ANSWERING REVIEWERS

May 8, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 17409-edited.doc).

Title: Lymphocyte-to-monocyte ratio predicts survival of patients with hepatocellular carcinoma after curative resection

Author: Ze-Xiao Lin, Dan-Yun Ruan, Yang Li, Dong-Hao Wu, Xiao-Kun Ma, Jie Chen, Zhan-Hong Chen, Xing Li, Tian-Tian Wang, Qu Lin, Jing-Yun Wen, Xiang-Yuan Wu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 17409

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

To Reviewer #1:

(1) Page 5, materials and methods "active infection" should be rephrased so as not to be confused with the hepatitis B infection.

The authors' answer: We followed the suggestion and changed the phrase "active infection" into "acute inflammation".

(2) The number of patients excluded because of involved surgical margins and the number of patients excluded after recurrence 1 month after resection should be stated separately. Were any of the included patients treated with ablation in addition to resection, or were these patients also excluded? How many were they?

The authors' answer: We apologize that the inclusion and exclusion criteria of this study was not clearly described. We defined curative resection as complete resection of tumor with clear microscopic margins. And negative margins were achieved in all the enrolled cases. Regarding OS as the primary endpoint, we have excluded patients who died within 30 days after surgery during recruitment of this study. Besides, to avoid confounding factors from different treatment modalities, we have also excluded patients treated with intraoperative radiofrequency ablation prior patient enrollment. These exclusion criteria were added in the article.

(3) Discussion page 9, delete the wording 'in' in the phrase 'These results were in consistent with'. Is there an influence on lymphocytes and monocytes by the hepatitis B infection?

The authors' answer: We followed the suggestion. The impairment of immune responses is an important feature of chronic HBV infection, which manifests in the peripheral blood as the change of leukocyte subset counts. As a result, hepatitis B infection may influence the concentration of lymphocytes and monocytes. However, concentrations of lymphocytes and monocytes had been found different between chronic HBV infection and HCC patients with HBV infection[1].

To Reviewer #2:

(1) In the abstract section, the authors should emphasize that prospective validation of their results is essentially required

The authors' answer: We followed the suggestion and made the emphasis in the last paragraph of the

Abstract section.

(2) Some grammar mistakes and punctuation errors are present and I recommend language and grammar editing for the manuscript before publication.

The authors' answer: We apologize for the grammar mistakes and punctuation errors in the manuscript. The language was edited by American Journal Experts prior to resubmission.

(3) The authors selected the HCC patients "based on the typical dynamic images evaluated by CT or MRI ". However they didn't mention which guidelines they follow. Please clarify.

The authors' answer: The preoperative diagnosis of HCC was made according to the APASL guideline, which we clarified in the MATERIALS AND METHODS section.

(4) The work up for HBV and HCV diagnosis is not mentioned, do you exclude HCV subjects based on HCV antibodies or HCV PCR?

The authors' answer: In China, most cases of HCC develop from chronic HBV infection, which is defined by the continued presence of HBsAg in the blood for longer than six months. And the diagnosis of HCV infection depends on the detection of anti-HCV antibodies. We excluded HCV subjects based on HCV antibodies. We simply mentioned that "All the patients were HBV-infected and hepatitis C virus-negative" in the MATERIALS AND METHODS section, as referencing the articles "A scoring model based on neutrophil to lymphocyte ratio predicts recurrence of HBV-associated hepatocellular carcinoma after liver transplantation" (Wang GY, Yang Y, Li H, et al. PLoS One. 2011; 6: e25295), and "Prognostic value of preoperative peripheral neutrophil-to-lymphocyte ratio in patients with HBV-associated hepatocellular carcinoma after radical hepatectomy" (Fu SJ, Shen SL, Li SQ, et al. Med Oncol. 2013; 30: 721). We have changed this expression into "All the patients had chronic HBV infection and were negative for hepatitis C virus antibody".

(5) How many patients actually received the antiviral therapy? I think that adding this factor the hazards model of prognostic factors affecting the OS and RFS could add value for such a cohort with HBV related HCC if the data is available.

The authors' answer: We totally agreed with your opinion that antiviral therapy might work as one prognostic factor affecting OS and RFS. But unluckily we could not achieve the specific data about antiviral therapy and we apologize for that.

(6) please verify how did you document cirrhosis ? Was it on radiological basis or pathological basis after resection?

