Mexico City, Mexico, December 15th, 2022

**RE: Response to Reviewers.**

Dear Editorial team,

*Circulation Cardiovascular Imaging*

We enclose the revised version of our manuscript, entitled “*Assessment of Left Ventricular Myocardial Fibrosis in Adult Patients with Ebstein’s Anomaly: A Retrospective Cohort Study Based on Cardiac Magnetic Resonance and Histopathological Samples*” for consideration as an Original Research Article by *Circulation Cardiovascular Imaging*. We appreciate the thoughtful input *provided* by both reviewers and by the editorial team, which has helped us to improve the quality of our manuscript. We append below a detailed point-by-point response to the reviewer’s comments along with the revised version of our manuscript.

We appreciate the opportunity to submit our work for consideration by *Circulation Cardiovascular Imaging*.

Sincerely,

Corresponding author

Dra. Nilda Espinola-Zavaleta

**Assessment of Left Ventricular Myocardial Fibrosis in Adult Patients with Ebstein’s Anomaly: A Retrospective Cohort Study Based on Cardiac Magnetic Resonance and Histopathological Samples**

**Reviewer #1**

1. **I suggest that the authors consider the possibility that LGE and T1 mapping yield different information in this cohort: One measures replacement fibrosis and the other interstitial fibrosis. While there may be overlap, we clearly do not understand the processes well enough to say how they are related and how they are different.**

R= Thank you for the comments. After careful considerations and having a comprehensive review of the medical literature, we want to make some clarifications.

1. We agree with the reviewer that the terminology can be confusing. Magnetic resonance imaging studies are based solely on LGE, *i.e*., replacement fibrosis. Therefore, we understand the importance of *clarifying* the type of fibrosis we are evaluating in our manuscript.
2. At our institute (National Institute of Cardiology), the T1 mapping method was introduced in 2016, *thus this technique was not available* to assess interstitial fibrosis by MRI *in* *all* *patients*. Therefore, we could only assess replacement fibrosis in our results. Furthermore, T1 mapping is a relatively novel technique, and considering that our study presents MRI studies since 2009, it was not possible for all patients to have this technique. We agree that the lack of T1 mapping *in* our results could be considered a limitation for our study, since T1 mapping is capable of evaluating interstitial fibrosis, which usually appears before replacement fibrosis.
3. However, for our histopathological samples, we were able to assess both replacement, interstitial and diffuse fibrosis, which we believe is a strength in our manuscript.

Overall, we have implemented several corrections throughout our manuscript. In the updated manuscript, we use replacement fibrosis as a synonym for LGE. We eliminated the term interstitial or diffuse fibrosis when we refer to LGE-CMR imaging since we do not evaluate T1 mapping or extracellular volume techniques. However, we maintain these terms *for* the histopathological study.

1. **Under 'clinical implications': This study raises the possibility of the creation of a standardized risk stratification system for patients with EA that includes the presence of fibrosis of the LV and lateral segment LGE, as well as other clinical and medical parameters. 60 patients should be a large enough sample size to propose an algorithm that includes clinical and imaging markers.**

R) We agree with the authors and have included this suggestion in our results. The working hypothesis of the model equation is that the main predictors of mortality in patients living with Ebstein’s anomaly are linked with clinical and medical parameters, and integrated with our findings, the presence of fibrosis. Using this approach, we performed a Cox-proportional hazard regression model that *included* sex, body surface area, right ventricular ejection fraction and the presence of LV fibrosis assessed by LGE-CMR as our main clinical, medical, and imaging predictors for mortality, which have been previously associated with this outcome. Based on this model, we used the linear combination of the β coefficient from the Cox proportional hazard regression model. The points given by each predictor were assigned by standardizing all β coefficients with the minimum absolute β coefficient obtained from Cox regression and divided by 10, for an easier interpretation.

