Children's Hospital Colorado

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October 8, 2017

Dear Editor:

We would like to thank the editorial staff at the World Journal of Cardiology for their consideration of our manuscript and communication through the submission process. We would further like to thank the reviewers for their time, diligence, and thoughtfulness in evaluating our manuscript. We found the comments made by each reviewer to be insightful and relevant. We appreciate the efforts of reviewer 03491752 and reviewer 00227375, and thank them for their time and kind comments regarding our manuscript. Since receiving comments from the reviewers, we have edited the manuscript to best address the concerns raised. Below, please find a point-by-point comment for each of those concerns.

Reviewer 01204088:

1. "ET-1 will not be a causative for coarctation of the aorta, and will be a peptide hormone that concentration change according to the circumstances."
    - We completely agree with the reviewer's assertion that we do not have evidence to support ET-1 as causative of coarctation of the aorta and thank the reviewer for pointing out this important distinction. In this study we discuss ET-1 concentration in patients undergoing coarctectomy to describe how the concentration of this hormone changes among different groups of patients undergoing repair as a reflection of the physiologic variability within the group. In this sense, our hypothesis agrees entirely with the reviewer's comment – that the physiologic derangement secondary to coarctation of the aorta may effect change in ET-1 concentration rather than vice versa. To clarify this point in the manuscript, we have added additional language to the introduction section: *"While ET-1 activation is not thought to be causative of coarctation of the aorta, defining ET-1 activation in the perioperative period could offer significant insight as a marker of the variable short and long term physiologic responses to coarctation seen in this population."*

2. "ET-1 level will change due to hemodynamic changes before and after surgery."
    - We thank the reviewer for noting this important point. The variable pattern of ET-1 concentration before and after surgery between neonates (higher pre-surgery, lower post-surgery) and older children (lower pre-surgery, unchanged post-surgery) reflecting the different physiologies is one of the major findings of this manuscript. To ensure this assertion is made clear we have added text to the introduction (quoted above) as well as the discussion: *"Those same patients also had a post-operative decline in*

ET-1 level associated with the acute physiologic change (rapid normalization of pulmonary hemodynamics)."

3. "Many peptides such as ET-1, BNP, NT-pro-BNP, sICAM-1, sVCAM-1, E-selectin, sFas-ligand, and IL-10 might be elevated in preoperative coarctation of the aorta. If possible, studies on changes of the other peptides will be appreciated. At least, showing changes of BNP or NT-pro-BNP levels will be required."
    - We appreciate the reviewer's comment regarding other relevant biomarkers that may be altered in this condition. *We have added data regarding BNP concentrations and their correlation with ET-1 levels pre- and post-surgery in our population to the manuscript.* We further agree that study of additional markers such as sICAM-1, sVCAM-1, E-selectin, sFas-ligand, and IL-10 will be interesting topics of future study. Unfortunately, limited stored plasma sample volume precludes the authors from completing this analysis in our current cohort of patients.

4. "Page 6, line 31-33. and Page 8, line 15-17. Echocardiogram after 24-72 hours will not be enough to evaluate left ventricular geometric change associated with changes of EF-1 level. At least, echocardiogram and ET-1 level after 7days and 1 month will be necessary for evaluation."
    - We acknowledge this entirely valid limitation raised by the reviewer. The prepared manuscript does not include sufficient time of follow up to allow comment about the role of ET-1 in chronic ventricular reverse remodeling after coarctation repair. Rather, the echocardiography data presented in this study reflect the pre-operative milieu and immediate hemodynamic effect of left ventricular outflow obstruction relief. A similar study with a longer period of monitoring will be an interesting future study to assess the chronic interaction between ET-1, coarctectomy, and left ventricular geometry. To ensure this point is clear within the manuscript, we have modified the text of the discussion: *"…raises the possibility that ET-1 could be not only a useful marker of LV remodeling but potentially an effector as well. Further, longitudinal studies will be needed to evaluate ET-1's role both in myocyte hypertrophy prior to repair and reverse remodeling after surgical correction."*

5. "Page 8, line 19-25. Patients with ET-1level less than 2 pg/ml is 1 in Fig 2 a) and 3 in Fig 2 b). Is it appropriate to statistically analyze the parameters in these small number of patients?"
    - We appreciate the reviewer's note about the statistical rigor and sample sizes. In figure 2a, only the subset of neonatal patients is included. In figure 2b, the Y axis represents difference between pre-operative and post-operative samples. In the analysis noted on page 8, there were 9 patients with ET-1 concentration >2 and 15 patients with ET-1 concentration <2. We have added language to the text of page 8 and the figure legend to clarify whether statistics refer to the entire group

or a sub-cohort: *"Including the entire cohort, RWT and LVMI were compared between patients with lower and higher levels of ET-1."*

More generally, we readily acknowledge that an inherent limitation to small studies is a greater risk of identifying chance findings. While many of our statistical tests have reached the threshold of significance, this data would undoubtedly benefit from validation by other centers with similar cohorts of patients. To that end, we have added text to the limitations section to more fully reflect this point: *"This study is prospective, single center, and targets a relatively rare patient population. As such, statistical power was limited, particularly in sub-cohort analysis. Biological heterogeneity, particularly among patients between 1 month and 1 year of age, also limited statistical analysis. Therefore, validation of these findings in similar cohorts at other centers will be of great importance."*

