Author's response to reviews

Title: Alpha-Fetoprotein Level as a Biomarker of Liver Fibrosis Status in 619 consecutive patients with Chronic Hepatitis B

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Author's response to reviews:

March 24, 2014

Dr. G. Cabbibo
Associate Editor,
BMC Gastroenterology

Re. Resubmission of manuscript entitled “Alpha-Fetoprotein Level as a Biomarker of Liver Fibrosis Status in 621 consecutive patients with Chronic Hepatitis B”

Dear Dr. Cabbibo,

We would like to thank the reviewers for their thoughtful and careful critique of our manuscript. Please find attached a revised copy of the manuscript and a point-by-point response to the reviewers’ comments.

We have addressed all the concerns raised by the reviewers. We have now included the median AST, ALT and GGT values for the study population, and eliminated the analysis using AFP cut off > 400 ng/ml. In addition to the existing table, we have now added a figure with all values of AFP for each stage of inflammation and fibrosis. We have added a multivariable ordinal regression model for inflammation and fibrosis stages (Table 4) and have clarified that...
serum ALT was excluded from the multivariable analysis due to its co-linearity to AST. We have also emphasized in the Discussion section that this is the first study to investigate the subclinical significance of low levels of AFP and that measurement of AFP levels has potential application in clinical assessment of inflammation and fibrosis. In addition, we have extensively revised the entire manuscript to improve the readability. We are confident that our results, demonstrating that increasing levels of inflammation and fibrosis in CHB patients were associated with increased serum AFP levels, are interesting and novel. We believe that the revised manuscript will be of interest to the readers of BMC Gastroenterology.

We thank you for your time and consideration and look forward to a favorable response from you at your earliest.

Sincerely yours,

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Point-by-point response to reviewers' comments

Referee 2:
Reviewer's report
Title: Alpha-Fetoprotein Level as a Biomarker of Liver Fibrosis Status in 621 consecutive patients with Chronic Hepatitis B
Version: 1 Date: 13 September 2013
Reviewer: Vincenza Calvaruso
Reviewer's report:
In this manuscript Liu and co-workers aimed at identifying the role of Alpha-Fetoprotein Level as a biomarker of liver fibrosis in a cohort of patients with chronic hepatitis B.
However, there are several limitations of the manuscript:
One of the main features of chronic hepatitis B is the occurrence of acute flares which have an important role in the progression of the disease. However this significant difference of the inflammation grade during the time, make extremely difficult the evaluation of performance of fibrosis biomarkers. This is particularly evident for a marker which is strongly influenced by inflammation like AFP. A repeated and dynamic evaluation of the biomarker may be more useful in this setting.

Response: We agree with the reviewer that it is important to perform a dynamic evaluation of biomarkers like AFP which are influenced by inflammation. We do plan to address this in our future studies, and have now listed this as a limitation of the present study.

The performance of AFP in the evaluation of the different grades and stages could be not considered satisfactory (AUROC always lower than 0.80).

A proof of the unreliability of the AFP in this setting is demonstrated also by the fact that the median values of AFP at liver inflammation stages null (6.1 ng/ml) was higher than the median value of stage G1-G3.

Response: We have now revised our Discussion section to state that measurement of AFP levels has potential application in clinical assessment of patient’s inflammation and fibrosis. However, AFP by itself is not useful for this assessment, and an important future goal will be the development of reliable mathematical models using a combination of different biomarkers.

Moreover the analysis using with AFP cut off > 400 ng/ml should be eliminated because a test with high specificity but so low sensitivity is not clinically relevant. I suppose that only a very few number of subjects had AFP higher than 400 ng/ml.

Response: We have now revised Table 5 and eliminated the analysis using AFP cut off > 400 ng/ml. We have revised the Results section to reflect the revised statistical data.
As the author stated in the discussion they applied the accepted pathological staging system used in China but there are no known comparisons between this system and other universal systems. This is an important limitation of the study.
Response: We thank the reviewer for this comment, and have now added this as a limitation of our present study.

The discussion is too long and the session explaining the performances of the available not invasive tests of liver fibrosis should be eliminated.
Response: We have made extensive revisions to the Discussion section to improve the readability.

All figures showing the histological features of the differences stages of liver fibrosis did not add any informations to the study results and should be eliminated.
Response: We have now deleted Figure 2 which showed the histological features of the different stages of fibrosis.

