May 18, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 18022-Review.doc).

Title: Prognosis of acute-on-chronic liver failure patients treated with artificial liver support system

Author: Piqi Zhou, Shaoping Zheng, Min Yu, Shengsong He, Zhihong Weng

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 18022

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) **Reviewed by 00053659**: The study is interesting if they could mention about liver transplantation and validation of their results. Indication of liver transplantation should be mentioned. The patient selection is unclear. What happen to transplant patients? The study is based on derivation study alone. The validation study should be performed.

Answers: In this study, patients who met the diagnostic criteria for acute-on-chronic liver failure (ACLF) were hospitalized, and they were treated with plasma exchange (PE) or plasma bilirubin adsorption (PBA) combined with PE. Liver transplantation was not available for patients owing to the shortage of liver and/or the high cost of the operation.

In the revised manuscript, another group of ACLF patients who were admitted to our hospital from 1 January 2014 to 31 December 2014 were enrolled into the validation cohort (n = 68). The performance of the new model based on derivation study was tested in the validation cohort by a receiver–operator curve (ROC).

(2) **Reviewed by 00069340:** Comments: 1. The title "Prediction of the prognosis of patients with acute-on-chronic liver failure treated with artificial liver support system" should be more specific. 2. The authors believe that "ACLF patients treated with PBA plus PE had better outcomes than patients treated with PE." To this end, the authors need to provide the indications for the patients to be treated with

plasma exchange or with plasma bilirubin adsorption combined with plasma exchange. 3. There are several "so on" and "etc" in the paper. The author need to specify not just state like these. 4. Statisticians should be consulted about the equation established by the authors. 5. There are many grammatical and writing problems in the paper, such as .....

Answers: a. The title was revised to "Prognosis of acute-on-chronic liver failure patients treated with artificial liver support system". It is difficult to specific because that the title should be no more than 12 words, as requested by *World Journal of Gastroenterology*.

b. The indications for the patients to be treated with PE or with PBA combined with PE: patients who met the diagnostic criteria for ACLF were hospitalized, and except standard medical therapy (SMT) they were treated with PE or PBA combined with PE. ACLF patients with persistent bleeding, circulatory shock, severe bacterial infection, pregnancy, international normalized ratio (INR) ≥ 3.0 or platelet count ≤ 30,000/μL were excluded for artificial liver support system (ALSS). The indications were stated in the revised manuscript in red.

c. The "so on" and "etc" in the paper were revised in the resubmitted manuscript.

d. The statistical methods of this study were reviewed by Dr. Hongbo Jiang from the Department of Epidemiology and Biostatistics, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, China.

e. The grammatical and writing problems in the paper were revised in the resubmitted manuscript.

(3) **Reviewed by 02445121:** There are some problems needed to be explained clarified by the authors. 1. The paper was relatively poor in English writing. So this paper must be rewritten in grammatical English with the help of a native English speaking-scientist or scientific English editing service. 2. In this study, the definition of ACLF is not sufficient, because there are conflicts about definition of ACLF. 3. How to define the criterion of scoring and how to choose the parameters for the novel model? How much were the heavyweight of different parameters? 4. How long time would the novel model be able to predict the prognosis of the patients with ACLF?

Answers: a. The paper was rewritten in grammatical English with the help of a scientific English editing service company (American Journal Experts).

b. In the revised manuscript, according to the recommendations generated by the Asian Pacific Association for the Study of the Liver, ACLF was defined as acute liver injury emerging as jaundice and coagulopathy, complicated by ascites and/or encephalopathy within 4 weeks in a patient with known or unknown chronic liver disease. And the definition of liver failure in ACLF: severe jaundice (total serum bilirubin ≥ 5mg/dl) and coagulopathy (INR ≥ 1.5 or prothrombin activity < 40%) which were indispensable; ascites and/or encephalopathy which diagnosed by physical examination (Sarin SK, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). Hepatol Int 2009; 3(1):269-282).

c. In this study, we investigated the correlation between survival and clinical parameters such as age, gender, etiology, the number of complication, the type of ALSS, serum biomarkers tested at

baseline including total bilirubin (TB), ALT, INR and creatinine, etc. In the bivariate analysis, some factors evaluated at baseline showed predictive impact on overall survival such as the type of ALSS, age, the number of complication, MELD score, TB, ALT, AST, and INR. However when variables with bivariate significance were selected into in a multivariate Cox regression model, the type of ALSS, age, the number of complications and MELD score were defined as independent predictive factors for survival.