This approach resulted in the following equation that we termed as Ebstein’s *Anomaly* Mortality Score:

𝑀𝑜𝑟𝑡𝑎𝑙𝑖𝑡𝑦-𝑆𝑐𝑜𝑟𝑒=17+2.0[𝑀𝑎𝑙𝑒-𝑆𝑒𝑥]−6.0[𝐵𝑆𝐴-𝑖𝑛-𝑚2/1.73]−0.1[𝑅𝑉𝐸𝐹-𝑖𝑛 %]+4.0[𝐿𝑉.𝐹𝑖𝑏𝑟𝑜𝑠𝑖𝑠]

Our equation explains 43.5% (R2=0.435) of the variance of our recorded deaths, with an overall good C-statistic (0.93). We further identified a threshold in our Ebstein’s *Anomaly* Mortality Score of 6.5 that could help clinicians to identify subjects with increased risk for mortality. Further analyses, derivation and results of the model are presented in **Supplementary Material**

Overall, we understand that this approach could have diverse limitations that could be part of further studies. Nevertheless, we consider this could be a broader approach by cardiologists to identify patients *at* with high *risk of* mortality in clinical practice.

1. **Some of the introduction provides a general overview of EA and can be more focused on the research question.**

R= Thank you for the observation. The introduction has been summarized and is focused on the objective of this research study.

1. **'LGE ... shows focal and diffuse myocardial fibrosis patterns in patients living with EA' the term 'diffuse fibrosis' is typically used for interstitial (non-patchy) fibrosis that is not picked up by LGE.**

R= We appreciate your observation. There may be some confusion throughout our manuscript regarding the use of terminology. Nevertheless, we want to clarify some important points:

1. According to Mewton et al. (1), replacement fibrosis can have a localized distribution (CAD, myocarditis, HCM, sarcoidosis) or a diffuse distribution (CKD, toxic cardiomyopathies, miscellaneous inflammatory disease). However, it is also true that most of the scientific literature usually *refers* to an interstitial pattern and not a replacement pattern when describing diffuse fibrosis.
2. Considering that our MRI study focuses only on replacement fibrosis (LGE) and therefore is unable to assess interstitial fibrosis using advanced MRI techniques, we have decided to adjust the manuscript, keeping the term *replacement fibrosis* when we referring to LGE and eliminating the term *diffuse fibrosis* to avoid confusion with interstitial fibrosis.
3. Finally, as we mentioned in response #1, We maintain the terms “replacement fibrosis” and “interstitial fibrosis” with regard to histopathology, since this study is capable of differentiating one from the other.
4. **The Celermajer index incorporates chamber areas, not volumes. Some experts have called this the 'severity index' instead.**

R= We appreciate your observation. Regarding the Celermajer Index, we have standardized this term as “*Severity Index*” throughout our manuscript, considering that this is the most accepted term in universal literature (2). Also, we have clarified that the Severity index uses the area of the *chambers* as an indirect measure to calculate the approximate volumes. We show the correction made in manuscript in lines 169 to 171 as stated below:

*“*Based on Fratz et al., we calculated the EA Severity Index by CMR, defined by the following chambers areas: (RA + aRV) / (fRV + LV + LA [left atrium])”

1. **What were the causes of death? Were only cardiac causes counted?**

The causes of death have been added to the main manuscript. Eleven of the twelve deaths were attributed to heart failure and one patient succumbed to sudden cardiac death due to a ventricular arrhythmia.

1. **In one place, the authors state that LGE imaging began 5-10 minutes after gadolinium injection and further down in the text they mention 10 minutes. 5 minutes would be unusually early for LGE imaging. Please clarify**

R= We appreciate your observation, and the text has been modified accordingly. Based on the international guidelines of the Society for Cardiovascular Magnetic Resonance (3), the MRI experts who are part of our study have clarified that late enhancement images were obtained 10 minutes after the injection of the contrast material (gadolinium).

