Author's response to reviews

Title: Microvascular invasion (MVI) is a poorer prognostic predictor for small hepatocellular carcinoma

Authors:

Min Du (vdumin@163.com)
Lingli Chen (chen.lingli@zs-hospital.sh.cn)
Jing Zhao (zhao.jinq@zs-hospital.sh.cn)
Feng Tian (11311210001@fudan.edu.cn)
Haiying Zeng (zeng.haiying@zs-hospital.sh.cn)
Yunshan Tan (tan.yunshan@zs-hospital.sh.cn)
Huichuan Sun (sun.huichuan@zs-hospital.sh.cn)
Jian Zhou (zhou.jian@zs-hospital.sh.cn)
Yuan Ji (ji.yuan@zs-hospital.sh.cn)

Version: 4 Date: 17 January 2014

Author's response to reviews: see over
Dear Dr Masahide Ikeguchi:

Thank you and all the reviewers for the critical comments. We feel very fortunate that our manuscript entitled “Microvascular invasion (MVI) is a prognostic predictor for small hepatocellular carcinoma (MS: 7041244110485920)” went to these reviewers as the positive and constructive comments from them helped us improve the manuscript greatly.

Accordingly, we have revised the manuscript and responded, point by point, to the comments as listed below. We have made minor modification in the title, the description of pre-operation and post-operation AFP level, tumor infiltrative lymphocytes and also added the incidence and proportion of HCC, SHCC patients in our institution. Meanwhile, we described the definition of progression survival, overall survival and microvascular invasion more explicitly, also made minor modification in the description of statistical analysis. All changes are underlined in red.

If you and/or the reviewers have any additional comments or questions, we are also happy to respond accordingly, to future improve our manuscript.

We would like to re-submit this revised manuscript titled “Microvascular invasion (MVI) is a poorer prognostic predictor for small hepatocellular carcinoma” to BMC Cancer, and hope that it is acceptable for publication in the journal.

Looking forward to hearing from you soon.

With kindest rewards,

Yours sincerely,
Min Du
E-mail:vdumin@163.com, Tel: 18721934096

Yuan Ji
E-mail:newera_ji@yahoo.com, Tel: 13681858366
Itemized response to reviewers’ comments

Replies to Professor Zhao-chong Zeng:

Question 1. Title: I have a suggestion, the title should add “a poorer prognostic predictor”.

Answer: Thank you very much for your suggestion, we have added “a poorer prognostic predictor” in the title.

Question 2. Abstract, results: One hundred forty-seven (33.0%) patients had serum alfa-fetoprotein (AFP) level ≥ 200 ug/ml and 178 (63.8%) patients had post-operation serum AFP level ≥ 20 ug/ml. This sentence is difficult understand. Please see point 6.

Answer: We are sorry for the misunderstanding due to unclear descriptions in our previous manuscript. According to your suggestion in Question 2 and 6, we have modified the sentence as the following “One hundred forty-seven of the 446 (33.0%) patients with pre-operation serum AFP level record had serum alfa-fetoprotein (AFP) level ≥ 200ug/ml before surgery and 178 of the 280 (63.8%) patients with post-operation serum AFP level record had AFP level ≥ 20ug/ml.” in the third line of the Results, page 2 in the revised manuscript.

Question 3. Methods in page3: from January 2006 to December 2008 in the authors’ institution were reviewed. Please tell readers how many HCC were treated in your hospital, and how many HCC patients received surgical resection. If you can provide this figure, readers will better understand the proportion of small HCC in the whole HCC group.

Answer: Thanks for your comment, we have added the number of HCC, SHCC
patients, proportion of SHCC and SHCC patients with MVI who had surgery in Zhongshan Hospital from 2006 to 2008 in Methods in page 5. The number of HCC patients underwent resection in Zhongshan Hospital from 2006 to 2008 is 3467 (1016, 1162, 1289 respectively) cases, of which 1376 cases (40%) were SHCC patients. Approximately 17% of SHCC patients (234) had MVI in these three years.

Question 4. The authors should provide the information on pathologic characteristics of resected specimens, especially HCC relative immune historychemistry.

Answer: Thanks for your suggestion. We have provided patients pathologic characteristics including tumor histological differentiation, tumor subtype, microvascular invasion and tumor infiltrative lymphocytes in Table1 in previous manuscript. Diagnosis of HCC largely rely on morphological features and supplemented by routine histochemical stains sometimes[1, 2]. In our study, most patients were moderate or poorly differentiated tumors, morphologic features are enough for the diagnosis. As such, using of immunohistochemical examination is dispensable for diagnosis of HCC.

