Author’s response to reviews

Title: Normative reference ranges for echocardiographic chamber dimensions in a healthy Central European population: Results from the Czech post-MONICA survey

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Author’s response to reviews:

Reviewer #1: The authors presented their data of normal reference ranges for echocardiographic chamber dimensions in a healthy Central European population. They analyzed echocardiographic images in the Czech post-MONICA survey. I agree that the definition of normal reference values is very important. However, there are several unsolved issues regarding this study.

1. The authors excluded 1275 persons in your study. The authors showed several reasons of exclusion. It would be better that the authors demonstrate the number of exclusion reasons in the figure 1.

We have added the number for each exclusion reason into Figure 1.

2. As you showed in the table 2 and 3, there can be significant difference in different age groups. However, the distribution of age of the participants is not evident. How about showing the distribution in the table 1?

We are thankful for this insightful comment, we added the age distribution into baseline table 1.

3. The authors showed mean difference of age and gender in the table 2 and 3. How about showing the number and standard deviation according to gender?
The effect of age and gender in Table 2 and 3 is not on mean, but on the upper or lower reference limit, as this value was the main interest. Upper/lower reference range can react differently than mean, typically due to variation of spread (either measured by standard deviation or percentiles). To address this issue, we:

I. As the reviewer suggests, have added Supplementary Tables 5-8 that show among other mean +/- SD, median [25th-75th percentile] stratified by age and gender for readers interested in mean values and parametric summaries. Significance of age and gender effect on median is now also shown in Supplemental Tables 7-8.

II. Have rephrased the text of the manuscript (Results section “Effects of age, gender and measurement method”) and tables title to make obvious that the effect in Table 2 and 3 is on upper/lower reference limit and not on mean value.

4. The authors set normal reference value of left atrial volume index (LAVI) as 42 in males and 40 in females. However, the authors included figure 2 showing the distribution of LAVI and set 42 as the reference. The authors should separate the distribution of LAVI according to both sex.

Figure 2 is used to show the methodology of deriving reference limits, the indexed LA volume distribution is shown just as an example. To address reviewer’s comment:

I. We have added Supplemental Figure 1 that shows distribution according to gender for both LAVi and LV mass.

II. We have edited the Figure 2 so that it more clearly shows that it is about methodology and not about LA volume results.

Moreover, the known reference of LAVI is 34mL/m2 in the previous studies. What is the difference between your study and the previous studies?

Our LA volumes are indeed larger than the guideline cited cutoff of 34ml/m2. However, our LA volumes cutoff are quite similar to the more recently published NORRE data (Kou et al, Eur Heart J Cardiovasc Imaging 2014;15:680-90.), where the upper limit for indexed LA volume using the same methodology was 40.3ml/m2 for both genders, which is quite comparable to our values. Epidemiological sampling of our population could lead to these larger reference limits. We have added this comment into the Discussion (section “LA volume, RV diameter and function”).

Reviewer #2: In this manuscript, the authors aim to derive values including reference range for echocardiographic measurements and degrees of abnormality from a randomly selected population sample in the Czech Republic.

They concluded that age, gender, and measurement method significantly affect cardiac dimensions and function and should be always taken into account.

I have several suggestions for this meaningful manuscript.

1. All the previous studies seeking normal reference range in a population such as NORRE study (ref. 9) provided average +/- standard deviation for reference range. Unfortunately, normal reference values for the chamber dimension and mass were not provided in this manuscript.

The authors only provided a cut-off number for degrees of abnormality in Table 4, 5.

I would like to recommend the authors to provide normal reference values for the echocardiographic
measurement in the form of average +/- standard deviation.

We are thankful for this important comment. We believe that nonparametric methods represent our real reference limits better (please see our reply to question 2). However, we understand the need for comparison and established standards. To address this:

I. We have added a Supplementary Tables 5 - 8 showing summary parameters for all the variables including mean ± SD and nonparametric median [25th,75th percentile], stratified by gender and by both gender and age.
II. We have also added a distribution graphs in Supplemental Figure 1 showing LV mass and LA volume measurements to give readers a visual idea of their distribution.

To provide a better sense of the normal reference range for the readers, the scattergram of each measurement in Figure 3 might be helpful.

We have redone Figure 3 as a scattergram and moved it as Supplemental Figure 2 to the general distribution plots in Supplemental Figure 1. Figure 3 is now an example of difference between 2D and M-mode measurement as reviewer suggested in minor comments.

2. It would be better for the authors to explain why they chose percentiles as the degree of abnormality instead of standard deviations.

We have used nonparametric quantile regression because the observed variables were not normally distributed. This has been done previously in the EchoNORMAL study. Actually, in our dataset, all the variables in both healthy and general population had significantly non-normal distributions (Shapiro Wilks p < 0.001 for all the tests). Furthermore, the non-normal distributions of LV mass and LA volume can be seen on the graphs in Supplemental Figure 1.

We have added this into the methods section, but we also now provide Supplemental Tables 5-8 which show parametric summaries for interested readers.

Also, please provide the rationale or clinical relevance for stratifying the degree of abnormality as mild, moderate, severe.

We understand any method trying to do this is potentially problematic, as is discussed in the 2005 Chamber quantification guidelines (Lang et al., J Am Soc Echocardiogr 2005;18:1440-63.) As we previously stated including the Limitations section, these cutoffs are mostly relevant in measurements where abnormality is present in general population (i.e, LV mass). They give a sense of how far away the value is to normal reference range. To update the manuscript:

I. We have explicitly included in the Methods section that our method has been used previously (Vasan Circulation 1997;96:1863-73 cited in our manuscript) and also discussed in previous Chamber quantification guidelines (Lang et al., J Am Soc Echocardiogr 2005;18:1440-63.)
II. We have updated Discussion section where we compare to the chamber quantification guidelines, which in some cases similarly use SD multiples to define degrees of abnormality (for example in LV mass, where mild moderate and severe abnormality is based on 2SD,3SD and 4SD deviation). Our method is nonparametric and can accommodate non-normal distributions.
III. We rephrased the Limitations section to make our method more understandable to the readers.

Minor comment
3. I would like to recommend the authors to provide representative images showing the discrepancy between 2D measurement and M-mode measurement of wall thickness.

We have added new Figure 3 to illustrate this. Original Figure 3 has been redone as a scatterplot and moved as Supplemental Figure 2 to address reviewers’ major comment.

4. Because height and weight have an influence on the chamber dimension, it would be interesting to have these data in the supplemental Table for each age by a decade.
   If there were differences in the height between generations, it might explain the influence of age on the echocardiographic measurement.

We have added the height and weight parameters into the Supplementary Table 9. Furthermore, we have already analyzed the effect of age on both unindexed and indexed variables, as is shown in Table 3 and Supplemental Tables 7-8. However, further analysis of proper mode of indexing and relationship of BSA, BMI and echocardiographic parameters was beyond the main scope of the manuscript. We added this comment into the limitations section.

5. Were all the echocardiographic measurements performed in the central core laboratory?
   Please specify in the method section.

   Yes, they were. We have added this statement into the methods section.

6. In page 6, line 10: pulse tissue Doppler --> pulsed wave tissue Doppler

   Thank you for noticing, we made this correction.