Editorial Office, *World Journal of Hepatology*

ESPS Manuscript no.: 29150

TITLE: The phase angle obtained by bioelectrical impedance analysis independently predicts mortality in patients with cirrhosis

Dear Mr. Lian-Sheng Ma,

We appreciate the opportunity to respond to the observations raised after the evaluation of our manuscript entitled "The phase angle obtained by bioelectrical impedance analysis independently predicts mortality in patients with cirrhosis," which demonstrate a deep critical analysis of our study and strong expertise in the issue covered. Our research group values these comments and considers them to be of great importance for discussion. The manuscript has been revised based on these comments, which has certainly improved its scientific quality. All changes are highlighted in the revised manuscript. We provide point-by-point responses to the reviewers' comments below.

Reviewer's code: 00039097

QUESTION 1. *Why choose only the male patients? Not female? Are there any differences in gender?*

RESPONSE: Thank you for this question. We choose to assess only male patients to make our sample as uniform as possible, as liver cirrhosis *per se* is a progressive disease and hepatic damage may differ, even slightly, among patients. In addition, cirrhosis is more common in men and malnutrition seems to have greater prognostic value for disease progression in men than in women.[1–3] The prognostic ability of the studied cutoff value for phase angle is associated directly with malnutrition. Thus, by evaluating only men, we were able to access not only a more uniform sample, but also the population most susceptible to the studied disease and its associated nutritional complications.

We have added this information to the manuscript:

"We assessed only male patients to make our sample as uniform as possible, as liver cirrhosis per se *is a progressive disease and hepatic damage may differ, even slightly,*

among patients. In addition, cirrhosis is more common in men and malnutrition seems to have greater prognostic value for disease progression in men than in women. The prognostic ability of the studied cutoff value for phase angle is associated directly with malnutrition. Thus, by evaluating only men, we were able to access not only a more uniform sample, but also the population most susceptible to the studied disease and its associated nutritional complications." (page 11, lines 296–304)

QUESTION 2. *The authors concluded that PA may be a useful and reliable bedside tool for prognosis predicition, however, it is just one non-validated variable not prediction model or system which can greatly increase the prediction abilities. Why not integrated valuable variables to form a model?*

RESPONSE: We appreciate this important question, which allows us to discuss our mortality model. Our study was designed to test whether the cutoff point for phase angle (PA) values recently identified as predictive of malnutrition-associated mortality in cirrhotic patients (≤4.9°) was adequate for this purpose in a cirrhotic population of different ethnicity than used for its initial identification. Among several prediction models proposed and validated for patients with advanced liver disease, the Child-Pugh (CP) score and the Model for End-Stage Liver Disease (MELD) are used most frequently to predict mortality in this population. The CP scale was originally designed to predict surgical outcomes in patients with cirrhosis, but its application was later extended to determine the prognosis, severity, and response to treatment.[4] The MELD system was initially developed to predict premature mortality in patients with cirrhosis who underwent transjugular intrahepatic portosystemic shunting procedures by using three easily accessible laboratory values: the international normalized ratio, the prothrombin time, and total bilirubin and serum creatinine levels. Subsequently, MELD application was shown to be useful for short-term mortality prediction in the general cirrhotic population, as well as for the disease severity estimation to better allocate patients for liver transplantation (LT). This mathematical model has been tested and validated to be a good predictor of the survival of adult patients on the LT list; it has also been shown to better predict short-term results than does the CP score.[5–7] Notably, the main clinical variables associated with poor prognosis in cirrhosis are embedded in the CP and MELD mathematical models. In this sense, CP and MELD scores may reflect the progression of liver damage and indirectly detect metabolic changes that may influence the cirrhosis prognosis. Based on these observations, CP and MELD scores were included in

our mathematical models used to test the ability of the studied PA cutoff to predict mortality in cirrhotic patients. The CP score showed no significant predictive value for mortality in our initial model (p = 0.25), and was excluded from the final model. In addition, as the studied PA cutoff covers mortality associated with malnutrition, we included age as a variable in our final model. Age is related to nutritional status (including sarcopenia, one of its most aggressive manifestations), it has been proposed as the main indicator for PA determination in women and men, and it had a significant effect in our initial mortality model. In short, to evaluate the isolated effect of PA on prognosis for mortality, our model was adjusted for MELD score and age, neutralizing the influence of these variables on the results.

