Author’s response to reviews

**Title:** Commercial 4-Dimensional Echocardiography for Murine Heart Volumetric Evaluation after Myocardial Infarction

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**Version:** 2  **Date:** 19 Feb 2020

**Author’s response to reviews:**

Response to reviewer comments:

We again thank the reviewers for their thoughtful comments about our revised manuscript. We have responded to the comments and have indicated the specific changes made to manuscript.

Reviewer #1: This is the second revision of the manuscript. The authors partially replied to the Major Issues. However, some Major Issues remain to be addressed.

Issue's number refers to first revision of the paper.

**MAJOR ISSUES**

Issue #1: Supplemental Table 2 should be provided with p-values for correlation coefficients. Furthermore, strain analysis should be extended to short axis views in order to obtain circumferential strain and a more accurate measurement of the radial strain (respect to the radial strain obtained from longitudinal long axis views).
We appreciate the suggestion regarding p-values and have included them in Supplemental Table 2 as requested. We have also performed both circumferential and radial strain measurements on the short axis images as requested by the reviewer. When compared to histology, we found moderate correlation between short axis radial strain ($r=0.68$) and short axis circumferential strain ($r=0.66$), and for this reason we included this data in the supplement but not in the main text. We have updated Supplemental Table 2 as well as the methods section to reflect these changes.

Issue #2: I appreciated the appropriate citation of the paper by Grune et al. However, I suggest to add this point (lack of control and sham mice) as a minor limitation of the study (thanks to the work presented by Grune et al.).

A note has been added in the potential caveats that no sham mice were visualized in this study.

Issue #5: Vevosonic should be changed in Visualsonics. Please correct.

We apologize for this oversight, the correction has been made. We have confirmed the naming of all VisualSonics related equipment and software.

Issue #10: I appreciated the check for normality of data. However, authors should add some details about the test (or algorithm) they used to check normality.

Normality was assessed using the D’Agostino-Pearson omnibus K2 testing. This test has been included in the methods section.

Issue #13: power of the study should be evaluated accurately and it should not be based on feeling of researchers. Furthermore, it seems that this important limitation was not still added to the potential caveats section.

We appreciate the author’s concerns about the sample size for our CMR group. We spoke at length with a biostatistician regarding this critique, but could not find a suitable method for calculating power based on the Bland Altman endpoints (bias or level of agreement). However, we did perform power analysis retrospectively on the linear regression data comparing volumetric measurements (reported in Supplemental Figure 1). We located the lowest $r$-value that was reported for assessment of EDV (2D-US vs MRI, $r^2=0.806$) and used this value to calculate power in Stata 16.0 (StataCorp). For a sample size of 12, our estimated power (based on the lowest $r$-value) is $>95\%$ (two-sided test, $\alpha=0.05$). We similarly performed a retrospective power analysis for the comparison of scar size estimates demonstrated in Figure 4. The lowest $r$-value in this comparison is $r=0.74$ (longitudinal strain rate vs histologic scar size). Similar power analysis for this comparison yields an estimated power of $>80\%$ with a sample size of 12 (two-sided test, $\alpha=0.05$). This description has been added to the methods and the biostatistician has been added as an author in this manuscript. We have included in the potential
caveats section highlighting that only 12 of the mice in this study underwent testing with all ECHO and CMR modalities.

Reviewer #2: The authors correctly answered the points I suggested, and in my opinion the manuscript can now be considered for publication.

We again appreciate the efforts of Reviewer 2 in the writing of this manuscript.