1. **Identification of LGE in the RV can be challenging. This is particularly true in the often-thin-walled RV that is present in EA. Please discuss the reliability of these measurements.**

R= Thank you for your observation. In the previous version of our manuscript, we considered the RV-LV insertion points and the tricuspid valve displacement areas as part of the LGE in the RV. After a broad review of the literature and discussion from our team, we have some points that we would want to clarify:

1. According to previous reports and the expertise from our MRI specialists, we agree that in Ebstein’s anomaly it is common to find LGE in the tricuspid valve displacement areas and in the insertion points of the right and left ventricles. However, the LGE observed within these areas usually is a non-specific finding in patients with EA, without a clear clinical translation, so, they are not being considered for this study.
2. In light of your valuable comment and regarding a similar issue raised by Reviewer 2, we decided to reassess each of the MRI studies from our patients with the objective *of confirming* the pathological LGE in each patient to avoid confusion, and it has been added to the main manuscript. Factors affecting the image quality were checked, and the potential pitfalls and artifacts (e.g., blood pool, epi- and pericardial fat, partial volume effects, vessels, or ghosting artifacts) mimicking myocardial scar in the delayed enhancement CMR imaging were also excluded.
3. With the observation made previously, in this new version of our manuscript we have considered in our definition of RV fibrosis as patients with a report of LGE found in the free wall of the right ventricle as a pathological finding.
4. As a result of this reassessment, we found that only 8 patients (14.04%, 95% CI: 7.29-25.35%) presented LGE in the RV free wall, which is in *agreement* with other research *reports* (4–6). We believe that this conceptualization could *result in* a more precise estimation of the prevalence of LV fibrosis within our population of study.
5. **'Coexisting cardiac anomalies were present in seven patients (11.6%) and included compacted myocardium (n=1), transposition of the great arteries (n=2),..' do the authors mean 'non-compacted' and 'congenitally corrected transposition'?**

R= We agree and thank you for the comment. We have corrected the terminology of these congenital heart diseases in the updated version of the manuscript, as shown below. It should be noted that Reviewer #2 suggested *excluding* patients living with tetralogy of Fallot and congenital corrected transposition of the great arteries as part of our final sample, as these conditions could alter the results. Therefore, we have excluded these patients and specified this information within the lines 262 to 265:

*“*Coexisting cardiac anomalies were present in 4 (7%) patients and included non-compacted myocardium (n=1), mitral valve prolapse (n=1), cleft mitral valve (n=1), and coarctation of the aorta (n=1)”.

Our final sample size estimation consisted of 57 adult subjects with unrepaired Ebstein’s Anomaly that have complete MRI and clinical data to be included in our manuscript. Overall, this sample size represents a significantly large sample considering previous reports.

1. **The authors adjusted for certain factors like LVEF and others when examining the association of LGE and mortality. It would be interesting to learn about the prognostic value of some of these other factors on mortality.**

R) Thank you for this kind suggestion. We attach the output and model diagnostic parameters:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Model Parameters** | **Predictor** | **Coefficients** | **HR** | **95% CI** | **P Value** |
| Unadjusted  R2=0.082  Dxy= 0.213  C-Statistic: 0.606  BIC: 69.08207  Wald Test (P-Value): <0.001 | LV-Fibrosis | 1.133 | 3.106 | 0.90-10.68 | 0.072 |
| Model 1  R2=0.281  Dxy= 0.599  C-Statistic: 0.884  BIC: 57.53123  Wald Test (P-Value): <0.001 | LV-Fibrosis | 1.431 | 4.181 | 1.14-15.30 | 0.030 |
| Male Sex | 2.676 | 14.539 | 2.02-104.1 | <0.001 |
| BSA | -3.810 | 0.022 | 0.003-0.993 | 0.042 |
| Model 2  R2=0.317  Dxy= 0.605  C-Statistic: 0.888  BIC: 62.93864  Wald Test (P-Value): <0.001 | LV-Fibrosis | 1.593 | 6.020 | 1.22-19.91 | 0.025 |
| Male Sex | 2.718 | 15.155 | 1.65-138.5 | 0.016 |
| BSA | -4.821 | 0.008 | 0.001-0.609 | 0.028 |
| Number of Comorbities | -0.147 | 0.863 | 0.389-1.91 | 0.717 |
| LVEF | -0.078 | 0.9246 | 0.881-0.969 | 0.001 |
| LV-Mass | -0.022 | 0.977 | 0.941-1.016 | 0.256 |
| RV-Atrialization | -0.027 | 0.972 | 0.918-1.030 | 0.344 |
| Time since arrival at the institute | -0.191 | 0.825 | 0.682-1.002 | 0.084 |

The models that were fitted in our section “*LV-LGE as a predictor of mortality in patients with EA*” are now fully displayed in the **Supplementary Material**.

