

Peer Review File

Article information: <http://dx.doi.org/10.21037/atm-20-7197>.

Comment 1: The section Introduction is largely un-referenced (e.g. the section: It remains a challenge to determine if initially asymptomatic carotid atherosclerotic plaque will become unstable or vulnerable. It is believed that vulnerable, non-stenotic plaques are an underestimated cause of stroke. Currently, the main topics of research on imaging of atherosclerotic lesions are turning towards the functional state of the plaque and the identification of high-risk groups. It is well known that stable plaques with a fibrous structure are less susceptible to rupture and related complications than unstable plaques in which an intense inflammatory process takes place (Table1). These two groups of plaques in typical, routinely performed imaging studies are difficult to distinguish. Currently, there are numerous novel imaging techniques that can detect potentially unstable plaques: magnetic resonance imaging, positron emission tomography and contrast-enhanced ultrasound. Processes indicating plaque activity (molecular imaging) such as: glucose absorption, protein degradation, exposure of adhesive molecules or content of markers for apoptosis are tested for use in the diagnosis of plaques at risk of destabilization.")

Reply 1: We agree with the comment.

Changes in the text: We have modified our text as advised. We have added 12 references to make the paragraph better referenced (references 4-15, highlighted in blue). We added some data to make the introduction to plaque instability more convincing (see page 2, line 7-16; text highlighted in blue).

Comment 2. Again, the section "Biology" comprises of long, un-referenced paragraphs. These paragraphs should be better divided and referenced. In other words, I believe the authors should pay attention to the structure of their paper. Although the content is good, the structure of the article needs some attention.

Reply 2: We agree with the comment.

Changes in the text: We have rebuild and shortened the "Biology" section. The paragraphs are now restructured and better referenced.

Comment 3. In the section "Treatment options", the authors mainly focus on the benefits of best medical treatment. I am not sure that patients with unstable plaques should solely be managed with antiplatelets, statins, etc., but instead should be considered for a prophylactic carotid endarterectomy. Please have a look and discuss the following article: Paraskevas KI, Veith FJ, Ricco JB. Best medical treatment alone may not be adequate for all patients with asymptomatic carotid stenosis. J Vasc Surg 2018;68: 572-5.

Reply 3: We agree with the comment. The authors also belong to the group of clinicians who strongly believe that a selected group of asymptomatic patients with plaque at risk of destabilization should be treated surgically.

Changes in the text: We added some data (see page 9, line 20-30; page 10, line 1-2; text highlighted in blue).

Comment 4. The second paragraph of the Concluding remarks needs some attention. The authors mention that "Of course, it would be more clinically useful to detect a plasma marker attesting to increased activity of the carotid atherosclerotic plaque. Such a marker, thanks to a simple blood sampling, could be used to identify patients with asymptomatic carotid plaques, which have a greater risk of becoming symptomatic in the near future, causing embolization into the cerebral vessels. Such patients, in addition to conservative treatment (pharmacological treatment and leveling of modifiable risk factors), could be good candidates for surgical, primary prevention of cerebral ischemia". I would rather write this as "candidates for a prophylactic carotid endarterectomy" not "prevention of cerebral ischemia".

Reply 4: We completely agree with the comment. Thank you for your suggestion.

Changes in the text: We have modified our text as advised (see page 11, line 1-2; text highlighted in blue).

Comment 5. Reference 46 is the same as reference 66.

Reply 5: We agree with the comment.

Changes in the text: References rearranged. In the new order it is reference 58 (highlighted in blue).

Comment 6. All abbreviations need to be expanded (e.g. NASCET, ECST, CRP, VCAM, IL, are not defined).

Reply 6: We agree with the comment.

Changes in the text: We have modified our text as advised. All abbreviations expanded (see page 1, line 25-26; page 3, line 15-18; page 8, line 21; text highlighted in blue)

Comment 7. The structure of the paper is often tiresome. There are huge paragraphs that are often quite dull and monotonous (e.g. in the section Biology). I am afraid many readers will get bored reading such long paragraphs.

