Author's response to reviews

Title: Decrease in shunt volume in patients with cryptogenic stroke and patent foramen ovale

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Author's response to reviews: see over
Reviewer 3 (Jill Jesurum)

Major Compulsory Revisions

Background

1. "PFO is permanent" - Clarify meaning. Spontaneous and at rest vs life-long trajectory, chronic. I think you mean physiologic trajectory of PFO throughout life-time.
   - The expression “permanent” means the physiologic trajectory of PFO throughout life-time. The sentence was changed as suggested.

2. State the hypothesis.
   - Our study is an explorative study; The primary objective was to test if a change in shunt volume occurs.
   - The information on the explorative character of the study was added.

3. Change "study was designed to" to "purpose of the study was...
   - determine changes in RLS volume overtime or longitudinally
   - The sentence was changed as suggested.

Patients and Methods

1. Change to Methods (delete "Patient")
   - The manuscript was changed as suggested

2. Clarify single-center?
   - Manuscript was changed as suggested; this information was added in the chapter “study design”

3. Clarify non probabability sampling technique.
   - The study was undertaken under the explorative character. The main criteria for the patients’ selection was stroke or TIA + TEE and ceTCD during hospitalisation. They all were contacted and invited to participate in the study. Among 130 eligible patients 102 participated in the study. Reasons for not taking part are described in the manuscript.
   - In the context of an explorative character, the present study does not demand being representative for a general stroke population, urging the need for verification of our results in other investigations!!! The latest was one of points of criticism in the last review and is therefore clearly stated in the discussion.

4. Consecutive enrollment?
   - The patients’ selection identified in the first step 169 consecutive patients! This information was added to the manuscript in the section Patients.
   - Out of these patients were finally 130 eligible for the study; 102 patients participated in the study.
   - The follow-up assessment was not conducted according to order the patients were treated for
5. Throughout manuscript when frequencies are stated need to insert (%) following frequency.

6. Clarify statistical and clinically important change in RLS volume between T0 and T1 and state rationale.

The manuscript was changed as requested.

Considering the explorative characteristics of the study and the difficulty in handling a curtain as a numeric value we applied the method of dichotomization. Furthermore it is difficult to state any conclusions on the clinical relevance as long our study is not a clinical trial. The potential value of a shut reduction on follow up need to be evaluated in further studies regarding stroke recurrence.

**STUDY DESIGN**

1. Clarify single group, observational design

This information was added.

Serial ceTCDs performed on two subsequent days in 18 individuals with PFO shunts in countable range revealed an intraindividual variability of ≤12 MES (unpublished data). Based on this observation, we pragmatically chose a cut-off ≥20MES. If this is the optimal approach remains a topic of debate; nevertheless considering the explorative approach of the study, this grading remains pragmatic decision.

We added the rationale behind the cut-off decision, indicating it is based on unpublished data. Further information on these 2 X 18 examinations was not added in order to avoid an overloading of the manuscript and considering this is not the main purpose of the present study.

This is very good suggestion, which provides more structure to the manuscript.

2. State rationale for why >= 20 MES was used as operational definition for change in shunt volume?

“evaluations graded differently” refers to the individual count of MES; in case the same examination revealed different counts of MES between the 2 experts. In this case they evaluated the examination in a consensus read and conducted the MES count together.

When “graded differently” was mentioned in the manuscript the information “(different MES count)” was added.

3. Include section "Setting"

ce-TCD

1. Clarify "consensus read" and operational definition of "evaluations graded differently"??
“consensus read” was change into “consensus count”

This is very god suggestion, which provides more structure to the manuscript.

STATISTICAL EVALUATION

1. Need to perform post-hoc power analysis

In a negative study, as the null hypothesis for the question of interest could not be rejected, a post hoc power analysis does not add information beyond the computed p value. In this case the post hoc power is calculated by solving the sample size equation for power after plugging the observed value in the study. The computed p value and power are depend as far the observed effect is concerned; thus they are inversely related to each other, so in this case a high p-value relates to lower power and vice versa.

In this context we chose not to perform and add any information on post hoc power analyses.

