Reviewer's report

Title: Urinary Trace Metals, Maternal Circulating Angiogenic Biomarkers, and Preeclampsia: A Single-Contaminant and Mixture-Based Approach

Version: 0 Date: 28 Apr 2019

Reviewer: Katherine Moon

Reviewer's report:

This manuscript describes an analysis of the relation between urine metals and preeclampsia, taking advantage of an existing case-control study of pre-term birth, itself nested within a longitudinal birth cohort. The study addresses important potentially modifiable risk factors for preeclampsia, a serious pregnancy-related cardiovascular condition whose etiology is not well understood. Although mixture-based approaches are gaining popularity, very few studies have examined mixtures of metals in relation to angiogenic biomarkers and risk of preeclampsia. The study is limited by the small sample size, and measurements of urine biomarkers of exposure at only a single time point. Additional details are needed to evaluate the PCA for urine metal mixtures.

0. Abstract

1. Background
   * Line 94-95: would "levels below the recommended range" be more precise than "levels" here, since essential metals can have a U-shaped dose-response?

2. Methods
   * Line 137-8: The inclusion/exclusion criteria (from the parent LIFECODES cohort, to the case-control study of preterm birth, to the present analysis) are not clear. A flow chart or diagram with sample sizes (in the supplement) to evaluate the potential for selection bias would be helpful. Were participants with urine samples in the 3rd visit similar to participants with urine collected at other study visits?

   * Line 157: What was the range of gestational ages at the 3rd study visit (median=26 weeks)? Later in the Discussion (Line 269) and in Table 2, the samples are referred to as "3rd trimester"; however, 26 weeks is the last week of the 2nd trimester. Please clarify what

   * Line 167-168: Did you consider or conduct any sensitivity analyses to examine whether imputing LOD/square root of 2 may have introduced bias or produced overly narrow confidence intervals? In Table 2, most of the metals had fairly low number of samples <LOD, Cd and Pb had relatively low proportion of values >LOD - 44% and 76%, respectively. These specific metals are also some of the more well-studied in relation to preeclampsia risk, making these assumptions potentially more influential for this analysis.

   * Was the Cox model's proportional hazards assumption satisfied?
* Line 187: Are there clinically relevant thresholds for these angiogenic biomarkers? If so, did you consider evaluating the biomarkers as categorical variables above a threshold?

* Assumptions about the relationship between variables using a directed acyclic graph (DAG) are mentioned twice (Line 192 and Line 201-202). It may be helpful, particularly for the question of whether BMI is a collider, to include the DAG in the paper.

3. Results
* Did you consider grouping the results in the tables by essential and non-essential metals?

* Change "significant" to "statistically significant" where it appears in the text. Non-statistically significant results may be meaningful, especially considering the exploratory nature of the aims and small sample size.

* It may help the reader to write in the tables the names of the metals with the highest loadings, instead of just the label. For example: "PC2: Toxic metals (Cd, Mn, Pb)"

* Line 304: Should be "Cd" not "As" in toxic metals PC

* Line 262: The absolute difference in risk by Ca supplementation is large and the sample size is relatively small, therefore, I recommend deleting the word "marginally". It does not add to the interpretation of the results and relies too much on statistical significance.

* Line 302-303: In the PCA, it would helpful to include information on the cumulative variance explained for each PC and the communalities for each item (metal variable) so that readers can assess how well the model fits the underlying data.

* Line 304-305: "toxic metals" and "seafood metals" should not be capitalized here

* Table 2:
  o Among the samples >LOD for the 4 metals where >70% were non-detectable, what was the distribution of values that were detected?

* Table 3 and Table 4:
  o In Table 3, I assume that the asterisk (*) indicating the adjustment variables applies to both the single contaminant and PCA models, correct? It looks here like the footnote is only applied to the single contaminant models in Table 3, but in Table 4, the asterisk is shown for both single and PCA models. Please clarify.

  o It's not clear whether the PCA models also report associations per IQR.

* Figure 1
It is a little hard to see the difference between the square and circular estimates in the figure. It is not critical, but perhaps closed and open circles would be easier to read.

4. Discussion

* Line 408: In addition to Pb and Zn, urine Se and Mn are also generally not considered very reliable exposure biomarkers.

* Can you add a citation or brief statement to support that As exposure likely to be seafood-related and therefore non-toxic?

* It is important to clarify that the "seafood-related" PC does not mean non-toxic. Even if arsenic is largely organic species, other components of "seafood-related" metals, like methylmercury, have been linked to adverse cardiovascular and pregnancy outcomes, although disentangling the adverse effects of methylmercury with the potentially beneficial effects of fatty acids requires careful assessment of potential confounding.

* Additional discussion of the strengths and limitations of PCA to evaluate environmental mixtures is warranted. For example, unsupervised PCA does not consider the outcome of interest, which may limit the generalizability between studies, and it does not allow for an assessment of the individual contributions to the mixture, or interaction between specific metals that may affect a common biologic pathway (Hamra & Buckley 2018 Current Epidemiology Reports).

* Line 405: The word "hormesis" often refers to a specific dose-response relationship characterized by stimulation at low doses and inhibition at high doses and has sometimes been used to argue that low doses of toxicants are likely to be beneficial (see Thayer 2006 EHP). I suggest using the general terminology "non-monotonic" here in the absence of specific hypotheses about hormesis.

5. Miscellaneous small typographical edits

* Line 54: Missing the "-1" after "sFlt"?

* Reference #31, #41-43 need editing; looks like reference software formatting issue.

* Line 127-128: "BWH" already defined above

* Line 283: Write out "preeclampsia"

Level of interest
Please indicate how interesting you found the manuscript:

An article whose findings are important to those with closely related research interests

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable
Declaration of competing interests

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.