The authors' answer: Diagnosis of cirrhosis was based on the pathological report after surgery. We have clarified this point in the first paragraph of the RESULTS section.

(7) 110 patients developed tumor recurrences, what was the Mean time to recurrence?

The authors' answer: The mean time to recurrence in this study was 28.3 months.

(8) The ROC curves analysis for LMR, ALC and AMC were performed. However the authors didn't mention the AUC for ALC or AMC and The positive predictive and Negative predictive values (PPV &NPV) for LMR.

The authors' answer: We followed the suggestion and added the AUC for ALC and AMC in the RESULTS section. The positive predictive and negative predictive values for LMR were 91.1% and 26.4% respectively, which were calculated based on the sensitivity and specificity.

(9) In the Discussion section, the authors mentioned CRP and NLR as hematological markers for patients with HCC. More updated references are available.

The authors' answer: We followed the suggestion and updated the references.

To Reviewer #3:

(1) The figure of ROC curve for LMR, ALC and AMC should be provided.

The authors' answer: We followed the suggestion and added the figure of ROC curve for LMR in the revised manuscript. The ROC curves for ALC and AMC are shown as below (Figure 1 and 2), which may be provided as Supplementary Material for this article. Because they were not the main marker investigated in this study.

Figure 1 Receiver operating characteristics (ROC) curve assessing the cutoff value of ALC for survival

analyses

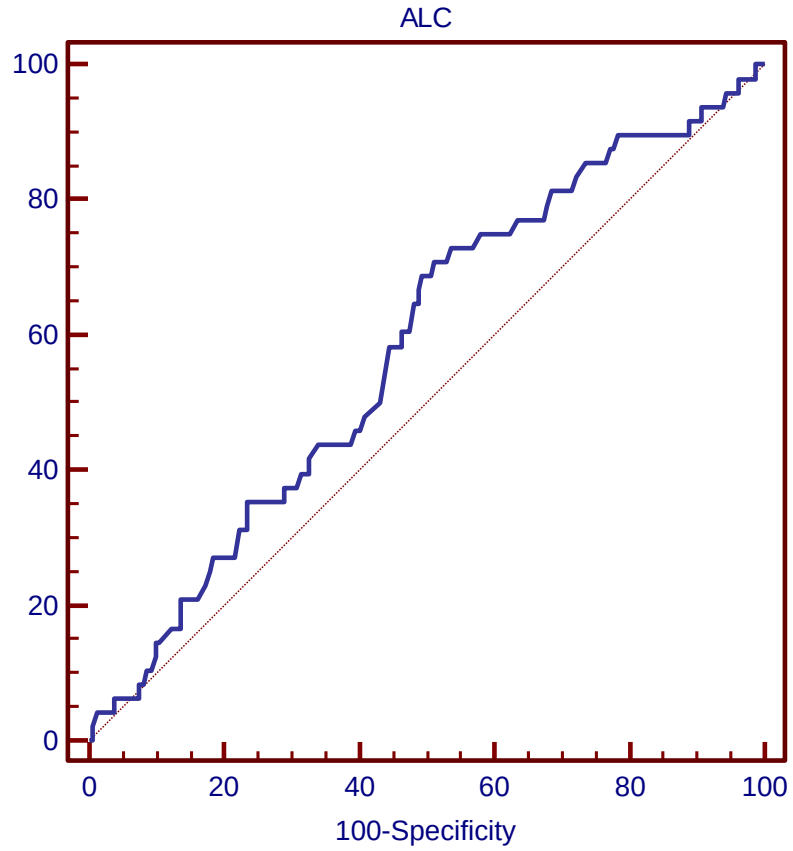
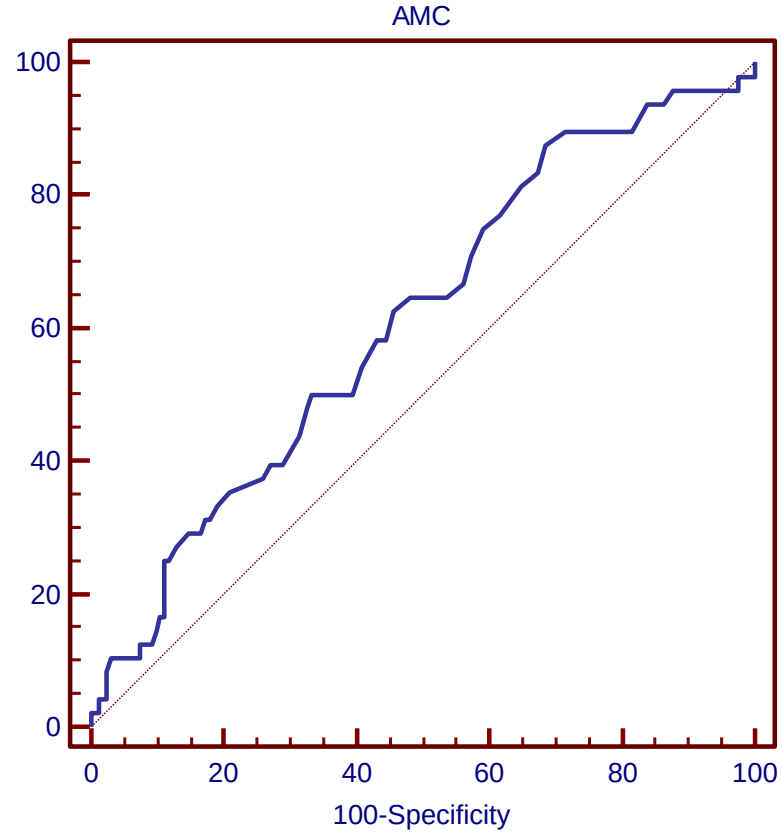


