JOURNAL OF Neuroscience Research

A Systematic Review of Non-Inflammatory Cerebrospinal Fluids Biomarkers For Clinical Outcome in Neonates With Perinatal Hypoxic Brain Injury That Could be Biologically Significant

Shi, Zhongjie; Luo, Kehuan; Deol, Saihaj; Tan, Sidhartha

Review timeline:

Submission date: 17 Nov 2020
Editorial Decision: Minor modification (22 Dec 2020)
Revision Received: 12 Jan 2021
Accepted: 14 Jan 2021

Editor 1: Eric Prager
Editor 2: Junie Warrington
Reviewer 1: Kasper Kyng
Reviewer 2: Steven Levison
Reviewer 3: Stephen Back

1st Editorial Decision

Decision letter

Dear Dr Shi:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We have now received the reviewer feedback and have appended those reviews below. I am glad to say that the reviewers are overall very enthusiastic and supportive of the study. They did raise some concerns and made some suggestions for clarification, but I expect that these points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I will be glad to discuss.

We ask that you return your manuscript within 30 days. Please explain in your cover letter how you have changed the present version. If you require longer than 30 days to make the revisions, please contact Dr Junie Warrington (jpwarrington@umc.edu). To submit your revised manuscript: Log in by clicking on the link below https://rex-prod.resxchange.com/submissionBoard/1/bd0d18c7-cf99-4fc2-8751-dd494a6ecd7c/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Nov-9220) where indicated. This will help us to link your revision to your original submission.)

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Eric Prager
Associate Editor, Journal of Neuroscience Research

Dr Junie Warrington
Editor-in-Chief, Journal of Neuroscience Research

JOURNAL OF Neuroscience Research

Associate Editor: Prager, Eric
Comments to the Author:
Thank you for submitting this excellent and timely manuscript. As you will see from the reviewer comments, they found the paper to be very informative, but and I agree with this completely, Reviewer 1 wants you to follow the Prsima guidelines completely. The reviewers also note that some grammatical errors are scattered throughout and some statements need to be tightened up some. I'd ask that you carefully review the manuscript fo these as well. We look forward to seeing a revised manuscript. Happy Holidays.

Reviewer: 1

Comments to the Author
Thank you for the opportunity to comment on this review. Clearly, a lot of work has gone into compiling the tables and I agree that a review on this important topic is needed. However, I have some major comments that should be addressed prior to an in-depth review.

Major comments
- Protocol not registered a priori (e.g. in PROSPERO)
- PRISMA checklist should be followed
- The scientific literature and knowledge of mechanisms of brain injury on term HIE are largely separate from that on hypoxia-ischemia in preterm. In this review term and preterm brain injury are largely mixed up. Suggest clearly separating data on these two separate populations throughout the review
- the objective/aim of the review should be clearly stated in the abstract and introduction
- Insufficient discussion of the populations in the included studies including the timing of sampling
- No evaluation or discussion of the risk of bias including publication bias
- No discussion of metaanalysis? Was it considered, why was it not done?
- Insufficient discussion of strengths and limitations in the review as performed
- Tables are very long, suggest helping the reader by synthesizing the data. Consider graphical presentation
- It is not obvious that "ranking the biomarkers according to the difference in the bounds of the confidence intervals" identifies biological significance. Please discuss. While I am not intimately familiar with 'the estimation method' it is possible to have overlapping CIs and statistically significant difference in means and equally the opposite is also possible

Additional
Title
- Title should identify that this is a systematic review
- suggest removing "That Could be Biologically Significant" which is implied
- what type of biomarkers? Should be stated in the title
- biomarkers of what? Should be stated in the title

Abstract
- 'we identified the best biomarkers' by what criteria?
- 'preterm HIE' is not a well-defined entity and if used it should be specified

Introduction
- Line 39: Cortical spinal fluid?

Materials and Methods
- Protocol not registered
- Why was the earliest test result extracted for multiple timepoints? How did this affect results?

Tables and Figures
- 'checkpoint' change heading to 'age at CSF sampling (days)' or similar
- Figure 1. It is not obvious what a 'doubtful' biomarker is

Reviewer: 2

Comments to the Author
Summary: In this systematic review article, the authors have evaluated articles that reported on levels of

non-inflammatory cerebrospinal fluid
biomarkers that are likely involved in hypoxic-ischemic encephalopathy. They estimated the biological significance by calculating a score based on the difference in the bounds of the confidence intervals and identified several prognostic CSF biomarkers for severe neonatal brain injury. The article represents a significant amount of work that will be valuable for the research community. The article is for the most part well written and the tables and figures are clearly presented. A few aspects of the article need revision.

1. At the outset of the article it would be helpful to state why it is important to identify biomarkers to identify and stratify those infants that might develop brain injury from HIE or asphyxia.

2. Pg. 6. Please provide the interval after HIE during which these CSF samples were taken to assist the reader in understanding whether these are biomarkers for acute, secondary or tertiary brain injury

3. Pg. 9. It would be helpful to indicate why CSF samples were obtained from non-HIE infants.

4. Pg. 9. Please consider replacing the word "indicating" with the phrase "classified as" for defining the categories for biomarkers.

5. Pg. 12. There are multiple isoforms of VEGF; therefore, please define which VEGF isoform has been studied on this page and in future sections of the review.

6. Pg. 14. The authors describe CK and CK-BB. If these are the same enzymes then please used one abbreviation through this review. If they are different then please define each enzyme.

7. Pg. 14. Please clarify the sentence that begins with "CK-BB increased with severity of impairment at 12 months of age". Was CK repeatedly measured or were CK levels positively correlated with severity of impairment?

8. Pg. 18. The sentence in the Discussion that begins with "Xanthine oxidase is a" needs to be re-written for accuracy.

9. Pg. 19. There are multiple forms of NSE with only the gamma gamma isoform specifically expressed in neurons. Therefore, this paragraph needs to be updated.

10. Pg. 21. Please define the tertiary phase of injury. Also, please indicate which therapeutics might be appropriate to prevent tertiary brain injury. Also, it is not clear that injury resulting from severe HIE will be reduced with treatments provided during the tertiary phase of injury. Therefore, the authors might want to emphasize that these biomarkers should be evaluated to see if they can be used to diagnose a moderate HIE injury vs. a severe HIE, as they then could be used to determine which treatments would be appropriate for infants during the tertiary phase of recovery.

11. Table legends: It would be helpful to define how the Score ($\Delta\% \bar{X}$) was calculated so that a reader won't need to consult the Methods to understand the table.

Reviewer: 3

Comments to the Author
In this manuscript, the authors undertake a literature analysis to identify potential biomarkers for various forms of preterm or full term neonatal hypoxic brain injury. Based on rigorous selection criteria, they selected 17 studies gleaned from an initial title/abstract screen of 993 studies that analyzed CSF non-inflammatory markers. They employed a rigorous unbiased series of analyses employing pooled mean differences and 95% confidence intervals. From this analysis they identified several promising CSF biomarkers for severe forms of HIE (creatine kinase, xanthine oxidase, vascular endothelial growth factor, neuron specific enolase, superoxide
dismutase and malondialdehyde).

The real strength of this article resides in the excellent discussion, which puts the potential biomarkers into the broader context of brain injury neurochemistry and biochemistry, which should be of particular interest to readers of JNR who focus on the pre-clinical analysis of hypoxia-ischemia mechanisms of brain injury or the identification of potential biomarkers. The article provides valuable insights into the challenges of translating from bench to bedside to ideally develop of panel of CSF biomarkers, which could be employed acutely for preterm neonates or subacutely for full term neonates after therapeutic hypothermia.

I have minor suggestions to enhance the accessibility of this data to readers of the JNR.

- A concise summary table would be value to pull together the take home messages for the patient populations for which each marker may be most valuable to pursue further. This could address different patient populations, the spectrum of severity of HIE (none, mild/moderate vs. severe) and utility as acute vs. delayed injury phase markers.
- Tables 1 to 4 are ideally supplemental data tables that could be stored on the JNR server or made accessible through an independent repository site.
- Although it may be beyond the scope of the article, it would be interesting to briefly discuss how robustly the findings from pre-clinical/experimental studies align with those from this clinical analysis.Authors' Response

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Comments to the Author:
Thank you for submitting this excellent and timely manuscript. As you will see from the reviewer comments, they found the paper to be very informative, but and I agree with this completely, Reviewer 1 wants you to follow the Prsima guidelines completely. The reviewers also note that some grammatical errors are scattered throughout and some statements need to be tightened up some. I'd ask that you carefully review the manuscript fo these as well. We look forward to seeing a revised manuscript. Happy Holidays.

Authors' response:

Dear Drs. Prager and Warrington,

We thank the editors so much for the opportunity to revise our manuscript. We really appreciate the insightful comments from the reviewers.

Reviewer 1:
Clearly, a lot of work has gone into compiling the tables and I agree that a review on this important topic is needed. However, I have some major comments that should be addressed prior to an in-depth review.
Major comments
- Protocol not registered a priori (e.g. in PROSPERO)

Authors' response: We have checked and found that reviews that have progressed beyond the point of completing data extraction are not eligible for inclusion in PROSPERO. We thank the reviewer for this valuable advice, and we will register our future review protocols.

- PRISMA checklist should be followed

Authors' response: The completed PRISMA checklist is now attached. We followed the journal format in dealing with the formatting issues

Journal: Abstracts should be written as a single, continuous paragraph

- The scientific literature and knowledge of mechanisms of brain injury on term HIE are largely separate from that on hypoxia-ischemia in preterm. In this review term and preterm brain injury are largely mixed up. Suggest clearly separating data on these two separate populations throughout the review

Authors' response: We agree that ideally term and preterm neonatal encephalopathy should be separately presented. We have also shown preterm vs term in Table 4. However, in reality, some studies do not mention gestational age, or combined term and preterm gestation together (Tables 1-3). Furthermore, scientifically, neonatal encephalopathy is actually a continuum from term to nearterm

to preterm to extreme preterm gestational ages. There simply are not enough studies in each category to parse out the differences.

- the objective/aim of the review should be clearly stated in the abstract and introduction

Authors' response: We have revised the abstract to state that "we focused on the non-inflammatory biomarkers in cerebrospinal fluid (CSF) that are involved in the development of possible brain injury in

asphyxia or HIE".

- Insufficient discussion of the populations in the included studies including the timing of sampling

Authors' response: There were only two studies that specifically looked at timing of the sampling. We had discussed the time-course patterns of two CSF markers in the original manuscript.

- No evaluation or discussion of the risk of bias including publication bias

Authors' response: We agree that bias should always be evaluated in systematic reviews. However, given that the included studies reported quite a lot of biomarkers, and each marker only had 1-2 studies at the most, it was very difficult to show publication bias or even use funnel plots in a metaanalysis.

We have added a supplementary table to show the quality of included studies and added a sentence explaining how we calculated risk of bias. If anything, we took a conservative approach and tried to focus on studies that not only showed statistical significance but biological significance as well. The logic of the argument being that for a biomarker that showed biological significance by our criteria of "strong", i.e. $\Delta\% \bar{X} > 100$, it was unlikely for studies replicating the original study to be nonsignificant

and be subject to publication bias.

- No discussion of metaanalysis? Was it considered, why was it not done?

Authors' response: In all our reviews, we start with the intent to perform a meta-analysis. The reviewer probably missed the number of studies that were available for review, at the most 1-2 studies for each biomarker. In our original manuscript, we had alluded to this problem in the first paragraph of discussion, that "The variation in the units of reported biomarkers and methods of measurement as well as the unfocused targets made it difficult to carry out a pooled analysis".

- Insufficient discussion of strengths and limitations in the review as performed

Authors' response: Limitations has been added in discussion.

- Tables are very long, suggest helping the reader by synthesizing the data. Consider graphical presentation

Authors' response: Unfortunately, there were a lot of biomarkers and quite a lot of studies were not comparable in terms of population studies or timing of sampling. We categorized studies by biological function. We opted to keep the tables in its present format for presenting a detailed picture of the state-of-art for the readers, so that they could come to their own conclusions.

- It is not obvious that "ranking the biomarkers according to the difference in the bounds of the confidence intervals" identifies biological significance. Please discuss. While I am not intimately familiar with 'the estimation method' it is possible to have overlapping CIs and statistically significant difference in means and equally the opposite is also possible

Authors' response: Perhaps, the reviewer missed the main contention that we are making of the difference between biological significance and statistical significance. The Estimation approach is a relatively new way of looking at the significance of the data and statistics (previous ref. 11). See the following table for the difference in philosophies (not included for brevity).

QUALITATIVE APPROACH QUANTITATIVE APPROACH

Testing approach Estimation approach

P value or Bayes factor Effect size with confidence intervals

Focus is on the plausibility of a specific null

hypothesis

Focuses on uncertainty and practical

significance

Seems definitive Encourages meta-analysis

Replications rare Encourages replication

To reiterate, the point we are making is that there may be statistical significance with overlapping confidence intervals but clinical utility is only if a biomarker has non-overlapping confidence intervals and by a wide margin. Even just non-overlapping 95% confidence intervals may not be good enough for a practicing clinician to make a momentous decision, for there may be a 2.5 x 2.5% or 5.5% chance of error. In our original manuscript, we had mentioned in the first paragraph of discussion, that "What was remarkable is that even though a lot of biomarkers were statistically significant, the biological significance using estimation approach showed only a few endpoints had clinical utility as a biomarker. An ideal biomarker should have a very low to zero false positive and false negative rate."

Additional

Title

- Title should identify that this is a systematic review

Authors' response: Done.

- suggest removing "That Could be Biologically Significant" which is implied

Authors' response: We would beg to disagree. We would like to retain the emphasis on the phrase "biologically significant". See argument above.

- what type of biomarkers? Should be stated in the title

Authors' response: We had mentioned "Non-Inflammatory Cerebrospinal Fluids Biomarkers" in the original title. The markers analyzed in this review were categorized in many biological, pathological, and developmental pathways, which would be too long to put in a title.

- biomarkers of what? Should be stated in the title

Authors' response: Added the phrase, "of clinical outcome'.

Abstract

- 'we identified the best biomarkers' by what criteria?

Authors' response: We have added "based on the estimation approach in evaluating the biological significance".

- 'preterm HIE' is not a well-defined entity and if used it should be specified

Authors' response: We beg to disagree. It is increasingly recognized that preterm brain injury from similar hypoxic-ischemic events does occur. While we ourselves do not like the term "HIE" and prefer "neonatal encephalopathy", the term "HIE" was used as a search term and the description of preterm HIE was a sub-category of those studies using preterm subjects.

Introduction

- Line 39: Cortical spinal fluid?

Authors' response: The typo has been corrected as "Cerebrospinal fluid".

Materials and Methods

- Protocol not registered

Authors' response: As explained previously, the protocol can only be registered before completing data extraction.

- Why was the earliest test result extracted for multiple timepoints? How did this affect results?

Authors' response: We have revised as "The earliest test result was compared between studies of the same biomarker if multiple time points were reported". The effect of different time points on CSF biomarkers was shown in the Results section.

Tables and Figures

- 'checkpoint' change heading to 'age at CSF sampling (days)' or similar

Authors' response: Done.

- Figure 1. It is not obvious what a 'doubtful' biomarker is

Authors' response: Figure 1 was meant to show our approach. We have added a phrase, "as meant for the clinician" to define "doubtful" in Fig 1B. An overlap in confidence intervals introduces too much

uncertainty for the clinician. Also, see the explanation above.

Reviewer 2:

Comments to the Author

Summary: In this systematic review article, the authors have evaluated articles that reported on levels of non-inflammatory cerebrospinal fluid
biomarkers that are likely involved in hypoxic-ischemic encephalopathy. They estimated the biological significance by calculating a score based on the difference in the bounds of the confidence intervals and identified several prognostic CSF biomarkers for severe neonatal brain injury. The article represents a significant amount of work that will be valuable for the research community. The article is for the most part well written and the tables and figures are clearly presented. A few aspects of the article need revision.

