Peer Review File

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Review Comments:

Reviewer A:

In this prospective study, 49 patients with severe AS and 20 controls were included. Native T1, post T1, partition coefficient, and extracellular volume fraction (ECV) were measured by 3.0T MOLLI T1 mapping in all subjects. Global strain and strain rate were measured by feature tracking. The highlights of this paper are as follows: 1) the first is that combined T1 mapping and feature tracking technique were used to asses changes of LV extracellular remodeling in patients with severe AS, which may be an interesting paper to add to the published knowledge database in similar areas. 2) the second is that the biopsy specimen (the gold standard of assessment of fibrosis) were obtained despite the sample size was limited.

There were some confusions and/or questions that need to be clarified by the author:

Comment 1: Methods / study population (Page5 line 103): It is not entirely accurate to say 49 AS patients were consecutively enrolled. The methods should likely state that patients with severe AS were consecutively enrolled between the time periods stated.

Reply 1: Thank you very much for the suggestion. We have now modified our text as advised (see Page 6, Lines 124-125).

Changes in the text: "Patients with severe AS were prospectively and consecutively recruited from January 2018 to June 2019 in a single tertiary center".

Comment 2: Methods / CMR imaging analysis (Page 7 line 147-152): Was LGE included in T1 measurements? Please clarify.

Reply 2: Thank you very much for the reminder. The T1 mapping was performed in a mid-ventricular slice, and LGE areas was included in the T1 and/or T1-derived measurements. We have now modified our text to clarify the technical details (see Page 9, Lines 179-180).

Changes in the text: "Areas with LGE were not excluded from T1 analysis".

Comment 3: Methods/ CMR imaging analysis (Page 7 line 150-152): the authors describe the deformation parameters but they did not describe whether they are using a 2D or 3D deformation values. Please explain this methodology.

clarify.

Reply 4: Thank you very much for the reminder. We have now added some data in the revised manuscript, Method section to clarify the detail (see Page 10, Lines 203-204).

Changes in the text: "Patients were divided into subgroups according to preserved LVEF (LVEF%≥50%) or reduced LVEF (LVEF <50%)".

Comment 5: Results/ Analysis of relationship (Page 10 line214): Are there any associations of the CMR findings (especially T1 mapping and feature tracking derived parameters) with the severity of AS (like the PPG, MPG and AVAi parameters measured by Echo).

Reply 5: Thank you very much for the comment. We have now made a further association analysis as suggested. The results are shown in the following table. According to the results of analysis, it seems that there were no any associations between the CMR findings (e.g. ECV, GLS and so on) and the severity of AS (the PPG, MPG, AVA, and AVAi parameters). Considering the result of this analysis does not add to any new conclusions in our study, it is not put into the manuscript. However, we're sincerely looking forward to your further opinion about it and we'll be pleased to follow your suggestion. Thank you.

Table Correlation analysis between CMR parameters and AS severity parameters

	MPG, mmHg	PPG, mmHg	AVA, cm^2	AVA index, cm^2/m^2
Native T1, ms	P=0.157	P=0.157	P=301	P=0.576
Post T1, ms	P=0.479	P=0.309	P=0.345	P=0.665
λ	P=0.235	P=0.171	P=0.892	P=0.517
ECV, %	P=0.753	P=0.833	P=0.195	P=0.196
GLS, %	P=0.812	P=0.577	P=0.600	P=0.222
GCS, %	P=0.372	P=0.280	P=0.600	P=0.545
GRS, %	P=0.859	P=0.858	P=0.169	P=0.145
GL strain rate	P=0.608	P=0.567	P=0.150	P=0.178
GC strain rate	P=0.317	P=0.688	P=0.842	P=0.468
GR strain rate	P=0.501	P=0.231	P=0.172	P=0.490

Note.—The correlation was presented as a Pearson correlation coefficient by using SPSS version 17.0 software (IBM Inc, IL, USA). A P value <0.05 was considered significant. ECV, extracellular volume; GLS, global longitudinal strain; GCS, global circumferential strain; GRS, global radial strain.

Changes in the text: No changes in the text.

Reviewer B:

This study investigated the ability of T1 mapping and FT to identify and assess the changes of ECV in patients with severe AS, and concluded that ECV is the structural marker of extracellular fibrosis burden and GLS is the early marker before the fibrosis burden intensifies. It is interesting study, main concerns as follows.

Comment 1: Abstract: please briefly describe how to define and measure CVF.

Reply 1: Thank you very much for the reminder. We have now added some data in the revised manuscript, Abstract section to briefly describe how to define and measure CVF (see Page 3, Lines 55-57).

Changes in the text: "The degree of myocardial fibrosis was quantified by using Masson trichrome stain in biopsy specimens obtained intraoperatively in 13 patients, and expressed as collagen volume fraction (CVF)".

Comment 2: There are some messy code of the ECV formulation, please check and revise.

Reply 2: Thank you very much for the reminder. We have now checked and modified the ECV formulation in the revised manuscript (see Page 9, Lines 186-187).