1. **The authors speculate that Hispanic and non-Hispanic patients with EA have different degrees and risks for fibrosis. However, without a head-to-head comparison with similar study design and methodology, this is uncertain.**

R= Thank you for your comment. There are some points that we would want to clarify.

1. Based on case reports and local research studies, we inappropriately speculated that Hispanic and non-Hispanic patients with EA have different degrees and risks for fibrosis. However, with further research, there is no study that compares EA in Hispanic and non-Hispanic patients, so we decided to remove this *comment* from the manuscript, considering that the evidence is not *sufficient* to make such an assertion.
2. Nevertheless, we consider that our manuscript brings novel insights regarding the situation of patients living with Ebstein’s Anomaly in the Mexican population. The results could provide important information and new areas of opportunity in the research of EA and the difference in the parameters obtained depending on the population in which the study is carried out.
3. **The authors discuss hct as a surrogate for cyanosis. While it is true that many cyanotic patients are polycytemic, hct is neither a sensitive nor a specific marker for oxygen saturation.**

R= This parameter was included in our analysis due to the latest research of Aly et al., where they observed that EA patients had a higher hematocrit compared with controls (44±4% versus 41±3%, P=0.03), in which hematocrit inversely correlated with oxygen saturation, the latter being associated with higher T1 time. The text has been modified accordingly to clarify this information.

**Reviewer #2**

1. **The main concern is that the imaging seems oversensitive to find nearly all patients have evidence of RV fibrosis and half with LV fibrosis. To this reviewer, the prevalence seems implausibly high, and therefore weakens the overall message of the paper. Line by line comments are given below as suggestions for improvement.**

R) We thank you and appreciate this valuable insight. The comments and issues raised by both reviewers have helped us to reframe and clarify certain information. We have *made* important changes *in* the manuscript to improve the overall message *of* our work.

1. **P. 15 ln 252 this degree of LGE in RV and LV seems exceptionally high, much higher than the prevalence found in other published series (see line 332), especially for a single dose of gadolinium. One suspects this reflects a significant amount of RV pooling or artifact being interpreted as LGE, or else a very skewed sample of the worst possible cases. Most would likely exclude inferior RV-LV junction enhancement. Suggest the investigators reevaluate the LGE imaging and determine if interpretation has been oversensitive.**

R= We appreciate your observation. We strongly agree with your comment. After a profound discussion, our research team decided to re-evaluate all the MRI studies included in our sample. For this reassessment, we *insured* that all patients had good quality images to avoid an erroneous diagnosis, as well as to confirm that the patients did not have another pathology that could bias or alter the LGE patterns. Factors affecting the image quality were checked, and the potential pitfalls and artifacts (e.g., blood pool, epi- and pericardial fat, partial volume effects, vessels, or ghosting artifacts) mimicking myocardial scar in the delayed enhancement CMR imaging were also excluded.

Considering these findings, our new estimations of the prevalence go in accordance with the studies that have been conducted in patients with EA and LGE. Briefly, some authors such as Ciepłucha A, et al. (5) have reported a 48.6% general prevalence in all chambers. Other authors such as Yang et al. (4) report that LGE was found in 22.7%, and Aly S et al. (6), reported a 16% prevalence in any chamber. These reports *demonstrate* the variability found throughout the different studies.

