Author’s response to reviews

Title: Biomarker Enhanced Risk Prediction for Development of AKI after Cardiac Surgery

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Author’s response to reviews:

Authors responses to reviewer’s comments.

Rakesh Malhotra (Reviewer 1) Comments Addressed:

1) It is unclear to me how the authors adjudicated incident AKI. It needs further explanation in the manuscript text. Was baseline sCR used and present in all subjects? Were baseline creatinine values imputed for missing values? If so how and what proportion had them? What reference sCr we use is critical to define AKI. Please consider what possible impact or sources of bias this could introduce.

• Baseline Scr was available in all cases as that was part of the study design. The diagnosis of AKI was based on an increase in serum creatinine above the baseline value obtained within 48 hr of surgery.
2) The AKI outcome used in this analysis also raises some issues. Although the authors use the serum creatinine criteria (not standard KDIEGO/AKIN criteria), they failed to use the urine criteria. This is a commonplace in retrospective studies, but it should not be in a prospective study like this one. Please acknowledge this limitation in the discussion, and the impact of it on the performance of the Biomarkers is not considered.

• While urine volume was collected this variable was not included in the original analysis due to the potential for urine output in a postsurgical setting to falsely classify patients as having AKI when in fact they are volume depleted. We have repeated the univariate and multivariate analysis considering both the KDIGO and the original more restrictive definition. These considerations have been included in the results tables, as well as the results and discussion sections.

• Thus, urine volume has been now included as a variable with the addition to the KDIGO classification standards. Those data are included in the results, discussion and tables of the revised manuscript.

3) I would like authors to provide some information about what to do with Biomarker information other than AKI prediction/detection? Does it change management? What is the next path forward? May be these studies will help inform to guide the development of future therapies. We need to ask these questions.

• The biomarkers explored were for the prediction of the development of AKI, similar to that provided by the clinical risk score. Enhanced prediction of risk of AKI will factor in clinical decision making of the risk:benefit ratio of surgery and identification of patients for clinical trials, presented in Introduction and Discussion. Although treatment of AKI is not currently available, the advance in prediction provided by biomarkers will assist in evaluating potential therapies.
4) There is discrepancy in the number of reported AKI events. In method section Page 5 line 28 authors reported 15 AKI events and in Results number is 16. Please correct.

- We have corrected this mistype and consistently show the number of cases as 15.

5) How was serum creatinine measured? Which assay was used? Please comment in the manuscript.

- The creatinine method used was the modified Jaffe method as performed in the clinical laboratory at the Jewish Hospital clinical site. The method used is not stated in the Materials and Methods.

6) Please provide rationale for using 9 subjects for proteomic analysis instead of all 16 subjects who developed AKI. Why not 16 AKI and 16 control?

- The discovery study was conducted to use a portion of the case and control subjects as proteomic study samples with the full cohorts for use in the validation studies by ELISA. The original proteomic study used an 8 x 8 study design. Following the proteomic analysis it was determined that one control sample was misselected and was reclassified as a case sample.

7) One of the limitations of database is small numbers (type 2 error?), no validation cohort and lack of racial diversity. Please acknowledged in limitation section.
• This limitation is acknowledged and addressed in the Discussion of the revised manuscript.

8) I would also encourage authors to provide details of AKI staging for incident AKI in the results section/Table 1.

• The stage of AKI is now provided in Table 1 of the revised manuscript.

9) What was the median time to incident AKI in 16 patients? It will be interesting approach to look at the longitudinal changes in biomarkers prior to AKI event and not rely on one-time measurement. Could the authors show the prospective temporality of the biomarkers and its variation over time prior to AKI?

• The study design identified AKI that developed within 72 hr of surgery. All patients with AKI had an elevated serum creatinine within 48hr. The current report focused on pre-operative urinary markers of post-operative risk. Post-surgical samples were not analyzed and do not currently exist for a secondary analysis. The reviewer raises an important question that will be addressed in future studies.

10) One can remove column of P-value as it does not add much to the table 1 content. It is only a suggestion.

• After discussion amongst the authors, we concluded that this information was valuable for understanding the data by the general readership.
11) As in all studies, issue of residual confounding (unmeasured or unknown confounders) play a role and thus may influence relationship between biomarkers and AKI.

- The unknown and confounders are in the error term in the statistical analysis. We were not able to control for these as they are not measured or known to measure

12) Please do comment on limitations of using NRI and IDI in the manuscript.

- The NRI and IDI are included to provide additional and specific information about the improvement in the prediction of AKI. Rigorous statistical methods were applied to determine the predictive models with associated p values. NRI and IDI were provided to give an indication of the utility of the proposed biomarkers in the prediction of AKI. The rationale for the use of NRI and IDI are provided in the Discussion.

13) Please comment on biomarker assay precision? Coefficient of variations?

- We have included information addressing the analysis of the standard curves for both linearity (R2) and coefficient of variation for this mean linearity in the results section. All standard curves were developed to exclude data points where replicate data had a CV in excess of the clinical chemistry standard of 20%.

14) I appreciate if further efforts are made to improve this manuscript grammatically correct. Please do spell check.
The revision to the manuscript included revisions for spelling and grammar.

Kelly Liang, M.D. (Reviewer 2) Comments Addressed:

15) -p. 5, Outcome Definitions and AKI definition

- As discussed in Reviewer 1 comment 1 we have revised our analysis to include a comparison of the more restrictive definition of AKI with the KDIGO definition of AKI.

16) -p. 5, Outcome Definitions and urine output.

- For the primary definition of AKI urine volume was not included in the original analysis. We agreed with Reviewer 2 that urine volume output as part of the AKI algorithm has the potential in a postsurgical setting to falsely classify patients as having AKI when in fact they are volume depleted. Urine volume has been now included as a variable with the addition to the KDIGO classification standards. Those data are included in the results, discussion and tables of the revised manuscript.

17) -p. 8, Proteomic Analysis- Any reason why Tissue Inhibitor of Metalloproteinases-2 (TIMP-2) or the Nephrocheck was not utilized?

- Our study addressed pre-surgical urine markers of post-surgical AKI risk and the focused on primary discovery approaches using a proteomic approach. While Nephrocheck has FDA approval we did not incorporate this test for two reasons: (1) it is has received approval and validation in a post-surgical setting and (2) while we did detect IGFBP7 preoperatively, we did not detect TIMP2.
18) -p. 12, Limitations Paragraph - use of other AKI biomarkers besides serum Cr (e.g., serum cystatin C levels or urine uromodulin to Cr ratio), as well as lack of a validation set to serve as controls. Were cystatin C levels not available? Are there any stored blood samples from these patients which could be tested for cystatin C to include in this analysis?

- We appreciate the reviewers discussion of other serum markers of eGFR and AKI. We utilized the serum Cr measures obtained from clinical data. Blood was not collected for cystatin C or Umod measurements. These suggestions are noted and valuable additions to the future study to confirm HRG and CFB as pre-surgical markers of post-cardiac surgery AKI.

19) -Table 1, What does CEI in "CEI/ARB" stand for? Also, there is no p-value listed for that row. Please include a footnote for all abbreviations used in this table. Also, I would swap 6 hr DBP with 6 hr SBP so that 6 hr SBP is listed above 6 hr DBP.

- We thank Reviewer 2 for these observations. Table 1 has been revised to denote the meaning of ACEi/ARB and to provide the p-value. Abbreviations were defined in the legend and the order of blood pressure made consistent.