Reviewer’s report

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

Version: 0 Date: 16 Dec 2019

Reviewer: Francesco Faita

Reviewer's report:

This study aimed to compare ultrasound systems for cardiac volumetric assessments in a murine model.

The paper has important limitations (described below in Major issues section) that limit its impact and interest. All following issued should be addressed before considering the paper for publication in Cardiovascular Ultrasound.

MAJOR ISSUES:

1) Study design 1: WMSI as obtained by 4D US images should be compared with strain analysis performed on 2D short and/or long axis. VevoStrain software should be the preferred platform for analysis because its high reliability and popularity. Please comment and add this point as a limitation of the study.

2) Study design 2: control and sham mice should be enrolled for the analysis in order to perform a full high-detailed comparison of the techniques.

3) Mice aged 12 weeks underwent baseline US (page 4, line 8) but these animals were 8 weeks old when surgery was performed (page 7, line 3). Please explain.

4) Isoflurane concentration and HR: it seems that adjustment of isoflurane concentration was different between US (variable according HR, page 4, line 18) and CMR (fixed range, page 5, line 9) procedures. This could have had a huge effect when comparing functional properties of the heart such as EF. Furthermore, HR measurements should be reported as acquired during both US and CMR procedures.

5) Page 4, line 18: more details about the US probe should be added.

6) Page 4, lines 32-34: more details about the Vevo software used for 2D-US analysis should be added.

7) Page 5, line 2: Figure 4 does not correspond to the description of the text.

8) Page 6, line 11: B and A were used in the equation but never described.
9) Operator variability: comparison of measurements obtained by different operators requires a more detailed description.

10) Statistical analysis: only parametric tests were used. However, the low number of subjects and the lack of normality check suggest to use a non-parametric approach.

11) Page 7, lines 32-37: 12 animals underwent US, CMR and histological analysis (line 34). However, the subset (!) was composed by 16 mice (line 37).

12) Page 7, lines 39-40: Table 2 should be Table 1.

13) Study design 3: the main aim of the paper is to compare cardiac US techniques using CMR and histological analysis as gold standard. However, as regards CMR technique, only few animals (12 of 37) and at a fixed time (4 weeks after intervention) underwent CMR thus highly limiting the reliability of the comparison and the strength of results. This point is critical and it should be recognized as a strong limitation of the paper.

14) Study design 4: comparison of week 1 to week 4 is confounding and not very useful even more in absence of CMR data at 1 week. As authors themselves already recognized (page 10, lines 13-16), the remodelling process could be quite different between animals in this model of infarction and there is no reason to expect a specific correlation between data at 1 and 4 weeks. Authors are encouraged to remove all sections related to comparison of week 1 and week 4 data limiting the paper to the comparison of US, CMR and histological data.

Level of interest
Please indicate how interesting you found the manuscript:

An article whose findings are important to those with closely related research interests

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?
3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal