Reviewer’s report

Title: Impact of Bubble Size in a Rat Model of Cerebral Air Microembolization

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Reviewer: Thomas Randsoe

Reviewer’s report:

This research is part of an ongoing research effort into the optimal prevention/treatment of iatrogenic CAM/AGE. The area is difficult to approach clinically and the authors are commended for expanding the body of knowledge into this field. Most interesting is the newly invented experimental model that with a high degree of reproducibility has the potential for providing valuable new data. The manuscript is nicely presented with a well defined issue, clear discussion and figures. For those of us that like in vivo observations of gas embolism the experimental set-up and data provides an interesting novel insight into a dynamic process.

Some minor remarks:

1. Discretionary Revisions

In both Background and Discussion the authors state a plausible relationship between increased bubble surface and increased noxiousness: (background) “increase the total bubble surface and, therefore, intensify the potentially hazardous gas-endothelium contact in turn”; (Discussion) “increase of bubble surface with smaller bubbles and the suspected noxiousness of the gas-endothelium-contact, we considered ischemic infarction to increase in a comparable order of magnitude”.

I believe this statement is actually the working hypothesis of the whole manuscript. If so, it seems appropriate with a clarification of the phrase stating a well defined hypothesis in the Background-section as well as a more in-depth discussion of the ideas encompassing the hypothesis in the Discussion section taking the results into consideration. Does the hazardous gas-endothelium contact rely on endothelial/glycocalyx alteration, immunological activation or ischemia? A mechanistic approach supporting the hypothesis that bubble surface area rather than volume inflicts ischemia is warranted.

If surface area rather than volume is a well known risk factor for ischemia then please add relevant ref.

2. Discretionary Revisions

Methods section 2.4. Could accidentally inlet of air into the ECA stump be a possibility during application of the bubble generator? This would cause an uneven amount of CAM volume and contemplate a major bias. If not, it appears appropriate to state, that accidentally inlet of AGE was not an issue.
3. Minor Essential Revisions

Section 2.7. Group III; “without detectable air bubbles”. Since no air bubbles were supposed to be injected in the shame operated group, it must be “without application of air bubbles”, or with the possibility of accidentally inlet of AGE.

4. Minor Essential Revisions

Results 3. Four animals were excluded. One in group I, two in group II and a fourth animal showing “severe hemodynamic instability as well as apneas during bubble application”. There seems to be an inconsistency, since this fourth animal has been subtracted from group III, the group in which no gas bubbles were suppose to bee implicated.

5. Discretionary Revisions

At the end of the discussion, the authors link the future aspects of the experimental set-up and “substructural” damage with spreading depression, migraine and aura. However, since only two animals suffered functional deficits without ischemic infarctions it seems a bit premature to draw this correlation, especially without revealing a description of the connection between lack of morphological changes verified by MR during CAM and spreading depression, migraine and aura. The authors support the correlation by adding relevant ref, however, each manuscript should contain enough information on its own and no additional information from other papers should be required to understand the point. Thus, if the authors’ future research involves the above mentioned neurological maladies based on the present experimental set-up, a detailed explanation is appropriate.

6. Discretionary Revisions

As debated in the Discussion, exclusion of animals brings the total amount of experimental animals down to an already critical low number. Specially giving the results, that animals in group I presented a Neuroscore with improvement (15 at 4h; 10 at 24h), that animals in group II ended up with a functional outcome after 24h of almost double the impact than group I (10 vs. 19) and that infarct size was more prevalent in group I than II (5.2 vs. 2.3). The fact that group I showed improvement in Neuroscore and ended up with half the score than group II stands in contrast to the ischemic impact verified by MR. It so appears, that the lack of significant difference could very well rely on the sparse number of animals (power calculation?) and that potentially underlying mechanisms causing the discrepancy in the results (Neuroscore/infarct size) may not reach clarification such as infarct size or predilection of infarct location conditioned by volume/surface area. Accordingly, in section 5. Conclusions, it seems reasonable to spend less effort on describing the quality of the bubble generator (leave the evaluation for the reader) and less effort on the potential future investigations regarding underlying pathology of gaseous microembolization (leave that for future reports) and instead conclude on/mention the uncertainties caused by the small study group as well as the discrepancies found in the results.
**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests