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February 24, 2021

Professor Lian-Sheng Ma, *Science Editor, Company Editor-in-Chief*

Dear Prof. Ma,

Thank you very much for your email communication regarding our manuscript "Development and validation of a prognostic model for patients with hepatorenal syndrome: a retrospective cohort study" (Manuscript NO. 60780, Retrospective Cohort Study). We are grateful for both you and the editors' very pertinent and constructive comments and suggestions, and would like to specifically address the points raised by them as follows:

Reviewers' Comments:

Reviewer #1:

The article by Xin-Yu Sheng, et al., Is interesting, well presented I suggest the following:

1. highlight the clinical importance of your conclusions.

Response: We would like to thank the reviewer for his/her valuable comments on our manuscript. We have added the highlight content in "DISCUSSION" section in the revised manuscript (Page 15, marked in red). We also show them as follows:

"Prognostic models for the short-term prognosis of HRS are not enough. Some commonly used prognostic models for end-stage liver disease, such as MELD, MELD-Na, COSSH-ACLF and CLIF-SOFA, are developed for the entire end-stage liver diseases including severe hepatitis and cirrhosis. The novel model, GIMNS,

takes HRS as the target disease. Moreover, our HRS patients were enrolled according to the latest diagnostic criteria of International Club of Ascites in 2015 and the sample size is larger than other researches, which can increase the credibility of the results. And we operated external validation in three other hospitals to prevent overfitting. Finally, GIMNS performed better in both derivation and validation cohort. As its prognostic efficacy is better than MELD, COSSH-ACLF and CLIF-SOFA, it has the value of clinical application."

2. How can your proposed method be validated against the gold standard?

Response:

First, after consulting a large amount of literature, we found that although the current diagnosis of HRS is based on the criteria of International Club of Ascites in 2015, there is no gold standard for predicting the short-term prognosis of HRS. Secondly, at present, clinicians still predict the prognosis of HRS patients based on the MELD or MELD-Na. Our prognostic model is developed directly for HRS, which is innovative. Finally, after rigorous statistics, we found that the new model is better than MELD, COSSH-ACLF and CLIF-SOFA in predicting the short-term prognosis of HRS in both derivation and validation cohort.

3. Is your method already applied in your hospital? what results have you had? help to improve the forecast?

Response:

It has not been used in our hospital. Since this is a retrospective study, although we have tried our best to increase the sample size, validated in external cohort and adopt strict statistical methods, there may still be selection bias. In this way, prospective studies which have higher levels of evidence are needed to verify our conclusions. This is what we are currently doing.

Reviewer #2:

This manuscript is well written. I have only one comment to make: 1.The finding of MCHC may be a statistical aberration rather than a true biological predictor of HRS, as firstly the reason behind its association is unclear, and its pathogenic role is even more doubtful. Secondly, the hazard ratio is the smallest of the 5 factors, and in absolute number it is also small, 1.014. Thus, before we accept the significance of MCHC, I would suggest the authors do a re-analysis by removing MCHC from its score and see if the conclusions are affected in any way.

Response:

We thank the reviewer for the time and effort expended in assessing our manuscript. When we discovered that MCHC, an indicator of auxiliary diagnosis of anemia, was related to the short-term prognosis of HRS, we also suspected that this was a statistical aberration. However, we then conducted a separate study on the prognosis of MCHC on HRS, and found this conclusion: After adjusted for age, sex, etiology, mean arterial pressure (MAP), neutrophils, alanine aminotransferase (ALT), After urea, potassium, bilirubin, sodium, platelets and international normalized ratio (INR), MCHC is indeed associated with 28-day mortality of HRS, and as MCHC level increases, HRS patients get worse outcome (Fig 1). Therefore, although the hazard ratio of MCHC is relatively small, it is still meaningful.

Following the reviewer's suggestion, we developed a model named GINS by removing MCHC from GIMNS. And comparison between GIMNS and GINS was showed at Table 1. Although there was no statistical difference between the two models, the area under ROC of GIMNS is larger than that of GINS. In addition, in the validation cohort, the Youden index of GINS is lower than that of GIMNS. The ROC curves result was consistent with Table 1 (Figs 2, 3).

In view of the value of MCHC, we ultimately plan not to remove MCHC.

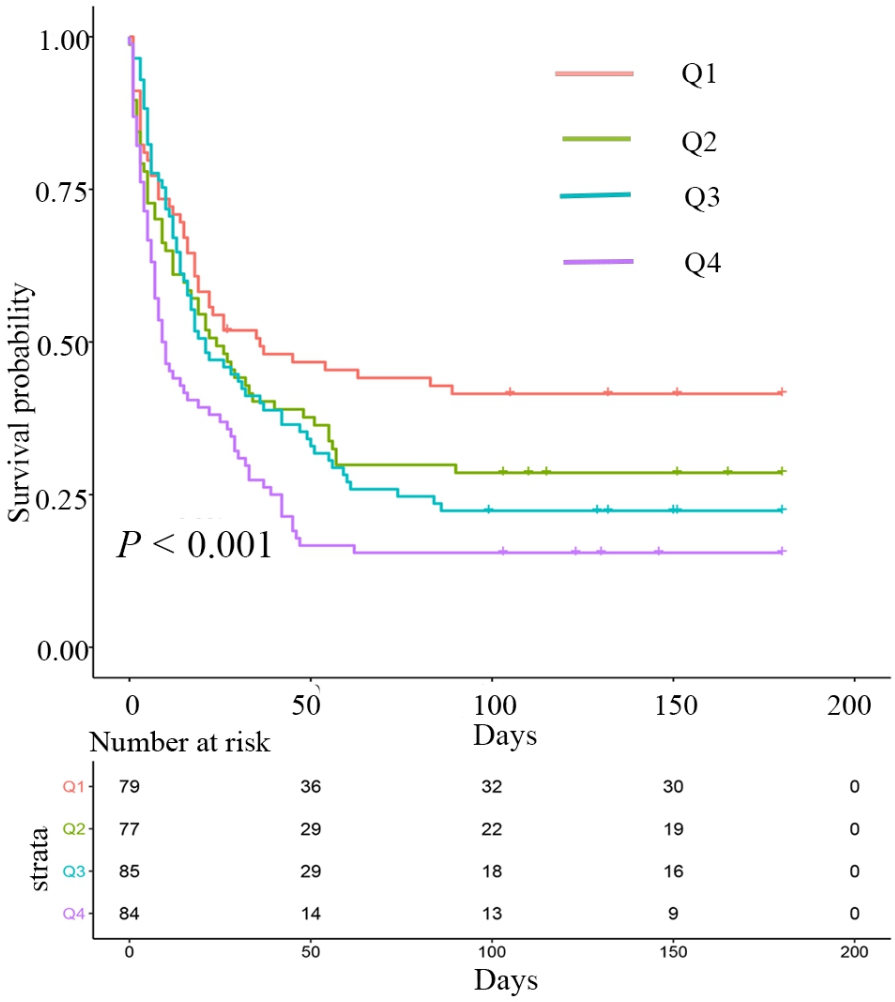


Fig 1. The survival probability of HRS decreases with increasing MCHC.

MCHC level was divided into quartiles. Q1 represents lower quartile while Q4 represents upper quartile.

Table 1. Accuracy of GIMNS and GINS in derivation and validation cohorts.

Models	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**Youden**	**AUROC (95% CI)**	**P value vs GIMNS**
Derivation cohort (n = 248)						
GIMNS	0.4	73.5	78.7	0.522	0.830 (0.778-0.882)	—

GINS	0.32	70.5	79.8	0.503	0.820 (0.743-0.874)	0.539
Validation cohort (n = 123)						
GIMNS	0.7	57.8	79.6	0.374	0.732 (0.642-0.821)	—
GINS	1.49	43.8	84.4	0.282	0.733 (0.647-0.824)	0.169

