Peer Review File

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Response to Reviewer A

Comment 1: There is a need in the manuscript for more motivation of why estimating the association of serum to tissue AFP is a desirable goal. How would a relationship between the two be useful to know?

Reply 1: Thank you for your valuable comment. AFP is a special protein produced by fetal liver cell and is a normal component of fetal serum. It gradually disappears after fetus birth and is extremely low in healthy people. Clinically, HCC cell can synthesize and secrete alpha-fetoprotein to serum. So, serum AFP is significantly increased in patients with HCC, and there is internal relationship between serum AFP and tissue AFP status. However, 20% patients with negative serum AFP, we would like to know the clinical association between serum AFP level and tissue AFP status.

Changes in the text: we add some quotations See Page 4, line 96-98, and Page 5, line 100-103.

Comment 2: It would be desirable to provide some justification clinically for why Youden's index (which equally weights sensitivity and specificity in picking a cutoff) is used by the authors. This should be related to the clinical motivation for estimating this relationship of serum and tissue AFP.

Reply 2: Thank you for pointing this out. Generally, serum AFP is elevated in HCC patients. However, we find that some HCC patients' tissue AFP status is positive but serum AFP is in normal level or tissue AFP status is negative and serum AFP is in normal level clinically. Therefore, we would like to identify the relationship of serum AFP level and tissue AFP status. The ROC curve can easily detect the ability to recognize disease at any threshold, with the lowest total number of false positives and false negatives. ROC curve was plotted to investigate the diagnostic role of serum AFP for tissue AFP status. Youden's index (sensitivity + specificity - 1) was used to determine the optimal cut-off value from the ROC plot.

Changes in the text: See Page 5, line 100-103.

Comment 3: There are some concerns with the multivariate analysis modelling that should be addressed – the authors show that serum AFP is extremely highly correlated with tissue AFP, and that there are some strong correlations with both of these with

different clinical covariates, but then go on to include them all in a model (Table 3) and to attempt to interpret the individual regression coefficients of the terms. The problem with this approach is that it ignores the extremely strong collinearity between the serum and tissue variables and many of the other covariates (they establish and summarize in Table 2). In models with collinear terms the regression coefficient estimates are not reliable to interpret on an individual basis, standard errors are likely to be inflated and individual tests of coefficients lose power to detect true associations. Only testing the overall association of the joint model to the response would be justified here. The hazard ratio estimates given here for the multivariate model are quite probably dependent on the order that these variables were introduced into the model and would change should the order be permuted or different covariates be included in the model that are not correlated with serum or tissue AFP. This issue can be somewhat seen in that if you look at the univariate models presented in the same table, both positive serum AFP and tissue AFP are associated with increased hazard (reduced survival time) but when they both factors into a model, they conclude neither tissue AFP nor serum AFP does not, although HBV positivity, which they are both highly correlated with, is claimed to have independent significance.

Reply 3: Thank you for your comment. We converted serum AFP value into categorical variables. Then, we included serum AFP (<92.33 and ≥ 92.33) and tissue AFP status (positive and negative) in univariate and multivariate models. Although they had association, but they're not collinear. Therefore, we consider the results to be reliable. The limitation is a small number of patients included.

Changes in the text: None

Comment 4: There should be some discussion of the multivariate modelling results and the fact that serum AFP is used as a prognostic marker currently in the field, as the authors mention, yet their results conclude that serum AFP is not associated "independently" with overall survival after adjustment of for clinical covariates.

Reply 4: Thank you for your suggestion. The variation trend of serum AFP can be used as an index to evaluate the efficacy for HCC after receiving treatment in previous studies. In our study, we just included the preoperative serum AFP value. We found the optimal cut-off value of serum AFP was 92.33 ng/ml to identify the negative and positive tissue AFP. In multivariate analysis, serum AFP (<92.33 and ≥ 92.33) was not an independently prognostic factor. The result means single serum AFP (<92.33 and ≥ 92.33) value cannot evaluate the survival of HCC. We add some discussion in the manuscript.