The value of immunohistochemical examination for diagnosis of HCC is limited due to the lack of sensitivity and specificity. Although Serum AFP levels are helpful for the diagnosis of HCC, its immunostains in a tumor is specific for hepatocellular differentiation if germ cell tumors can be excluded; however, staining tends to be patchy, and sensitivity is 30% to 50%[3-5]. HepPar1 has emerged as the most sensitive and specific immunohistochemical marker for HCC, but yields a diffuse cytoplasmic granular staining pattern in normal and neoplastic hepatocytes and also tends to lack
of expression in poorly differentiated HCCs\textsuperscript{[3, 6]}. GPC-3 is normally expressed in fetal liver and placenta but not in normal adult liver\textsuperscript{[7]}, however, the sensitivity and specificity of GPC3 for the diagnosis of small HCC were 77\% and 96\% respectively in resected cases\textsuperscript{[8]}.

We are very pleased to summarize and provide the information on pathologic characteristics of SHCC patients as well as HCC relative immune histochemistry if you still deem its necessity.

Question 5. Follow-up: the last date of follow-up.

Answer: Thanks for the suggestion. The last date of follow-up is July 6\textsuperscript{th}, 2012. Mean follow-up was 54 months (4~75 months). We had added it in the fourth line in page 6 in revised manuscript.

Question 6. Results of Clinical pathological characteristic in page4: Please add the patient number with AFP # 20 µg/L because the authors present the patient number who AFP # 20 µg/L for those with post-operation. It is easy to confuse that AFP failure is higher as point out in comment2.

Answer: Thank you very much for explaining this question so carefully, we have modified the sentence in the third line of the Results, page 2 in the revised manuscript.

Question 7. Figure1. The authors can merge figure1 a and b to be 1, i.e. the survival curves of OS and DFS in the same figure.

Answer: We are sorry for making a misunderstanding in Figure 1 and thanks very much for your suggestion. I have corrected Figure 1 using SPSS software and added p
value in the revised manuscript.

Replies to Professor Lijian Hui:

Minor Essential Revisions:

Question 1. Currently there are still some debates on the definition of microvascular invasion (MVI), what’s the criteria for MVI except for “presence of tumor emboli in a portal radicle vein, large capsule vessel or in a vascular space lined by endothelial cells” described in this manuscript?

Answer: We agree with your important viewpoint. Microvascular invasion has repeatedly been identified as a risk factor for recurrence and death after resection of HCC\textsuperscript{[9]}, which can encompass a wide spectrum, ranging anywhere from invasion of a single small vessel near the tumor capsule to just sort of gross vascular invasion. ROAYAIE et al proposed a classification system for vascular invasion based on a risk score linked with prognosis, which includes invasion of a vessel with a muscular wall and invasion of a vessel that is more than 1cm from the tumor\textsuperscript{[10]}. In our study, we
analyzed the vessels in the peritumor area which includes vessels with or without muscular wall and had been confirmed by immunostains of CD34, however, we didn’t record the distance of the vessels from the tumor. Maybe in the future, we will classify the microvascular invasion more precise.

Question 2. The authors demonstrate that except for MVI, all the other pathological factors have no prognostic value for SHCC patients. However, there are several previous studies show that tumor histological differentiation, AFP and some other factors were predictors for HCC patients, why these factors did not correlate with the survival in SHCC patients?

Answer: Thank you for raising this important issue. Indeed, we can’t figure out whether these factors are not associated with the survival of SHCC patients or MVI accounting for a significant proportion in prognosis that leading to a false unrelated result for these clinicopathological factors. So we analyzed the univariate and multivariate analysis of clinicopathological factors in 375 patients without microvascular invasion. Multivariate analysis demonstrated that tumor border and capsular formation predicted recurrence and survival of SHCC patients. None of the AFP, histologic differentiation, cirrhosis, tumor infiltrative lymphocytes predicted the survival of SHCC patients in univariate and multivariate analysis. It is demonstrated that tumor size can predict histologic grade and microvascular invasion and high histologic grade, alpha-fetoprotein level, multiple tumor nodules each predicted vascular invasion in tumors larger than 5cm[5, 11, 12]. As discussed in previous manuscript, we can conclude that due to SHCC is small in tumor volume, it is
reasonable that tumor histological differentiation, AFP and some other factors were not prognostic predictors.

Question 3. There is a mistake in Figure 1. The figure did not show the comparison between patients with MVI and without MVI. Moreover the authors should add the p value for Cox analysis.

Answer: Sorry for making such a mistake and thanks very much for your suggestion. We have altered Figure 1 and added p value on the Figure in revised manuscript.

Discretionary Revisions:

Question 1. The author described “Tumor inflammation” as the number of lymphocytes in tumor areas. However, inflammation is more than lymphocytes, may be tumor infiltrative lymphocytes more appropriate than tumor inflammation.

Answer: We agree with your comment and have altered all “tumor inflammation” into “tumor infiltrative lymphocytes (TIL)” in line 11 in page 2, line 6 in page 3; line 10 and the third line from the bottom in page 5, line 6 in page 8, Legends in Figure 2, Table 1 and Table 2.
References


7. Shafizadeh N, Ferrell L D, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the


