Title: Clinical implication of disturbed left atrial phasic functions in the heterogeneous population associated with hypertension or atrial fibrillation

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Author’s response to reviews:

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To: Cardiovascular Ultrasound

Dear Professor Jong-Won Ha,

Thank you for your help in our manuscript, entitled: "Clinical implication of disturbed left atrial phasic functions in the heterogeneous population associated with hypertension or atrial fibrillation" (CARU-D-19-00023). Each of the reviewers' comments was carefully considered and the manuscript was revised as suggested. Below you will find the explanation to each of the reviewers' comments and the revision we made.

Reviewer #1:

Zhu et al studied LA phasic strains and strain rates using STE in 4 cohorts - HT without AF, HT with AF, lone AF, and healthy subjects. They found that (1) the passive conduit and reservoir LA strains are impaired, with preserved pump functions, in HT subjects without AF; (2) LA pump function and synchrony were impaired in HT subjects with AF; (3) LA strains were impaired despite normal LA volume in lone AF patients; (4) ROC curve analysis of various phasic LA strain parameters show they could be used to discriminate HT subjects with vs without AF, or lone AF vs healthy patients.
This is an interesting paper, and of clinical relevance. It provides important data on the alteration in the LA phasic function in relation to occurrence of AF in HT patients and lone AF. The diagnostic cut-off values proposed by this paper is of potential diagnostic relevance in patients with suspected atrial arrhythmia in the setting of hypertension.

However, I have the following comments that would need the authors to address:

- page 4 line 73. please define new-onset AF in this study
Response: Thanks for your kind suggestions. We would like to accept your suggestions and add the definition, as you can see according to the 'tracked changes' in the marked copy of revision.

- page 6 line 129-152: this is the main part of the results, with a lot of parameters and data that sometimes may appear confusing and difficult to follow. I would suggest the authors to more concisely summaries the major findings in the text, in a more logical sense that readers can more easily follow, while referring to the tables for detail data.
Response: Thanks for your kind suggestions. We would like to accept your suggestions and revise original statement as concisely as possible, as you can see according to the 'tracked changes' in the marked copy of revision.

- please clarify whether patients are prospectively or retrospectively recruited.
Response: Thanks for your kind suggestions. We would like to accept your suggestions and add the statement in the first sentence, first paragraph of the Study Population, Methods section, as you can see according to the 'tracked changes' in the marked copy of revision.

- page 5 line 101-108: the authors calculate global strain and SR by averaging 12 segments from the 4 and 2 chamber views, but calculate the dyssynchrony by SD of the time to peak strain/SR of 15 segments of the 4, 3, and 2 chamber views - what is the rationale behind the different views and LA wall segments used in strain/SR vs dyssynchrony?
Response: Thanks for your kind suggestions. Because dedicated software for LA strain analysis has not yet been released, we used the software for LV analysis to study LA strain. We would like to accept your suggestions and list it as a limitation, as you can see according to the 'tracked changes' in the marked copy of revision. The software divided the LA wall into 6 segments in each view, and automatically generated longitudinal strain and strain rate curves of 6 segments and the average curve of 6 segments. The recent consensus statement from the ASE and EACVI on chamber quantification recommended that LA volume should be computed using a biplane algorithm, which includes the apical four-chamber and two-chamber views [1]. Most publications on LA strain have used the same two views. Despite the fact that LA muscle bundles or strands running from superior to inferior have been identified only in the posterior wall of the left atrium (as imaged in the apical long-axis or four-chamber view) [2, 3], the apical long-axis view can be difficult for LA strain analysis because the ascending aorta is difficult to separate from the LA wall due to the limitation of software and it may confound accurate LA strain measurements. Therefore, the use of the biplane LA strain which includes data obtained from both apical four- and two-chamber views is acceptable. However, the time to peak longitudinal strain/strain rate of all segments can be available. When calculating the LA dyssynchrony, the 3 segments of posterior wall in the apical long-axis view were also included for analysis.


- Strain/SR ratio indices: why chose these ratio indices? while 3 ratios were analyzed for strain (pump/res, pump/cond, cond/res), only one ratio (SRpump/cond) was calculated? authors should add a paragraph explaining the physiological implication of these ratios they used.
Response: Thanks for your kind suggestions. We would like to accept your suggestions. The reason why chose these ratio indices have been mentioned in the last sentence, the second paragraph of Two-dimensional Speckle Tracking Analyses, Methods section and the second paragraph of Discussion section. Now we add a more detailed explanation in the end of the second paragraph of Two-dimensional Speckle Tracking Analyses, Methods section and the second paragraph of Discussion section in order to give readers a better understanding, as you can see according to the 'tracked changes' in the marked copy of revision.