According to our findings, the studied PA cutoff was able to independently predict mortality in patients with cirrhosis, potentially associated with malnutrition. Several prognostic markers were altered in patients with PA values equal to or below the studied cutoff value, compared with those with PA values above the cutoff: mid-arm muscle circumference, albumin, and handgrip-strength values were lower, and severe ascites and encephalopathy incidences, interleukin (IL)-6/IL-10 ratios, and C-reactive protein levels were higher. These findings supported the utility of PA ≤ 4.9° as a good prognostic marker for cirrhosis, enabling us to develop models or systems to increase its predictive ability. The improvement of clinical tools is always of great interest, and this approach can be developed in the future, but it was not the focus of the present study and was not required to meet its purpose.

We have revised the manuscript as follows:

"In this study, we aimed to test whether this PA cutoff (≤4.9°) had prognostic value for mortality in a population of patients with cirrhosis of different ethnicity than used for its initial identification." (page 6, lines 121–124)

"The prognostic value of the PA for mortality prediction was evaluated in mortality models adjusted for variables potentially impacting nutritional status and/or cirrhosis severity (age, Child-Pugh (CP) and MELD scores).[8]" (page 7, lines 181–184)

"The Child-Pugh score had no significant effect in the initial mortality model and was not included in the final model (Table 2)." (page 9, lines 220–221)

"The Child-Pugh score was added to our initial mortality model because it may reflect the progression of liver damage and indirectly detect metabolic changes that may influence the prognosis of the disease.[6] However, it had no significant effect on mortality prediction.

Notably, the MELD score has been validated as a good predictor of the survival of adult patients on the LT list, and has been found to better predict short-term results than does the CP score.[6] This difference in performance may explain the significant value of the MELD score, and not the CP score, for mortality prediction in our initial model." (pages 9–10, lines 249–256)

QUESTION 3. *The authors only use one single-timepoint of PA value. If there is several timepoints of PAs and the changing of PAs may be more useful than the recent one.*

RESPONSE: Thank you for this comment. As with any biological marker, the PA has specific cutoff values for given populations. Recently, the 4.9º cutoff point for PA was identified as a prognostic marker for cirrhotic patients; for this reason, it was used in the current study. We tested four different frequencies of bioimpedance to evaluate the prognostic value of this cutoff point. Notably, in agreement with the authors who established the cutoff point, only the frequency of 50 kHz showed significance in the final prognostic model (please see the Kaplan–Meier survival curves developed for the other frequencies below, figure 1,2,3).

As discussed in the response to question 2, the previously established PA cutoff of 4.9º independently predicted mortality in patients with cirrhosis, and worse prognostic markers were identified in patients presenting PA values at and below this cutoff. These findings support the utility of PA ≤ 4.9° as a good prognostic marker for cirrhosis, enabling the testing of other PA cutoffs for this purpose. We have improved the description of the previous identification of the studied cutoff value, as follows:

"Recently, the 4.9º PA value was identified as the best cut-off for malnutrition associated with the severity of liver cirrhosis, and was shown to have important prognostic value for malnutrition-associated mortality in this patient population." (pages 5–6, lines 118–121)

Figure 1. Kaplan–Meier survival curves for 134 patients with cirrhosis, obtained using cutoff values based on a phase angle of 5 kHz, determined by bioelectrical impedance analysis

