Peer Review File

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Reviewer A

The authors have an interesting approach to using a biomarker to better assess the course of patients with congenital heart defects after correction with the use of a cardiopulmonary bypass. If it is possible to influence the biomarker therapeutically, the care of patients with congenital heart defects could be further improved in the future.

Unfortunately, a few points remain unclear. It is recommended to complete and discuss them further.

1. **Comment 1**: The authors found 86 patients who met the described inclusion criteria. Based on the duration of ventilation, the group was divided into quartiles with a study group of those having longer ventilation times and a control group with shorter ventilation times. It is assumed that each group contained statistically the same number of patients, i.e. 21.5? Of 43 patients, the plasma samples were thus not studied further at all? Otherwise, it is recommended to indicate how many patients were in the control group and how many were in the study group, from each of which 20 patients were randomly selected.

Reply 1: Thanks for the comment and suggestion to clarify our included patients. In patient selection section under Method, we explained the inclusion and exclusion criteria. 86 patients met the inclusion criteria. The duration of mechanical ventilation is one of the relatively objective measures that is used to indicate lung injury. We divided our included patients in 4 quartiles based on the duration of mechanical ventilation percentile. 21 patients were in the control group (< 25th percentile) and 21 patients were in the study group (>75th percentile). Randomly selected 20 patients from the control and study groups and tested their serum samples. We did not test the remaining patients' samples. We hope that will add clarity to our patient selection.

Changes in the text: in the patient selection section, we added the following statemen (line 94-98) : *We had 21 patients in the control group (< 25th percentile of mechanical ventilation duration) and 21 patients in the study group (>75th percentile of mechanical ventilation duration).* And the following statement: *We did not test the remaining the samples of the remaining patients (number =46) who met the inclusion criteria.*

2. This also raises the question of why not all patients in the two groups were studied. Why do the authors possibly limit the statistical power of their work?

Reply 2: Thank you for the comments. We understand that 20 patients in each group seems to be random number. At this stage of our discovery and validation, we are limited by the fund that allow us to do our study. We wanted to do this step of validation prior to expanding our research to include larger number of patients in the next step when we apply for research grant. We are currently to the process of doing similar testing on 200 patients using the finding of the data presented in this paper.

Changes in the text: in the patient selection section, we added the following statement (line 104-105: *The fund of our study allowed us to test only 40 patients for PRG4 (two sample for each patients)*

3. The two groups include children with very different heart defects. The control group has a significantly higher proportion of simpler heart defects (VSD) and a higher proportion of heart defects that lead to increased pulmonary perfusion (VSD and AVSD). In particular, patients with VSD come out of the operating room without ventilation in many centers and do not require ventilation at all postoperatively in the intensive care unit. It remains open whether such patients were also included in the control group.

Reply 3: Thank you for the comment. All our patients and regardless of their surgery, arrives to the cardiac intensive unit intubated and get extubated after admission. Reviewer A raise very important point in relation to the pathophysiology of the cardiac defect in our cohort. It is very accurate that patients with VSD and AVSD with increased pulmonary blood flow (left to right shunt) compared to patients with tetralogy of Fallot in general. However, our patients were selected to be in the control vs study group based on their level of lung injury and need of invasive mechanical ventilation. We tried to investigate the association of PRG4 level with length of mechanical ventilation, rather than certain type of cardiac defect and its association with lung injury. It is true that the study and control group are not homogenous in terms of cardiac defects, however they are classified in similar STAT category in terms of complexity. Length of cardiopulmonary bypass was indifferent among both groups.

Changes in the text: in the patient characteristic section, we added the following statement (line 144-146): *All our patients are admitted to the cardiac intensive care unit intubated and on mechanical ventilation. Patients are extubated after admission whenever they are ready from the respiratory and hemodynamic standpoint.*

4. Factors that may or are known to influence plasma levels of PRG4 (age, gender, ethnicity, etc.) are not mentioned. Thus, whether a heart defect in itself has an impact on the plasma level of PRG4 is missing from the discussion.