Regarding the right ventricle (RV), we considered the RV-LV insertion points and the tricuspid valve displacement areas as part of the RV-enhanced areas. The MRI specialists explained that in Ebstein’s anomaly (EA) it is common to find LGE in the previously mentioned areas; however, this LGE usually is a non-specific finding in patients with EA without a clear clinical translation, and, although it is reported in our hospital imaging studies reports, it is not being considered for this study. In the updated manuscript only LGE found in the RV-free wall was considered significant. As a result of this reassessment, we found that only 8 patients (14%) presented LGE in the RV, which is in accordance with what has been reported in other research (Aly S et al., 2021, reports 16% in the RV; Ciepłucha A, et al., 2018 reports 32.4% in the atrialized RV, and 5.4% in the functional RV) and, in turn, corrects the overestimated prevalence of LGE presented in the previous manuscript.

On the other hand, the evaluation of the left ventricle (LV) was more detailed and deeper. The typical LGE pattern was linear or patchy, and despite the fact that the LGE found was not abundant or highly conspicuous as in other pathologies, it was visible enough for our MRI experts to detect it and report it as present. In this reevaluation we found that 17 patients (29.8%) presented fibrosis in any of the 17 LV segments. At first glance, this prevalence seems high when compared to other studies, and even we were surprised by the results. However, we present several points that we believe are essential to understand this in greater depth:

1. Breaking it down by LV segments, we found that the prevalence decreases significantly depending on the affected site. We found some of the areas with the greatest enhancement are those of the septal wall, principally in the basal and mid cavity segments. This is not so unreasonable, considering that this area is close to the RV, so it is more likely to present a higher degree of enhancement, due to ventricular interdependence and the abnormalities of the interventricular septum already described in EA. We conclude then that LGE prevalence in the LV in general increased significantly due to the septal segments; however, as we explained above, this is expected. Secondly, the prevalence found in the LV free wall is low, with a mid-inferolateral and basal inferolateral *predominance* (7.02%). At first glance, it seems insignificant, but what is striking and novel about our study is that despite the fact that the prevalence was low, its presence is an indicator of a greater probability of death. This has been added to the main manuscript.
2. This is the first study to describe in depth the implication of replacement fibrosis located exclusively in the LV evaluated by MRI in patients with uncorrected EA, analyzing each one of the 17 segments of the LV, despite the fact that this zone model has already been widely described. It should be noted that the different studies evaluating fibrosis by MRI in EA have *used* a global approach, with a certain bias toward the RV. This is understandable since EA has always been considered as a right heart disease. But, if we consider that heart failure (HF) is the main cause of death in EA (studies report that up to 50% are due to it) and HF *results in part from* a substratum *of* fibrosis and inflammation *that has* not *been* completely clarified, and, considering that LV is the most relate chamber to HF, we believe that it is crucial to continue investigating *it*.
3. It is known that interstitial fibrosis is reversible, *unlike* replacement fibrosis, which occurs following myocyte apoptosis or necrosis, and therefore is irreversible (7). Mewton et al. (1) state that interstitial fibrosis and infiltrative fibrosis ultimately lead to replacement fibrosis in the later stages of cardiac diseases.
4. There are histopathological studies carried out in the last century (Celermajer 1992, Lee 1995, Daliento 1997) (8–10) *in* *which* the characteristics of hearts with EA were described, both in the right and left ventricle. All of them describe fibrosis in the RV, but also in the LV of almost all patients. Most of the subjects had interstitial fibrosis, although with patches of replacement fibrosis.
5. The recent studies that evaluated T1 mapping or extracellular volume (ECV) by MRI in EA showed that the LV does present a higher prevalence of interstitial fibrosis in comparison with healthy controls. Yang et al. found that the average myocardial ECV was significantly increased in patients with Ebstein’s anomaly (P < 0.001). The septal ECV was higher than that of the free ventricular wall in Ebstein’s anomaly patients, and free wall ECV was significantly higher than that of the controls (P < 0.001). Twenty (45.5%) patients had an ECV higher than 30%, which is above the upper limits of normal. Aly et al., found that EA patients had higher ECV compared with controls, and 5 of them (40%) had abnormally high ECVs.
6. The etiology and pathophysiology of the fibrosis found in the left ventricle (either by histopathology or by MRI) are not yet known and in fact represent a challenge for today's researchers. Lee et al. (9) suggest that the interstitial fibrosis seen in children and adults with EA is acquired, not part of the congenital malformation. The different histological changes with age may represent differing responses of the heart with age to stress.

