

## Peer Review Comments and Author Responses

### Reviewer 1

1. *Perhaps the title could be more representative of the chosen design, explaining that the study is a proposal.*

Response: We thank the reviewer for this excellent suggestion, and we changed the title accordingly, to clarify that this is a protocol study (line 2).

2. *In the “Study design” section, I suggest that the authors cite here that the design of the proposed study will be a cohort and the follow-up time to all the assessments.*

Response: We appreciate the reviewer’s recommendation and we included the information about the cohort design in the study design section (line 103). We also added that patients will undergo cross-sectional analysis in this protocol (line 110).

3. *Specifically, in the “Genetic-molecular investigations” and “Quality of Life Assessment” subsections, I do not think the author should explain here why they will be conducting these assessments, as in the other subsections they only describe the methodology. Also, which genes will be searched after sequencing the DNA samples, and how would it be done (equipment, extraction kits, software, etc.)?*

Response: Thank you for this interesting contribution. We moved the explanation about the importance of genetic-molecular investigations to the discussion section (lines 300-305), and we improved the description of the methodology regarding genetic-molecular investigation. Regarding Quality of life, as suggested, we removed the explanation about the reason for this analysis and included the associations we intend to analyze.

4. *Regarding Figure 1, the different arrow colors can be explained in the subtitle.*

Response: We included the indication of arrow color in the subtitle, as suggested (lines 336-337).

### Reviewer 2

5. *While the topic is compelling, the study suffers from significant issues, particularly regarding the clarity of its design.*

Response: To make it clearer that this is a study protocol, we have included this information in the title.

6. *It appears to describe an experimental study, yet the results are not provided.*

Response: This is an observational study protocol and for this reason there are no results yet to be provided.

7. *Additionally, a figure included in the paper is not referenced in the text, raising questions about its relevance.*

Response: Figure 1 is referenced in the text in line 237, in the “Prediction of covariates and confounders” section. This figure is theoretical DAG (directed acyclic graph), a useful tool for understanding causal relationships and controlling for confounders. The authors used this tool to analyze the factors possibly associated with the neurodevelopment performance of TOF patients.

8. *This disconnect is further highlighted by the misalignment between the stated objectives and the conclusions drawn.*

Response: This is a protocol of an observational study, and for this reason a misunderstanding may have occurred. As the study has not been performed yet, we cannot draw final conclusion at this moment, only the expected results.

### **Reviewer 3**

9. *Firstly, congratulations for trying to answer a complex question regarding a prevalent condition when we are dealing with cyanotic congenital heart disease. Without a doubt, a better understanding of the association of this pathology can result in better practices, which ultimately culminates in improved care for this population.*

Response: We appreciate the kind words of the reviewer very much.

10. *During what period will neuropsychological tests be carried out? I understand that they will be done in patients with TOF between 7 and 18 years old, but I would like to clarify whether the rehabilitation time (be it with neuropediatricians and psychologists, for example) could not alter the performance findings of these patients if applied at different times and in patients with different accompaniments over time.*

Response: Neuropsychological tests will be carried out just after recruitment. Although we have a cohort of TOF patients that will allow us to assess the previous history and the factors that may affect the neurodevelopment, we will also perform a cross-sectional analysis to understand the associations between cardiac, brain MR imaging and neuropsychological status. We thank the reviewer for raising this issue and we improved the description of the study design to make this point clearer (line 110).

11. *In addition to the cross clamp and bypass time, do you intend to detail those who required reintervention surgery or presented complications in the immediate postoperative period? Perhaps these patients have an “extra insult” that could negatively affect future evaluation.*

Response: We agree with the reviewer that several clinical and post-operative issues can influence results in this complex disease, and all these factors will be considered in our analysis. We appreciate this comment, and we made this point clearer in the protocol (lines 107-108).

*12. How do you intend to differentiate changes in brain imaging in terms of temporal division (intrauterine x postoperative)? I did not find in the paper a description of the period in which the images will be taken. If measured only after surgical correction, how do you know if they are not already prior to the intervention?*

Response: All MR exams will be performed prospectively, just after the recruitment in a cross-sectional evaluation. We made this point clearer in the study design section (line 111). Although we do not have previous imaging to assess the temporal aspect of the findings, we will be able to differentiate ischemic lesions from malformations to better understand the associations between these findings and the neuropsychological abnormalities.

*13. Again: I congratulate you on the originality of the study and relevance of the topic. I will appreciate your considerations.*

Response: We are grateful for the reviewer's kind words and recognition of the originality and relevance of our study.

#### **Reviewer 4**

*14. Very good manuscript, I only suggest to explain the reason for exclude patient under 7 years of age, the form to evaluate the socioeconomic status of the patient, if you want to include the patient with severe adverse effects in the perioperative period like cardiopulmonary arrest, take care with the possibility of missing data*

Response: We decided to exclude patients under seven years of age for two main reasons: first, the neuropsychological evaluations would differ between those under and over seven years of age, making it challenging to compare results across these age groups. Secondly, patients under seven years of age usually require sedation to remain still during the procedure, increasing the risks and raising ethical concerns. The socioeconomic status will be assessed through a structured questionnaire. Regarding patients with severe adverse effects in the perioperative period we will assess all previous complications through questionnaires and medical records. Patients who died in the post-operative period will not be included in our study as the cross-sectional analysis with MRI and neuropsychological tests will be prospective.

