Author's response to reviews

Title: A novel scoring system for prognostic prediction in d-galactosamine/lipopolysaccharide-induced fulminant hepatic failure BALB/c mice

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Author's response to reviews: see over
Dear Dr. Rikki Graham
Senior Assistant Editors
BMC series Journals

We do appreciate for your instruction and advices, which let us to view our submitted manuscript more carefully. We think the three reviewers’ comments are very important. We have revised our manuscript in some places accordingly. Followings are answers with some important discussions for a clear understanding.

Table 1. List of changes made to the first reviewer’s comments

<table>
<thead>
<tr>
<th>Original questions</th>
<th>Corresponding answers</th>
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<tr>
<td>Authors should compare their score using small molecules with conventional parameters such as ALT, AST, LDH etc. Is their score superior to conventional parameters?</td>
<td>It is a very good question, which can make our manuscript more value. When we carried out this investigation, we detected ALT, AST, and Bilirubin, the data about their plasma levels were supplemented in “Methods”, “Results” and Table 1 in this revised manuscript. Unfortunately, we did not detect LDH due to limited sera.</td>
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<tr>
<td>As a minor point, in Materials and methods, what is &quot;10 microL EDTA&quot;?</td>
<td>It should be “10µL solution of 4% EDTA”.</td>
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Table 2. List of changes made to the second reviewer’s comments

<table>
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<tr>
<th>Original questions</th>
<th>Corresponding answers</th>
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<tr>
<td>In the multivariate logistic regression analysis used to derive the DSI, the authors state that “After 3 steps, lactate and glucose were removed, and the other variables (HB, urea, and phosphate) were used to calculate the nomogram”. The authors should explain why lactate and glucose were removed in calculating the DSI.</td>
<td>For explaining this, we changed “Backward stepwise was used in the multivariate logistic regression analysis.” to “Backward stepwise, which is one of three main approaches to automating the selection of explanatory variables for inclusion in a regression model, was used in the multivariate logistic regression analysis. In backward selection, all variables are included in the initial model, and then removed sequentially until a final model is produced.”</td>
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<td>The authors should state this as a potential limitation of the study in the Discussion section.</td>
<td>It is a good suggestion. We supplemented “Potential limitations” in the Discussion section as follow.</td>
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</table>
Various biological factors affect the metabolic composition of biological fluids, and these factors might be involved in human to different extend. Although our previous pilot result showed that changes of plasma phosphate, HB, urea, glucose and lactate levels in FHF patients were consistent with those of FHF mouse model, it is unknown whether the scoring system based on the five metabolites would be applicable to predicting the prognosis FHF seen in clinical practice. In addition, etiology of FHF patients is complex, which can influence metabonomics of body fluids. All of these need a further investigation.

| There are several grammatical errors throughout the text that should be corrected. | 1. In the third sentence of the third paragraph of “Introduction”, we changed “with d-galactosamine/lipopolysaccharide (GalN/LPS)-treated BALB/c mouse model of FHF,” to “in d-galactosamine/lipopolysaccharide (GalN/LPS)-treated BALB/c mouse model”.  
2. In the 2nd sentence of the 1st paragraph of “Methods”, we deleted “were” before “approved by the Subcommittee on Research Animal Care and Laboratory Animal Resources”.  
3. In the forth sentence of the 2nd paragraph of “Methods”, we added “at” before “5 h after treatment from 23 mice of the survival group”.  
4. In the fifth sentence of the 2nd paragraph of “Methods”, we changed “10 µL EDTA” to “10 µL solution of 4% EDTA”.  
5. In the forth subtitle of “Results”, we added “the” before “survival and dead groups”.  
6. In the 2nd sentence of the third paragraph of “Discussion”, “more effectively than more traditional and inexpensive laboratory values” was changed to “more effectively than those traditional and inexpensive laboratory values”. |

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<th>Table 3. List of changes made to the third reviewer’s comments</th>
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<td>Original questions</td>
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<td>Many institutions do not allow the use of “dead” of the study animals</td>
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to be the endpoints. Would you please clarify how you define the premortum characteristics including clinical behaviors to select the surviving and dead animals in your study? predicting the prognosis of FHF mouse model. And early prognostic prediction is very important for decision whether medical treatment or liver transplantation. So, we use whether “dead” or not as the endpoint. Similar method can be found in some papers (Yokoyama T, et al. Metab Eng, 2005, 7:88–103. Arai K, et al. Hepatology, 2001, 34:360-371. Arvelo MB, et al. Hepatology, 2002, 5:535-543.). Additionally, the study was carried out in accordance with the Chinese National Research Council guidelines and approved by the Subcommittee on Research Animal Care and Laboratory Animal Resources of the Peking University People’s Hospital.

2. As for manifestations of the GalN/LPS mouse model
   At 4 and 5 hours after treatment, there were no differences between mice treated with GalN/LPS and control mice. Six hours after GalN/LPS treatment, there were no significant differences between the survival group and the control group, but the dead group exhibited slow movements and reactions and very tumescent livers.(Feng B, et al. Liver transpl, 2008, 14:1620-1631)
   In addition, after single retro-orbital blood, the mice are still surviving and can be investigated until the endpoint.

DSI appears to be potentially promising. However, at what time points in the clinical course of the patients with FHF would the equation be applicable? Would you predict that is there a possible time point in the course of FHF that DSI would not be effective or predictive such as if the equation is applied after 12 or 24 hours since the induction or onset of FHF? Are there any other limitations of this study? Yes, it is a good question. In revised manuscript, we supplemented “Potential limitations” in the Discussion section as follow.
   Various biological factors affect the metabolic composition of biological fluids, and these factors might be involved in humans to different extend. Although our previous pilot result showed that changes of plasma phosphate, HB, urea, glucose and lactate levels in FHF patients were consistent with those of FHF mouse model, it is unknown whether the scoring system based on the five metabolites would be applicable to predicting the prognosis FHF seen in clinical practice. In addition, etiology of FHF patients is complex, which can influence metabolomics of body fluids. All of these need a
I would like to see a table documenting the biochemical profile including ALT, INR, Glucose, T-bil, urea, phosphate, and b-hydroxybutyrate levels of the 2 surviving and dead groups at 6 hours after Gal-LPS induction. In the table, the Cliché and Kings’ College Hospital criteria should be included.

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<th>Further investigation.</th>
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<td>Plasma levels of ALT, AST, and TBIL were added to the manuscript (Table 1). Here, levels of plasma phosphate, HB, urea, glucose and lactate were not showed because related data were published in our previous paper (Feng B, et al. Liver Transpl, 2008, 14:1620-1631). The frequently used clinical tools to predict mortality in FHF are King’s College Hospital criteria and Clichy criteria (Chung PY, et al. Liver Transpl, 2003, 9: 248-253). Unfortunately, some parameters such as arterial pH, PT, INR among others are difficult to detect in BALB/c mice due to limited sera. Because of these, we added “Potential limitations” in revised manuscript. It is unknown that the scoring system based on the FHF mouse model would be applicable to predicting the prognosis FHF seen in clinical practice. By a further investigation, based on metabonomics and multivariate logistic regression, we will understand whether a new scoring system is superior to Cliché and King’s College Hospital criteria in FHF patients.</td>
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We hope you will find our revised manuscript acceptable for publication. If you still have some questions on revision, please contact us without any hesitation.

Thank you again and Best wishes

Sincerely yours

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