Reply 7: We agree with the comment.

Changes in the text: We have rebuild and shortened the "Biology" section. The paragraphs are now restructured and better referenced.

Comment 8: In the section Treatment options, I did not see any recommendations from e.g. the European Society for Vascular Surgery 2018 guidelines. It is imperative that the recommendations from international guidelines are included.

Reply 8: We agree with the comment.

Changes in the text: We added some data (see page 7, line 29; page 8 lines 1-2, 10-13, 19-20; page 9, lines 20-30, page 10, lines 1-2; text highlighted in blue).

Comment 9: The authors briefly discuss carotid endarterectomy and stenting in the Introduction, but these interventions are not mentioned at all when discussing treatment options for carotid artery stenosis. Please elaborate.

Reply 9: We agree with the comment.

Changes in the text: We added some data (page 9, lines 20-30, page 10, lines 1-2; text highlighted in blue).

Comment 10. Since the authors discuss the importance of unstable carotid plaques, I would expect an expert opinion section where the authors summarize issues that need to be addressed in future guidelines.

Reply 10: We agree with the comment. The issue was widely discussed in other articles of the ATM supplement. We strived to make our work focused on slightly different elements of carotid plaque instability. Anyway, we agree that our work, as an expert review, should convey a clear message.

Changes in the text: We added some data (page 11, line 15-18 text highlighted in blue).

Comment 11. Introduction, 1st paragraph line 4: "there is a general agreement that carotid endarterectomy". The authors mention the term "carotid endarterectomy" here for the first time. They mention it again another 4 or 5 times in the text before page 9, last paragraph, where they decide that they should use an abbreviation (CEA). Subsequently, they continue to use "carotid endarterectomy" despite the fact that they have defined the abbreviation.

Reply 11: We agree with the comment.

Changes in the text: from the moment we defined the CEA abbreviation (see page 2, line 1), we only use the abbreviation in the text (highlighted in blue).

Comment 12: The same applies to "asymptomatic carotid stenosis". The authors to use the abbreviation "ACS" on page 2, line 10, and then continue to use the full term "asymptomatic carotid stenosis" 7 or 8 times in the text.

Reply 2: We agree with the comment.

Changes in the text: from the moment we defined the ACS abbreviation (see page 2, line 10), we only use the abbreviation in the text (highlighted in blue).

Comment 13: The text is still not well-referenced in several occasions. For instance, on page 9, lines 1-5, the authors write "Some substances with known anti-inflammatory effects, such as methotrexate (inhibitor IL-6, TNF, CRP), have been proved ineffective in reducing

cardiovascular events. On the other hand, others, such as colchicine (an anti-inflammatory substance that inhibits the activity of neutrophils) are still under research. The first reports are promising, as colchicine, in addition to high doses of statins, lowers the risk of recurrent cardiovascular episodes (69-71). "The first sentence discussing methotrexate (NOT metothrexate) is not referenced. Reference 69 is appropriate here, but the authors have placed reference 69 together with reference 70 and 71 for the second sentence discussing colchicine. This is inappropriate. Each reference should be placed in the appropriate section of text, not at the end of the paragraph.

Reply 13: We agree with the comment.

Changes in the text: Corrected (see page 9, line 1-4; highlighted in blue).

Comment 14: The next paragraph (page 9, beginning with "Taking into account...") is not well-referenced either. There are 7 sentences without a single reference and then the authors use a single reference (Nr. 31). I strongly advise the authors to go through their manuscript once again very carefully and correct all the minor and very frustrating errors and omissions. Another example is reference 31. The title of the journal is not abbreviated, but provided in full [Advances in Pharmacology]. Did the authors not see on PubMed/MedLine that this should be "Adv Pharmacol" instead of "Advances in Pharmacology"?

Reply 14: We agree with the comment.

Changes in the text: Corrected (see page 9; line 5-13; see page 13, reference 31).