Peer Review File

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Reviewer A

It concerns a very important issues of inflammatory response in cardiac pediatric surgery. I do congratulate on brilliant idea and well performed study design.

My minor comment concerns NLR which value occurred not significant statistically in evaluation of postoperative outcomes (MV duration, vasoactive drugs, LOS, AKI..) - did you analyze it based on the whole group or separately for both subgroups? it looks like it should be presented in Table 4 but the one is missing.

Replay: Thank you so much for your attentive review. I apologize for the missing one of Table 4. We have included Table 4 in manuscript R1 (see Page 29, lines 673-674).

Reviewer B

I have a few comments for the authors to consider.

Essentially, this study describes some baseline immunologic data in cyanotic TOF vs acyanotic VSD patients before CPB exposure. Specifically, right atrial RNA expression and NLR.

1) The two groups differ in several variables that influence clinical outcomes (NLR, cyanosis, CPB time, ischemia time is 20% longer that didn't reach statistical significance in the small sample size). Therefore, it is difficult to conclude that there is a direct link between NLR and prognosis without other statistical approaches. It looks like Table 4 could be missing from the paper, where it seems you have addressed this, although the limitation and conclusion sections might be altered to reflect the results more accurately.

Replay 1: We apologize for the missing one of Table 4. We have included Table 4 in manuscript R1 (see Page 29, lines 673-674).

Replay 2: We agree with you about our conclusion. We think that the study design and the small sample size don't respond to the clinical issue, but the potential pathophysiology of NLR comparing and correlating with mRNA. We have altered the conclusion to be more accurate (see Page 4, lines 88-89, and Page 20, lines 437-438).

2) One possible explanation of similar mRNA profiles between TOF and VSD is that anesthesia induction, line insertion, sternotomy, etc., could contribute to the immunologic profile observed and make two otherwise different groups more similar.

Replay: We agree with your interpretation. It makes perfect sense. In the new version of the manuscript, we have added your contribution (see Page 18, lines 400-402). Thank you.

3) It might be interesting to consider and elaborate on the relationship between lower IL10 and higher NLR as part of the cyanotic pro-inflammatory phenotype.

Replay: We appreciate your suggestion. We've added a paragraph regarding the Pro-inflammatory phenotype role of IL-10 and higher NLR. (see Page 18, lines 422-425).

Reviewer C

I read with interest the article "Myocardial Tissue Expression of mRNA and Preoperative Neutrophil-Lymphocyte Ratio in Children Undergoing Congenital Heart Surgery". In this study, neutrophil-lymphocyte ratio (NLR) was measured preoperatively in patients with ToF and VSD, and right atrial biopsies were examined for TNF-a, IL-1b, IL-6, and IL-10 in a small prospective translational study. Patients with ToF had a higher NLR of 0.46 vs 0.28 and experienced longer days on mechanical ventilation, ICU LOS, hospital LOS, etc. without NLR as independent risk factor. Interestingly, ToF patients had a lower expression of IL-10 mRNA and this correlated with SaO2, but a similar finding was not observed in other inflammatory markers. Also to note, IL-1b, TNF-a, and IL-6 were not isolated in every patient with VSD. I am curious to hear what the authors consider a "normal" NLR in a healthy, acyanotic patient.

As mentioned, there are no normative values for this ratio in pediatrics, and an NLR of 0.46, while significant, does not appear elevated compared to the cited literature. The authors should be commended for their work in attempting to find myocardial-level expression of inflammatory marker mRNAs and in making the comparison to NLR and outcomes. For Figure 4, were the VSD patients in whom mRNA wasn't expressed included in creating this?
Replay: Thanks for the question. To create Figure 4, all patients who did not express the mRNA were excluded from the analysis. We clarify this in manuscript R1 (see Page 29, lines 673-674).

I am also curious whether the differences observed in the postoperative period for ToF and VSD are inherent to surgeon/institution preferences, specifically whether VIS and mechanical ventilation are expected routinely in ToF patients postop. Would have been interesting to compare/include acyanotic patients with ToF to those with cyanosis.
Replay: The duration of mechanical ventilation was not due to the surgeon's preference, it depended on the patient's clinical condition. We agree with your observation regarding the MV and VIS time being longer in the TOF group. In the present study, this question is not answered for two reasons: sample size and the type of patient selected as described in the study limitations (see Page 10, lines 212-213).

How are AKI and LCOS defined, and how do the values observed in this study compare to those routinely observed in your institution?
Replay: Very good observation, we have added in the text, the section "Definition of variables (see Pages 9-10, lines 208-211)." Thank you a lot.

Again, interesting work supporting other studies that suggest patients with cyanosis lack certain protective mechanisms from typically inflammatory response pathways. This study would be stronger with postoperative data as well as measurement of markers of inflammation in the serum, as mentioned in the limitations.
Replay: Thank you. Absolutely, it would be better. Next studies must cover these limitations.