Peer Review File

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Reviewer A

Voortman and colleagues report a small study assessing the rotational position of the aortic root relative to the base of the left ventricle as viewed inferiorly by cardiac MRI in 50 consecutive health adults. They report good to excellent intra- and interobserver variability in both the assessment of the rotational position and assessment of the angle of the atrial septum relative to the posterior left atrial wall. This small study is important in establishing the feasibility and reliability in a normal cohort of adults as assessed by cardiac MRI, and the authors should be congratulated.
Please respond to the following:

Comment A1. The authors state this is the first CMR-based study to assess this metric in a series of patients. The authors should refer to the following study as aortic root rotation in patients with history of transposition of the great arteries status post arterial switch operation, and modify their related statements in introduction, limitations and conclusion:
Tseng SY, et al. Aortic root rotation position associates with aortic valvar incompetence and aortic dilation after arterial switch operation for transposition of the great arteries. Int J Cardiovasc Imaging 2023;39:1013-1021.
Reply A1: We appreciate your valuable comment and the reference to the study by Tseng SY, et al. concerning aortic root rotation in patients post-arterial switch operation for transposition of the great arteries.
In our statement, we aimed to emphasize that our study is the first to assess aortic root rotation using cardiovascular magnetic resonance (CMR). While we acknowledge the pioneering work by Tseng SY, et al., we wish to clarify that their measurements were primarily based on computed tomography (CT), and the novelty of our research lies in demonstrating the reliability of these measurements specifically using CMR.
Changes in the text: To address this distinction, we have refined our statements in the conclusion and discussion sections of our manuscript (see Page 2, lines 57-58; Page 7, lines 174-175; Page 11, lines 269-271). Furthermore, in the introduction, we have acknowledged the previous study by Tseng SY, et al., and its contributions (see Page 4, lines 89-95).

Comment A2. The authors state that the interatrial septum serves as a key anatomical landmark for the assessment of the rotational position, and rightfully note bowing of the primary atrial septum as an unreliable landmark. From an anatomical standpoint, the authors are using the muscular buttress of the atrial septum, and the posterior interatrial infolding, or what many may refer to as part of the secondary septum (though not a septal structure). The studies referenced using CT and echo (reference #10, 12, 13) and the study mentioned above using MRI (Tseng et al) were performed by the same core authors, and used the triangular thickening of the right fibrous trigone and muscular buttress of the atrial septum as the reference point to the aortic root, drawing a line down the long axis of this

triangular structure. The authors are referred to the following paper for a detailed understanding of this anatomy which is being assessed: Mori S, et al. Demonstration of living anatomy clarifies the morphology of interatrial communication. Heart 2018;104:2003-2009. This difference in methods may partly account for the differences the authors discuss between this normal study and the normal studies referenced using CT, and presumably, using the posterior interatrial septum may add an additional variable to the measurement used in this study, though the authors attempt to assess this by their second measure of the atrial septum and posterior left atrial wall.

Reply A2: We extend our gratitude to the reviewer for careful evaluation of our method and insights in anatomical standpoints with the reference to the paper of Mori et al. We acknowledge the potential variation in aortic root rotation angles between studies, particularly those utilizing the axis of the right fibrous trigone compared to our emphasis on the axis of the atrial septum.

While we recognize the differences in methods between our study and those referenced (#10, 12, and 13), we would like to point out that the CT examinations in studies #7 and 9, based on the descriptions in their methodologies, appear to utilize the axis of the atrial septum. This aligns with our approach, emphasizing the atrial septum as a key anatomical landmark for aortic root rotation assessment.

Changes in text: we have modified our text in the method section to explicitly clarify the anatomical landmarks employed in our study (see Page 5, lines 124-127). Additionally, we have incorporated a paragraph in the discussion, emphasizing that differences in methods may contribute to disparities between our study and those using CT as a reference (see Page 9, lines 216-226).

We believe these refinements address your concerns and provide a clearer context for our methodological approach.

3. Page 8, CRM should be changed to CMR.

Changes in text: Minor changes in English writing are addressed (see Page 5, line 123)

4. Discussion, page 9, the authors state “there is a lack of analysis of reliability of these measurements.” and make further statements that this is the first study to assess this. Reference #10 (Tretter et al) assessed intra- and interobserver variability in their CT-based study.

Reply: Upon a thorough examination, we found that Tretter et al. indeed assessed intra- and interobserver variability in their CT-based study, utilizing the relationship of the interleaflet triangle between the left and non-coronary leaflets to the aortic leaflet of the mitral valve as a marker for aortic root rotation.

The reported intraobserver and interobserver reliability coefficients of 0.71 and 0.64, respectively, underscore the robustness of their measurements. While we missed explicit details on the method of measurement, we appreciate the clarification provided by the reviewer.

Changes to text: To address this methodological aspect, we have incorporated a paragraph in the discussion, emphasizing the reliability analysis performed by Tretter et al. in their CT-based study (see Page 9, lines 220-226). This addition aims to provide a more comprehensive perspective on the existing literature and the efforts made in assessing the reliability of aortic root rotation measurements. We hope this clarification and modification align with your expectations.

5. Discussion: there are additional important studies assessing the rotational position that are not reviewed, including the previous study by Tseng et al which had assessed this by CMR. The following studies should be reviewed and considered in their application to your study:

a. Toh H et al. Living anatomy of the ventricular myocardial crescents supporting the coronary aortic sinuses. Semin Thoracic Surg 32:230-241.

i. This CT-based study demonstrated a statistical correlation in the amount of myocardial crescents incorporated into the coronary sinuses depending on the rotational position of the aortic root.

b. Tretter JT et al. Vulnerability of the ventricular conduction axis during transcatheter aortic valvar implantation: a translational pathologic study. Clin Anat 2023;36:836-846.

i. This study demonstrated a predictable relationship of the conduction axis relative to the aortic root depending on the rotational position of the aortic root.

c. Oishi K et al. Rotational position of the aortic valve: implications for valve-sparing aortic root replacement. Eur J Cardiothorac Surg 2022

Reply: We would like to express our gratitude to the reviewer for highlighting the importance of additional studies assessing the rotational position of the aortic root. We have carefully reviewed and incorporated the suggested studies into our manuscript to provide a more comprehensive discussion.

Changes to text: To address this, we have added the following articles as references in the discussion:

See Page 9, lines 230-233: "We have added the following article: Tseng et al. as a reference."

See Page 9-10, lines 236-240: "We have added the following articles: Toh et al., Tretter et al., and Oishi et al. as references."

These additions aim to acknowledge and integrate the findings from these studies into the context of our research. We believe this enhances the overall completeness and relevance of our manuscript.

6. Discussion, page 10: The authors discuss that measurement discrepancies found between their normal values reported by CMR assessment vs echo and CT. The CT methods reported using multiplanar reformatting ensure assessing the short axis of the aortic root relative to the right fibrous trigone and muscular buttress of the atrial septum (i.e. reference #10). In the current study, the authors are presumably using long axis

images of the left ventricle, left ventricular outflow tract, and aortic root, which does not ensure the precision afforded in the methods using multiplanar reformatting of the short axis of the aortic root. This is a limitation which should be acknowledged as a potential discrepancy between these values, in addition to the chosen method of the atrial septum reference line.

Reply: In response to the insightful comment from the reviewer regarding potential measurement discrepancies between our reported normal values assessed by CMR and previously published values by echo and CT, we appreciate the thorough consideration of our methodology. The CT methods, as referenced in #10, use multiplanar reformatting to assess the short axis of the aortic root relative to the right fibrous trigone and muscular buttress of the atrial septum. It is important to note that in our study, we did not utilize multiplanar reformatting of the short axis of the aortic root, and we acknowledge this as a potential limitation.

Changes in text: We concur with the reviewer's observation, and we have emphasized this limitation in our revised text (see Page 10, lines 256-260)

Finally, we would like to express our gratitude to Reviewer A for the meticulous evaluation of our manuscript. We believe that the suggestions have enhanced the quality of the manuscript, and we have adequately addressed all raised concerns.

Reviewer B

An interesting and intriguing study. The paper is well written. I have a few questions and constructive suggestions.

The premise, to my mind, is the main issue here. The proposed measurements and the correlations observed are technically sound but I think we should start with first principles. The evidence for dissection in dilated roots is quite weak, based on refs [7,9]. It is not clear if rotation may cause dissection or, conversely, dilatation can lead to rotation because of the way the heart is tethered at the base. The best known example of root rotation is seen in tetralogy of Fallot and other cono-truncal abnormalities. Furthermore, the aorta in Fallot pts is dilated in all ages, perhaps related to fetal and early life flows with pulmonary pathway obstruction. Having said that, dissection in dilated Fallot roots is exceedingly rare!

In terms of methods, this study is done on healthy subjects. If the atria or the aorta start to dilate, will the relationship hold? I understand that the septal angle does not matter, the measurements are based on septal insertions. What if an elderly patient develops atrial fibrillation and massive LA dilatation? The posterior part of the atrial mass is quite 'mobile' and one can picture a change of the proposed angle without an actual rotation of the aortic root. Another example - some patients have bicuspid aortic valves and/or the commissures are not at 120 degrees from each other. This is another potential problem with the reference points chosen.

Finally, we know from morphologic studies of congenital heart disease that the cardiac tube

can have major anomalies in its segments which are independent of one another. With hearts that have congenital anomalies or progressive acquired problems, the interpretation of these angles could become increasingly difficult and potentially not very useful. I question the value of offering prophylactic surgery for root aneurysms based on root rotation, among other features. We are not there yet and we have a way to go to acquire this type of indication, we should simply proceed cautiously.

None of this is meant as a criticism. I wonder if the authors could tone down a bit the potential clinical utility of this index and expand the limitations section.

Reply: We want to express our sincere appreciation for your thorough evaluation of our manuscript and the constructive feedback you provided. In response to your insightful comments, we have made several important adjustments to enhance the clarity and context of our study.

