Author’s response to reviews

Title: Comparison of short axis and long axis acquisitions of T1 and extracellular volume mapping using MOLLI and SASHA in patients with myocardial infarction and healthy volunteers

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Author’s response to reviews:

We would like to thank the editor and the reviewers for their comments. We have addressed each of the editor’s and reviewers’ concerns and believe that the manuscript is now improved as a result of the review process. Editor’s and reviewers’ comments are shown in bullet-points. The changes in the manuscript are tagged according to a specific comment and highlighted.

Editor Comments:

• E1. Please include the email addresses of all authors on the title page.
  Per Editor’s suggestion, we have now added the email addresses of all authors on the title page.

• E2. Please describe the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.
  We have now added the following text in the manuscript:
  “These funding sources had no role in the design of this study and in the collection, analysis, and interpretation of data and in writing the manuscript.”

• E3. Please remove the figure titles embedded within the figures and re-upload the corrected versions. All figure titles/legends should be placed at the end of the main manuscript, after the References, and not within any of the figure files. Please upload each Figure and Table individually, as separate files.
  Per Editor’s request, we have now removed the figure titles embedded within the figures and re-uploaded the corrected versions of the figure files individually, as separate files.

• E4. You have not referenced figure 8 in the manuscript.
  We thank the editor for pointing this out. We have now added the following text in the manuscript, under the Section “SAX vs LAX T1 and ECV values in healthy volunteers”: 
“The corresponding Bland-Altman plots for ECV measurement in SAX view against the 3-chamber LAX view using SASHA is shown in figure 8.”

Reviewer #1

• **R1.1** I am not sure that SASHA are a most common sequence for T1 mapping. This is true only for MOLLI. Please modify the text.

Per reviewer’s request, we have now modified the “Introduction” to read like:

“Today, the most commonly used T1 mapping technique in CMR is the MOdified Look-Locker Inversion recovery (MOLLI) [6] whereas the Saturation recovery single-shot acquisition (SASHA) [7] T1 mapping technique has been proposed as a means of mitigating the T1-underestimation in MOLLI [7, 8].”

Moreover, we also modified “Discussion” to read like:

“Nowadays, MOLLI is mainly used as the preferred technique in cardiac T1 and ECV mapping [20] whereas SASHA has been proposed in the literature as a means of mitigating the T1-underestimation in MOLLI [7, 8].”

• **R1.2** It is not very clear if you want to compare MOLLI vs SASHA or short vs long axis or both. Please specify better the design of this study.

The main aim of this study was to compare short axis and long axis acquisitions of T1 and extracellular volume mapping. MOLLI and SASHA were utilized in patients with myocardial infarction and healthy volunteers as a means to investigate the impact of slice position and orientation on T1 and ECV measurements. This has now been explicitly elucidated in Introduction:

“MOLLI and SASHA sequences were utilized in healthy volunteers and in patients with myocardial infarction to investigate the impact of slice position and orientation on T1 and ECV measurements.”

• **R1.3** Please use native T1 instead of derived T1 in all text.

Per reviewer’s request, we have now modified the manuscript.

• **R1.4** Methods: Did you measure the same wall in short axis and long axis plane or did you consider the global value of native T1 and ECV?

In this study, the same region of interest on the myocardium was measured in short axis and long axis images. Compared to previous studies (refs 15 and 16) the current study did not measure the native T1 and ECV values of the entire myocardium within the slice to evaluate the performance of long-axis acquisitions against the short-axis acquisitions. This is explicitly stated in Methods and Discussion and is shown in Figure 1:

“T1 measurements were performed by drawing same size regions of interest (ROIs) on the myocardium (ROI area: 0.1 cm2) as well in the blood pool (ROI area: 0.8 cm2) at the intersections of the short axis and long axis images (Figure 1).” (Methods)
“Compared to the previous two studies [15, 16], the current study did not measure the mean T1 and ECV values of the entire myocardium within the slice to evaluate the performance of long-axis acquisitions against the short-axis acquisitions. In this study, T1 measurements and ECV calculations were performed using an ROI-based analysis by drawing same size ROIs on the myocardium as well in the blood pool at the intersections of the short axis and long axis images. This approach was considered more representative for evaluating the differences between different slice orientations.” (Discussion)

We have now modified Section “Image analysis” in Methods to read like:

“T1 measurements were performed by drawing same size regions of interest (ROIs) on the myocardium (ROI area: 0.1 cm²) as well in the blood pool (ROI area: 0.8 cm²) at the intersections of the short axis and long axis images (Figure 1), therefore the same tissue area was evaluated twice (once in short axis and once in long axis view). In healthy volunteers, the ROIs were placed at the center of the myocardial wall and special care was taken so as to avoid signal contamination from adjacent blood. In patients, ROIs of infarction and myocardium-at-risk (MaR) were considered as affected myocardium. Contrast enhanced SSFP (CE-SSFP) and late gadolinium enhancement (LGE) images were used to detect regional myocardial edema and fibrosis. Special care was taken so as to place the ROIs within a single tissue type area (remote, edema or fibrosis) and avoid signal contamination from adjacent tissue types. The placement of the ROIs was performed by an experienced reviewer (DN: 5 years of CMR experience).”