We used the ratio indices as indexed parameters of conduit and pump function for further assessment of phasic functions. The absolute value of total deformation is reservoir strain, which is the sum of the absolute value of conduit and pump strain. Calculated the proportion of reservoir deformation (PALSres) contributed to conduit phase (PALScond/PALSres ratio) and pump phase (PALSpump/PALSres ratio), as well as the ratio of the pump phasic component and the conduit phasic component (PALSpump/PALScond ratio and PALSRpump/PALSRcond ratio). Both three strain ratios (pump/res, pump/cond, cond/res) and one SR ratio (pump/cond) were calculated for comparing two components of LA emptying function (early passive and later active) among four groups, while other SR ratio (pump/res, cond/res) have no this kind of capability.

LA function is known to be divided into three parts: reservoir, conduit, and booster pump function. The three components are mutual interdependence and can be redistributed to compensate for each other in order to maintain cardiac output in early stage of some pathophysiological conditions. Structural and functional remodeling of the left ventricle caused by hypertension changes both ventricular compliance and relaxation, influencing the balance between early and late filling. The fall in passive volume shift from atrium into the left ventricle after early filling and diastasis forces the atrium to both increase its stroke volume during contraction and recruit more contractile force to work against the increased ventricular pressure. On the one hand, the absolute value of total deformation (reservoir strain) differed between hypertensive patients and controls while no significant differences were observed in pump strain. On the other hand, no significant differences were observed in the absolute value of total deformation (reservoir strain) and conduit strain among three groups with hypertension, PAF or both. Therefore, we used the ratio indices as indexed parameters of conduit and pump function for the assessment of phasic functions.

- page 7-8, line 178-180: "Our findings suggest that (1) in early stage of hypertension, conduit function is first and most severely impaired, followed by reservoir function, while booster pump function is still preserved and contribution proportion of pump phase shows a compensatory increase". This statement is not supported by data as this is not a longitudinal study.
Response: Thanks for your kind suggestions. We would like to accept your suggestions and revise original statement, as you can see according to the 'tracked changes' in the marked copy of revision.

- page 8, line 192-193: "In our study, the depression in PALSres, PALScond and PALSRcond shown in hypertensive patients suggested that LA conduit function was first and most severely impaired, followed by reservoir function." Again, a natural history of LA phasic function impairment with conduit strain impairment taking place first before reservoir strain is implied by the authors but not supported by data. Although the chronicity of HT is longer in the HT+AF group than the isolated HT group in this study (by merely 3 years and +/-4.5y SD), the comparison is still only cross-sectional between the two cohorts rather than longitudinal within the same cohort. One cannot conclude or imply that the isolated HT group will "progress" later to HT+AF group.

Response: Thanks for your kind suggestions. We would like to accept your suggestions and revise original statement, as you can see according to the 'tracked changes' in the marked copy of revision.

- LA reservoir strain is passive and primarily a reflection of the LV longitudinal systolic strain -- systolic LV longitudinal deformation pulls down the mitral annulus and thus stretch the LA. The impairment of LA reservoir strain and SR are likely reflecting impaired LV longitudinal strain in hypertensive subjects. The relations of LV GLS and LA passive strains as described by recent studies (Tang et al J Am Soc Echocardiogr 2019;32:503-513) and (Ersboll M et al. Circ Cardiovasc Imaging 2013;6:26-33) should be discussed.

Response: Thanks for your kind suggestions. We would like to accept your suggestions and discussed the relations of LA function and LV function in hypertensive subjects in the second paragraph of Discussion section, as you can see according to the 'tracked changes' in the marked copy of revision.
Reviewer #2:

The present manuscript, "Clinical implication of disturbed left atrial phasic functions in the heterogeneous population associated with hypertension or atrial fibrillation" is an original article exploring the diagnostic value of LA phasic functions measured by 2-dimensional (2D) speckle tracking echocardiography (STE) in heterogeneous populations with hypertension and/or atrial fibrillation (AF). This study suggests the potential utility of 2D STE-based assessment of LA phasic functions for the purpose of differentiating between patients with lone AF and healthy individuals or hypertensive patients with concomitant AF and those with only hypertension. Their efforts deserve praise, but the reviewer has major concerns regarding this article.

Major comments

Comment #1

1. First of all, are the absolute differences in measurements clinically relevant? How do the differences between groups compare to inter-observer and intra-observer variability for these indices? For example, if the mean PALSRes in patients with long AF was 1.43 and the mean PARSRres in healthy controls was 1.71, is this difference (0.28) greater than the variability in PARSRes measurements?

At least the authors should provide data on inter-observer and intra-observer variability for the measurements.