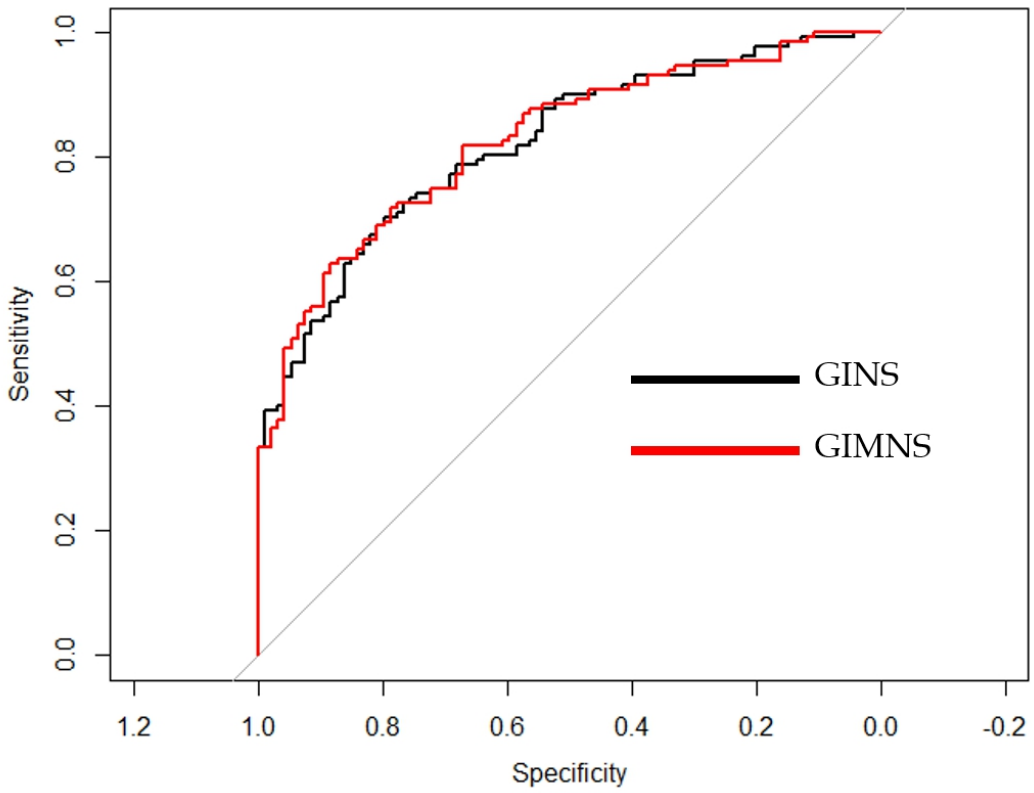


Fig 2. ROC curves of GINS and GIMNS in derivation cohort.

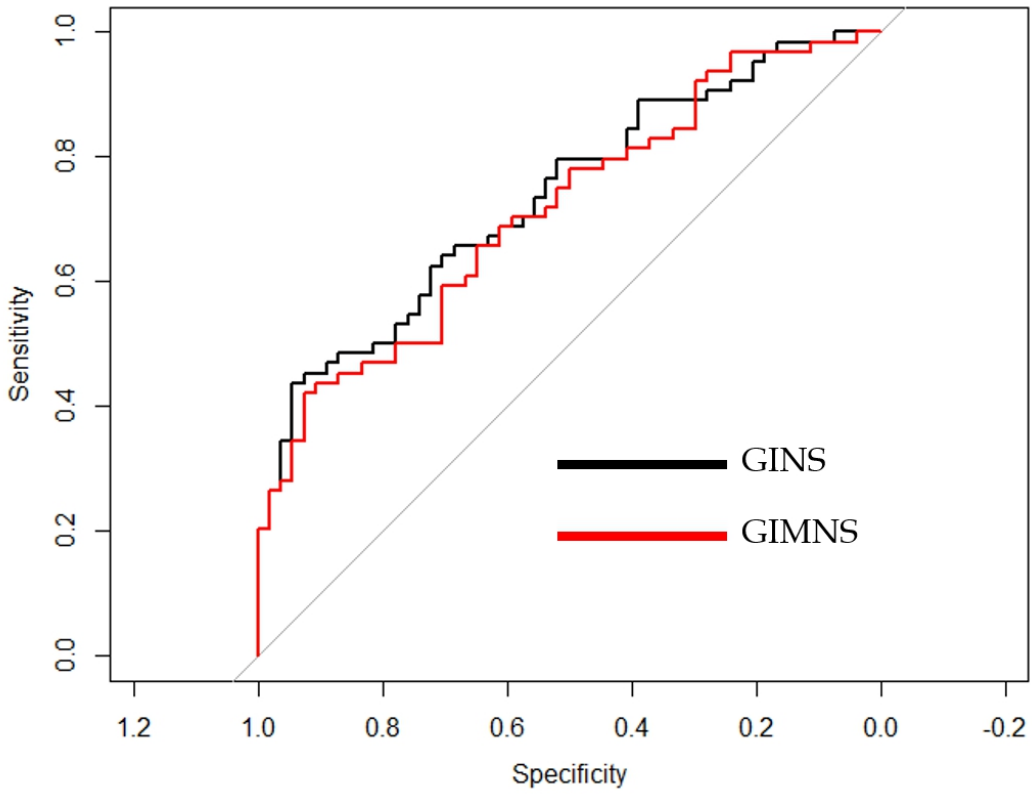


Fig 3. ROC curves of GINS and GIMNS in validation cohort.