- R1.5 The long axis measurement means in 3, 2 or 4 chamber view depending on the short axis wall? Please describe in details the methods of measurement because is not clear.

This has been addressed previously in the answer of the reviewer’s comment R1.4.

- R1.6 You didn't use the colored T1 mapping maps derived from MOLLI sequences?

It is not clear to the authors whether the reviewer refers to the images presented in Figure 1 or to the methodology that was used in this study for measuring the T1 values.

In the first case, Figure 1 in the manuscript presents single-shot bSSFP images and not T1 maps. The single-shot bSSFP images were extracted from the MOLLI pulse sequence for the time-point on the recovery curve that presented the highest contrast between the blood pool and the myocardium. We have now modified the caption of figure 1 to read like:

“Myocardial and blood regions of interest on single-shot bSSFP images extracted from the MOLLI pulse sequence. (A) shows a midventricular short axis image, (B) shows a two-chamber long axis view image, (C) shows a three-chamber long axis view image and (D) shows a four-chamber long axis view image. The native T1 anatomical images (single-shot bSSFP images) have been extracted from the MOLLI pulse sequence for the time-point that presented the highest contrast between the blood pool and the myocardium.”

In the second case, the single-shot bSSFP images were used for estimating the relaxation time parameter through a ROI-based curve fitting. In this study, the motion-corrected T1 maps were
derived anew from both pre- and post-contrast MOLLI and SASHA images. We have now modified the manuscript in section “Image analysis” to read like:

“The relaxation time parameters were estimated through a ROI-based curve fitting on the in-line, motion-corrected image series derived from both pre- and post-contrast MOLLI and SASHA acquisitions.”

- R1.7 Results: It is not clear if you compare short and long axis native T1 and ECV in MOLLI sequences or both MOLLI and SASHA.

We have now modified the titles of the two sections in “Results” so as to clarify:

“SAX vs LAX T1 and ECV values in healthy volunteers using MOLLI and SASHA”
and “SAX vs LAX T1 and ECV values in patients using MOLLI and SASHA”

- R1.8 Figure 8 ok
The reviewer’s comment is unclear to the authors. We are assuming that it refers to the comment that the Editor made that figure 8 had not been referenced in the manuscript text. We have now added the following text in the manuscript, under the Section “SAX vs LAX T1 and ECV values in healthy volunteers”:

“The corresponding Bland-Altman plots for ECV measurement in SAX view against the 3-chamber LAX view using SASHA is shown in figure 8.”

Reviewer #2

- R2.1 Please be more specific about reporting the p values. P> 0.05 could be 0.06 or 0.9, two numbers which are totally different with two different significance for the readers. P<0.05 also could be 0.04 or 0.0001, two numbers which are totally different with two different significance for the readers.

Per reviewer’s request, we have now added the p-values in the manuscript.

- R2.2 More information regarding the placement of region of interest (ROI) on myocardial images is needed. Who drew the ROIs?

In this study, the same tissue area was evaluated twice, once in short axis and once in long axis view. The placement of the ROIs was performed by Dr. David Nordlund, an experienced reviewer with more than 5 years of CMR experience. In healthy volunteers, the ROIs were placed at the center of the myocardial wall and special care was taken so as to avoid signal contamination from adjacent blood. In patients, CE-SSFP and LGE images were used to detect regional myocardial edema and fibrosis and special care was taken so as to place the ROIs within a single tissue type area (remote, edema or fibrosis) and avoid signal contamination from adjacent tissue types.

We have now modified the text in the section “Image analysis” in the manuscript so as to clarify:
“The relaxation time parameters were estimated through a ROI-based curve fitting on the in-line, motion-corrected image series derived from both pre- and post-contrast MOLLI and SASHA acquisitions. All images were analyzed using the software Segment, version 2.0R5453 (http://segment.heiberg.se) [19]. T1 measurements were performed by drawing same size regions of interest (ROIs) on the myocardium (ROI area: 0.1 cm²) as well in the blood pool (ROI area: 0.8 cm²) at the intersections of the short axis and long axis images (Figure 1), therefore the same tissue area was evaluated twice (once in short axis and once in long axis view). In healthy volunteers, the ROIs were placed at the center of the myocardial wall and special care was taken so as to avoid signal contamination from adjacent blood. In patients, ROIs of infarction and myocardium-at-risk (MaR) were considered as affected myocardium. Contrast enhanced SSFP (CE-SSFP) and late gadolinium enhancement (LGE) images were used to detect regional myocardial edema and fibrosis. Special care was taken so as to place the ROIs within a single tissue type area (remote, edema or fibrosis) and avoid signal contamination from adjacent tissue types. The placement of the ROIs was performed by an experienced reviewer (DN: 5 years of CMR experience).”

- R2.3 I believe one of the most important confounding factors in this study is the significant age difference between the cases and controls. The patients with myocardial infarction are significantly older, which may affect their CMR and ECV. This should be discussed in the section of the study limitations. Also, they have to try to correct this confounding factor. I am not sure but other factors, such as patient’s heart rate at the time of image acquisition or body size can also be considered as confounding factors.

The main aim of this study was to investigate the variation of myocardial T1 and ECV estimates due to the position and orientation of the slice of interest at the same subjects and not to compare the T1 and ECV estimates among human groups with different characteristics. Therefore, no comparison of T1 and ECV values among groups with different characteristics is performed in this study.

However, to address the reviewer’s concerns, a previous multicentre study (Dabir et al., JCMR 2014) has already shown no correlation between age and native T1 with the use of MOLLI. Moreover, Kellman et al. (JCMR, 2014) showed that the heart rate sensitivity of MOLLI can be significantly reduced by modification of protocols and that the T1 accuracy of MOLLI 5s(3s)3s and MOLLI 4s(1s)3s(1s)2s is not HR dependent. Chow et al. (MRM, 2013) has already showed that the accuracy of measured T1 values with SASHA is independent of heart rate.

We have now added the following sentence in the “MR protocol” section in the manuscript: “Previous studies [7, 8] have shown that these pulse sequences are heart-rate independent.”

- R2.4 As it stands, the conclusion is quite brief and would benefit from further elaboration on the potential for future research based on your results as well the overall implications of your results. I suggest adding a statement that mentions the overarching clinical implications of your findings and explains why your findings are relevant/important and how they can shape future research.

Per reviewer’s request we have now modified the “Conclusions” in the manuscript:
“In conclusion, long axis measurements of myocardial T1 and ECV using MOLLI and SASHA exhibit good agreement with the corresponding short axis measurements allowing for fast and reliable myocardial tissue characterization. This may be of high importance in clinical cases where shortening of the overall imaging acquisition is required. Moreover, the ROI-based design of the current study may be utilized in other studies that are focused on myocardial tissue characterization in order to evaluate the differences between different slice orientations, especially in cases with focal native T1 abnormalities.”

• R2.5 Please ensure that all abbreviations used in tables and figures are defined in full, as figure/table footnote. Each figure/table should be independently descriptive of all its content. Per reviewer’s request we have now added at the end of every figure legend a full description of all the abbreviations used in the figure.

• R2.6 Was the patient renal function one of your inclusion/exclusion criteria?

The reviewer is correct. We have now added this in the manuscript:

“Eight (8) healthy volunteers with no medical history (5 men, 3 women, age 25 ± 5 years) and eight (8) patients (7 men, 1 woman, age 66 ± 10 years) with myocardial infarction and without any renal impairment were included in this study.”

• R2.7 Abbreviations and acronyms (such as CMR and ECV) are often defined the first time they are used within the abstract main text and then used throughout the remainder of the manuscript. Please consider adhering to this convention. This is specially important regarding your abstract, as the abstract readers may not have access to the full text.

Per reviewer’s request, we have now modified the abstract in the manuscript.

• R2.8 The results section of the abstract is does not provide an adequate description of the main outcomes of the research. Please provide more objective data for your abstract readers.

Per reviewer’s request, we have now modified the “Results” section of the abstract:

“In healthy volunteers, there were no statistically significant differences in native T1 and ECV values between short axis and long axis acquisitions using MOLLI (two-chamber, three-chamber and four-chamber) and SASHA (three-chamber). In patients, there were no statistically significant differences in native T1 and ECV values between short axis and 3-chamber long axis acquisitions in both remote and affected myocardium using MOLLI and SASHA.”