#### **Reviewer 5**

*15. I congratulate the authors on conducting this important study, searching for the impact of Tetralogy of Fallot on neuropsychological performance. The causal framework is a major strength of this study, which aims to relate physiological factors such as hypoxemia to the outcome. However, I have a few concerns regarding study design and manuscript structure.*

Response: We sincerely appreciate your kind words and constructive feedback.

*16. Concerning sampling strategy, it is stated that patients referred by cardiologists were screened for eligibility. What were criteria used by cardiologists to refer these patients to the study? Cardiologists' discretion could be a source of selection bias. How were cardiologists aware of the inclusion criteria? Ideally, a referral rate (percentage of patients who were referred for the study) from outpatients' clinics should be reported. The possibility of a selection bias due to differential referral should be cited as a limitation in the discussion section.*

Response: It is indeed an important concern. Cardiologists will refer all patients between seven and 18 years old with TOF consecutively. Then, the inclusion and exclusion criteria will be analyzed by the research team through the patient's medical records and online interview. The referring pediatric cardiologists are not aware of inclusion/exclusion criteria. This procedure intends to minimize sampling bias. We appreciate the feedback, and we improved the description of the sampling strategy in the methods stating that all patients who met the inclusion/exclusion criteria would be included (line 106). Nevertheless, we understand that any convenience sampling method may imply a risk of bias, so a paragraph with this limitation was added to the discussion section (lines 318-319).

*17. How were controls approached? Telephone, email, phone book? Please describe with more detail sampling strategy for controls.*

Response: We understand that age and sociocultural differences may affect strongly the results of the neuropsychological tests. So, to ensure the matching of patients and controls we decided to invite controls from the same social environment of the enrolled patients, such as siblings, neighbors or schoolmates. Accordingly, this selection will occur during the online screening consultation with the parents of the patient, and the invitation will also be made through online consultation. We improved the description of the sampling strategy for controls, as recommended (lines 109-110 and 121-123).

*18. This study was conducted with a convenience sample and thus presents a high risk of selection bias (decreased generalizability), in contrast to a probability sampling strategy.*

*This should be listed as a limitation in the discussion section.*

Response: We agree with the reviewer and this limitation was included in the limitations section, as addressed in the first comment (lines 318-319).

*19. Methods section, "Hypoxemia, arrhythmia, and surgery will be considered as mediators ... the inclusion of these factors on the final statistical model to establish an appropriate adjustment for these variables is highly recommended". In fact, one should not adjust for mediators, only with you are conducting a mediation analysis and want to see the effect without considering these mediators. You should adjust for confounders, not for mediator variables. Please, revise accordingly.*

Response: We agree with the reviewer that we used the word "adjustment"

inappropriately, as we were dealing with mediators. In fact, we aim to understand the role of these mediators in the primary outcome. As we included in the text, we plan to understand the direct and indirect effect of these variables.

*20. Regarding the DAG: Educational quality and socioeconomic status should probably be considered as confounders (potentially associated with both exposure and outcome); Genetic factor is considered a confounder, and that seems appropriate if you want to address effect of hypoxemia, arrhythmia and surgery only, not with genetical background. I would suggest reviewing this framework to look for more confounders, such as other comorbidities, smoking, smoking of the parents (especially mother during pregnancy), to name a few.*

Response: After discussing the important points highlighted by the reviewer, we reanalyzed the factors included in DAG and considered that educational quality and socioeconomic status do not increase the risk of the exposure (to be born with Tetralogy of Fallot). Although not associated with the exposure, these factors may affect the outcome and for this reason we will include controls that are matched according to these factors. Considering the epidemiological distribution of congenital heart disease (Zaidi & Brueckner, 2017), we believe that genetic factors are the main contributors to the presence of Tetralogy of Fallot. Although environmental factors can also be associated with congenital heart disease, this possibly occurs through epigenetic routes, with modification of gene expression (Zhang et al., 2021), which will also be assessed through genetic analysis. We did not include comorbidities and smoking because these factors are very uncommon in our young population. Most previous studies of neurodevelopment in patients with TOF did not evaluate environmental factors in fetal life, and we believe this information would be difficult to obtain without recall bias, so we decided to not include them in the DAG.

*21. Please add a section in the discussion addressing strengths and limitations of your study, with a rationale of how limitations will be dealt with.*

Response: We appreciate the reviewer's suggestion, and we added a section addressing the study's strengths and limitations lines 313 – 321).

#### Minor comments

*22. Introduction, 3rd paragraph: "has impacted the interpretation of findings from this research". Since you are referring to a few articles, I would change "from this research" to "from previous studies".*

Response: The suggested correction was done at line 80.

*23. Introduction 4th paragraph: "have not, to our knowledge, been systematically explored". I would suggest to use direct language to make Reading of the manuscript easier whenever possible.*

Response: The suggestion about the direct language was included (lines 83-85).

*24. I believe the Methods section could be shortened by moving technical aspects of imaging and study procedures to the appendix. This will allow including more details in the discussion section (such as a section for strengths and limitations of the study).*

Response: We chose to maintain the detailed study procedures to ensure the transparency of our methods; however, we also included a section on strengths and limitations, as suggested (lines 313-321).