Reviewer #3:

Sheng et al. developed a model for predicting mortality of patients with HRS. The strengths of the study are that there was a large number of enrolled patients, and the performance of the developed model was validated in another independent cohort. There are some major concerns:

1. We appreciate the reviewer's suggestions and comments. Previous studies have shown that patients with type 1 HRS had worse prognosis than those with type 2 HRS. What was the proportion of patients with type 1 and 2 HRS in the derivation and validation cohort? Was there any difference in mortality of patients with type 1 and 2 HRS? Was the type of HRS associated with survival in these cohorts? Can the GIMNS score be applied to both type 1 and 2 HRS patients?

Response:

According to the latest criteria of International Club of Ascites in 2015, HRS is no longer classified into type 1 and type 2. Previous definition of type 1 HRS was "Doubling of serum creatinine to a concentration ⩾2.5 mg/dL within 2 weeks" while definition of type 2 HRS was "Gradual increase in serum creatinine, not meeting criteria of type 1 HRS". However, current definitions of HRS were "Increase in serum creatinine of ⩾0.3 mg/dL within 48 hours or Increase in serum creatinine ⩾1.5 times from baseline" and "No signs of structural kidney injury" [1]. Therefore, most of type 2 HRS patients were not included in our cohort, and since this is a retrospective study, we cannot increase sample size of type 2 HRS patients for further analysis. Finally, type 1 and type 2 HRS were not discussed in our cohort.

Reference:
[1] Simonetto DA, Gines P, Kamath PS. Hepatorenal syndrome: pathophysiology, diagnosis, and management. BMJ. 2020 Sep 14;370:m2687. doi: 10.1136/bmj.m2687. PMID: 32928750.

2-3. Can you please give more details about organ failure, e.g. which organs were affected, what was the proportion of patients with each failed organ? Did each failed organ have a similar magnitude of impact to mortality?

Response:

We concerned five types of organ failure, including liver failure, coagulation failure, brain failure, respiratory failure, and circulatory failure. We respectively compared these five organ failures in the survivor cohort and non-survivor cohort and finally found that the proportion of liver failure, coagulation failure and brain failure in patients from non-survivor cohort was significantly higher than those from survivor cohort. At the same time, the proportion of respiratory failure and circulatory failure remained the same in the two cohorts (Table 1). Next, Cox regression was calculated to balance the confounding factors. Finally, after adjusted for age, sex, etiology, mean arterial pressure (MAP), neutrophils, alanine aminotransferase (ALT), urea, potassium, bilirubin, sodium, platelets and international normalized ratio (INR), it was found that brain failure and respiratory failure were independent risk factors for the 28-day mortality of HRS patients. The mortality of patients with respiratory failure is 4.2 times that of those without respiratory failure while the mortality of patients with

brain failure is 1.6 times that of those without brain failure. Other organ failures were not associated with the prognosis of HRS (Table 2). Considering the potential impact among various organ failures, we finally decided to include the number of organ failures as an independent variable into the prognosis model [1].

Reference:

[1] Davenport A, Sheikh MF, Lamb E, Agarwal B, Jalan R. Acute kidney injury in acute-on-chronic liver failure: where does hepatorenal syndrome fit? Kidney Int. 2017 Nov;92(5):1058-1070. doi: 10.1016/j.kint.2017.04.048. Epub 2017 Aug 23. PMID: 28844314.

Table 1. Characteristics of HRS patients.

