Author's response to reviews

Title: Left ventricular systolic function changes in hypertrophic cardiomyopathy patients detected by the strain of different myocardium layers and longitudinal rotation

Authors:

Jun Huang (305669112@qq.com)
Zi-Ning Yan (docyanzining@163.com)
Li Fan (1900883927@qq.com)
Yi-Fei Rui (2318472619@qq.com)
Xiang-Ting Song (docsongxiangting@hotmail.com)

Version: 1 Date: 25 Jun 2017

Author’s response to reviews:

Dear Prof. Michel Noutsias,

Thank you very much for giving us an opportunity to revise our manuscript. We would also like to thank the reviewers for the critical but constructive comments. We have extensively revised our manuscript, according to the comments and suggestions of the reviewers and editor, and responded, point by point to, the comments as listed below, and all changes made to the text are highlighted in red so that the reviewers may be easily identified. Although we acknowledge that the overall priority score assigned to our previously submitted manuscript may not be sufficient for publication in your journal, we believe that our work is of merit and our manuscript has been further improved by incorporating and implementing the comments of the reviewers. I would like to submit this revised manuscript to BMC Cardiovascular Disorders. I hope it is acceptable for publication in the journal.

Looking forward to hearing from you.

With kindest regards,

Yours sincerely

Jun Huang, Department of Echocardiography, ChangZhou No. 2 People's Hospital Affiliated to NanJing Medical University, ChangZhou, China
Email: 305669112@qq.com
F Schnell (Reviewer 1)

In overall, the paper it is written in suboptimal English language, with many grammatical mistakes. The paper might benefit from general editing.

Answer: Thanks to the reviewer. We are sorry for our poor English because we are not native English speakers. This manuscript has been edited and proofread by Medjaden Bioscience Limited.

Major comments

(1) A decrease in longitudinal function in HCM patients has already be proven. The authors should explain in the introduction why they chose to study multilayer strain and longitudinal rotation.

Answer: The anatomy of normal myocardium consists of subendocardial, middle wall and subepicardial myocardial fibers. Using multilayer strain to analysis the LV function is a new method. It can evaluate the LV function from the anatomy of the heart.

Longitudinal rotation (LR) as a new marker has received little attention. The LR means the rotational motion in the long axis of heart, but the origin of LR is still unclear. Some researchers considered that in the LV, there was clockwise LR in the systolic period in patients with heart failure. Our laboratory also did some research in patients with hypertension and dilated cardiomyopathy, and we found longitudinal clockwise rotation motion in the heart.

(2) In the discussion, the authors should explain how their findings might be clinically relevant. In other words, what does the study of multilayer strain or longitudinal rotation adds to the study of the global longitudinal strain for the clinician?

Answer: The changes of peak systolic longitudinal strain of the subendocardial, midmyocardial, and subepicardial layers, and the longitudinal rotation detected by 2D-STE can reflect the LV systolic dysfunction in HCM patients. In clinician, early detection of LV dysfunction in HCM patients can make us to understand the pathophysiology of HCM better, and it also can help the physician to have an earlier symptomatic treatment and then compare the efficacy of the different drugs.

(3) The authors state that the longitudinal strain in altered in the hypertrophied LV myocardium and in the non-hypertrophied myocardium. Which results support this statement? The fact that longitudinal strain is altered in the septum (which is hypertrophied) and in the posterior wall (which is not hypertrophied) as compared to healthy controls? But, at the same time the authors demonstrate that there is a correlation between the diastolic inter ventricular septal thickness and peak longitudinal systolic strain. I would suggest that the authors perform a correlation between the regional strain and the corresponding wall thickness (and not solely the septal wall thickness).
Answer: In table 2, we known, In HCM patients, the LV peak systolic longitudinal strain was lower than in the normal subjects, not only in hypertrophied LV myocardium, but also in non-hypertrophied myocardium.

The diagnosis of HCM was based on the following M-mode and 2D echocardiographic evidence of wall thickness ≥ 15 mm in one or more LV myocardial segments and non-dilated left ventricle (LV). In addition to the absence of another cardiac or systemic disease capable of producing the magnitude of hypertrophy evident in patients with HCM, such as the valve diseases valve stenosis, hypertensive heart diseases, and coronary heart disease. Apical HCM patients were excluded for the study. All enrolled HCM patients were non-obstructive, based on the degree of LV outflow tract obstruction, there was no obstruction at rest or provocation (peak gradient < 30 mmHg). All enrolled HCM patients were had septal wall hypertrophy and with/without other LV walls hypertrophy. So we made the correlation between the end-diastolic interventricular septal thickness and peak longitudinal systolic strain.

Minor comments

(1) In the introduction, the authors state that "the interventricular septum is always involved (in HCM)" (p3): I do not agree with this statement, in type IV of Maron's classification (published in the Am J Cardiol in 1981) the septum is not involved (i.e. apical HCM). What is the rational to include only patients with a ratio of interventricular septum-to-LV posterior wall thickness >1.3 and to exclude patients why a LV obstruction. This might be relevant, but this has to be explained to the reader.