Response: Thanks for your kind suggestions. We would like to accept your suggestions and add the inter- and intra-observer variability of LA strain and strain ratio indexes measurements in the Method section, as you can see in the Figure 3.

2. The authors defined paroxysmal AF as AF episodes confirmed by at least one ECG within a year, that terminates spontaneously or with intervention within 7 days of onset according to the current guidelines. In this setting, how the authors can assure that healthy controls enrolled in the present study did not have asymptomatic paroxysmal atrial fibrillation? More aggressive efforts to diagnose paroxysmal atrial fibrillation, such as 24-hour holter ECG, are usually needed to conduct studies involving patients with paroxysmal AF. The lack of such efforts to verify the presence or absence of paroxysmal atrial fibrillation is a major limitation of this study.

Response: Thanks for your kind suggestions. We would like to accept your suggestions and list it as a limitation, as you can see according to the 'tracked changes' in the marked copy of revision.
3. I am not sure that you can say the controls were age and sex matched. There was no significant difference because the sample size was small. Furthermore, among the three groups consisting of isolated hypertension group, lone AF group, and hypertension with paroxysmal AF group, which one was used for the matching process? Perhaps, say "healthy control" and "no significant difference in age and sex". To match, you really need to have excluded some individuals.

Response: Thanks for your kind suggestions. We would like to accept your suggestions and revise original statement, as you can see according to the 'tracked changes' in the marked copy of revision.

4. One-way analysis of variance (ANOVA) was used to perform between-group comparisons of continuous variables in the present study. Considering the small sample size of this study, the author provide data on whether values follow a normal distribution.

Response: Thanks for your kind suggestions. The Levene’s test was used in order to check the homogeneity of variance and Kolmogorov-Smirnov test was applied to check the normal distribution. Between-group comparisons of continuous variables were performed using one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test to adjust for multiple comparisons, when normality and homogeneity of variance assumptions are satisfied; otherwise the equivalent non-parametric tests were used when Kolmogorov-Smirnov was in favor of absence of normal distribution or the Levene’s test was in favor of absence of homogeneity of variance.

3. This study protocol did not include assessment of the presence or severity of valvular heart disease, such as mitral stenosis or regurgitation, which can affect the risk of LAAT as well as the degree of left atrial enlargement. The authors should provide comprehensive information on valvular heart disease, including the presence/absence, type, and severity.

Response: The exclusion criteria of this present study included moderate or severe valvular disease, as you can see the statement in the first paragraph of the Study Population, Methods section. We added the information about mild mitral or aortic regurgitation in Table 1, as you can see according to the 'tracked changes' in the marked copy of revision.

4. Data on body weight, height, body mass index, body surface area, smoking status, and alcohol consumption should be in Table 1.
Response: Thanks for your kind suggestions. We would like to accept your suggestions and add the data on body weight, height, body mass index, body surface area, smoking status, and alcohol consumption in Table 1, as you can see according to the 'tracked changes' in the marked copy of revision.

5. Although the author provided data on medications used in the study population, data on other medications, such as diuretics, digoxin, and statins should be added.

Response: Thanks for your kind suggestions. We would like to accept your suggestions and add the data on other medications, such as diuretics, digoxin, and statins in Table 1, as you can see according to the 'tracked changes' in the marked copy of revision.

6. Who performed transesophageal echocardiography (TEE) for the detection of LAAT? Who conducted LA strain analysis? Is there any pre-specified sequence of performing tests, such as TEE first, and then LA strain analysis or vice versa? The authors should also clarify whether the examiner(s) was blinded to the results of prior measurements and clinical data.

Response: We didn’t performed TEE for the detection of LAAT in this study.

7. Figure legends do not describe detailed information about analyses performed in this study. For example, in figure legend 3, there is even no description for what is observed in Figure 3a-b, Figure 3c, and Figure 3d-e.

Response: Thanks for your kind suggestions. We would like to accept your suggestions and add a more detailed explanation in the corresponding Figure legends in order to give readers a better understanding of the figures, as you can see according to the 'tracked changes' in the marked copy of revision.

Minor comments:

Comments #1

Grammar, punctuation, and typographical errors should be fixed.

Response: The English language have been improved by a professional colleague who is fluent in English.
Comments #2

Please check abbreviations through manuscript and tables. First of all, define an abbreviation at its first use in text and then simply use the abbreviation throughout the text. For instance, the abbreviation "AF" was used to denote "atrial fibrillation", but the full name "atrial fibrillation" was used thereafter.

Response: Abbreviations through manuscript and tables have been checked.

Above is the explanation and changes we have made. If you have more suggestions, we would like to accept. Thanks for all you have done for our manuscript. We hope this manuscript can be helpful in clinical practice.

Sincerely,

Mengruo Zhu, MD