Author's response to reviews

Title: Serum hyaluronate as a Non-invasive Marker of Hepatic Fibrosis and Inflammation in HBeAg-negative Chronic Hepatitis B

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Version: 4 Date: 3 July 2005

Author's response to reviews: see over
Dear editors

Thank you very much for your kind message informing us of the encouraging decision of the editorial board regarding our manuscript. We are very thankful to the reviewers for their thoughtful and precise critics, observing which has helped us to improve the quality of the manuscript substantially. Attached please find a thoroughly revised version of our work. My co-authors and I have tried our best to go through and include all the points raised by the reviewers. You will find below the point by point response to reviewers’ comments.

Best regards
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Reviewer 1:

Thank you very much for your constructive comments.

1) The manuscript was completely revised. The data concerning patients with decompensated cirrhosis was omitted from our analysis. Furthermore, because of small number of HBeAg positive chronic hepatitis B (CHB) (14 patients), we omitted data of HBeAg positive patients, and focused on HBeAg negative CHB.

2) We agree with the referee 1 that number of our patients with extensive fibrosis was relatively small. We added this point as one of limitations of the study in the discussion section of the revised manuscript.
3) Our aim was to find out the level of hyaluronate to discriminate mild from severe fibrosis and inflammation. The cut off point for this purpose was identified (126.4 ng/ml), which could discriminate subgroups of chronic hepatitis B. In this study serum hyaluronate was higher in CHB patients (as a whole) compared to normal volunteers. Also, our data showed that hyaluronate level was not statistically different between CHB patients with mild fibrosis than normal volunteers. However, its level increases with advancement of fibrosis.

4) We had one patient with stage 6 fibrosis with hyaluronate level of 1260ng/ml. In the process of this work patient developed decompensated cirrhosis and therefore was omitted from our study.

5) We agree that we do not have stage 6 patient in this study. It would be better if we could have number of patients with stage 6. Presence of well defined regenerative nodules which differentiate stage 6 from 5 are not appreciated by our pathologist. Therefore, it is not unfair to call stage 5 as compensated cirrhosis. As a matter of fact stage 5 and 6 are considered compensated cirrhosis by some investigators (1).

6) Different levels of hyaluronate in subgroup of patients with chronic hepatitis B are real and are not by chance. However further work with a bigger sample size is running in our institution to validate and reaffirm this finding.

**Reviewer 2:**

Thank you very much for your constructive comments.

1) The manuscript was completely revised. The data concerning patients with decompensated cirrhosis was omitted from our analysis.
2) Our aim was to discriminate between subgroups of patients with chronic hepatitis B by the serum hyaluronate level. Therefore we have not used normal values in our ROC curve.

3) The work of Myers et al was discussed in the discussion.

4) We were looking for a simple, cheap and noninvasive test which could be used routinely in our laboratory.

5) Fibro test and Acti test have been shown to be valuable but because of high expense and complexity, we are not able to use it routinely in our laboratory.

6) Our primary aim was to discriminate the levels of fibrosis but our secondary endpoint was to discriminate inflammation too. Because the process of fibrogenesis and inflammation are inseparable in vivo.

7) Each biopsy sample had 10 mm length, 1.4 mm width and contained 4 or more portal spaces.

8) Using cut off points of 126.4 for serum hyaluronate, only one out of 54 patients with mild fibrosis would be misclassified as having extensive fibrosis. This patient had stage 2 of liver fibrosis and had serum hyaluronate level of 138.0 ng/ml. Conversely, only one of 11 patients with extensive fibrosis had low serum hyaluronate. That patient with stage 4 of fibrosis had serum hyaluronate level of 37.7 ng/ml.

9) Mean ± SD of serum hyaluronate in the normal volunteers was 20.4 ±15.4. The upper limit of normal (as defined by the 95th percentile of the variable) in the normal volunteers was 58.6 ng/ml. This point was added to the revised version of the manuscript.

Reviewer 3:
Thank you very much for your constructive comments.

1) The manuscript was completely revised. The data concerning patients with decompensated cirrhosis was omitted from our analysis.

2) Because of small number of HBeAg positive CHB (14 patients), we omitted data of HBeAg positive patients, and focused on HBeAg negative CHB.

3) We have not used METAVIR classification because our pathologists are used to Ishak scoring system (2). This system has been validated and widely used world wide. In a comparative study, there was excellent correlation with regard to liver fibrosis (weighted kappa 0.998) between Ishak and Metavir systems (3). Furthermore, many previously performed noninvasive fibrosis markers studies have used Ishak system for scoring liver fibrosis (1,3).

4) We used hyaluronate, because it is cheap and simple. Our data reaffirm that it is a useful test .This does not devaluate the Acti test and Fibro test.

References:

