Reviewer’s report

Title: MR Imaging Features of Orbital Langerhans Cell Histiocytosis

Version: 4 Date: 08 Sep 2019

Reviewer: Anton Lennikov

Reviewer's report:

Manuscript BOPH-D-19-00089R4 "MR Imaging Features of Orbital Langerhans Cell Histiocytosis" have further improved following fourth revision cycles. Authors addressed majority of my comments, with the exemption of IHC materials and methods description, that require further clarification, please see below.

I understand that authors did not perform the IHC staining themselves, but instead requested histology service of their institution. Therefore I suggested a rewrite of the of IHC materials and methods part. I also added comments within the text indicated by [] that need to be addressed for clarity and reproducibility of IHC described by authors.

[Excised post-surgery material or biopate?] specimens were embedded in paraffin and cut into 4 µm sections. Sections were then deparaffinized by xylene followed by decreasing concentration of ethanol (100%-50%) and finally washed in cold tap water. Endogenous peroxidase activity was neutralized by incubation with 0.5% hydrogen peroxide in methanol. Antigen retrieval used enzymatic method [which enzymatic method specifically Proteinase K?] in pH 9 Tris-EDTA buffer. Following antigen retrieval, [Blocking step and blocking buffer is missing] samples were incubated with primary antibody to S-100 (Biocare, U.K., 16090216, 1:150); CD 68 (Zeta, US, 16081622, 1:200) and CD1a (Epitomics, US, 16030516, 1:200). [I could not find antibody information based on authors description, please verify the lot numbers and companies described] Following primary antibody incubation slides were washed and secondary [which secondary, source, dilution?] antibody was applied. After another washing, DAB (3,3'Diaminobenzidine) substrate was applied. Slides were counterstained with hematoxylin. Positive controls [what was used as positive control?] and negative controls (sections without added primary antibody) was used to validate the specificity of the signals.

Minor comments:
"The IHC staining shows positive signals for S - 100, CD 68 and CD1a (fig.4,5)". - Please replace the comma , and , are not the same characters.
Figure 3a - there is a residual text in the background from the MRI image that should be removed.
Figure 4 - missing the scale bar.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

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