Variables	**Non-survivor n=278**	**survivor n=93**	***P* value**
Liver failure (%)	171 (61.5)	27 (29.0)	<0.001
Coagulation failure (%)	79 (28.4)	5 (5.4)	<0.001
Brain failure (%)	73 (26.3)	7 (7.5)	<0.001
Respiratory failure (%)	5 (1.8)	0 (0.0)	0.434
Circulatory failure (%)	63 (32.1)	28 (30.1)	0.932

Adjusted for age, sex, etiology, mean arterial pressure (MAP), neutrophils, alanine aminotransferase (ALT), urea, potassium, bilirubin, sodium, platelets and international normalized ratio (INR).

Table 2. Multivariate Cox regression of each failed organ.

Variables	**Multivariate Cox regression**	
	Adjusted HR (95% CI)	***P* value**
Liver failure (%)	1.205 (0.901-1.610)	0.209
Coagulation failure (%)	0.972 (0.620-1.523)	0.900
Brain failure (%)	1.620 (1.192-2.201)	0.002
Respiratory failure (%)	4.244 (1.689-10.663)	0.002
circulatory failure (%)	0.869 (0.666-1.134)	0.302

Adjusted for age, sex, etiology, mean arterial pressure (MAP), neutrophils, alanine aminotransferase (ALT), urea, potassium, bilirubin, sodium, platelets and international normalized ratio (INR).

4. It is very interesting that MCHC but not Hb and MCH was associated with survival. What would be the possible explanation of this phenomenon? Supposed Hb,

MCH, and MCHC was put in the multivariate model one at a time, would each of them be independently associated with mortality?

Response:

The mechanisms underlying the prognostic role of MCHC are not fully understood. However, some hypotheses can be proposed. One potential explanation is that ascites, a common complication of decompensated cirrhosis and a characteristic of HRS, reduces effective circulating blood volume; therefore, leads to hemoconcentration, which relatively increases MCHC level. Another hypothesis is an uncompensated increase in erythropoietin caused by hepatic and renal insufficiency. Further studies are needed to determine the pathophysiology between MCHC and HRS.

Because there is a strong correlation between MHC and MCHC (correlation coefficient: 0.447, $P<0.001$) and the collinearity analysis Kappa test (k=106.3), suggesting that there is collinearity, MHC and MCHC cannot be included together into Cox regression model, otherwise it will affect the accuracy of the model.

From table 1, after multivariate Cox regression adjusted by confounding factors, among the three indicators, MCHC, MHC and Hb, only MCHC is related to the prognosis of HRS, which is a prognostic indicator. Whether including MCHC and Hb together, or MHC and Hb together, the results indicate that MCHC is an independent risk factor for the 28-day mortality of HRS, and MHC and Hb are not associated with prognosis of HRS

.

Table 1. Multivariate Cox regression of MCHC, MHC and Hb.

Variables	**Multivariate Cox regression**	
	Adjusted HR (95% CI)	***P* value**
MCHC	1.012 (1.005-1.019)	0.001
MHC	1.026 (0.992-1.061)	0.130
Hb	1.003 (0.998-1.007)	0.203
MCHC+Hb	MCHC: 1.012 (1.004-1.102)	0.002
	Hb: 1.000 (0.995-1.005)	0.962
MHC+Hb	MHC: 1.032 (0.996-1.068)	0.082
	Hb: 1.004 (0.999-1.008)	0.123

Adjusted for age, sex, etiology, mean arterial pressure (MAP), neutrophils, alanine aminotransferase (ALT), urea, potassium, bilirubin, sodium, platelets and international normalized ratio (INR).

5. The AUROCs of MELD, CLIF-SOFA, and COSSH-ACLF scores in the validation cohort were much lower than those in the derivation cohort. The sensitivity of CLIF-SOFA in the validation cohort was also very much lower than that in the derivation cohort, i.e. 29.7% vs. 72.7%. Can you please give the reasons for these findings?

Response:

This just shows that the three prognostic scores including MELD, CLIF-SOFA, and COSSH-ACLF are not suitable for HRS patients. Although they are widely used in the evaluation of the severity of end-stage liver diseases, there has been no accurate proof whether they are suitable for HRS patients. The reduction of accuracy and sensitivity in the validation cohort indicates that they are not universal and are not suitable as a prognostic scoring model for HRS patients.

5. A very recent study (Zulian Terres et al. GastroHep 2020) reported that Child-Pugh score was better than CLIF-SOFA and MELD score in predicting 30-days mortality. What was the prognostic performance of Child-Pugh score in the derivation and validation cohort?