Specifically, in the Clinical and Research Implications section, we have revised the language related to the clinical utility of AoR rotation. We recognize the importance of approaching this aspect with caution, considering the uncertainties surrounding the role of AoR rotation in aortic disease. We have also removed the section discussing the possibility of four-dimensional flow CMR, aligning with your suggestion to tone down the potential clinical utility of this index.

Additionally, in response to your considerations regarding our methods, we have included a clarifying paragraph in our Limitations section. This addition aims to highlight that our study's measurements were conducted on a cohort of healthy subjects without anatomical anomalies, utilizing the intra-atrial septum as a reliable reference point. While recognizing the potential influence of variations in the anatomy of the atrial septum, especially in cases involving congenital abnormalities or conditions like atrial dilation, we concur with your suggestion to exercise caution. We support a vigilant approach in monitoring potential variations in the reference point, such as by measuring the angle of the intra-atrial septum (IAS).

Changes in text: In response to the reviewer's feedback, we have incorporated the suggested changes into our manuscript. Specifically, in the Clinical and Research Implications section, we have revised our language to align with the recommendation (see Page 9-10, lines 230-240).
Additionally, we have addressed concerns about our methodology in the Limitations section by adding a clarifying paragraph (see Page 10-11, lines 261-268).

We firmly believe that these refinements contribute to a more comprehensive and cautious interpretation of our study's findings. Once again, we are grateful for your insightful feedback, which has undoubtedly improved the overall quality of our manuscript.

Reviewer C

This study is very useful because it demonstrates that cardiac MRI can be used to analyze aortic valve rotation. In this study, MR images obtained from 50 patients were measured and averaged. Tho results are consistent with previous reports. However, there are some issues that should be clarified. My comments are as following.

1. The author calculated an average of 50 people. What was the variation in the rotation of each case? In previous reports, early half of the aortic valves were reported to rotate clockwise or counterclockwise. There must have been some subjects in the present study in which the aortic valve was rotated. What is the variation in the degree of rotation angle?
Reply: We appreciate your inquiry regarding the variation in aortic valve rotation in our study and the terminology used for classification. In our study, we measured the angles from all three coronary cusps, and the obtained rotation angles from each cusp demonstrated variability, with an average standard deviation of 12-15 degrees. This finding aligns with observations in previous studies, where the angle of the non-coronary cusp (NCC) is commonly used to represent the rotation angle of the aortic root.
We approached the mentioned terminology with caution and refrained from utilizing specific categories such as clockwise, neutral, or counterclockwise rotation. The terminology in question seems to have originated from a study conducted by Tretter et al. (#reference 10), where classification is based on the fibrous trigone's position relative to the NCC, resulting in categories such as A) rotated clockwise (rightward), B) occupying a central neutral position, or C) rotated counterclockwise (leftward).
We believe these terms carry a subjective connotation, and to date, there is limited research establishing widespread normal values for what constitutes a "normal angle" and determining when an angle is considered rotated. To illustrate, one could alternatively use classifications like A) extreme clockwise rotation, B) clockwise rotation, and C) normal. We aim to convey the nuances in rotation without imposing subjective judgments on the extent of rotation.

2. In Tetralogy of Fallot, the aortic root is overriding rather than rotating. It is difficult to equate them when discussing rotational position of the aortic base, and we think they are different diseases. We think that it is difficult to compare tetralogy of Fallot with rotational position of the aortic valve.

Reply: We express our gratitude to the reviewer for raising this noteworthy concern. To the best of our knowledge, the comparison between the spatial position of the aortic root in children with Tetralogy of Fallot and healthy subjects has been predominantly based an 2D echocardiographic study from 1986. We acknowledge that evaluating aortic root rotation with overriding in Tetralogy of Fallot might pose challenges. Importantly, we recognize the need for further research to assess whether aortic root rotation, particularly in conditions like Tetralogy of Fallot, is indeed a component of overriding of the aorta. To date, such a comprehensive investigation has not been conducted, and we believe future research endeavors could provide valuable insights into this aspect.

3. I think it is a great idea to use IAS as an indicator to calculate rotations. By the way,

there are reports that the size of each coronary sinus changes in relation to the rotational position of the aortic valve (Oishi K, Arai H, Oi K, Nagaoka E, Yashima M, Fujiwara T, Takeshita M, Mizuno T. The rotational position of the aortic valve: implications for valve-sparing aortic root replacement. Eur J Cardiothorac Surg. 2022 Aug 3;62(3):ezac179.). The angle to be measured would also be affected by the change in the size of the coronary sinus. How was the size of each Valsalva sinus in this study?

Reply: We thank the reviewer for highlighting the intriguing study by Oishi et al., which discusses the relationship between the rotational position of the aortic valve and changes in the size of each coronary sinus, with potential implications for valve-sparing aortic root replacement.

In our study, we did not incorporate measurements of the size of each Valsalva sinus, as the methodology utilized by Oishi and colleagues relies on multiplanar reformatting (MPR) and is primarily validated for computed tomographic analysis. We acknowledge the potential relevance of investigating the size of Valsalva sinuses in relation to aortic valve rotation, and we believe future research could explore the feasibility and validation of such measurements using CMR.