

Novel circulating- and imaging- based biomarkers to enhance the mechanistic understanding of human drug-induced liver injury

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1st Editorial Decision

Date: 29 Nov, 2016

Ref.: Ms. No. JCTRes-D-16-00038

NOVEL CIRCULATING- AND IMAGING- BASED BIOMARKERS TO ENHANCE THE MECHANISTIC UNDERSTANDING OF HUMAN DRUG-INDUCED LIVER INJURY

Journal of Clinical and Translational Research

Dear Dr. Antoine,

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at <http://www.jctres.com/en/author-guidelines/> under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Mar 29, 2017.

To submit a revision, go to <http://jctres.edmgr.com/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Hartmut Jaeschke, PhD
Associate Editor
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This is an excellent review from Clarke et al. The review gives a detailed summary of the current status of an array of biomarkers that have been studied extensively in liver disease. Some minor changes are suggested below.

- * Page 7 lines 48-50. This sentence is a bit of a run-on and could use some punctuation.
- * "Once the inflammatory cells are activated they can liberate ROS which will act as a turnover, as disulphide HMGB1 has higher affinity for TLR and RAGE, increasing cytokines release and inducing more inflammatory cells recruitment [42]."

I am confused by the word turnover here. Another phrase may make this point more clearly.

- * The referenced Huebener paper is only on two differences diseases. It would be beneficial to point out it is unknown if this effect is ubiquitous or limited to APAP and Gal/LPS.

- * "Circulating elevated levels of HMGB1, acetylated HMGB1 and K18 (keratin-18) were identified in the serum of patients with cholestatic liver injury (n=17), compared to patients without any concurrent injury (n=10), confirming inflammatory necrosis as an underlying mechanism of obstructive cholestasis. Overall these data suggest that the underlying mechanisms in humans are fundamentally different to those previously reported in equivalent diagnoses within pre-clinical rodent species [53]."

It may beneficial to note that the authors found these same circulating molecules in another pre-clinical rodent study, making these markers translational in nature in the model, see Woolbright et al., 2013.

- * The section on full length K18 is incredibly limited, especially given that dozens of liver diseases have elevations in K18. This section should be expanded, even if it only to mention the near ubiquitous increases seen in FL-K18.

Reviewer #2: This is a useful and informative review of DILI biomarkers. A few issues should be addressed:

- 1) The quality of the writing is poor, which is surprising considering the high quality of other recent publications by the senior author. I would suggest that the senior author review and revise the text to improve readability. For example, on page 4, line 47, I do not believe that "haematological" is the correct word, as the biomarkers being discussed have nothing to do with hematology or coagulation. Other sections are simply hard to follow due to the writing.
- 2) In the final paragraph on page 6, it would be useful to more fully discuss the implications of the Ward et al. paper in PNAS (2014). The authors of that paper found that miRNA profiles could differentiate between APAP-induced liver injury and hypoxic hepatitis in patients. That is a critical point for diagnosis that should be discussed.
- 3) At the top of page 8, the authors present the role of inflammation in DILI and specifically APAP-induced liver injury as if it is well-established. However, there is considerable controversy. For an overview of the controversy surrounding HMGB1 specifically and inflammation in general, see Jaeschke et al. Liver Int. 2012. These controversies should be briefly discussed by the authors of the present manuscript as well.
- 4) In the acylcarnitines section on page 13, the authors should also discuss the results in Bhattacharyya et al. Biomark Med. 2014., in which it was found that acylcarnitines are significantly increased in some patients after APAP overdose (those who received NAC at later time points).

Monday 19th December 2016

RE: Manuscript submission – JCTRes-D-16-00038

Dear Editors,

We would like to thank the reviewers for their constructive comments which we have found very useful. We have updated the manuscript based on this advice and we believe it is improved as a result. Below we detail our responses to the reviewer's comments and indicate specific changes made to the manuscript.

Reviewers' comments:

Reviewer #1: This is an excellent review from Clarke et al. The review gives a detailed summary of the current status of an array of biomarkers that have been studied extensively in liver disease. Some minor changes are suggested below.

→ Thank you very much for these comments.

1. Page 7 lines 48-50. This sentence is a bit of a run-on and could use some punctuation.

→ We have rephrased this sentence to ameliorate the reading.

2. "*Once the inflammatory cells are activated they can liberate ROS which will act as a turnover, as disulphide HMGB1 has higher affinity for TLR and RAGE, increasing cytokines release and inducing more inflammatory cells recruitment [42].*" I am confused by the word turnover here. Another phrase may make this point more clearly.

→ We have changed the word turnover which was confusing. This paragraph has also been edited to make it more understandable.

3. The referenced Huebener paper is only on two differences diseases. It would be beneficial to point out it is unknown if this effect is ubiquitous or limited to APAP and Gal/LPS.