Finally, it was necessary to show all of the above to conclude that we most likely found a higher prevalence of LGE in our study compared to other studies because our study population consisted *exclusively of* adults. *This was* unlike most of the studies, such as *that of* Yang and Aly, which include*d* children and adolescents *and* interstitial fibrosis *rather than* replacement fibrosis *could be expected*. It should be considered that age and adverse events throughout life possibly play a fundamental role in the transition from interstitial fibrosis to replacement fibrosis. Our hypothesis is supported by our histopathologic findings, where we described fibrosis in almost all of these patients, mainly interstitial but also replacement. Of course, this *explanation for our results* remains as a hypothesis, and more studies are definitely required to *determine* the origin of fibrosis in the LV and to know the prevalence in other populations.

1. **That said, this reviewer agrees with the strategy of only analyzing associations with LV LGE, which is more reliable to image and more interesting from a pathophysiology perspective.**

R) Thank you for the invaluable comment. We agree that our findings are focused on the evaluation of the prevalence of fibrosis *in* different heart segments and chambers. Nevertheless, the most important contribution of our manuscript is the characterization of LV fibrosis, and in particular, its *characteristic* as a predictor of mortality in patients living with Ebstein’s anomaly. Furthermore, we integrated our findings into a mortality score that uses clinical and imaging parameters to identify subjects at risk for greater mortality. We believe that our findings have clinical relevance and bring novel insights into the study of patients living with Ebstein’s anomaly in Mexico that could serve as an opportunity for further research in other countries.

1. **P 16, ln 275. Please state what LGE patterns were found by imaging on the 4 patients that had histopathology. Did the imaging and path specimens show fibrosis in the same locations (ie septum vs. free wall, mid wall, etc).**

R= Thank you for your observation. As we mentioned in the previous paragraphs, after reanalyzing each one of the CMR imaging studies of our patients and reevaluating the areas of enhancement, eliminating those that are not clinically significant (displacement areas, RV-LV insertion points), we want to clarify certain points:

1. We identified through MRI images that only one patient (of the four who had histopathology) had LGE. This LGE was found in the LV basal-anterolateral and LV mid-anterolateral segments, as well as in the anteroseptal basal segment. Regarding histopathology, the four patients presented fibrosis in the samples analyzed (free biventricular walls). This fibrosis was mostly interstitial, with patches of replacement fibrosis; replacement fibrosis was significant *in only one patient* (shown in Figure 3). Unfortunately, we did not have the opportunity to evaluate the histology of the interventricular septum of the different patients, we only focused on the free walls.
2. Making the CMR-histopathology comparison, we found that CMR was able to detect significant replacement fibrosis in the same places that were found by histopathology. However, it is also necessary to point out that MRI-LGE missed the minimal changes (small patches) of replacement fibrosis, which were visible in histopathology, and probably appeared before they are clinically significant.

The foregoing only confirms that the best way to evaluate a patient by MRI is to combine LGE techniques with T1 mapping and extracellular volume, to detect all patterns of fibrosis, and enable the early establishment of *appropriate* treatment.