Reply 4: Thank you for the comment, Reviewer A raise important points in relation to impact of certain demographic characteristics on the level of PRG4. In the patient chrematistics section under Results, we reported the gender and ethnicity distribution among our group. In general, our cohort had more white than other race (65%), however distribution in study vs control was indifferent (table 1). There were more male in our group but that difference was statistically insignificant (55% vs 40%). in terms of age and weight, study group had younger and smaller age compared to control group (table 1). To investigate the impact of age and weight on the level of PRG4, we repeated our analysis with adjustment for weight and age, and presented our results in table 5 and result section (line 159-169). Due to small number of our cohort, we cannot make conclusion in regard to the gender and ethnicity impact on PRG4 levels.

Changes in the text: we added the following statement to the limitation of our study (line 207-208): *In addition, we were unable to discover the impact of certain demographic characteristics on the PRG4 levels.*

5. Different heart defects could also affect plasma levels of PRG4 in different ways. For example, a heart defect with increased pulmonary blood flow (e.g. VSD or AVSD) could affect plasma levels of PRG4 differently than a heart defect with decreased pulmonary blood flow (e.g. Tetralogy of Fallot).

Reply 5: We agree with the author in regards to the impact of different cardiac lesion and physiology on acute lung injury. We mentioned that factor as one limitation of our study (line 192-193).

Changes in the text: we added the impact of the cardiac lesion on acute lung injury in the discussion section (line 204-208): *Our study did not investigate the association of different cardiac lesions with the length of mechanical ventilation. We divided our patients based on the length of mechanical ventilation rather than the cardiac lesion. Thus, we are unable to have any conclusion relating to the association of cardiac defect and PRG4 levels.*

6. **The authors discuss PRG4 as a possible biomarker to predict the postoperative course after heart surgery with the use of heart-lung machine. It should be stated how much time the laboratory test procedure takes to obtain a meaningful test result.**

Reply 6: Thank you for the suggestion. At the current time, PRG4 testing is not available commercially. The ELISA testing for PRG4 takes typical time for bench analysis, up to one day, which includes overnight incubation and 7 hours of work after then. Future platform development would shorten this period.

Changes in the text: we added the following statement to the methods section (line 119-123) and reference was added (n=17): *The plate-based ELISA assay employed here requires an overnight incubation step and then approximately 7 hours of work, which is a typical duration for bench ELISA analysis. However, PRG4 has also been quantified using a homogenous bead-based assay employing the AlphaLISA platform, which has the potential for high-throughput analysis of clinical samples.*

7. The limitations of the study are certainly not only limited to the number of patients and should be discussed in more detail.

Reply 7: we agree with reviewer, more details about limitation were added to the discussion. Thank you for the suggestion.

Changes in text: we added the following statements to discussions in relation to the limitations (line 201-212) : *At this stage and as evident in our study, lower levels of PRG4 are correlated with worse clinical and respiratory outcomes. We were limited by the number of patients that had matching cardiac defects and available samples in our study, thus we were not able to discover the association of longer CPB time with the postoperative levels of PRG4. Our study did not investigate the association of different cardiac lesions with the length of mechanical ventilation. We divided our patients based on the length of mechanical ventilation rather than the cardiac lesion. Thus, we are unable to have any conclusion relating to the association of cardiac defect and PRG4 levels. In addition, we were unable to discover the impact of certain demographic characteristics on the PRG4 levels. Study group had lower postoperative levels of PRG4 compared to preoperative levels, but that was statistically insignificant. The duration of inotropic support was clinically and statistically longer in the study group, due to the retrospective nature of our study, we are unable to determine if this hemodynamic variation is related to acute lung injury or heart failure or both.*

Reviewer B

Asfari et al describe the use of a Novel biomarker for ALI after cardiac surgery in pediatric patients. In children who underwent select cardiac surgical repairs those who had lower PRG-4 after surgery were found to have lower lung compliance, higher O2 demand and longer duration of mechanical ventilation. Authors conclude that PRG-4 as a potential biomarker for ALI after CPB. Overall, interesting and important results with potential clinical implications.

Suggestions:

Comment 1: Methods: Clearly define the selected "clinical" outcomes (line 112).

Reply 1: Thank you for the suggestion and comment. We believe that will add

more clarity to our paper. We added section in the method to define the clinical outcome

Changes in the text: we added the following paragraph to methods: *Clinical outcomes:*
The secondary outcomes of our analysis included ICU and hospital length of stay (day), duration of inotropic support (hours) rather than vasopressor inotrope score, duration of inotropic support was defined as the total duration of inotropic infusion start (or admission time if they are admitted on inotropic support) to the time of weaning infusion off. Lactate level at admission and Creatinine level at post-operative day 1 and 2

Comment 2: Include 95% CI intervals as appropriate. Example: results in Line 130-132; 135-137; etc.