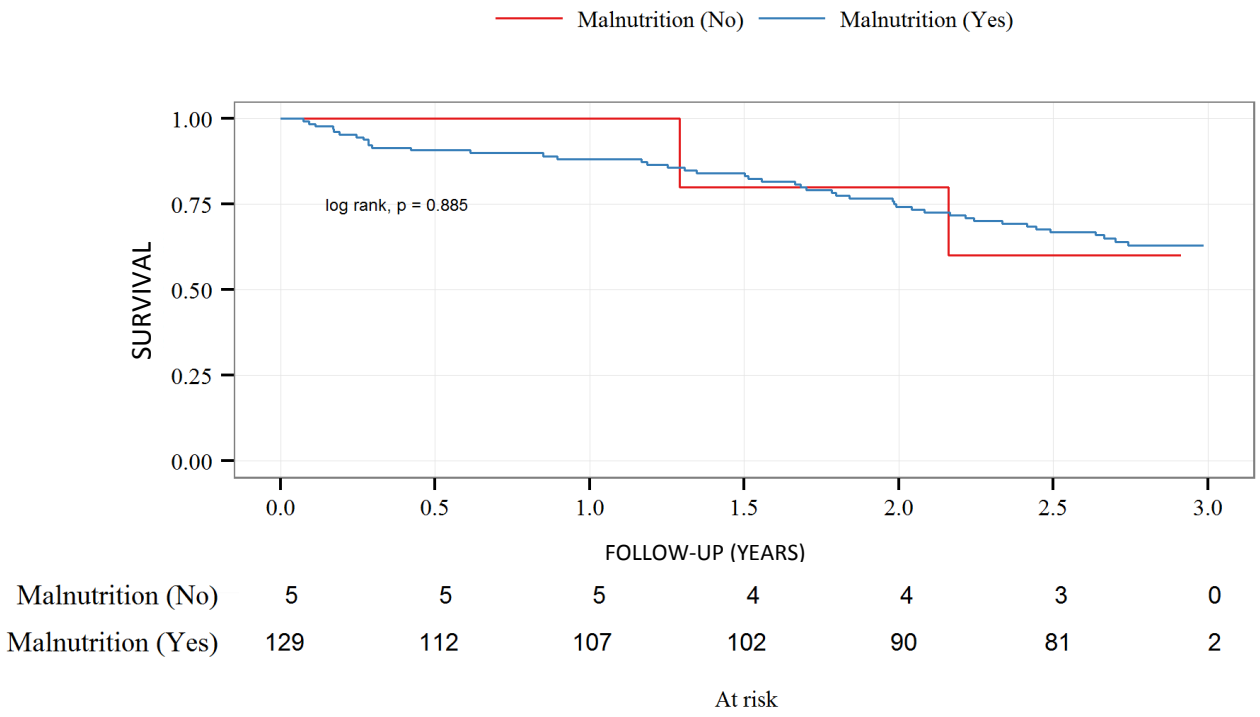


Figure 2. Kaplan–Meier survival curves for 134 patients with cirrhosis, obtained using cutoff values based on a phase angle of 100 kHz, determined by bioelectrical impedance analysis

