers (AFP, AFP -L3%, DCP) could be determined as follows. Each marker did not differ significantly between patients with recurrent typical HCC and those without recurrent typical HCC, according to a Mann-Whitney U-test (P = 0.893, 0.750, and 0.563, respectively). The tumor marker levels of patients with one recurrent early HCC were as follow: AFP, 15 ng/ml; AFP -L3, 50 %; and DCP, 7.5 mAU/ml. The median tumor marker levels of patients without recurrent pathologically early HCC were as follows: AFP, 20 ng/ml; AFP -L3, 25 %; and DCP, 26 mAU/ml. We considered these tumor markers to show no obvious differences between patients with and those without recurrent pathologically early HCC in this study, but these issues should be studied using a larger number of cases. In this study, as our goal was to focus on local tumor progression, we did not incorporate these sentences regarding the relationship between recurrence and tumor markers in the text.

Minor Comments

1 <The tumor showed in Figure 3 is recognized as hyper-vascular tumor by a contrast-enhanced US. Is this not a typical HCC? If not only dynamic study of CT and /or MRI but also anigiography/CTHA/CTAP was performed, I think this tumor could be diagnosed as a typical HCC. >

Thank you so much for the valuable comment. In Fig. 3 in the original manuscript, the lesion appeared to be slightly hyper-vascular throughout the whole area during the arterial phase of contrast-enhanced US and to be iso-echoic during the post-vascular phase of contrast-enhanced US. In contrast, this lesion appeared to be partially hyper-vascular during the arterial phase of contrast-enhanced CT. Consequently, this lesion did not exhibit a typical HCC enhancement pattern using contrast-enhanced CT. This lesion was not examined using anigiography/CTHA/CTAP. As we described in the limitations, the pathological diagnoses based on needle biopsied specimens might have resulted in an underestimation of the histological grade; however, the nodule was certainly diagnosed as pathologically early HCC according to the presence of stromal invasion using a needle biopsy. According to the recommendation of Reviewer 2, we have changed Figure 3 to depict a case of recurrent pathologically early HCC, and this recurrent case was similarly recognized as a hyper-vascular tumor using contrast-enhanced US and dynamic contrast-enhanced MRI with Gd-EOB-DTPA.

2 <Legend of Figure 1 A, Figure 2A, Figure 3A: Is this a fusion image? It looks like just conventional US and hepatobiliary phase of EOB-MRI. >

Thank you for your valuable comment. Trimming the images made it difficult to understand them as fusion images. We have shown the images before trimming, as follows. We have also made the images larger. In Figure 3, we changed the image to one depicting a recurrent case based on the comment of Reviewer 2.

Figure 1 A

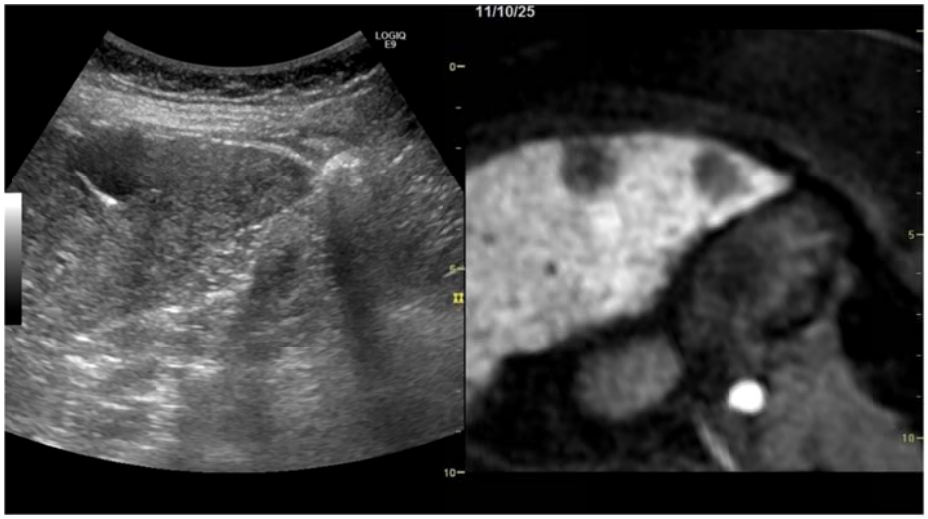
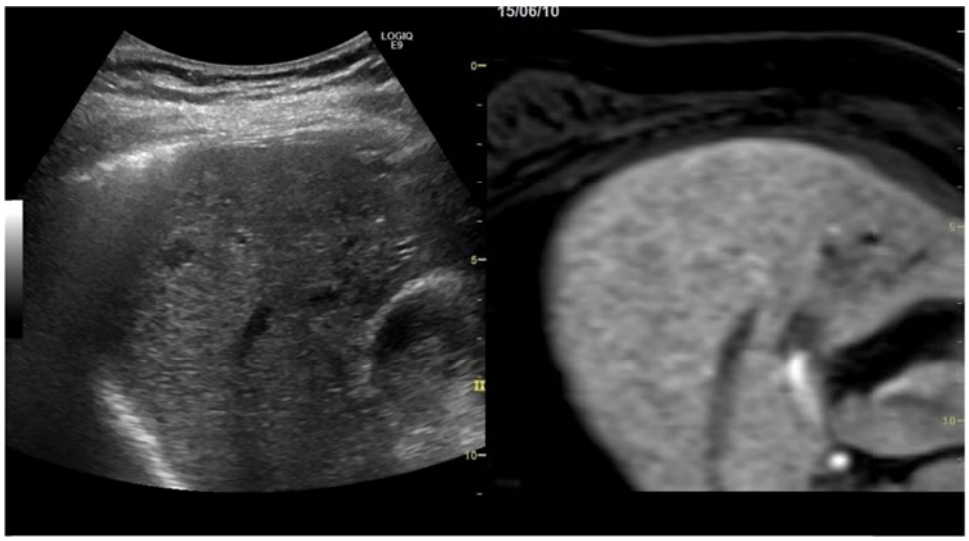


Figure 2A



3 <Legend of Figure 1 B, Figure 2B, Figure 3B: What does “One-day” mean?>

Thank you for your valuable comment. To avoid confusion, we have deleted “One-day” and have added “obtained one day after RFA” (Legend of Figure 1B, 1C, 2B).

Others:

We apologize, but unfortunately we made a mistake regarding the diagnosis of typical HCC using contrast-enhanced CT or dynamic contrast-enhanced MR imaging with Gd-EOB-DTPA. We have changed the sentence “When a lesion was visualized as a high-attenuation area during the arterial phase of contrast-enhanced CT or dynamic

contrast-enhanced MR imaging with Gd-EOB-DTPA and as a low-attenuation area (washout) during the hepatic venous phase or equilibrium phase or in both phases" in the original manuscript to "When a lesion was visualized as a hyper-vascular area during the arterial phase of contrast-enhanced CT or dynamic contrast-enhanced MRI with Gd-EOB-DTPA and as a hypo-vascular area (washout) during the hepatic venous phase or equilibrium phase or during both phases of contrast-enhanced CT or the delayed phase of dynamic contrast-enhanced MRI with Gd-EOB-DTPA" in the revised manuscript (page 9, Materials and Methods section of the revised manuscript). We believe that this modification does not change the meaning of the manuscript.

We trust that these revisions are satisfactory and that our manuscript will now be accepted for publication in the *World Journal of Gastroenterology.* Again, thank you very much for reviewing our manuscript. We hope to receive an early and favorable reply.

Sincerely yours,

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