June 15, 2016

Ze-Mao Gong,
Science Editor, Editorial Office
Baishideng Publishing Group Inc.

RE: Ms. No. 25258

Dear Professor Gong,

We thank you for the additional opportunity to revise our manuscript, entitled **"Practice guidelines for the pathological diagnosis of primary liver cancer: 2015 update".**

Detailed point-by-point replies to the reviewers' comments in addition to our revised manuscript have been resubmitted. All changes made in the revised manuscript were indicated using track changes in the revised manuscript and by line number in the response letter.

We hope our revised manuscript could be acceptable for publication in *World Journal of Gastroenterology.* We thank you once more for your time and consideration.

Sincerely,

Wen−Ming Cong, M.D., Ph.D.
Professor of Pathology
Director of Department of Pathology
Eastern Hepatobiliary Surgery Hospital
The Second Military Medical University
Shanghai, 200438, China
Tel.: +86 21−81875191
Fax: +86 21−81875191
E-mail: wmcong@smmu.edu.cn

REVIEWERS' COMMENTS

Reviewer #1

COMMENTS TO AUTHORS
The Authors report an update of guidelines for pathologic diagnosis of primary liver cancer. A few revisions are needed

Major Comments

Comment 1) It is not clear why the Authors defined Small HCC (SHCC) tumor less than 3 cm. Actually, according to the Barcellona staging system accepted by the EASL and AASLD, difference in outcome and treatment are mainly based between "very early" and "early stage" which is less than 2 cm and less than 3 cm, respectively. Since the Authors state that there is a different pathologic behaviour and outcome between small HCC and HCC, I suggest to the Authors to use "2 cm" as a cut off for small HCC and not 3 cm

Response: Thank you very much for your suggestions and comments. However, we still hope to keep the original definition. As we mentioned in the manuscript on lines 246-253 and 258-271, the definition of SHCC with the Chinese characteristics is ≤3 cm in diameter by the Chinese Pathology Working Group for Liver Cancer [12], and the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer (2011 Edition) proposed by the National Health and Family Planning Commission of China [13] for the following reasons:

(1) Studies indicating that a SHCC growing near to or larger than 3 cm in diameter is an important turning point in the transformation from relatively benign features to more aggressive tumor behaviors [16,17]. However, the unique genetic changes in those with SHCC ≤3 cm in diameter during the early stage have been reported [18,19];
(2) Patients with tumors >3 cm have an increased risk for microvascular invasion (MVI) and the presence of satellite nodules, as well as poor prognosis [17,20]. Specifically, the overall postoperative 5-year survival and recurrence-free survival of patients with SHCC ≤3 cm are 67.8% and 52%, respectively, which are significantly higher than that of 42.3% and 29.3%, respectively in patients with HCC >3 cm ($P < 0.001$) [17,21]; and
(3) Most studies of patients with SHCC ≤2 cm are based on multi-center joint studies with long-term data collection (Table 1, [16]) because too few surgical cases in a single center exist. At present, there are almost no systematic studies or knowledge based on a large series of cases that describe the pathobiological characteristics of SHCC ≤2 cm [16,19,22].

Table 1 Information about studies on ≤2-cm SHCCs in the literature

Years	Authors	SHCC/total	Survey periods	5-year survival	No. of units
1987	Kondo et al. [52]	15/–	10 years	–	2
1992	Nagao et al. [53]	23/–	10 years	61 %	1
1995	Nakashima et al. [54]	27/–	8 years	–	1
1998	Takayama et al. [55]	80/1,172	10 years	93–54 %	2
2000	Arii et al. [51]	1,318[a]/8,010	8 years	71.5 %	≈800 (LCSGJ)
2002	Vauthey et al. [15]	57/591	18 years	59–50 %	4
2004	Ikai et al. [56]	2,320/12,118	10 years	–	≈800 (LCSGJ)
2005	Wu et al. [36]	45/–	17 years	Median: 138 months	1
2006	Ando et al. [57]	91[a]/574	6 years	55.2 %	1
2007	Minagawa et al. [58]	2,767/63,736	7 years	70 %	829 (LCSGJ)
2008	Forner et al. [59]	60[a]/89[a]	4 years	–	2
2008	Livraghi et al. [60]	218/– (RFA)	11 years	68.5 %	5
2009	Farinati et al. [61]	65[a]/1,834[a]	18 years	Median: 60 months	10 (ITA.LI.CA)
2009	International Consensus Group for Hepatocellular Neoplasia (ICGHN) [62]	23/– (in 2002)[b] + 22/– (in 2004)[b]	–	–	3 ?
2010	Takayama et al [63]	1,235/– (surgery)	4 years	2-year: 94 %	≈800(LCSGJ)
2011	Di Tommaso et al [64]	47/86 (biopsy)	5 years	–	2
2012	Yamashita et al [65]	149/–	16 years	67–87 %	2

[a] Some patients were pathologically confirmed

[b] Small hepatic nodular lesions, including low- and high-grade dysplastic nodules and HCCs