Reply 2: Thank you for the comment and suggestion. We added 95% confidence interval to the results. We believe this is a better way to present the result and improve our paper.

Changes in the text: we added the confidence interval to our results. We inserted the CI next to the p value. The addition is mainly in the PRG4 plasma level analysis after adjustment for weight and age. The section changes in line 167-179:
PRG4 plasma levels as predictor of prolonged mechanical ventilation with adjustment for weight and age

Using logistic regression, we studied the ability of PRG4 plasma levels in predicting prolonged mechanical ventilation as shown in Table 5. For each 1 unit decrease in preoperative PRG4 level, there was 14% higher likelihood of prolonged mechanical ventilation (odds ratio: 0.86, 95% confidence interval (0.751-0.991), p=0.037). For each 1 unit decrease in postoperative PRG4 level, there was 20% higher likelihood of prolonged mechanical ventilation (odds ratio 0.804, 95% confidence interval (0.689-0.939), p=0.006). After adjustment for weight and age, preoperative PRG4 levels were not able to predict prolonged mechanical ventilation (95% confidence interval (0.745-1), p= 0.058). However, each one unit decrease in the postoperative PRG4 level, there was 20% higher likelihood of prolonged mechanical ventilation (95% confidence interval (0.668-0.96), p=0.0167) as shown in Table 5. The receiver operative curve for postoperative PRG4 level in predicting prolonged mechanical ventilation after adjustment for weight and age is shown in supplemental Figure 1.

Comment 3: Expand limitations: clinical vs statistical significance. Lactates of 2.5 vs 1.4? How does this really impact clinical care? Duration of "inotropic support" (Define intropic use. Was inotropic/vasoactives score used?)

Reply 3: Thank you for the comment. Based on your suggestion and reviewer A suggestion, we expanded on the limitation of our study.
We included other limiting factors. We agree with the reviewer comment about the lactate outcome. The difference of lactate median among both groups was statistically significant, however the difference in the median value or quartiles are not clinically significant. This can implicate that at admission time, patient was in relatively similar hemodynamic state, however the median duration of inotropic support for the study group is longer (8.5 hours vs 1 hour), which is statistically and clinically significant. The difference in the hemodynamic state among both groups can be the primary issue or the sequence of the acute lung injury or both. Patients in the study group had significant worse respiratory indices compared to the control group as shown in table 3. We added this detail to the limitation section under discussion.
We added statement in the method section to define the clinical outcome including duration of inotropic support. We did not use vasopressor inotrope score.

Changes in the text: in regards to the limitation, we added more details into the limitation paragraph. The new paragraph is as follow (201-212):

At this stage and as evident in our study, lower levels of PRG4 are correlated with worse clinical and respiratory outcomes. We were limited by the number of patients that had matching cardiac defects and available samples in our study, thus we were not able to discover the association of longer CPB time with the postoperative levels of PRG4. Our study did not investigate the association of different cardiac lesions with the length of mechanical ventilation. We divided our patients based on the length of mechanical ventilation rather than the cardiac lesion. Thus, we are unable to have any conclusion relating to the association of cardiac defect and PRG4 levels. In addition, we were unable to discover the impact of certain demographic characteristics on the PRG4 levels. Study group had lower postoperative levels of PRG4 compared to preoperative levels, but that was statistically insignificant. The duration of inotropic support was clinically and statistically longer in the study group, due to the retrospective nature of our study, we are unable to determine if this hemodynamic variation is related to acute lung injury or heart failure or both.

Definition of duration of inotropic support was added in the following section under methods (131-136)

The secondary outcomes of our analysis included ICU and hospital length of stay (day), duration of inotropic support (hours) rather than vasopressor inotrope score, duration of inotropic support was defined as the total duration of inotropic infusion start (or admission time if they are admitted on inotropic support) to the time of weaning infusion off. Lactate level at admission and Creatinine level at post-operative day 1 and 2

Reviewer C

Comment: This was a well written manuscript with clear data and nice visual graphs.

Reply: Thanks for reviewing our paper. Thanks for the feedback.