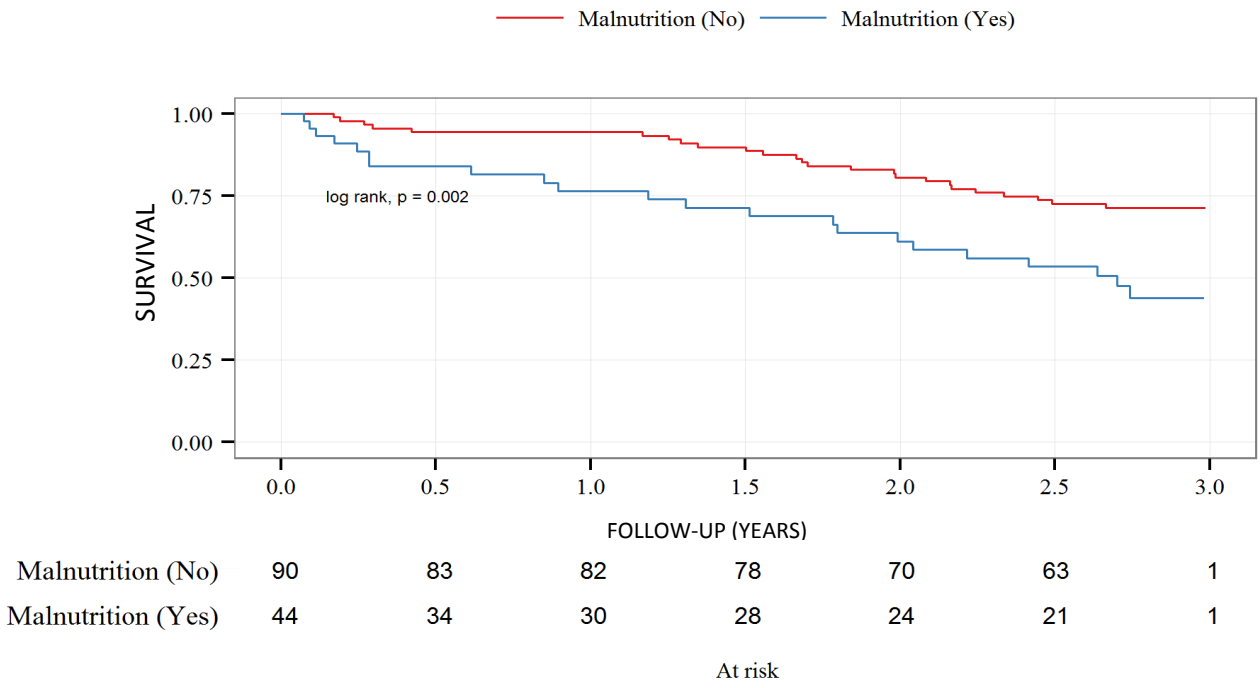
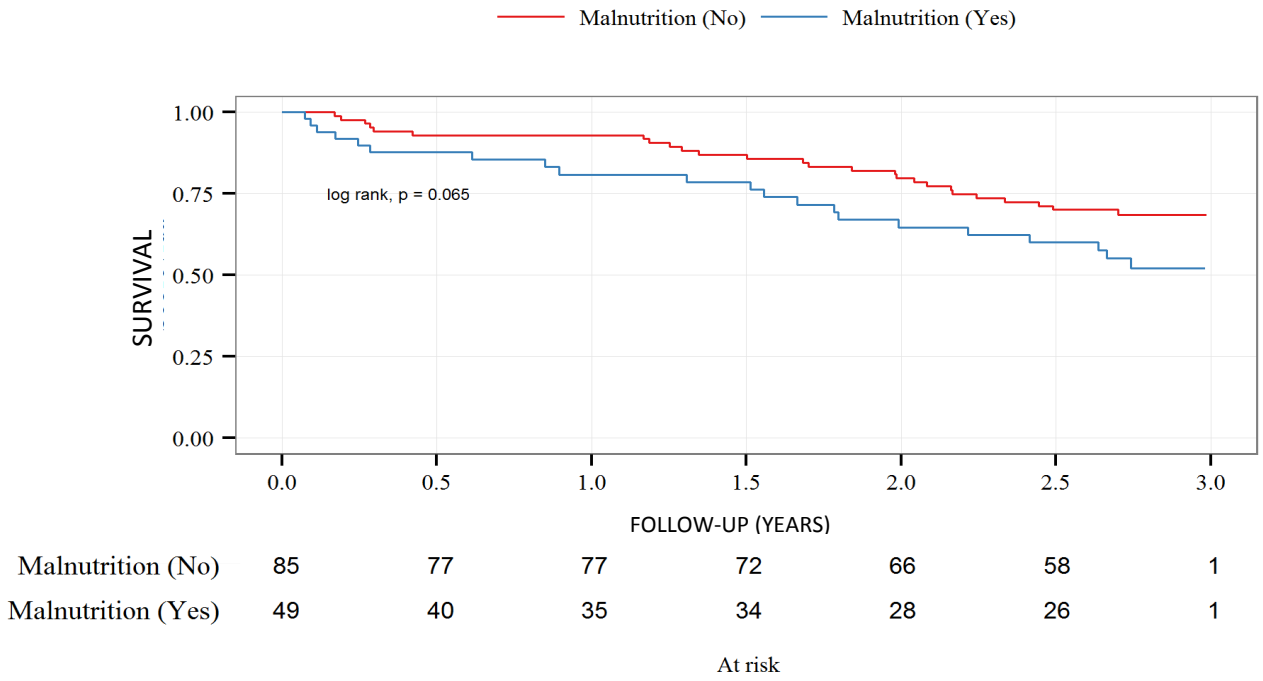


FIGURE 3. Kaplan–Meier survival curves for 134 patients with cirrhosis, obtained using cutoff values based on a phase angle of 200 kHz, determined by bioelectrical impedance analysis



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6. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with endstage liver disease. Hepatology. 2001; 33: 464-70.

7. Luca A, Angermayr B, Bertolini G, Koenig F, Vizzini G, Ploner M, Peck-adosavljevic M, Gridelli B, Bosch J. An integrated MELD model including serum sodium and age improves the prediction of early mortality in patients with cirrhosis. Liver Transpl. 2007; 13(8):1174-80.

8. Ruiz-Margáina A, Macías-Rodrígueza RU, Duarte-Rojoc A,,Ríos-Torresa SL, Espinosa-Cuevasb A, Torrea A. Malnutrition assessed through phase angle and its relation to prognosis in patients with compensated liver cirrhosis: A prospective cohort study. Digestive and Liver Disease. 2015;47:309–314.