References

16. Cong WM, Wu MC. Small hepatocellular carcinoma: current and future approaches. Hepatology international 2013; 7(3):805-812. [PMID: 26201917. doi: 10.1007/s12072-013-9454-z]
17. Lu XY, Xi T, Lau WY, Dong H, Xian ZH, Yu H, Zhu Z, Shen F, Wu MC, Cong WM. Pathobiological features of small hepatocellular carcinoma: correlation between tumor size and biological behavior. Journal of cancer research and clinical oncology 2011; 137(4):567-575. [PMID: 20508947. doi: 10.1007/s00432-010-0909-5]
18. Moribe T, Iizuka N, Miura T, Kimura N, Tamatsukuri S, Ishitsuka H, Hamamoto Y, Sakamoto K, Tamesa T, Oka M. Methylation of multiple genes as molecular markers for diagnosis of a small, well-differentiated hepatocellular carcinoma. International journal of cancer. Journal international du cancer 2009; 125 (2):388-397. [PMID: 19384946. doi: 10.1002/ijc.24394]
19. Llovet JM, Chen Y, Wurmbach E, Roayaie S, Fiel MI, Schwartz M, Thung SN, Khitrov G, Zhang W, Villanueva A, Battiston C, Mazzaferro V, Bruix J, Waxman S, Friedman SL. A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. Gastroenterology 2006; 131 (6):1758-1767. [PMID: 17087938]
20. Ueno S, Kubo F, Sakoda M, Hiwatashi K, Tateno T, Mataki Y, Maemura K, Shinchi H, Natsugoe S, Aikou T. Efficacy of anatomic resection vs nonanatomic resection for small nodular hepatocellular carcinoma based on gross classification. Journal of hepato-biliary-pancreatic surgery 2008; 15(5):493-500. [PMID: 18836803. doi: 10.1007/s00534-007-1312-8]
21. Wu MC. Attention should be paid to the diagnosis and treatment of small hepatocellular carcinoma. National Medical Journal of China. 2007; 87:2089-2091.
22. Farinati F, Sergio A, Baldan A, Giacomin A, Di Nolfo MA, Del Poggio P, Benvegnu L, Rapaccini G, Zoli M, Borzio F, Giannini EG, Caturelli E, Trevisani F. Early and very early hepatocellular carcinoma: when and how much do staging and choice of treatment really matter? A multi-center study. BMC Cancer 2009; 9:33. [PMID: 19171074. doi: 10.1186/1471-

2407-9-33]

Comment 2) The Authors clearly defined pathologic examination (MVI, Satellite nodule, combined HCC-CCC, etc), but there was few details about cholangiocarcinoma. It would be better to define that these guidelines are for hepatocellular carcinoma.

Response: Thank you very much for your suggestions and comments. As we mentioned in the Introduction, primary liver cancers (PLC) include malignancies that originate from the hepatocytes (hepatocellular carcinoma, HCC), which account for the majority of PLC, and intrahepatic cholangiocytes (intrahepatic cholangiocarcinoma, ICC). Thus, the present guidelines, including “Sample collection and tissue fixation and processing”, “Description of microscopic tissue characteristics”, “Description of precancerous lesions and Immunohistochemical diagnosis”, etc., are applicable for HCC as well as ICC. Therefore, we chose the title, “Practice guidelines for the pathological diagnosis of primary liver cancer”.

Minor Comments

Comment 1) On page 15, classification of hepatocellular adenoma is not related to this manuscript; it should be eliminated (high grade displasia is completely another disease)

Response: Thank you very much for your suggestions and comments. We mentioned the molecular pathological subtypes of hepatocellular adenoma in the “Description of precancerous lesions” subsection because (1) hepatocellular adenoma (HCA) is a precancerous lesion, (2) β-catenin-activated HCA may have a higher risk of malignant transformation than the other types, and (3) HCA should be subtyped into four molecular pathological subtypes.

Comment 2) On page 16, difference in MVI is not only due to "sample collection and diagnostic criteria" but also to different type of tumor included in the analysis; if you analyze series with resected tumor from 2 cm up to more than 10 cm is obvious that MVI differs significantly (as the Authors report Pawlik's paper). This statement should be changed

Response: Thank you very much for your suggestions and comments. We changed the statement and emphasize that there is a partial correlation with the sample collection protocol and diagnostic criteria (line 342). For example, the 7-point sampling protocol aimed to increase the positive detection rate of MVI, which would be difficult in cases where the number of sampling tissues is too small irrespective of the tumor size.

Reviewer#2

COMMENTS TO AUTHORS

It is of great importance to update the guidelines of the pathological diagnosis for the primary liver cancers. Thus, the Expert Committee organized several seminars for guideline formulation, mainly focusing on the following topics: gross specimen sampling, concepts and diagnostic criteria of small HCC, microvascular invasion, satellite nodules, immunohistochemical and molecular diagnosis .The final version of the 2015 guidelines had been approved at the last Expert Committee meeting, held in April 11, 2015 in Shanghai, China. It is an interesting work, however, the written language should be modified by a native English speaker. Moreover, the authors would better to list the biomarkers for diagnosis, differential diagnosis, prognosis and therapy in a table.

Response: Thank you very much for your suggestions and comments. As suggested by the reviewer, the revised manuscript has been edited by a native English speaker to remove the grammatical and typographical errors.

Regarding the suggestion to list the biomarkers for diagnosis, differential diagnosis, prognosis and therapy in a table, we did not include the suggested table because (1) some immunohistochemical markers mentioned in the guidelines are not used for evaluating the prognosis of a patient or differential therapy and (2) some markers are simply used to confirm the hepatocyte or cholangiocyte origins, which can be easily explained in text.