Reviewer's report

Title: Activation of Calpain-1 in Human Carotid Artery Atherosclerotic Lesions

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Reviewer: Wei Li

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The manuscript by Gonçalves and colleagues demonstrate the active form of calpain in human carotid atherosclerotic lesions, which is significantly decreased in the plaques from symptomatic patients. Furthermore, active calpain appears in the same region of the lesion where TUNEL is positive as well. Based on the findings, authors conclude that calpain-1, inversely associated with symptoms, is commonly active and colocalized with apoptosis in human carotid atherosclerotic lesions.

Major issues
1. The conclusion regarding calpain activation is only based on western blot results by using active form of antibodies. Other techniques should be considered since proteolysis detected in Figure 1 might be due to the artifacts from the processing of the samples, as authors stated. Please note artifacts, not artefacts.

2. The co-localization results of calpain by immunohistochemistry with TUNEL positivity on sequential slices of the same specimens appear less convinced and double staining is needed.

3. The authors claim that “all plaques contained the calpain-generated 150 kDa #-fodrin breakdown product (Figure 1A)”. However, I can hardly see any band from sample 31.

4. Are the samples shown in Figure 1A from both symptomatic and asymptomatic groups? It would be interesting to see whether the two groups differ in the cleavage form of calpain-1.

5. The quality of Figure 2 is low and should be better prepared.

Minor issues,
1. The detailed information of primary antibodies used for immunoblotting should be given.

2. It would be more proper to present the data in Figure 3 as OD#/fodrin/ODtublin than calpain activity.

3. In Statistical Analysis, it is stated that values are presented as mean ± SD. However, Figure 3 is a box plot that is not suitable to show mean ± SD.
**Level of interest:** An article whose findings are important to those with closely related research interests

**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.