Reviewer's code: 02860895

QUESTION: *The multivariate analysis was done to clarify the real predictive potency of PA. Besides PA, age and MELD score were included in this analysis (Table 3). I think that Child-Pugh should be included as a variable because PA correlated to Child-Pugh (Table 1). This is a very critical point of the study.*

RESPONSE: Thank you for this relevant comment, which demonstrates a deep knowledge of the area covered in our study. Among several prediction models proposed and validated for patients with advanced liver disease, not only the Model for End-Stage Liver Disease (MELD), but also the Child-Pugh (CP) score, is used most frequently to predict mortality. The CP score may reflect the progression of liver damage and indirectly detect metabolic changes that may influence the prognosis of the disease. We included the CP score in our initial mortality model, but it showed no significant effect (p = 0.25) and was not included in the final model. Notably, the MELD score has been validated as a good predictor of the survival of adult patients on the liver transplantation list; it has also been shown to better predict short-term results than does the CP score.[1–3] This difference in performance may explain the significant value of the MELD score, and not the CP score, for mortality prediction in our initial model.

We have further discussed the use of the CP score in the revised manuscript, as follows:

"The prognostic value of the PA for mortality prediction was evaluated in mortality models adjusted for variables potentially impacting nutritional status and/or cirrhosis severity (age, Child-Pugh and MELD scores).[4]" (page 7, lines 181–184)

"The Child-Pugh score had no significant effect in the initial mortality model and was not included in the final model (Table 2)." (page 9, lines 220–221)

"The Child-Pugh score was added to our initial mortality model because it may reflect the progression of liver damage and indirectly detect metabolic changes that may influence the prognosis of the disease.[5] However, it had no significant effect on mortality prediction. Notably, the MELD score has been validated as a good predictor of the survival of adult patients on the LT list, and has been found to better predict short-term results than does the CP score.[5] This difference in performance may explain the significant value of the MELD score, and not the CP score, for mortality prediction in our initial model." (pages 9–10, lines 249–256)

References

1. Kamath PS, Kim W. The model for end-stage liver disease (MELD). *Hepatology.* 2007;45:797-805.
2. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with endstage liver disease. *Hepatology.* 2001; 33: 464-70.
3. Luca A, Angermayr B, Bertolini G, Koenig F, Vizzini G, Ploner M, Peck-adosavljevic M, Gridelli B, Bosch J. An integrated MELD model including serum sodium and age improves the prediction of early mortality in patients with cirrhosis. Liver Transpl. 2007; 13(8):1174-80.
4. Ruiz-Margáina A, Macías-Rodrígueza RU, Duarte-Rojoc A,,Ríos-Torresa SL, Espinosa-Cuevasb A, Torrea A. Malnutrition assessed through phase angle and its relation to prognosis in patients with compensated liver cirrhosis: A prospective cohort study. Digestive and Liver Disease. 2015;47:309–314.
5. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with endstage liver disease. Hepatology. 2001; 33: 464-70.

In this study, we aimed to test whether this PA cutoff (≤ 4.9º) had prognostic value for mortality in a population of patients with cirrhosis of different ethnicity than used for its initial identification.

The Child-Pugh score had no significant effect in the initial mortality model and was not included in the final model (Table 2).

The Child-Pugh score was added to our initial mortality model because it may reflect the progression of liver damage and indirectly detect metabolic changes that may influence the prognosis of the disease.[ref] However, it had no significant effect on mortality prediction. Notably, the MELD score has been validated as a good predictor of the survival of adult patients on the LT list, and has been found to better predict short-term results than does the CP score.[ref] This difference in performance may explain the significant value of the MELD score, and not the CP score, for mortality prediction in our initial model. Data from the final mortality model support the prognostic value of PA ≤ 4.9°, as it was associated independently with mortality.

We assessed only male patients to make our sample as uniform as possible, as liver cirrhosis *per se* is a progressive disease and hepatic damage may differ, even slightly, among patients. In addition, cirrhosis is more common in men and malnutrition seems to have greater prognostic value for disease progression in men than in women. The prognostic ability of the studied cutoff value for phase angle is associated directly with malnutrition. Thus, by evaluating only men, we were able to

access not only a more uniform sample, but also the population most susceptible to the studied disease and its associated nutritional complications.