Referee 1:
Reviewer’s report
Title: Alpha-Fetoprotein Level as a Biomarker of Liver Fibrosis Status in 621 consecutive patients with Chronic Hepatitis B
Version: 1 Date: 10 September 2013
Reviewer: Anna Pecorelli
Reviewer’s report:
Major Compulsory Revisions:
1) Materials and Methods, Patients, second paragraph: there are few data about study populations’ characteristics, for example: median value of ALT and AST, percentage of obese patients or patients with metabolic syndrome, diabetes or dislipedemia are included (these patients in fact could be affected by NASH
which can contribute to liver damage.)

Response: In addition to median AFP values, we have now included the median AST and ALT and GGT values in Table 2. We have also clarified that in this study, we excluded patients with moderate to severe fatty liver (as revealed by B-mode ultrasound) to prevent any possible bias.

2) Materials and Methods, Patients, second paragraph: how HCC has been excluded? Which imaging techniques have been used? And which criteria has been adopted?

Response: Based on the 2009 AASLD recommendations, initial screening for HCC should be done using B-mode ultrasound, and MRI scan and enhancement should be adopted to exclude cancerration of liver nodules when necessary. In this study, all study subjects underwent at least one B-mode ultrasound examination prior to liver biopsy. Patients with AFP levels > 200 ng/ml underwent CT/MRI scan with enhancement. We have now included this information in the Methods section.

3) Materials and Methods, Methods, Liver biopsy and histopathology, first paragraph: it is no specified if biopsies were made by different clinicians. Different experience could influence for example the length of the tissue samples.

Response: We have now clarified that all biopsies were performed by different experienced doctors, each of whom had performed the procedure more than 20 times. All procedures were under the guidance of B-mode ultrasound.

4) Materials and Methods, Methods, Liver biopsy and histopathology, first paragraph: if possible specify the median length of tissue samples. 15 mm is too small (a length of at least 25 mm is necessary to evaluate fibrosis accurately).

Response: We have now clarified that the length of the tissue samples obtained were 1.5-2 cm or longer.

5) Results, Demographic characteristics, first paragraph: patients < 12 years old should be excluded because the possible different behavior of AFP in child.
Response: We have now revised our analyses after excluding the two subjects who were younger than 12 years old.

6) Results, Demographic characteristics, first paragraph: no data are available on the HBV infection duration. Specify the median duration of CHB.
Response: We thank the reviewer for this comment. However, it is difficult to estimate the median duration of CHB infection, because 70% of patients in China are infected via mother-to-child vertical transmission. We have now revised our Discussion section to state that the initial detection of CHB infection does not represent the start of infection and we therefore used age to replace the duration of infection.

7) Results, Demographic characteristics, first paragraph: there are too many patients in inflammation and fibrosis stage 3-4 (more than half).
Response: Our data (Table 2) showed that Stages 1, 2 and 3 had 24.4%, 22% and 20.8% of study patients, respectively, while Stage 4 had 32% of patients. These data suggest that there was no significant bias in patient selection.

8) Results, Correlation of AFP versus liver inflammation and fibrosis stage: insert a figure with all values of AFP for each stage of inflammation and fibrosis, not just a table.
Response: We thank the reviewer for this comment and have now included these data in the new Figure 2.

9) Discussion, first paragraph: it’s mandatory to evaluate the correlation between AFP and ALT, AST and Gamma-GT.
Response: We have now revised this section to state that AFP had significant weak to moderate correlation with AST, ALT and GGT values (Spearman’s correlation coefficients were 0.22, 0.331, and 0.445, respectively; all p values < 0.001).

10) Discussion, first paragraph: it’s mandatory to evaluate the reproducibility of AFP test dosages.
Response: As we have stated in our Methods section, all patients in this study were hospitalized patients, and all serum and liver biopsy samples were obtained within 7 days after hospital admission. Due to the characteristics of CHB, AFP may decline significantly or change in these patients after treatment. The reproducibility of AFP values could therefore not be determined, and all AFP values in this study were the pre-treatment baseline levels. Since the major objective of this study was to explore the clinical significance of low levels of AFP, we did not discuss other indicators in detail.

11) Discussion, first paragraph: the discussion about age and gender should be included in the results not in the discussion.
Response: We have now moved the information about age and gender from the Discussion to the Results section.

12) Discussion, first paragraph: it is mandatory a multivariate analysis of age and sex.
Response: We have now added a multivariable ordinal regression model for inflammation and fibrosis stages (Table 4) and have included these data in the Results section.

13) Discussion, fourth paragraph: it is unacceptable not include ALT values for each patient, which is an inclusion criterion for the diagnosis of CHB as specified in the text.
Response: We have now clarified in the Discussion section that serum ALT was excluded from the multivariable analysis (Table 4) due to its colinearity to AST. Other variables including AST and GGT were analyzed.

Quality of written English: Needs some language corrections before being published
Response: The entire manuscript has been extensively revised to improve the readability.