Reviewer #3

• R3.1 The present study differs from previously published reports on inter-orientation reproducibility by the areas the T1/ECV is assessed. The analysis in the present paper is confined to the area of intersection. Hence, the same tissue is evaluated twice. This provides means to evaluate reproducibility of the measurement technique in the presence of changed imaging plane orientations. In comparison previous studies evaluated the reproducibility of
T1 measured across the entire myocardium in different orientations. Hence, the present study provides data on technical variability in in-vivo measurements, while other studies provide data that is confounded by biological variability of the measured (and different) tissue between the slices. Hence, I would encourage the authors to highlight this in the discussion. Additionally, the authors should elaborate in the discussion on the inter-slice vs. intra-slice variability. For example in Figure 4 the intra-slice variability for ECV seems large compared to the inter-slice variability. Furthermore, this kind of reproducibility data will be illustrative to compare the methods. Hence, I would encourage the authors to include additional analysis to compare inter-slice reproducibility between the two techniques and include a corresponding figure.

We thank the reviewer for pointing these out. We have now extended the section “Discussion”. Regarding the reviewer’s comment on the difference of the current study compared to previous studies, we have now added the following text in the manuscript:

“This approach was considered more representative for evaluating the differences between different slice orientations since it eliminates any T1 variability caused by biological focal abnormalities in the myocardium and enhances the investigation of any T1 variability caused by the technical design of the quantitative approach.”

Regarding the reviewer’s comment on the difference of the inter-slice variability against the intra-slice variability, we have now added the following text in the manuscript:

“In the current study, although no significant differences on T1 and ECV measurements were shown between short and long axis slices using MOLLI, figures 2 and 4 presented a larger intra-slice variability of the MOLLI-based T1 and ECV estimates within the midventricular short axis image compared to the inter-slice variability (short axis vs long axis). In a similar manner, previous studies [11, 21, 22] have demonstrated significant regional variations of native T1 values in SAX slices of normal subjects. These differences were not considered representative of a true difference in tissue composition but were attributed to other factors, such as inadequate B0-shimming around the heart (off-resonance issues) [9], receiver coil sensitivity and distance of the receiver coil elements from the region of interest [23].”

Regarding the reviewer’s request to compare the two techniques, we would like to highlight that in the current study the SASHA sequence was used to acquire a midventricular SAX image and a single LAX (three-chamber) image. A comparison of the inter-slice reproducibility between the two techniques would require a re-design of the study so as to include in the MR protocol the SASHA sequence for the acquisition of the two-chamber and four-chamber LAX images. We acknowledge this limitation of the current study. Therefore, we have now added the following text in “Limitations”:

“Moreover, the design of the current study does not allow for a direct comparison between MOLLI and SASHA on the inter-slice variability of the T1 and ECV estimates using the two-chamber and four-chamber LAX images since these images were not acquired neither in healthy volunteers nor in patients.”
Today, the most commonly used T1 mapping techniques in CMR is the MOdified Look-Locker Inversion recovery (MOLLI) [6] whereas the Saturation recovery single-shot acquisition (SASHA)[7] T1 mapping technique has been proposed as a means of mitigating the T1-underestimation in MOLLI [7, 8].
Moreover, we have modified the text in this manuscript in Results so as to clarify:

“No registration distortion [20] was observed in the motion corrected, T1-weighted image series derived from both MOLLI and SASHA acquisitions in healthy volunteers.” (Paragraph “SAX vs LAX T1 and ECV values in healthy volunteers using MOLLI and SASHA”)

And

“No registration distortion [20] was observed in the motion corrected, T1-weighted image series derived from both MOLLI and SASHA acquisitions in patients.” (Paragraph “SAX vs LAX T1 and ECV values in patients using MOLLI and SASHA”)

Ref. 20 refers to the study “Myocardial T1-mapping at 3T using saturation-recovery: reference values, precision and comparison with MOLLI.” by Weingärtner et al. (JCMR, 2016).

- **R3.9** Page 7, Line 55: Please consider replacing "Last" by, "Lastly" or "Finally" throughout the manuscript
  Per reviewer’s request, we have now replaced “Last” with “Lastly” in three parts in the manuscript.

- **R3.10** Page 8, Line 27: Should read "…presented the lowest agreement…"
  Per reviewer’s request, we have now modified the manuscript.

- **R3.11** Figures 3, 5 and 8: Please add dashed lines to indicate the level of agreement in the Bland-Altman plots.
  Per reviewer’s request, we have now increased the thickness of the dashed lines to indicate better the level of agreement in the Bland-Altman plots (figures 3, 5 and 8).