Changes in the text:

$$\lambda = [\frac{1}{myo\ post\ T1} - \frac{1}{myo\ native\ T1}]/[\frac{1}{blood\ post\ T1} - \frac{1}{blood\ native\ T1}]$$

$$ECV = (1 - hematocrit) * [\frac{1}{myo\ post\ T1} - \frac{1}{myo\ native\ T1}]/[\frac{1}{blood\ post\ T1} - \frac{1}{blood\ native\ T1}]$$

Comment 3: For the CMR-FT analysis, which software did authors use? Please provide more detail of measurement of FT parameters.

Reply 3: Thank you very much for the reminder. For the CMR-FT analysis, we used the tissue tracking module of Medis software (QStrain 2.0, Medis, Leiden, the Netherlands). In addition, we have now added some data to provide more detail of measurement of FT parameters in the revised manuscript (see Page 9, Lines 189-196 to Page 10, Lines 197-199).

Changes in the text: "LV myocardial strain and strain rate analysis were evaluated by loading cine SSFP images into the tissue tracking module (QStrain 2.0, Medis, Leiden, the Netherlands) using two-dimensional (2D) FT technique. The endocardial and epicardial borders of the LV were manually sketched in the end-diastolic and end-systolic phases respectively (Supplemental Figure 2), and trabeculations were all excluded from the endocardial borders. Global longitudinal strain (GLS) and strain rate were obtained from two- , three-, and four-chamber views for LV. Global circumferential strain, (GCS), global radial strain (GRS), and strain rate were obtained from the basal, middle, and apical levels of the LV in the short-axis view".

Comment 4: For the biopsy, how to register with CMR results? any focal lesion? LGE positive/negative in the biopsy position?

Reply 4: Thank you very much for the comment. This is a prevailing situation for all papers reporting correlations between imaging findings and histology, which the spatial heterogeneity of the disease is not taken in account. Biopsy specimens from patients with severe AS were only obtained from the basal LV septum at the time of AVR surgery in the clinical situation. However, the AS is diffuse disease which the extracellular fibrosis can be detected and assessed by drawn a single ROI in the septum on mid-cavity short-axis maps as the JCMR guideline advised (1). So, regarding the diffuse extracellular volume alteration of AS, the T1-derived parameters (e.g. ECV and so on) of CMR results are almost matched the

results of the biopsy specimens quantified and expressed as CVF in our study. We believe that this is more in line with clinical practice in cases who complete heart specimens are not available. Since the CMR findings is blinded to the surgeon, the LGE may be positive and/or negative in the biopsy position. Hopefully this explanation is acceptable to you and we're sincerely looking forward to your further opinions. Thank you.

Changes in the text: No changes in the text.

Comment 5: What's the pattern of LGE of patients?

Reply 5: Thank you very much for the comment. Twenty-eight patients (28/49, 57%) had LGE positive in our severe AS cohort, and half of them were with preserved LVEF (manuscript, Table 2). The pattern of LGE of patients included the non-infarct (patchy mid-wall or patchy transmural) and/or infarct LGE (patchy subendocardial) in line with previous study (2). We have now added some data in the revised manuscript to briefly clarify the pattern of LGE of patients (see Page 12, Lines 256-258).

Changes in the text: "Regarding the parameters of fibrosis imaging, focal displacement fibrosis measured by LGE was detected in 28 patients (28/49, 57%) with infarct-like and/or non-infarct two patterns".

Comment 6: Analysis of relationship, why only GLS? how about GRS/GCS relationship with ECV/CVF?

Reply 6: Thank you very much for the comment. The main reasons for analysis of relationship between only GLS and other parameters are listed as follows. **First**, as the reported technical limitations, validation issues and intervendor agreement pertain particularly to GCS and GRS, and less to GLS, the preferred global strain parameter should be GLS (3). **Second**, according to the published data, the GLS is the most studied and reliable parameter in the AS area utilizing STE and/or CMR-FT technique (4,5). **Third,** as stated in the manuscript Discussion, Paragraph 2, Lines 267-268, the GLS is the sensitive parameter to assess the subclinical function during the early stage of course of AS, which myocardial fibrosis starts in the subendocardial layers leading to a reduction in longitudinal LV mechanics. **As mentioned above, we mainly assess the relationship between GLS and other parameters.**

Moreover, we also make an analysis about GRS/GCS relationship with ECV/CVF as suggested. And the results are shown in the following figure. Considering the result of this analysis does not add to any new conclusions in our study, it is not put into the manuscript. However, we're sincerely looking forward to your further opinion about it and we'll be pleased to follow your suggestion. Thank you.