Response:

We thank the reviewer for raising this advice. We did the comparison of the efficacy of CTP score and GIMNS in HRS patients.

Thus we compared the prognostic performance of GIMNS and CTP score in predicting the 28-day mortality of HRS in both derivation and validation cohort. The results are as follows: GIMNS performed better in derivation cohort (P=0.001) (Fig 1) and remained the same in validation cohort (P=0.579) (Fig 2). Totally, GIMNS performed better.

Finally, CTP score was not selected in our analysis because the CTP score is relatively subjective and may not be suitable for use as an objective index in predicting prognosis of HRS patients.

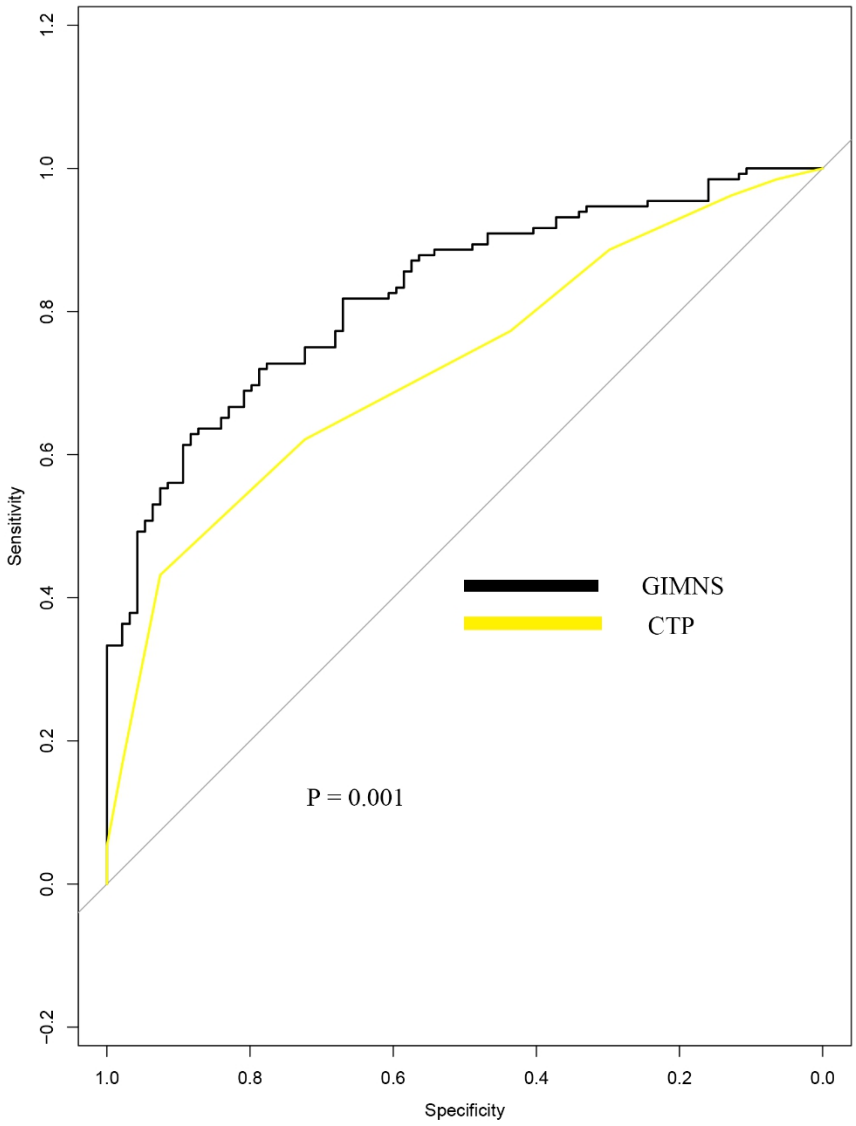


Fig 1. ROC curves in derivation cohort

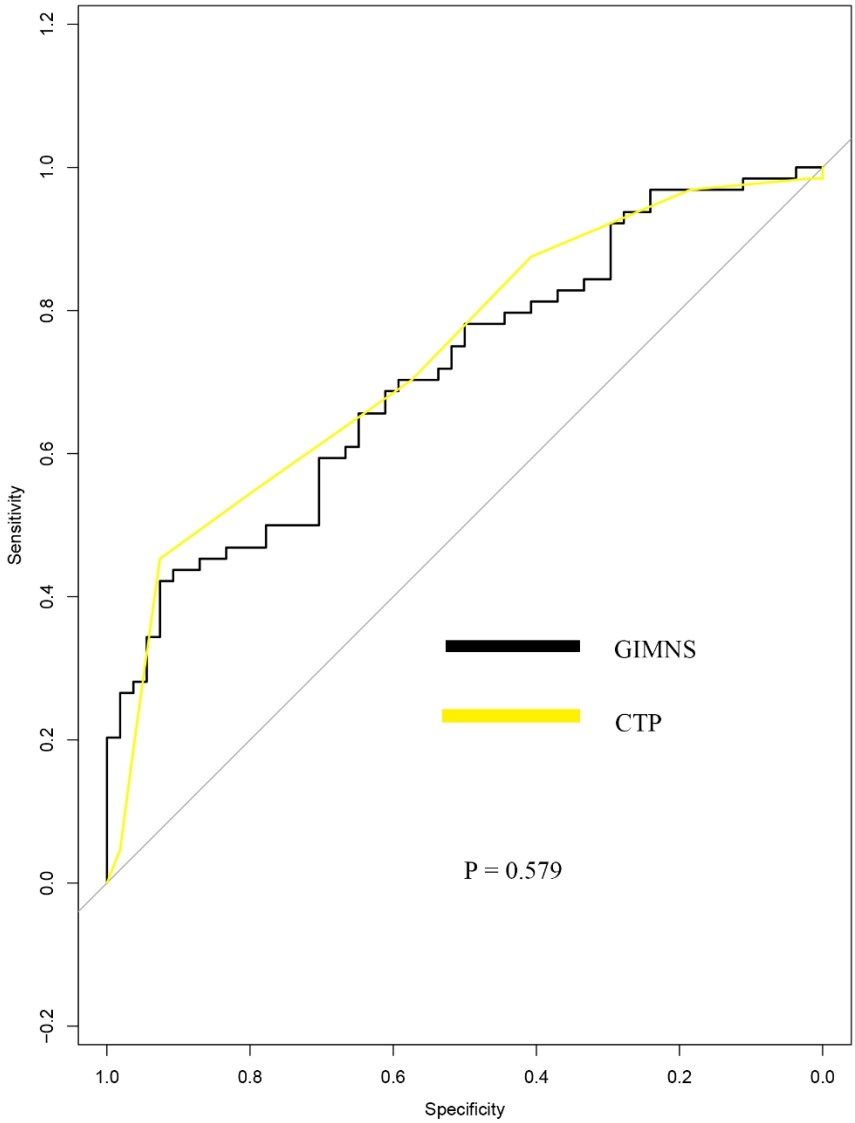


Fig 2. ROC curves in validation cohort.

Minor concerns:

1. In the result section on page 8, it was stated that "survivors had higher levels of INR and ALT compared to non-survivors". This is inconsistent with the results shown in Table 1. Please correct.

Response:

We thank the reviewer for carefully examining our data. We have corrected it as "Non-survivors had higher levels of INR and ALT compared with survivors." (Page 9, marked in red).

2. In the discussion section on page 15, there is a typo in the sentence “Pathological sections re needed to separate HRS from ATN”.

Response： Thank you for pointing it out. We have corrected it as “Pathological sections are needed to separate HRS from ATN.” (Page 16, marked in red).

Reviewer #4:

This is a very interesting study on development and validation of a novel prognostic score named GIMNS for patients with HRS. The paper, in its actual form, is worth publishing. It meets the high quality of papers published in WJG.

Response： Thank you very much for the positive comment on our work as a resource for understanding prognostic score for patients with HRS.

Reviewer #5:

This is a very interesting approach in this relevant topic. In my opinion, this is a very interesting manuscript for our readers.

Response： Thank you very much for your compliments on our work.

Reviewer #6:

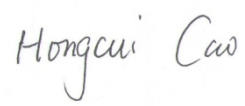
No comment.

Response： Thank you for your comments.

Taken together, we feel that your comments and suggestions are very helpful in improving our manuscript, and therefore have made corresponding additions and modifications (marked in red in the re-submitted version). Please let us know if you need further clarification. Thank you once again for your professional and timely assistance.

Sincerely,

Hongcui Cao, M.D.



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