Changes in the text: See Page 11, line 247-249

Comment 5: The authors should comment on the big disparities seen in the estimated association of the clinical covariates between the two types of AFP measurements, e.g. Tumor number, tumor size, tumor stage.

Reply 5: Thank you for pointing this out. We found that serum and tissue AFP were both associated with tumor stage, but serum AFP was associated with tumor size and tissue AFP was associated with tumor number. The two types of AFP measurements have different relationship of clinical covariates. We have made more discussions about their relationship according to the comment.

Changes in the text: See Page 10, line 227-233

Comment 6: More extensive specification of whether they are talking about serum AFP or tissue AFP throughout the paper, particularly when summarizing earlier results from other sources such as in the Introduction and Discussion would be helpful to the reader.

Reply 6: Thank you for your valuable comment. The previous reports about serum AFP or tissue AFP are important for our study, so we try our best to show the earlier results in our manuscript. We have retrieved literatures from Pubmed again to enrich the contents. However, there are few studies about tissue AFP. That is the significance of our study.

Changes in the text: See Page 5, line 100-103, Page line 227-233.

Response to Reviewer B

Comment 1: It has been already well-known that serum AFP levels are pivotal for diagnosis, prognostic value, and prediction of therapeutic effect. Therefore, you should not explain the utility of the serum AFP in the Introduction and Discussion a lot. Rather, tissue AFP should be enhanced, because the novel point of the present study is evaluating the tissue AFP. In addition, the contents described in Introduction and Discussion are messy and there is a lot of duplication. It should be concise and evade duplication. (Line. 85-108, L. 210-219)

Reply 1: Thank you for pointing this out. We totally agree with you. The previous reports about serum AFP or tissue AFP are important for our study and the earlier results of tissue AFP should be displayed importantly in the manuscript. We add some discussion about tissue AFP in the manuscript and delete the duplication according to your comment.

Changes in the text: See Page 4-5, line 93-104.

Comment 2: You should describe the protocol of ECLI and IHC briefly. Clone name, and dilution, and so on.
"with a range of 0-7ng/ml" I could not understand this meaning. Normal range? Measuring range?
(L. 131-134)
Reply 2: Thank you for your comment. We are sorry for ignoring the protocol of IHC, and we added the relevant contents in the manuscript. Serum AFP was measured by ECLI in Department of laboratory, but the value could be collected from preoperative results, we did not participate in the serum test.
Then, "a range of 0-7ng/ml" means the reference range of ECLI kits of serum AFP, so it is the normal range for serum AFP in our study. We make a clear explanation in the manuscript.
Changes in the text: See Page 6, Line 128, 131-135

Comment 3: The median age was 57.3 years (Interquartile range [IQR]:49.5-63.9 years), and more than half patients were less than 60 years old.
You wrote as above in the manuscript. However, "The median age was 57.3 years" means more than half Pts are included less than 57.3 years. Therefore, you should not write "more than half patients were less than 60 years old". (L. 159)
Reply 3: Thank you for your suggestion. We apologize for the repeat sentences.
Changes in the text: We have modified our text as advised and we deleted "more than half patients were less than 60 years old". See Page 7, Line 161.

Comment 4: More than 90% patients had only one tumor. (L. 160)
I think "single tumor" or "solitary tumor" is better.
Reply 4: Thank you for pointing this out. We are sorry for the poor standard English word.
Changes in the text: We have changed the word in the manuscript. See Page 7, Line 161.

Comment 5: The Kaplan-Meier curve was used to compare survival of patients with different serum and tissue AFP. I could not understand this sentence. What is "different"? (L. 183)
Reply 5: Thank you for pointing this out. We are sorry for the ambiguous sentences. What we would like to say is the different serum AFP level (< 92.33 and ≥92.33) and different tissue AFP (Negative and positive).

Changes in the text: We have modified our text in Page 8, Line 183-184.

Comment 6: Lastly, I recommend you should take the English editing again.

Reply 6: Thank you for your comment. We apologize for the poor standard of English language and grammar.

Changes in the text: We have tried our best to reedit the spellings and polish the English language in the manuscript. The changes were stained in yellow.