2. Descriptive data should be presented as mean, SD, range and/or 95% CI.

As parameters of interest are not normally distributed, indicating the distribution by mean and SD are not meaningful. In this case median, range or the interquartile ranges add the appropriate information on the location and the dispersion of the distribution. Nevertheless to be able to compare the results the mean and the SD were mentioned in the manuscript.

3. State level of significance, 2 tail or 1 tail.

As we did not expect a reduction or an increase of shunt volume two tailed tests were used.

As the level of significance is concerned, it needs to be underlined the study was planed and conducted with an exploratory design. In this context the p value need to be regarded as exploratory. It indicates a level of the reproducibility of our results under the assumption there is no change between the compared parameters, rather than an level of significance.

4. Statistical software program used?

For statistical analysis the SPSS Software (Statistical Package for Social Sciences) release 15.0 (SPSS Inc., Chicago, IL, USA) was used.

This information was added in the manuscript.

5. How were assumptions evaluated? Especiaially for logistic regression?

The explorative nature of the study we started the evaluation without any expectations. Our study was not a clinical trial. No assumptions were
formulated in advance.

From the methodological point of view the analysis was conducted using an explorative approach. In the first step a univariate analysis was conducted. Parameters associated in the univariate analysis and parameters appearing relevant in a clinical context were than entered into the logistical regression analysis. Continuous variables were considered by building quartiles. In this context it needs to be underline that the p-value should be regarded in explorative sense.

The study and the analysis of data were paned and conducted in an exploratory sense. Therefore the calculated p-values need to be regarded in an exploratory way.

The exploratory character of the study was stated in the manuscript.

RESULTS

1. Add section "Sample" - include demographics, age, gender, diagnosis.

The section “Sample” was included as requested.

2. Needs significant reformatting, restructuring, editing and use of secondary titles to improve readability.

The chapter Results was restructured as follows:

- Sample
- Initial PFO and RLS assessment
- Follow-up RLS assessment
- Inferential statistics

See comment on 2. (above)

3. Add section "Initial RLS Measurments" - divide into TEE and TCD sections. Add details on septal morphology characteristics in TEE section.

See comment on Statistical evaluation point 2.

4. Need to be consistent throughout manuscript with descriptive data reported. Instead of median, use mean, standard deviation, range, and 95% CI.

Among these 5 patients 2 suffered of transient ischemic attack and 3 of stroke.

This information was added to the manuscript as required.

5. During follow up, 5 patients had a "recurrent event" - clarify what this means, use diagnosis. Define “recurrence”??

The manuscript was corrected as required.

6. Again, frequencies are used but need to include (%) after all references to frequency.

The time delay was calculated in month from index event.

The manuscript was corrected as required.

7. Clarify "time delay" - what is the unit of measurement weeks, months from index event? operational definition.
8. Clarify that with only 5 patients having "recurrent events" - inferences can not be made because of lack of power. Calculate power and report.

9. 12 patients had a small increase in RLS. Report mean, SD, range of MES of these 12 patients.

DISCUSSION

1. Temper statements using "true and accurate" words. Does this study have adequate power to state this degree of evidence. Post hoc power has not been included but should be.

The statements “true and accurate” were used for discussing the findings in other investigations which compared the shunt size as analysed by ceTCD compared to TEE. We do not use these statements to characterize our study findings.

As already recommended in the last review we clearly state, that our results need verification and should be regarded as explorative.

2. Define "time delay" in units of measurement.

The manuscript was changed as suggested.

3. Change "sustain" to "support".

The manuscript was changed as suggested.

4. For future research, describe what you recommends from a methodological standpoint.

This is a very important point. It was mentioned in the new version of the manuscript.

We added that for planning further investigations a rigorous time schedule for the follow-up assessment and standardized settings for RLS evaluation are major methodological issues which need to be taken into consideration.

5. Temper "remarkable result" to "intriguing".

The manuscript was changed as suggested.

TABLE 2 and 3.

1. Include N in title.

The manuscript was changed as suggested.

TABLE 4.

1. Report post hoc power of cryptogenic stroke patients and RLS volume change.

See comment on chapter statistical evaluation point 1.

2. Define unit of measurement (days, weeks, months) between T0 and T1.

The manuscript was added as suggested.

Report mean, SD, range, 95% CI. (for Table 5 as well)

See comment on chapter statistical evaluation point 2.