The MELD score can be calculated using the equation: 9.57x ln[creatinine (mg/dl)] + 3.78 x ln[bilirubin (mg/dl)] + 11.2 x ln(international normalized ratio) + 6.43(etiology: 0 if cholestatic or alcoholic, 1 otherwise). The complications include hepatic encephalopathy (HE), spontaneous bacterial peritonitis (SBP), hepatorenal syndrome (HRS), upper gastrointestinal hemorrhage, and electrolyte disturbance. The type of ALSS include PE and PE plus PBA (we assigned 0=PE and 1=PE plus PBA). According to the multivariate model, a risk score (R) can be calculated using the formula: R = 0.03*(age) + 0.097*(MELD score) + 0.527*(the number of complications) - 0.79*(the type of ALSS), then the heavyweights of different parameters were 0.03, 0.097, 0.527 and 0.79, respectively.

d. In this study, we generated a multivariate Cox proportional hazards model by SPSS software. Based on this model, a risk score (R) can be calculated by the formula: R = 0.03*(age) + 0.097*(MELD score) + 0.527*(the number of complications) - 0.79*(the type of ALSS). Using the means of covariate (age, MELD score, the number of complications and the type of ALSS) of the derivation cohort in this study showed by SPSS, the mean risk score of 3.7 can be calculated according to the formula. At the same time, the survival table generated by the SPSS showed the estimated survival probabilities for a patient with a risk score of 3.7. We defined these survival probabilities as $S_0(t)$. To calculate the probability of survival at t days of a patient could use the following equation: $S(t)=S_0(t)^{\exp(score-3.7)}$, as described previously (Yang JD, et al. Model to estimate survival in ambulatory patients with hepatocellular carcinoma. Hepatology 2012; 56:614-621). However, the novel model can't tell the **exact** survival time of the patients with ACLF.

(4) **Reviewed by 00503572:** I have some comments: 1.- Authors obtain a multivariate Cox model whose AUROC is better than MELD for prediction. I think the description of how to calculate Risk score (R) is difficult to understand. I Suggest better describe this parameter in order to reproduce it . 2.- Limitations of the study are not discussed. One of the main one in my opinion is its retrospective design. I suggest discuss about this issue. 3.- The clinical characteristics of the cohort may limit its application in other populations such as western countries patients where alcohol and drugs are the main cause of ACLF. Make comments at this regard. 4.- The manuscript has too many figures. I think figures 1 and 4 may be suppressed. 5.- In Table 1, the number size of the cohort was not given 6.- In Figure 2 Add p value on the figure 7.- In Figure 5, give the Z value on the figure. 8.- Generally English is good. Nevertheless the text needs a deep revision to correct mistakes.

Answers: a. In this study, we investigated the correlation between survival and clinical parameters such as age, gender, etiology, the number of complication, the type of ALSS, serum

biomarkers tested at baseline including total bilirubin (TB), ALT, INR and creatinine, etc. In the bivariate analysis, some factors evaluated at baseline showed predictive impact on overall survival such as the type of ALSS, age, the number of complication, MELD score, TB, ALT, AST, and INR. However when variables with bivariate significance were selected into in a multivariate Cox regression model, the type of ALSS, age, the number of complications and MELD score were defined as independent predictive factors for survival.

The MELD score can be calculated using the equation: 9.57x ln[creatinine (mg/dl)] + 3.78 x ln[bilirubin (mg/dl)] + 11.2 x ln(international normalized ratio) + 6.43(etiology: 0 if cholestatic or alcoholic, 1 otherwise). The complications include hepatic encephalopathy (HE), spontaneous bacterial peritonitis (SBP), hepatorenal syndrome (HRS), upper gastrointestinal hemorrhage, and electrolyte disturbance. The type of ALSS include PE and PE plus PBA (we assigned 0=PE and 1=PE plus PBA). According to the multivariate model, a risk score (R) can be calculated using the formula: R = 0.03*(age) + 0.097*(MELD score) + 0.527*(the number of complications) - 0.79*(the type of ALSS).

b. In the revised manuscript, the discussion about limitations of the study were added into the "Discussion" part and marked in red.

c. The comment "The clinical characteristics of the cohort may limit its application in other populations such as western countries patients where alcohol and drugs are the main cause of ACLF" was added into the "Discussion" part and marked in red.

d. Figure 1 was suppressed, however figure 4 was important and retained. In the revised manuscript the performance of the novel model based on the derivation cohort (shown in figure 3A and 4A) was tested in the validation cohort (shown in figure 3B and 4B).

e. In Table 1, the number size of the cohort was added into the revised manuscript.

f. The p value on the figure 1 (former figure 2) was added into the revised manuscript.

g. The Z value on the figure 4 (former figure 5) was added into the revised manuscript.

h. The paper was rewritten in grammatical English with the help of a scientific English editing service company (American Journal Experts).