→ We agree with this suggestion and have made a note of this ambiguity within the text.

4. "*Circulating elevated levels of HMGB1, acetylated HMGB1 and K18 (keratin-18) were identified in the serum of patients with cholestatic liver injury (n=17), compared to patients without any*

concurrent injury (n=10), confirming inflammatory necrosis as an underlying mechanism of obstructive cholestasis. Overall these data suggest that the underlying mechanisms in humans are fundamentally different to those previously reported in equivalent diagnoses within pre-clinical rodent species [53]." It may be beneficial to note that the authors found these same circulating molecules in another pre-clinical rodent study, making these markers translational in nature in the model, see Woolbright et al., 2013.

→ We have included Woolbright reference and explained the findings in pre-clinical model.

5. The section on full length K18 is incredibly limited, especially given that dozens of liver diseases have elevations in K18. This section should be expanded, even if it only mentions the near ubiquitous increases seen in FL-K18.

→ We agree and have expanded on this section. Our aim was to keep recap from other reviews to a minimum and focus here on novel findings and a mechanistic basis. We also discuss K18 in more detail, later in the review alongside cK18 where it makes more sense to discuss the two markers together to avoid repetition and improve readability.

Reviewer #2: This is a useful and informative review of DILI biomarkers. A few issues should be addressed:

1. The quality of the writing is poor, which is surprising considering the high quality of other recent publications by the senior author. I would suggest that the senior author review and revise the text to improve readability. For example, on page 4, line 47, I do not believe that "haematological" is the correct word, as the biomarkers being discussed have nothing to do with hematology or coagulation. Other sections are simply hard to follow due to the writing.

→ We thank the reviewer for this advice and have removed the term "haematological" from the text in reference to the biomarkers, and replaced with "circulating".

→ In other sections we hope to have also improved readability.

2. In the final paragraph on page 6, it would be useful to more fully discuss the implications of the Ward et al. paper in PNAS (2014). The authors of that paper found that miRNA profiles could differentiate between APAP-induced liver injury and hypoxic hepatitis in patients. That is a critical point for diagnosis that should be discussed.

→ We have included miRNA profile and discrimination between APAP-induced liver injury and ischemia from Ward et al.

3. At the top of page 8, the authors present the role of inflammation in DILI and specifically APAP-induced liver injury as if it is well-established. However, there is considerable controversy. For an overview of the controversy surrounding HMGB1 specifically and inflammation in general, see Jaeschke et al. Liver Int. 2012. These controversies should be briefly discussed by the authors of the present manuscript as well.

→ We have added a brief statement on the beneficial effects of the inflammatory response reviewed by Jaeschke et al in 2012.

4. In the acylcarnitines section on page 13, the authors should also discuss the results in Bhattacharyya et al. Biomark Med. 2014., in which it was found that acylcarnitines are significantly increased in some patients after APAP overdose (those who received NAC at later time points).

→ We have used Bhattacharyya et al. reference to show the utility of ACs in diagnosing APAP-induced liver injury and the need of further investigation in mitochondrial biomarkers field.

We would like to thank the editors for the opportunity to submit a revised manuscript.

Thank you for your consideration.

Yours sincerely,

A handwritten signature in black ink, appearing to read "D. Antoine". The signature is fluid and cursive, with a large initial "D" and a trailing flourish.

Daniel Antoine, PhD

Journal of Clinical and Translational Research
Review process file 03.2017S1.005

2nd Editorial decision
Date: 19 Dec, 2016

Ref.: Ms. No. JCTRes-D-16-00038R1
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Dear Dr. Antoine,

Thank you for submitting your revision and addressing the reviewers' comments. A few minor technical issues need to be addressed.

To submit a revision, go to <http://jctres.edmgr.com/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Hartmut Jaeschke, PhD
Associate Editor
Journal of Clinical and Translational Research

Reviewers' comments:

The authors addressed all comments of the reviewers. However, a few issues with the references need to be addressed:

ref. 48 is the same as ref. 64

The following references are incomplete (mainly volume and page numbers are missing): 29, 39, 44, 94, 96

3rd Editorial decision
Date: 20 Dec, 2016

Ref.: Ms. No. JCTRes-D-16-00038R2
NOVEL CIRCULATING- AND IMAGING- BASED BIOMARKERS TO ENHANCE THE
MECHANISTIC UNDERSTANDING OF HUMAN DRUG-INDUCED LIVER INJURY
Journal of Clinical and Translational Research

Dear Dr. Antoine,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

Comments from the editor and reviewers can be found below.

Journal of Clinical and Translational Research
Review process file 03.2017S1.005

Thank you for submitting your work to JCTR.

Kindest regards,

Hartmut Jaeschke, PhD
Associate Editor
Journal of Clinical and Translational Research

Comments from the editors and reviewers:
