Reviewer's report

Title: An assessment of the vulnerability of carotid plaques: a comparative study between intraplaque neovascularization and plaque echogenicity

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Reviewer: Andrew Degnan

Reviewer's report:

The authors have submitted an article that details an important topic in the carotid atherosclerosis literature looking at whether detection of contrast-enhancing neovascularization within carotid plaque on US correlates better with embolic signals on TCD than does mere plaque morphology (echogenic vs. echolucent) on CDUS. They conclude that CEUS has great value based on a good association between CEUS grading and MES detection. Nevertheless, the study is a limited sample of essentially only male patients without non-US imaging studies or post-CEA histology performed for comparison to other modalities. There are a few points which need to be clarified and the writing could be improved upon substantially, but the paper offers some good preliminary evidence to support the association between microemboli and the presence of CEUS-detected neovascularization.

- Major Compulsory Revisions

1. (pg. 4) More information needs to be provided as far as how recruitment was carried out, what kind of institution patients came from, etc. Were all the patients asymptomatic? This is the biggest distinction that needs to be made - are we looking at only asymptomatic moderate to severe carotid disease in individuals who never had a stroke? Or are there people with TIA or stroke included in the patients you examined?

2. On what imaging study was the cut-off of 50% performed? It needs to be specified more specifically: i.e. Was it individuals who had a stenosis of 50% or greater on screening US who then underwent CEUS?

3. How were results agreed upon by the two neuroradiologists? Was it consensus, for instance? How was disagreement handled? Also, were neuroradiologists blinded to each of the other studies - i.e. were the CEUS, MES, CDUS reviewed independently without knowledge of the interpretation of other studies? You mentioned blinding for the CEUS, but not in the CDUS section, so there should be more clarification perhaps before the modality-specific methods sections.

- Minor Essential Revisions

The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.
1. Within the abstract:

There is a fragment within the Background section: "To compare CEUS and CDUS in the assessment of plaque vulnerability using transcranial color Doppler (TCD) monitoring of microembolic signals (MESs) as a reference technique." Rephrase to make it a sentence.

2. Within the methods portion of the abstract:

It is unclear what is meant by within 3 days. I am assuming within 3 days of the CDUS study for each patient? If so, I don't think this aspect nor the mention of P-value cutoffs really need to be included within the abstract.

3. In the results section of abstract:

You mention stroke risk factors but don't mention within the methods what data you are using (i.e. lipids, age, etc.)

You write, "Using CEUS, MESs were found in 2 patients (12.5%) in class 1 (non-neovascularization) and in 15 patients (50.0%) in class 2 (neovascularization) (P=0.023)" It would be better phrased as with class X as opposed to in.

4. In the conclusion portion of abstract:

I would argue that a more accurate conclusion would be that "This study provides preliminary evidence to suggest that intraplaque neovascularization detected by CEUS is associated with the presence of MESs, whereas plaque echogenicity on traditional CDUS does not. These findings argue that CEUS may better identify high-risk plaque."

5. (pg. 3) Whenever mentioning names of contrast media, the generic and trade name and manufacturer should be provided. (see here for details for Levovist (http://www.us-tip.com/serv1.php?type=db1&dbs=Levovist).

6. (pg. 3) Rephrase for grammatical purposes: "Previous studies[12, 13] have shown echolucent plaques with a greater contrast-agent enhancement compared to echogenic plaques." Better written as: Previous studies demonstrate that echoluent plaques tend to have greater contrast enhancement compared to echogenic plaques.

7. (pg. 4) The aim of your study was to compare CEUS-detected neovascularization with plaque morphology on CDUS regarding their correlation with MESs.

8. Where does the use of MRA come into play in this study, you mention 50% stenosis on MRA in the exclusionary criteria, but there wasn’t any mention of how MRA fits into the study design.

9. (pg. 7) How many studies were excluded because of image quality issues? It would be important to know as this has an impact on feasibility in applying this method for routine use in the future.

10. (pg. 8) You write in the first sentence of the discussion "we examined a direct correlation between CEUS and CDUS"
characteristics of the carotid plaque and MESs." It's really more appropriately described as an association you examined rather than a correlation. There needs to be more careful use of statistical terms as your writing exaggerates the things you actually tested. The following sentence from that one states "However, there was a significant difference between the MES monitoring and CDUS (P=0.2368)." This is more accurately written as "we did not observe a significant association between MES results and CDUS properties."

11. (pg. 8) Again, the use of the term 'objective' is imprecise. You are trying to say that CEUS appears to be more accurate at assessing plaque vulnerability.

12. (pg. 8) "digitalized angiography" should be Digital subtraction angiography.

13. (pg. 10) "Our observation of a positive relationship between neovascularization in plaques and MESs and plaque echolucency did not correlate with MES and is consistent with this report." This sentence needs to be rephrased.

- Discretionary Revisions

These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

1. (pg. 3) "Color Doppler ultrasonography (CDUS) has historically been employed as the first-choice technique for the diagnosis of carotid plaques" A better way of putting this would be to say it is the screening test of choice for assessing carotid atherosclerosis.

2. (pg. 4 and elsewhere) Occasionally used is the mention of determining which method is more objective. It's better said that you are trying to assess which method correlates better with known vulnerable plaque features. Both methods can be 'objective' but one can be less accurate or less correlated than the other.

3. (pg. 5) Either chose to exclude the formula or better write it with definition of variables. I would argue for its omission.

4. (pg. 8) There is the use of "echo poor" - simply stick with echogenic or echolucent, in this case you are referring to echolucent - this will clear up any confusion, especially as echolucent is used throughout the literature that you cite.

5. (pg. 8) I am not sure where the sentence "In this study, we compared all of these risk factors and found no significant difference between the groups with and without ischemic stroke." fits into the discussion.

Level of interest

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- An article whose findings are important to those with closely related research interests

This article is of interest to those in the field of carotid imaging. It offers evidence to support the use of CEUS - a presently under-developed imaging method to
look at neovascularization. It's clinical impact is as yet unclear and this particular study does not employ a broad array of imaging modalities for comparison to merit the next level of interest. It is, nevertheless, a starting point for further inquiry in the field of non-invasive carotid atherosclerosis imaging.

Quality of written English
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- Needs some language corrections before publication

There is frequent use of fragments and poorly worded sentences. As an example, within the abstract itself there is an obvious fragment "To compare CEUS and CDUS in the assessment of plaque vulnerability using transcranial color Doppler (TCD) monitoring of microembolic signals (MESs) as a reference technique." The writing quality unfortunately detracts from the content of the article.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**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