Answer: We are sorry for that we did not introduce the enrollment of HCM patients clearly. The diagnosis of HCM was based on the following M-mode and 2D echocardiographic evidence of wall thickness ≥ 15 mm in one or more LV myocardial segments and non-dilated left ventricle (LV). In addition to the absence of another cardiac or systemic disease capable of producing the magnitude of hypertrophy evident in patients with HCM, such as the valve diseases valve stenosis, hypertensive heart diseases, and coronary heart disease. Apical HCM patients were excluded for the study. All enrolled HCM patients were non-obstructive, based on the degree of LV outflow tract obstruction, there was no obstruction at rest or provocation (peak gradient < 30 mmHg). All enrolled HCM patients were had septal wall hypertrophy and with/without other LV walls hypertrophy. The reason we excluded the apical HCM and obstructive HCM patients were:
(1) Apical HCM patient is lack of representative because the LV hypertrophy was in the apical.
(2) In clinical, non-obstructive HCM patients are more than obstructive HCM patients.

(2) "Written informed consent was obtained from the each couple enrolled in the study (p4); "Consents of all the patients were accepted" (p5). I would suggest not repeat this information twice.

Answer: We have deleted the sentence “Consents of all the patients were accepted”.

(3) In the results, the authors state that "the longitudinal rotation degrees in normal subjects was <3°, around the zero baseline for a small angle movement. In HCM patients, the clockwise
longitudinal rotation was found in the heart (Table 4, Figure 3). I agree that it seems that there is a difference between HCM and controls, but there is no statistical comparison to confirm this statement. Indeed, statistical comparison were only done between the peak segmental longitudinal rotational degrees but not for the global longitudinal rotation.

Answer: Thanks to the reviewer, we have added the statistical comparison for the global longitudinal rotation in Table 3.

Table 3 Comparison of the peak segmental and global longitudinal rotational degrees in the systolic period between HCM patients and normal subjects (mean±s.d.)

<table>
<thead>
<tr>
<th></th>
<th>Base-Septal(°)</th>
<th>Mid-Septal(°)</th>
<th>Apex(°)</th>
<th>Mid-lateral(°)</th>
<th>Base-lateral(°)</th>
<th>Global</th>
<th>Base-Septal(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCM (36)</td>
<td>-9.45±2.65</td>
<td>-7.90±3.08</td>
<td>-5.07±3.61</td>
<td>-2.49±4.85</td>
<td>0.15±6.14</td>
<td>-4.92±2.65</td>
<td></td>
</tr>
<tr>
<td>Normal (36)</td>
<td>-9.21±3.11</td>
<td>-4.52±4.01</td>
<td>1.28±3.42</td>
<td>6.38±3.63</td>
<td>9.66±3.63</td>
<td>0.02±2.42</td>
<td></td>
</tr>
<tr>
<td>P-Value</td>
<td>0.687</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Base-Septal: the base of the septal wall, Mid-Septal: the middle of the septal wall, Apex: the apex of the left ventricular, Mid-lateral: the middle of the lateral wall, Base-lateral: the base of the lateral wall.

(4) In the discussion, the authors state that "hypertrophy of the LV in HCM patient likely leads to an increase in the after-load of the heart". I am afraid that I do not understand this statement. Afterload might be increased by a LV obstruction in HCM patients, but these patients were excluded from the study.

Answer: We are sorry for this mistake. We have deleted the sentence.

(5) The authors also state "that neural and humoral regulation mechanisms may underlie the orientation of the longitudinal rotation". That is purely speculative, the authors should support their assumption with at least some references.

Answer: Thanks to the reviewer, because the orientation of LR is unclear, we considered neural and humoral regulation mechanisms may underlie the orientation of the longitudinal rotation is just our hypothesis. Further researches are necessary to confirm this hypothesis.

P Collier (Reviewer 2)

(1) Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors. In general, strain can be difficult to measure in HCM patients due to varying thickness of the septum vs. free wall so that
the automated ROI needs to be significantly modified. I would request that the authors at least include some examples of their region of interests, how they traced the layers and provide examples showing adequate tracking.

Answer: Thanks to the reviewer, we explained the analysis in the next page with a figure.

(2) No reproducibility data is provided.

Answer: The results for the intraobserver and interobserver variabilities for the peak systolic global strain of subendocardial, midmyocardial and subepicardial layers and longitudinal rotation degrees upon repeated measurements in all study patients were shown in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Interobserver</th>
<th>Intraobserver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCM</td>
<td>Normal</td>
</tr>
<tr>
<td>Global subendocardial</td>
<td>-16.94±4.47</td>
<td>-24.08±3.41</td>
</tr>
<tr>
<td>Strain midmyocardial</td>
<td>-13.60±3.90</td>
<td>-21.01±2.99</td>
</tr>
<tr>
<td>(%) subepicardial</td>
<td>-10.98±3.48</td>
<td>-18.43±2.70</td>
</tr>
<tr>
<td>Global LR(°)</td>
<td>-4.97±2.66</td>
<td>-0.05±2.44</td>
</tr>
</tbody>
</table>

(3) Prior studies have shown that strain reduces proportionately with the degree of hypertrophy/fibrosis in HCM. How many of the HCM patients had LBBB which may account for the abnormal rotation?

Answer: In the research, we knew that LBBB may cause abnormal rotation, so if the HCM patient’s ECG showed LBBB, we would exclude the patient from the research.