August 22, 2013

Dear Editor,

Please find the enclosed edited manuscript in Word format (file name: 4242-Answering Reviewers.doc).

Title: Differentiation of dysplastic nodule from early stage HCC: utility of conventional MR imaging

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

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

(1) Reviewer (02444850): Into the discussion section the role of contrast enhanced ultrasound (CEUS), for differentiation between dysplastic nodule and HCC has to be mentioned.
Answer: Thanks for your comments. We have added a paragraph describing the role CEUS plays in differentiating between dysplastic nodules and HCC in the Discussion section..

(2) Reviewer (01713316): In methods section: please define 2 grades of Edmonston-steiner grades of well differentiated HCC
Answer: Thanks for your comments. We have modified our description to make it more clear and have added two references. The presence of high cellularity, diffuse capillarization, abnormal biliary canaliculi and stromal invasion are considered features of well-differentiated HCC, Edmondson-Steiner grade I (w1-HCC). The presence of nuclear pleomorphism and thicker trabecular growth are features characteristic of Edmondson-Steiner grade II, but are also characteristic of well-differentiated HCC in the WHO system (w2-HCC)..

The grading system of Edmondson and Steiner
Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. Cancer. 1954;7:462-503.

Grade I Reserved for those hepatocellular carcinomas where the difference between the tumor cells and hyperplastic liver cells is so minor that a diagnosis of carcinoma rests upon the demonstration of more aggressive growths in other parts of the neoplasm.
Grade II Cells show marked resemblance to normal hepatic cells. Nuclei are larger and more hyperchromatic than normal cells. Cytoplasm is abundant and acidophilic. Cell borders are sharp and clear cut. Acini are frequent and variable in size. Lumina are often filled with bile or protein precipitate.
Grade III Nuclei are larger and more hyperchromatic than grade II cells. The nuclei occupy a relatively greater proportion of the cell (high N:C ratio). Cytoplasm is granular and acidophilic, but less so than grade II tumors. Acini are less frequent and not as often filled with bile or protein precipitate. More single cell growth in vascular channels is seen than in grade II.
Grade IV Nuclei are intensely hyperchromatic. Nuclei occupy a high percentage of the cell. Cytoplasm is

variable in amount, often scanty. Cytoplasm contains fewer granules. The growth pattern is medullary in character, trabeculae are difficult to find, and cell masses seem to lie loosely without cohesion in vascular channels. Only rare acini are seen. Spindle cell areas have been seen in some tumors. Short plump cell forms, resembling “small cell” carcinoma of the lung are seen in some grade IV tumors.

The grading system of WHO

Hepatocellular carcinoma. In: Hamilton SR, Aaltonen LA, eds. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Digestive System. Lyon, France: IARC Press; 2000.

Well-differentiated hepatocellular carcinomas: The lesions are composed of cells with mild atypia and increased nucleus-to-cytoplasm ratio in a thin trabecular pattern, with frequent psuedoglandular or acinar structures.

Moderately differentiated hepatocellular carcinomas: The lesions are characterized by trabecular growth of three or more cells in thickness. Tumor cells have abundant eosinophilic cytoplasm and round nuclei with distinct nucleoli. A pseudoglandular pattern is frequent, and pseudoglands often contain bile or proteinaceous fluid.

Poorly differentiated hepatocellular carcinomas: It grows in a solid pattern without distinct sinusoid-like blood spaces; only slit-like blood vessels are observed in large tumor nests. The neoplastic cells show an increased nucleus, cytoplasm ratio, and frequently pleomorphisms, including bizarre giant cells.

(3) Reviewer (01713316): what is the protocol on screening for HCC in the author's institution? Is MR the initial imaging modality? If not when is the MR used in the algorithm? If the initial imaging modality is different from MR, then what are the characteristics and initial diagnosis of lesions on this imaging

Answer: Thanks for your comments. In our institute, ultrasonography is the imaging modality used for screening patients at risk of HCC. In patients with ultrasonographic evidence of HCC, Gd-DTPA-enhanced MR imaging is used for further diagnostic workup.

(4) Reviewer (01713316): How many of 73 patients had AFP > 100 and how many were above 200

Answer: Thanks for your comments. Of the 73 patients, one patient with w2-HCC had AFP > 100 ng/mL and six patients (w1-HCC, 5; w2-HCC, 1) had AFP > 200 ng/mL. None of the patients with dysplastic nodules had AFP > 100 ng/mL. The data have been added to Table 1 in the revised manuscript.

(5) Reviewer (01713316): It would strengthen the paper if authors can provide an attrition diagram on their selection cohort from the initial sample group

Answer: Thanks for your comments. We have added a flowchart describing how patients were selected.

(6) Reviewer (01560772): In the title of the manuscript, it is written as “early stage HCC”. However, in Table 1, mean tumor size was as large as 1.8 cm in the DN group, 2.1 cm in the w1-HCC group and 2.9 cm in the w2-HCC group. As shown in the Hepatology Vol 49, pages 658–664, 2009 (Kojiro et al), if tumors reach 1.5 to 2 cm in diameter, they tend to be de-differentiated resulting in moderately differentiated HCCs. Generically, an early stage HCC is considered as an nodule smaller than 1.5cm. Thus it may be inadequate to use the term of “early stage HCC” in this manuscript.

Answer: Thanks for your comments. Although early HCCs are usually <2 cm in diameter, some early HCCs and nodule-in-nodule-type HCCs measuring 5 cm have been encountered[a,b]. Several studies use the term “early stage HCC” to describe small, well-differentiated HCC[c].

[a]Fujii et al, Imaging of large early and early advanced hepatocellular carcinomas of more than 5 cm in diameter: report of two cases. Hepatogastroenterology 1998;45:1085–92.

[b]Kudo M. Atypical large well-differentiated hepatocellular carcinoma with benign nature: a new clinical entity. Intervirology 2004;47:227–37.
[c]Haradome et al. Additional value of gadoxetic acid-DTPA-enhanced hepatobiliary phase MR imaging in the diagnosis of early-stage hepatocellular carcinoma: comparison with dynamic triple-phase multidetector CT imaging. J Magn Reson Imaging. 2011 Jul;34(1):69-78.

(7) Reviewer (01560772): In Table 3, arterial enhancement was observed in 12/40 (30%) patients of the w1-HCC group and in 13/19 (68%) patients of the w2-HCC group. However, such large nodules (size: 2-5cm) could have much higher arterial enhancement rate, especially in w2-HCC group. Therefore, it seems insufficient to judge arterial enhancement only by dynamic MR imaging in those large-sized tumors. To evaluate the arterial enhancement of the tumors more accurately, the authors should analyze arterial phase with other imaging modalities (i.e., conventional dynamic CT, CT angiography or contrast enhanced ultrasonography, etc.).
Answer: Thanks for your comments. Our study was a retrospectively study. It is impossible to evaluate the study tumors with other imaging modalities. According to the diagnostic criteria for HCC used in our institute, patients with tumors demonstrating atypical features in dynamic MRI should undergo dynamic CT study for further evaluation. CT angiography and contrast-enhanced ultrasound are not unavailable in our institute.

(8) Reviewer (01560772): Previous MRI studies reported that hyperintensity on DWI was also related to HCC de-differentiation in addition to that on T2-WI. The authors had better add DWI imaging analysis to this manuscript.
Answer: Thanks for your comments. The DWI was not a standard imaging protocol during the study period. Because this study is retrospective, it is impossible to evaluate the study tumors with DWI. We agreed with you that DWI might help differentiate dysplastic nodules from HCC.

(9) Reviewer (01560772): Generically, late phase washout is seen more frequently than arterial enhancement in early stage HCCs. However, the rate of late phase washout was lower than that of arterial enhancement in the w1-HCC and w2-HCC groups [w1-HCC: 9/40(22.5%) vs. 12/40(30%) and w2-HCC: 9/19(47%) vs. 13/19(68%)] in the manuscript. The author should clarify the reason.
Answer: Thanks for your comments. We re-checked all images of the 73 study tumors and the results were the same. One possible reason is that many of the well-differentiated HCCs in our study were hyperintense on precontrast T1WI, resulting in hyperintense/isointense images in the late phase of the dynamic study. Another reason might differences in patient populations being examined. We hope answer addresses your concern.

(10) Reviewer (01560772): In Table 3, arterial enhancement was observed in 5 DNs. To the best of my knowledge, no DNs show arterial enhancement. Therefore, the author should explain the reasons. ?
Answer: Thanks for your comments. Many studies have reported that DNs can depict arterial enhancement[a,b,c].
[a]Freeny et al. Significance of hyperattenuating and contrast-enhancing hepatic nodules detected in the cirrhotic liver during arterial phase helical CT in pre-liver transplant patients: radiologic-histopathologic correlation of explanted livers. abdom Imaging. 2003;28:333-46.
[b]Krinsky et al. Dysplastic nodules in cirrhotic liver: arterial phase enhancement at CT and MR imaging--a case report. Radiology 1998;209:461-4.
[c]Taouli et al. Magnetic resonance imaging of hepatocellular carcinoma. Gastroenterology 2004;127 Suppl 1:S144-52.

(11) Reviewer (01560772): In case of the existence of multiple nodules in one patient, which nodule did the authors select for the analysis? The authors should clarify the selection criteria.

Answer: Thanks for your comments. In our study design, only tumors that underwent histological examination were selected for imaging analysis.

(12) Reviewer (01560772): In Table 1, unit for the size of nodules is lacking, might be “cm”?

Thanks for your comments. We have corrected the mistake.

3 References and typesetting have been corrected

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

Sincerely yours,

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