(5) **Reviewed by 00503430:** This is the very interesting issue but this research has some methodological problem and need major revision. Selection of patients and study design sections in PATIENTS AND METHOD is very disorganize and confusing and should be rewrite. Some of the RESULTS is mentioned in PATIENTS AND METHOD section. How the authors classify the R value to Poor, Fair, and Good? What are the criteria for this? (<3; 3-5; >5) The comparison of data between two groups is helpful.

Answers: In the resubmitted manuscript, the PATIENTS AND METHOD and RESULTS were revised and rewritten in red.

According to the multivariate model, a risk score (R) can be calculated using the formula: R = 0.03*(age) + 0.097*(MELD score) + 0.527*(the number of complications) - 0.79*(the type of ALSS). To calculate the probability of survival at t days of a patient could use the equation: $S(t)=S_0(t)^{\exp(score-3.7)}$. $S_0(t)$ gives the estimated survival probabilities for a patient with a risk score of 3.7 which is the mean risk

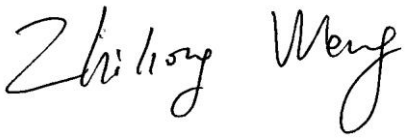
score of the ACLF patients in derivation cohort. Specially, based on the score, the expected survival probability in individual patient can be calculated. For example, the 90- and 270-day survival probabilities in patient in the lowest quartile (R=1.9) were 96.3% and 93.3%, respectively. However in the highest quartile (R=5.6), the survival probabilities sharply decreased to 21.9% and 6.0% at 90- and 270-day, respectively.

The new prognostic model showed that ACLF patients with lower R value consistently had a better long-term survival probability. So, we could classify the outcome of ACLF patients as Good, Fair, and Poor according their R values, as described previously (Yang JD, et al. Model to estimate survival in ambulatory patients with hepatocellular carcinoma. Hepatology 2012; 56:614-621). The "<3; 3-5; >5" is an example to differentiate the prognosis of ACLF patients.

3. References and typesetting were corrected

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

Sincerely yours,



Zhihong Weng, Associate Professor
Department of Infectious Disease
Union Hospital, Tongji Medical College
Huazhong University of Science & Technology
Wuhan 430022, China
E-mail: wzh941@126.com

June 30, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 18022-Review-2015.6.30.doc).

Title: Prognosis of acute-on-chronic liver failure patients treated with artificial liver support system

Author: Piqi Zhou, Shaoping Zheng, Min Yu, Shengsong He, Zhihong Weng

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 18022

The manuscript has been improved according to the suggestions of Journal Editor-in-Chief:

1. "Though the new model could predict the patient's survival better than MELD score, it can only be applied to patients who undergo ALSS. The basic problem of this study is that ALSS is not actually proven to be effective in prolonging the patient's survival by a well-designed randomized controlled trials. Therefore, it should be included in the discussion session."

Answers: In the revised manuscript, the comment "**ALSS is not actually proven to be effective in prolonging the patient's survival by a well-designed randomized controlled trial. Though the new model could predict the patient's survival better than MELD, it can only be applied to ACLF patients who undergo ALSS besides SMT.**" was added into the discussion session and marked in red.

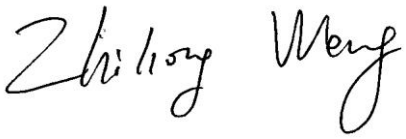
2. "Another minor point is that when developing MELD score, any variables that could be subjective including HEP was discarded intentionally. MELD score was originally developed to determine the priority of LT objectively. Therefore, only laboratory variables were included in the variables. So, it is natural that the performance would be better if we include other clinical variables, but it could result in a fake case for determining the priority of LT. Please insert some comment in the discussion that MELD score was made with only subjective parameters and that could be one reason why this new model had a better performance."

Answers: In the revised manuscript, the comment "**MELD scoring system was originally developed to determine the priority of liver transplantation objectively and was made with only subjective parameters. Therefore, this new prognostic model including some other clinical variables besides MELD had a better performance than MELD scoring system.**" was added into the discussion

session and marked in red.

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

Sincerely yours,



Zhihong Weng, Associate Professor
Department of Infectious Disease
Union Hospital, Tongji Medical College
Huazhong University of Science & Technology
Wuhan 430022, China
E-mail: wzh941@126.com