Author’s response to reviews

Title: MR Imaging Features of Orbital Langerhans Cell Histiocytosis

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Response to the comments:

Anton Lennikov, M.D., Ph.D. (Reviewer 3): 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 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.

Response:
Thanks for your detailed comments. Because I am a radiologist, I’m sorry I really don’t know the specific process of immunohistochemistry, and I have asked the pathology department for help. I consulted professionals again for this revision.

We have rephrase this section as follow:

Excised post-surgery material 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 high temperature and high pressure method in pH 8 EDTA buffer. Following antigen retrieval, put in the 3% hydrogen peroxide solution for 5-10 minutes, remove the endogenous peroxidase, and then put in PBS buffer 3 minutes to wash three times, samples were incubated with primary antibody to S-100 (Biocare, U.K., ZM-0224, 1:150); CD 68 (Zeta, US, ZM-0060, 1:200) and CD1a (Epitomics, US, ZA-0544, 1:200). Following primary antibody incubation slides were
washed and secondary antibody PV-6000 (Beijing Zhongshan Golden Bridge Biotechnology Co. LTD, Beijing, China) was applied. After another washing, DAB (3,3’Diaminobenzidine) substrate was applied. Slides were counterstained with hematoxylin.

Note:
[S-100, CD 68 and CD1a were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co. LTD (Beijing, China), their website is www.zsbio.com. The technicist of the company told me the origin of the three antibody: S-100 from Biocare, U.K., CD 68 from Zeta, US and CD1a from Epitomics, US, and the number are the article numbers.]

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.
Response:
In the revised manuscript, we have corrected it.

Figure 3a - there is a residual text in the background from the MRI image that should be removed.
Response:
In the revised manuscript, we have corrected it.

Figure 4 - missing the scale bar.
Response:
In the revised manuscript, we add the scale bar.