Figure 2 Receiver operating characteristics (ROC) curve assessing the cutoff value of ALC for survival analyses



(2) What additional value does the LMR value have in prognosis over the other independent predictors of survival (tumor size, encapsulation, multiple tumors) other than cirrhosis?

The authors' answer: Clinicopathological factors such as poor histological differentiation, advanced BCLC stage, elevated serum ALP level and microvascular invasion were identified as independent indicators of inferior prognosis. As we clarified in the INTRODUCTION section, clinical tumor parameters can only partially explain the prognostic heterogeneity of HCC. LMR is a novel inflammatory biomarker combining estimates of host immune homeostasis (i.e., absolute lymphocyte count) and tumor microenvironment (i.e., absolute monocyte count), which is different from other clinicopathological parameters. And results of multivariate Cox proportional hazard analysis also showed that LMR appeared to be the strongest predictor for survival.

(3) Would a low preoperative LMR perhaps lead to consideration of neoadjuvant therapies? Would following the LMR postoperatively aid in identifying recurrence earlier?

The authors' answer: Surgical resection and liver transplantation remain the cornerstones of curative treatment for HCC patients . After consideration of both tumor stage and the preserved liver function, if a HCC patient is suitable for surgery, he should receive the potential curative treatment in the first place. Our study revealed that low preoperative LMR level was an independent predictor of inferior OS and RFS. Patients with a low preoperative LMR may benefit from postoperative therapies. But further prospective studies are awaited to validate the clinical usages of LMR.

We totally agreed with your opinion that postoperative LMR might also help to identify early recurrence. And we have planned to expand the study cohort and investigate the impact of dynamic changes in the LMR before and after hepatectomy on prognosis in HCC.

(4) Does the author have any plans to assess patients in the future on a prospective basis or in a clinical trial looking at adjuvant therapies?

The authors' answer: Our study showed that LMR could be used to identifying HCC patients with poorer survival, who may benefit from adjuvant therapies. Before starting clinical trial on LMR based adjuvant treatment, we need to expand the study cohort prospectively to validate the clinical usages of LMR as a prognostic marker for HCC. That's our plan, as we addressed in the DISCUSSION section.

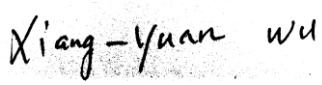
(5) The size of font don't match in Table 2 and 3.

The authors' answer: We apologize for the typesetting errors. And we have corrected them.

3 References and typesetting were corrected

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

Sincerely yours,



Department of Medical Oncology
The Third Affiliated Hospital of Sun Yat-sen University
600 Tianhe Road,
Guangzhou 510630, China
2015-05-08
E-mail: wuxiangy@mail.sysu.edu.cn

REFERENCES

1 **Li J**, Li J, Bao Y, Pan K, Lin X, Liu X, Wang H, Xu Y, Luo X, Li H, Duan C. Low Frequency of Peripheral Lymphocyte in Chronic Hepatitis B Patients Predicts Poor Progression to Hepatocellular Carcinoma. J Clin Lab Anal 2015 Jan 19. [PMID: 25600684 DOI: 10.1002/jcla.21838]