For a better understanding of our manuscript, we have adjusted the text in lines 295 to 306 as shown below:

*“*As previously described, 12 patients from our cohort succumbed during our study period, 4 of whom underwent necropsy at our Institute. The 4 heart samples were analyzed and compared with their respective CMR study in order to look for a descriptive CMR-histopathology correlation characteristic of EA **(Figure 3)**. Regarding CMR findings, we found that only one of the four patients showed intense LGE (in the septum and lateral wall of the LV). The rest of the patients did not show LGE in any ventricle. However, the histological study found that the four patients had biventricular fibrosis in the analyzed areas (free walls). In all cases, the fibrosis was characterized by a mid-wall location, with a predominantly diffuse interstitial pattern, accompanied by small patches of replacement fibrosis, except in one patient, where this replacement fibrosis was highly stained. The comparison of the histopathology and CMR findings of each patient are presented in **Table 2”**

1. **P 17 ln 290. The "3-fold increased risk" is not statistically significant. Model is "adjusted for" and Model 2 conferred up to 6-fold. It is not clear to readers what variables exactly are in these models. If all of the variables in line 294-295 are included, there are far too many variables for the number of patients/events in the study to be stable. Ln 304 says "same covariates as above" but these are not clear. The methods should be stated in manuscript text, not just in table 3.**

R= We appreciate this comment. We want to clarify that the Cox proportional Hazard regression models displayed in section “*LV-LGE as a predictor of mortality in patients with EA*” were performed with the purpose *of evaluating* the hazard effect that the LV fibrosis assessed by LGE could have *on* all-cause mortality. To assess this, we fitted three models. The first model was the unadjusted and univariable association between LV fibrosis and our outcome. The second model was then adjusted for sex and body surface area to consider the relative effect of sex (as male subjects living with Ebstein’s *anomaly* tend to have increased risk for mortality) and differences in body composition. Finally, a third model was fitted *taking* the variables included in model 2 *into consideration.*

1. **P 12 ln 199, it is not clear whether the 4 patients with histopathology are the only patients who died.**

R= Thank you for your observation. Our study included a total of 57 patients, all of whom underwent MRI, and 12 of them died in the subsequent years after the MRI study. Of the patients who died, only 4 underwent necropsy, which are the histological samples we had the opportunity to analyze and compare with their respective MRI studies. For a better understanding of our manuscript, we have adjusted the text in lines 192 to 195 as shown below:

*“*An objective of our study was to correlate the findings by CMR with histopathological studies~~,~~ for a better understanding and characterization of the myocardial fibrosis patterns in patients with EA. To achieve this, we performed histopathological studies on four patients who were part of our study and died after the CMR”.

1. **Follow up details such as median length of follow-up and number of deceased vs. number lost to follow up etc. are not given but are important for the reader.**

R) Thank you for the observation. We want to clarify some points within our study design.

1. For some contexts it is important to *bear* *in mind* that most of the cases with Ebstein’s anomaly observed in our institute are diagnosed in childhood in diverse pediatric hospitals all across Mexico (for which *it* was impossible to obtain information at diagnosis), and then, when they reach adulthood, are referred to our institute for follow-up consultations. Shortly after *admission to the institute*, most of the cases that have unrepaired conditions are evaluated by MRI. The reason for not performing MRI on all adult cases that arrive at our institute is *for* economic reasons.
2. With this context, we designed this study as a retrospective cohort that included 57 adult patients with unrepaired Ebstein’s anomaly *who* had complete MRI *study* information. *With this in mind*, we identified *the patients* in our hospital registries *who* have had this condition *from birth* up to 2021. Then, we extracted the information of the MRI at the arrival at our institute (and in most cases, the only MRI available) and clinical status at the last clinical visit.

Using this information, and understanding the potential limitations of our design, we used age as our time-to-event follow-up in our Cox proportional hazard regression models and we further adjusted for time since arriving at the National Institute of Cardiology as a confounding variable. The approach, as suggested by Manav V. Vyas (11), of using age as a time-to-event has been suggested as an alternative when there is uncertainty regarding the beginning of exposure (in this case, myocardial fibrosis). We hypothesize that *the patients in* our *cohort* could have developed fibrosis *from* the early stages of childhood as reported previously by Celermajer et al. (8,9,12). Therefore, using age could be a reasonable approximation.

These comments have been clarified in the new version of our manuscript lines 138 to 141:

*“*To model the risk associated with LV-fibrosis we used age as our time-to-event follow-up. This approach was reported by Manav V. Vyas as an alternative when uncertainty exists regarding the beginning of exposure (in this case, myocardial fibrosis)”.