1. At the outset of the article it would be helpful to state why it is important to identify biomarkers to identify and stratify those infants that might develop brain injury from HIE or asphyxia.

Authors' response: This has been added in the Introduction.

2. Pg. 6. Please provide the interval after HIE during which these CSF samples were taken to assist the reader in understanding whether these are biomarkers for acute, secondary or tertiary brain injury …

Authors' response: The reviewer raises a very important issue that we had tried to address in the Discussion. In most cases, the timing of the insult is not known. Thus, it becomes impossible to categorize whether the time after delivery is reflective of acute, secondary or tertiary insults. We had indicated timing of sample collection in the Tables of the original manuscript, but caution the readers in not putting too much emphasis on the fidelity of the time to the stage of primary vs. secondary vs tertiary injury. If the insult is at delivery, the days after delivery would give an idea of the stage, but in most of the cases of perinatal brain injury, this is not obvious and could be remote from the time of delivery. We have published a previous review and a manuscript emphasizing this point:

Tan, S. (2014). Fault and blame, insults to the perinatal brain may be remote from time of birth. Clin Perinatol, 41(1), 105-117. doi:10.1016/j.clp.2013.10.006

Derrick, M., Englof, I., Drobyshevsky, A., Luo, K., Yu, L., & Tan, S. (2012). Intrauterine fetal demise can be remote from the inciting insult in an animal model of hypoxia-ischemia. Pediatr Res, 72(2), 154-160. doi:10.1038/pr.2012.65

3. Pg. 9. It would be helpful to indicate why CSF samples were obtained from non-HIE infants.

Authors' response: We have added that information to the Results section, "All of the nonasphyxiated cases were cases of suspected meningitis or sepsis based on clinical conditions, but were negative for bacterial cultures".

4. Pg. 9. Please consider replacing the word "indicating" with the phrase "classified as" for defining the categories for biomarkers.

Authors' response: Done.

5. Pg. 12. There are multiple isoforms of VEGF; therefore, please define which VEGF isoform has been studied on this page and in future sections of the review.

Authors' response: In the original ref 25 VEGF165 was mentioned. We have updated this in the revision.

6. Pg. 14. The authors describe CK and CK-BB. If these are the same enzymes then please used one abbreviation through this review. If they are different then please define each enzyme.

Authors' response: CK-BB was Creatine kinase brain isoenzyme in the original ref 27, while CK was Creatine kinase in the original ref 23. CPK was creatine phosphokinase in the original ref 16, which was the same enzyme as CK. The full names were shown in abbreviations under Tables.

7. Pg. 14. Please clarify the sentence that begins with "CK-BB increased with severity of impairment at 12 months of age". Was CK repeatedly measured or were CK levels positively correlated with severity of

impairment?

Authors' response: We have revised it as "CK-BB collected at postnatal day 2-5 was significantly increased with severity of impairment diagnosed at 12 months of age".

8. Pg. 18. The sentence in the Discussion that begins with "Xanthine oxidase is a" needs to be rewritten for accuracy.

Authors' response: Done

9. Pg. 19. There are multiple forms of NSE with only the gamma gamma isoform specifically expressed in neurons. Therefore, this paragraph needs to be updated.

Authors' response: We have revised it as "The gamma isoform of NSE".

10. Pg. 21. Please define the tertiary phase of injury. Also, please indicate which therapeutics might be appropriate to prevent tertiary brain injury. Also, it is not clear that injury resulting from severe HIE will be reduced with treatments provided during the tertiary phase of injury. Therefore, the authors might want to emphasize that these biomarkers should be evaluated to see if they can be used to diagnose a moderate HIE injury vs. a severe HIE, as they then could be used to determine which treatments would be appropriate for infants during the tertiary phase of recovery.

Authors' response: Tertiary phase of injury is now explained with added references. We presently do not have any treatments other than cooling for term HIE. We have added our references from our preclinical work using human umbilical cord stem cells and tetrahydrobiopterin. The paragraph has now been expanded to incorporate the reviewer's comment.

11. Table legends: It would be helpful to define how the Score ($\Delta\% \bar{X}$) was calculated so that a reader won't need to consult the Methods to understand the table.

Authors' response: Added to all Table Legends.

Reviewer 3:

Comments to the Author

In this manuscript, the authors undertake a literature analysis to identify potential biomarkers for various forms of preterm or full term neonatal hypoxic brain injury. Based on rigorous selection criteria, they selected 17 studies gleaned from an initial title/abstract screen of 993 studies that analyzed CSF non-inflammatory markers. They employed a rigorous unbiased series of analyses employing pooled mean differences and 95% confidence intervals. From this analysis they identified several promising CSF biomarkers for severe forms of HIE (creatine kinase, xanthine oxidase, vascular endothelial growth factor, neuron specific enolase, superoxide dismutase and malondialdehyde).

The real strength of this article resides in the excellent discussion, which puts the potential biomarkers into the broader context of brain injury neurochemistry and biochemistry, which should be of particular interest to readers of JNR who focus on the pre-clinical analysis of hypoxia-ischemia mechanisms of brain injury or the identification of potential biomarkers. The article provides valuable insights into the challenges of translating from bench to bedside to ideally develop of panel of CSF biomarkers, which could be employed acutely for preterm neonates or subacutely for full term neonates after therapeutic hypothermia.

• A concise summary table would be value to pull together the take home messages for the patient populations for which each marker may be most valuable to pursue further. This could address different patient populations, the spectrum of severity of HIE (none, mild/moderate vs. severe) and utility as acute

vs. delayed injury phase markers.

Authors' response: We tried to comply with the reviewer's request but a concise summary table or a figure was impossible to present as a logical table or figure with individual markers, especially as some

of the strong biomarkers were for death and not acceptably strong for differentiating severe from mild
injury. Our conclusions are that we need 1) definitely more data from studies, and 2) a combination of biomarkers would be more suitable to distinguish from severe vs. moderate vs. normal injury.

• Tables 1 to 4 are ideally supplemental data tables that could be stored on the JNR server or made accessible through an independent repository site.

Authors' response: We will follow the decision of the editor to see if those tables should be supplementary or not.

• Although it may be beyond the scope of the article, it would be interesting to briefly discuss how robustly the findings from pre-clinical/experimental studies align with those from this clinical analysis.

Authors' response: There are even fewer studies studying CSF biomarkers AND eventual neurobehavioral outcome in preclinical animal studies. This is because of the paucity of survival studies,
and more importantly because most animal models do not have the severe phenotype of neurobehavioral deficits. Our rabbit model of CP would be a good starting point but we are still trying to miniaturize the biochemical assays to tackle the minute amounts of CSF available in newborn animals. We have added a sentence on preclinical studies in the Discussion.

2nd Editorial Decision

Decision Letter
Dear Dr Shi:

Thank you for submitting your manuscript "A Systematic Review of Non-Inflammatory Cerebrospinal Fluids Biomarkers For Clinical Outcome in Neonates With Perinatal Hypoxic Brain Injury That Could be Biologically Significant" by Shi, Zhongjie; Luo, Kehuan; Deol, Saihaj; Tan, Sidhartha.

You will be pleased to know that your manuscript has been accepted for publication. Thank you for submitting this excellent work to our journal.

In the coming weeks, the Production Department will contact you regarding a copyright transfer agreement and they will then send an electronic proof file of your article to you for your review and approval.

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Congratulations on your results, and thank you for choosing the Journal of Neuroscience Research for publishing your work. I hope you will consider us for the publication of your future manuscripts.

Sincerely,

Dr Eric Prager
Associate Editor, Journal of Neuroscience Research

Dr Junie Warrington
Editor-in-Chief, Journal of Neuroscience Research

Associate Editor: Prager, Eric
Comments to the Author:
Congratulations on the great paper!

Reviewer: 3

JOURNAL OF Neuroscience Research

Comments to the Author
The authors have fully addressed my concerns

Reviewer: 2

Comments to the Author
The authors have addressed all of my comments and concerns.