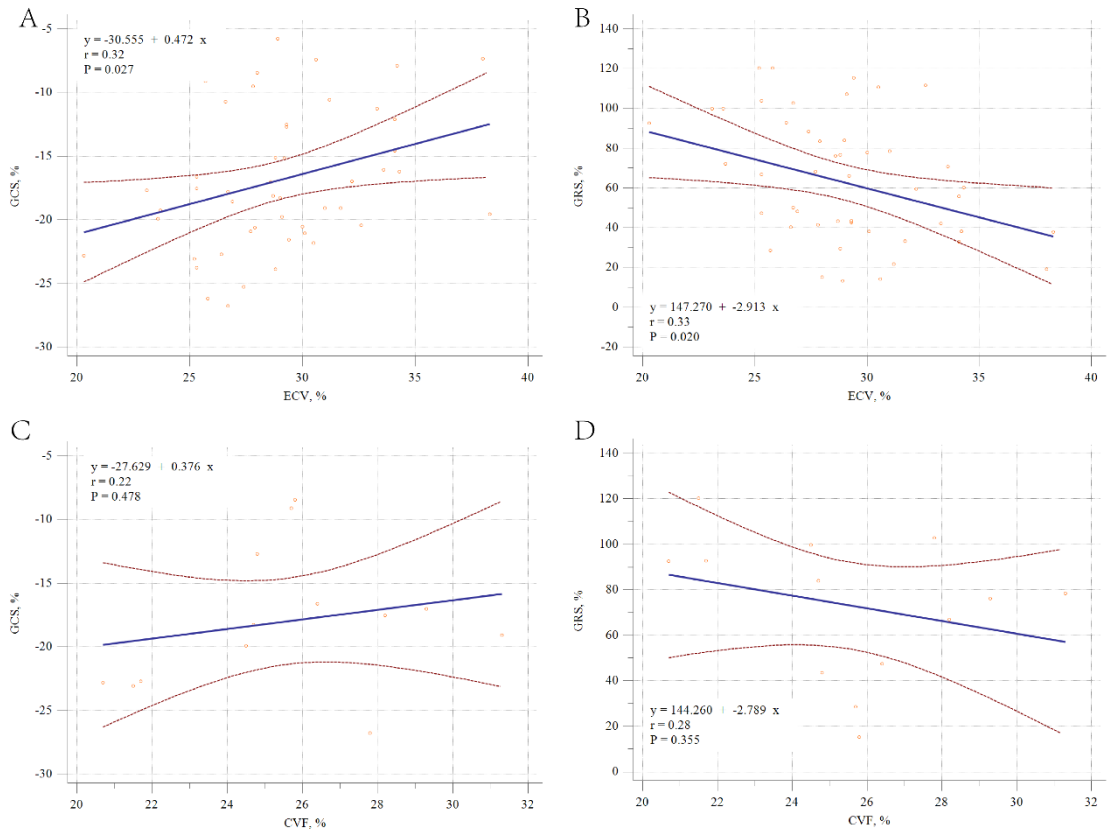

Figure: The correlation GRS/GCS with ECV/CVF. There was mild relation between ECV and GCS/GRS. However, no significant relation between CVF and GRS/GCS. ECV, extracellular volume; CVF, collagen volume fraction; GLS, global longitudinal strain; GCS, global circumferential strain; GRS, global radial strain. Note that only 13 patients with severe AS obtained the biopsy specimen who could be assessed the CVF in histology.

Changes in the text: No changes in the text.

Comment 7: Authors concluded that GLS is the early marker of myocardial mechanics related to disease state. How to conclude GLS is a early maker? How long these patients suffered from disease since patients with severe AS?

Reply 7: Thank you very much for the comment and valuable reminder. The patients suffered from disease since patients with severe AS varied from six months to three years. Indeed, we must acknowledge that such a conclusion is not objective and rigorous based on the results of our study. Thank you again. We have now deleted 'early' throughout the manuscript related to concluded expression (see Page 4, Lines 77; Page 15, Line 310; and Page18, Line 380).

Changes in the text: "GLS is the functional marker before the fibrosis burden intensifies".

References:

1. Messroghli DR, Moon JC, Ferreira VM, et al. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2* and extracellular volume: A consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imaging (EACVI). J Cardiovasc Magn Reson 2017;19:75.

2. Treibel TA, Lopez B, Gonzalez A, et al. Reappraising myocardial fibrosis in severe aortic stenosis: an invasive and non-invasive study in 133 patients. Eur Heart J 2018;39:699-709.
3. Amzulescu MS, De Craene M, Langet H, et al. Myocardial strain imaging: review of general principles, validation, and sources of discrepancies. Eur Heart J Cardiovasc Imaging 2019;20:605-19.
4. Agoston-Coldea L, Bheecarry K, Cionca C, et al. Incremental Predictive Value of Longitudinal Axis Strain and Late Gadolinium Enhancement Using Standard CMR Imaging in Patients with Aortic Stenosis. Journal Of Clinical Medicine 2019;8.
5. Kearney LG, Lu K, Ord M, et al. Global longitudinal strain is a strong independent predictor of all-cause mortality in patients with aortic stenosis. Eur Heart J Cardiovasc Imaging 2012;13:827-33.