1. **Intro is long; could omit first portion describing Ebstein’s, as interested readers will already be familiar with these points.**

R= Thank you for your comment. We have removed the first part of the introduction, where we covered the generalities of EA. The introduction has been summarized and improved, focusing on the objective of this research study.

1. **P 8 ln 115, "records over 12 years" is a bit misleading. The study doesn't necessarily cover 12 years of follow up (though the follow up is not given).**

R= Thank you for your very insightful comment. We agree that the title may be a bit misleading, considering that despite the fact that our study evaluated patients with MRI studies from 2009 to 2021 (12 years), it is true that not all patients have 12 years of follow–up since not all the patients were evaluated in 2009, but throughout the period already mentioned, so some have less follow-up time. We have removed this part of the title from the manuscript, so the corrected title is shown below:

*“Assessment of Left Ventricular Myocardial Fibrosis in Adult Patients with Ebstein’s Anomaly: A Retrospective Cohort Study Based on Cardiac Magnetic Resonance and Histopathological Samples”*

1. **P 9 ln 131, informed consent was obtained as for a prospective study, but is not necessarily ideal for a retrospective study that includes deceased patients (no way to consent the dead). Please reconcile this statement with the retrospective vs. prospective nature of the study.**

R= Thank you for your very pertinent observation. You are undoubtedly right, considering that we conducted a retrospective cohort study, based on previously performed MRI studies, without requiring informed consent from us to patients to participate in our study. We have removed this part of the manuscript.

1. **P 15 ln 242. Male predominance mentioned, but is just 51.6%, thus not really meaningful.**

R= We agree with you. This adjustment has been made in the script, as shown below, lines 256 to 257:

*“*Briefly, our sample had roughly equal numbers of males and females (male=30; 52.6%), with a mean age of 29.52 (IQR: 21.24-39.17”.

1. **P 15. Ln 245. WPW is not an arrhythmia but a structural abnormality.**

R= Thank you for your comment, we agree with you. The adjustment has been made in the script, as shown below, lines 259 to 261:

*“*At the last recorded visit, palpitations were the most common clinical manifestation (31.5%), Wolff-Parkinson White was the most frequent structural anomaly (29.8%), atrial fibrillation was the most common arrhythmia (14%)”.

1. **P 15, ln 248. Patients with transposition and tetralogy of Fallot should likely be excluded since the loading conditions (such as pulm valve dysfunction or systemic RV) are significantly different.**

R= Thank you for your comment. Considering that patients with tetralogy of Fallot and correction of transposition of the great vessels have a different pathophysiology than Ebstein's anomaly, as well as a higher risk of presenting some degree of fibrosis, we have decided to eliminate these 3 patients from our study, in order to focus exclusively on Ebstein's anomaly. Our final sample has remained with 57 patients. The statistical adjustments that this loss of patients supposes have been corrected throughout the manuscript.

1. **P 18, ln 319 and 321 did not seem to be part of the study. Would omit.**

R) Thank you for this observation. We have removed these sections and emphasized the current knowledge of the disease in the literature.

1. **Table 1, time of treatment refers to what treatment? Could this be age at time of MRI scan?**

R= Thank you for your comment. This variable indeed means the time since the arrival at our institute. As previously mentioned, most of the cases with Ebstein’s anomaly observed in our institute are diagnosed at childhood in diverse pediatric hospitals all across Mexico (for which was impossible to obtain information at diagnosis), and then, when they reach adulthood, they are referred to our institute for follow-up consultations. Nevertheless, we have renamed this variable as “Time since the arrival at our institute”

1. **Figure 3, Please specify whether these images are all from the same patient, or different patients.**

R= The MRI and histopathology images shown in Figure 3 belong to the same patient. Our purpose was to compare the results of both studies and to correlate how sensitive the LGE by MRI is *for* identifying replacement fibrosis. In the description of Figure 3 as well as in Table 2 the results obtained are further specified.

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