6. "Page 8, line 1-12. Showing normal vlues of ET-1 in naonates, older infants, and older children in your fascility will be appreciated. Table 2. Showing normal values of each parameters will be appreciated."
    - We agree entirely with the reviewer's request for normal values in our population. Unfortunately, as testing for ET-1 concentration is not readily clinically available, true normal values at different ages are not well published. We make reference to the control group in the paper by Tavli et al (2010) out of interest but readily acknowledge this limitation in our analysis. Creation of normal values for ET-1 will be a critical component of transitioning ET-1 level monitoring from the research to the clinical setting. For table 2 (the echocardiographic metrics), we have included a note in the manuscript regarding normal relative wall thickness values. Normal mass values are included, as referenced in the materials and methods section, in the paper by Daniels et al (1995).

Reviewer 02519915:

7. "A recently published study has showed new insight in the topic and should be cited in the current manuscript (Cardiovascular Research (2017) 113, 1329-337 Enhanced endothelin-1/Rho-kinase signalling and coronary microvascular dysfunction in Hypertensive myocardial hypertrophy)"
    - We deeply thank the reviewer for bringing this important article to our attention. Indeed, we are in complete agreement that this important article brings valuable insight to the topic at hand. We have noted the key finding in the introduction section of the manuscript and included the specific reference as well: *"Additionally, a recent study showed increased ET-1 blood concentration was associated with left ventricular hypertrophy in a mouse model of coarctation of the aorta."*

8. "In Materials and Method the primary endpoint of the study should be clarified."

- We agree entirely with this point of clarification. Text has been added to the materials and methods section to identify the primary outcome of the study: *"The primary outcome for analysis was change in ET-1 concentration from the pre-operative to the post-operative sample. Other associations were tested as secondary outcomes."*

9. "Authors describe subgroup analysis looking at Pulmonary Artery Pressure correlation with serum ET1 levels. However, PAPS is not shown in tables and figures. I suggest PAPS to be added in table 2 with others echocardiographic parameters."… "Page 9, line 10-14. Showing presence or absence of PDA, PA pressure, and LA pressure in each subgroup will be appreciated."
    - We fully appreciate this point and agree that pulmonary artery systolic pressure would be a useful value to know. Unfortunately, for the majority of our patients an accurate estimate of pulmonary artery pressure is not available (due to a combination of an insufficient envelope of tricuspid regurgitation to measure peak velocity, an absent ductus arteriosus, or the limitations of applying the Bernoulli principle to flow through a tubular structure like the ductus arteriosus).

      The comment we make in the discussion regarding high pressure in the pulmonary artery pre-operatively refers to the physiologic principle that, in neonates with coarctation of the aorta and patent ductus arteriosus, the flow through the ductus arteriosus is generally from pulmonary artery to aorta during ventricular systole. This pattern of flow mandates at least iso-systemic pressure in the pulmonary arteries, thereby suggesting the pulmonary artery pressure is far above normal while not providing a specific value for that pressure. We have added text to the manuscript to reflect this point: *"Six patients (all in the neonatal cohort) had evidence of a patent arterial duct on echocardiogram and were on prostaglandin infusion at the time of repair. In each of those six patients, ductal flow was right to left in systole, indicating that pressure in the pulmonary artery was equal to or greater than pressure in the aorta."*

      The physiologic principle is further noted in the discussion: *"The youngest subset of patients demonstrated the highest pre-operative ET-1 levels; these patients, in addition to manifesting high LV afterload, all demonstrated elevated pressure in the pulmonary arteries due either to ductal dependent systemic blood flow or as an upstream consequence of left atrial hypertension."* We further acknowledge that, while we hypothesize a role for left atrial hypertension in a subset of this population, small patient size and safety concerns preclude routine monitoring of left atrial pressure in the peri-operative period, particularly for research-only purposes. Future animal models will be highly useful to directly assess the role of atrial hypertension in this population. We have added text to the limitations to reflect this point as well.

10. "Measure units should be specified in tables. In table 1 weight preop is 7,9 whether wieght postop is 80."
    - We thank the reviewer for pointing out this error. Indeed the weight pre-op is 7.9 and post-op is 8.0. We have fixed this error in the revised manuscript.

11. "Figure 1 shows mean serum ET 1 concentrations and superior SD. I suggest to use boxplot graphic with median and interquartile values."
    - We appreciate this note from the reviewer. Review by our statistician indicated that ET-1 was approximately normally distributed in our population; hence our choice to use mean (SD) as the measure of central tendency.

12. "No control population could be a strong limitation of the study results."
    - We agree entirely that not having a control population is a limitation to the study. The logistical and regulatory challenges associated with performing research blood draws on healthy infants made inclusion of a control arm in this study challenging, especially with no preliminary data to assist in appropriately powering a matched study design. Based on the findings presented in this manuscript, we now have preliminary data to help design and power a future cohort to include control infants that would be acceptable to our local IRB.

We would like to, once again, thank the authors and editors at the World Journal of Cardiology for their effort and time in reviewing our manuscript. To the best of our ability, the comments and changes outlined above and in the manuscript address the concerns raised by the reviewers. We look forward to your reply regarding this submission.

Sincerely,

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