

October 8, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3741-Review.doc).

Title: The Neutrophil-Lymphocyte Ratio Predicts the Prognosis of Hepatocellular Carcinoma after Liver Transplantation

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**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 3741

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

**Reviewer 00068443**

*(1) This is an very interesting paper on a prognostic factor for liver cancer recurrence after transplantation. A possible discrepancy, yet quite important detail for diagnostic sensitivity and specificity, is that the neutrophil and lymphocyte counts are affected by several factors. This would not appear plausible and requires a bit of explanation. 1 Preoperative adjuvant therapy including transcatheter arterial chemoembolization and radio frequency, causes neutrophil and lymphocyte decrease or increase. 2 Hypersplenism leads leukocytopenia, which kind of leukocyte is affected deeply?*

- a. In our center, the HCC patients received LT at least one month after they had received preoperative adjuvant therapy when their blood test became normal.
- b. Patients who had hypersplenism were excluded from our study.

**Reviewer 00054001**

*(2) The authors stated that neutrophil-lymphocyte ratio (NLR) is a strong prognostic factor for outcome of liver transplantation for HCC from their experience. This manuscript is easy to understand and well organized. I consider this manuscript should be published with minor revisions. My concerns are as follows. 1. Did the authors analyze the relationship between outcome of LT and actual count of neutrophils or lymphocytes? In the Discussion session, the authors stated that high number of neutrophil is considered to be the abundant resource of VEGF that has been reported to be associated with vigorous tumor proliferation. Furthermore, it was also stated that high number of lymphocyte is suggested to reflect the strong anti-tumor immunity. I wonder which is critical. 2. The authors should refer to impact of hypersplenism on NLR or outcome of LT. In cirrhotic patients who are listed for liver transplantation, hypersplenism is common. It was reported that hypersplenism affects each blood cell not equally; i.e., it is likely to reduce rather*

neutrophil count than lymphocyte count. Furthermore, impact of splenectomy should be analyzed if available.

- a. Generally, the white blood cell counts of all patients included in our study were in the normal range. Patients with higher NLRs had higher actual counts of neutrophils and relatively lower lymphocyte counts. Whether neutrophils or lymphocytes have a larger impact on the tumor recurrence of HCC after LT is unclear, and the mechanism has not been clearly explored. Therefore, we need to perform more clinical and basic studies to further understand this phenomenon.
- b. Patients who had hypersplenism were excluded from our study.

#### **Reviewer 00069788**

(3) *This paper is a retrospective registry-based epidemiological study of HCC in China, attempting to examine the role of NLR as a predictor of long-term outcome. Due to the retrospective methodology and the mono-regional population results obtained in the paper cannot be safely generalized and multicenter confirmatory studies are needed. Nevertheless, the methodology applied is accurate and the scientific background sufficiently solid to justify publication after revision. Points for revision: 1. Consider revising the term retrospective cohort in the title. Cohort traditionally implies on-going research. In this case, the data is historical and therefore retrospective study seems more appropriate. 2. Review the use of the English language throughout the text, preferably by an English speaker. Language polishing is of particular importance, since the text becomes in a few cases confusing due to expressive weaknesses. Pay particular attention to the use of "significance". P values > 0.05 imply no statistically significant difference among the compared groups, not lack of significance of the survival values themselves (this misconception being repeated various times). 3. Please provide background on the average waiting time prior to LT in China in general and in the study population in particular. This is a major predictive factor for outcome not currently discussed in the text. 4. Explain the paradoxical lack of difference in terms of outcome between the 3 Child groups. Cirrhosis severity is known to be clearly associated to morbidity and mortality. Would there be any difference by using a more detailed system, such as the MELD score? What if specific indicators were examined separately (e.g. ascites, albumin or bilirubin). It would also be important to examine separately HCC patients without any evidence of cirrhosis, especially in a background of chronic HBV infection. 5. With regard to HBV status, the authors provide very limited information. For instance, no feedback is provided with regard to e antigen status, HBV DNA upon listing and pre-LT or medication provided. Although various oral agents are mentioned, it is not clear what the current local policy for drug administration selection is. Moreover, HCV status of the population is not commented in the study, nor is the presence of other pro-carcinogenic factors, such as aflatoxins, ALD or NASH background. Moreover, it would be useful to provide recent epidemiological data on overall HBV contribution to cirrhosis / HCC in China (to justify its high prevalence in the sample). 6. The authors take the position that Milan criteria should be expanded. This is indeed a controversial issue with many supporters and critics around the world. However, the study cannot provide evidence in this direction because in fact total tumor size was confirmed as an independent risk factor in the multifactorial model. Therefore, it seems more logical to rephrase the text so that the message is that additional factors other than tumor size may also be of predictive value, such as microvascular invasion and NLR and these could be further evaluated for inclusion in future, more comprehensive criteria. 7. NLR would be more reliable as a marker if more details could be provided with regard to recent use of antibiotics, previous hospitalizations for SBP / other infections and use of medication affecting leucocyte number and type. Remember that in immunocompromized patients (as are those with HCC and cirrhosis) infections take longer to resolve and occasionally emerge with atypical presentations, including cognitive impairment, weight loss or non-GI bleeding. If this information cannot be obtained via the registry, it should be discussed in detail in the limitations section to be taken into account in future studies.*

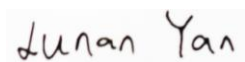
- a. The title of this manuscript has been revised.
- b. The language of this manuscript has been polished by language editing company. And we have revised the errors in the paper.

- c. In our center, the median waiting times for living donor and deceased donor LT were 0.9 and 1.6 months, respectively.
- d. In our study, we found that the Child score did not affect tumor recurrence and overall survival of HCC after LT. Perhaps the number of patients in this study was too low to illustrate this issue.
- e. The e antigen status, which represents HBV-DNA, of several HBV-related HCC patients was positive, and these patients received anti-viral drugs, such as Lamivudine, Adefovir, Telbivudine and Entecavir, prior to or after transplantation. The HCV-positive patients were excluded from this study. Of the 280 patients included in this study, 263 (93.9%) were HBV(+). The carcinogenic factors of 17 HCC patients may have been alcohol because they had a history of alcohol abuse.
- f. We have revised our manuscript to make it more logical.
- g. In the limitations section, we have presented more factors that affect the NLR.

3 References and typesetting were corrected

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

Sincerely yours,



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