Author’s response to reviews

Title: MR Imaging Features of Orbital Langerhans Cell Histiocytosis

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Version: 3 Date: 25 Jul 2019

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Response to the comments:
Jian Li, MD, PhD (Reviewer 1): Thanks for the authors' responses. I appreciate the authors' effort on documenting the MRI features of LCH patients. It could be valuable to provide further insight on diagnosis of the LCH rare disease. Thanks for your encouragement. I will keep working hard.

Sentaro Kusuhara, M.D., Ph.D. (Reviewer 2): The manuscript (#BOPH-D-19-00089R2, by Wu C, et al.) is well revised, but there are still concerns some of which I cannot condone. Therefore, I do not recommend for publication as is.
Many thanks for your comments once more, and I apologized for my mistakes.
1) In Results of Abstract, what modality was used to detect the appearance of hemorrhage and cystic change? As I pointed before, MRI can display various images resulting from hemorrhage. However, we cannot know whether there is hemorrhage at the lesion until we do surgery.
Response:
I am sorry that it was not revised accurately last time. We have revised it again. Please review it. The points we are trying to make are that, by contrasted with the HE staining, we consider that the hyperintense signals on T1WI is the appearance of hemorrhage in the lesion; and the markedly hyperintense signal on T2WI which is similar to the signal of water is the appearance of cystic change.
2) In Table 2, the characteristic of lesions can be evaluated by the combination of MRI images. Table 2 only shows the number of cases on each MRI imaging technique, which I think is non-informative. What we know is the number of cases whose MRI images were, for example, T1w high/T2w low/Gd enhancec(-), T1w high/T2w low/Gd enhancec(+), T1w iso/T2w high/Gd enhancec(+), etc.
Response:
In the revised manuscript, we have corrected the table 2. We combined the presentations of T1WI, T2WI and Gd enhance.

3) On Page 6, line 33-36: grammatical mistakes should be amended. Plus, please show the values of visual acuity in unaffected patients.

Response:
In the revised manuscript, we have corrected the grammar mistakes. And add the information of the values of visual acuity in unaffected patients.

4) I guess Figure 4 is representative tissue images. If so, there is no novel information because this kind of image is reported elsewhere. In addition, given that all tissue images were like those in Figure 4, what makes different MRI images?

Response:
Figure 4 is a representative image of HE stain. We have corrected the figure legend.

We used this figure for three aims:
First, it were used to confirm the pathological diagnosis of our cases, it presented a large proliferation of Langerhans cells, eosinophils and neutrophils.
Second, it showed many red blood cells, a large amount of hemosiderin and macrophages in the lesions, this indicated that there were hemorrhage in lesions, these manifestation were match with the hyperintense signals on T1WI.
Third, it showed a large amount of necrotic tissue in the lesion, which is match with the appearance on MRI, paly the role of mutual verification

5) In Results of Abstract, the words "majority" and "several" are still ambiguous. Description such as "x out of y (?%)") is suitable for a scientific report.

Response:
I’m so sorry. This time, we have corrected those to "x out of y”.

Anton Lennikov, M.D., Ph.D. (Reviewer 3): Manuscript BOPH-D-19-00089R2 "MR Imaging Features of Orbital Langerhans Cell Histiocytosis" by Wu et al. have significantly improved in terms of clarity and presentation. Authors appropriately conducted additional experiments and figures. However, the manuscript still require additional English proofreading to improve the readership experience; authors advised using different scientific editing/proofreading service than AJE. Also, materials and methods and results for immunohistochemistry data are not present in the manuscript text.

Many thanks for your comments once more.
In the revised manuscript, we have corrected the typos and grammar mistakes once again by a new proofreading service. We add the information of IHC data at materials and methods and results.

Comments:
1. Good IHC data, authors may consider enlarging portions of the images to emphasize the macrophages phagocytosis of hemosiderin, but overall, I am satisfied with the new figure. Authors must include materials and methods for their IHC process and refer to Figure 5 in the results portion of the manuscript. I understand that authors primary focus is MRI, but histology is contributing to authors specificity in diagnosis that it is Langerhans Cell Histiocytosis. In this version of the manuscript, the figure is only referred in the discussion. Please add the section in the results section of the manuscript that describes histological findings of the study. In the materials and methods, please specify the antibodies sources and dilutions used.

Response:
In the revised manuscript, we add the information of IHC data in the materials and methods and results.

2. Is figure 7 represents the CT 3D scans of the individuals presented in Figure 6? If so, I suggest merging it into a single figure and clarify this in the text. If the photographs and CT scans are unrelated its also useful to clarify it in the figure legend using words such as "representative photographs" and "representative CT scan."

Response:
The photographs (fig.6) and CT 3D image (fig.7) are unrelated. In the revised manuscript, we have corrected the figure legend.

3. Add arrows to Figure 7, while in figure 7B, the bone erosion is visible; in Figure 7a, it might take a moment for non-specialist to understand what features authors are referring to. Otherwise, it is a good addition to the manuscript narrative and scientific value as some clinicians may only have CT scan on hands.

Response:
In the revised manuscript, we add the arrow, and added the description of the 3D reconstructions of CT.

Suggestions
1. I understand that microscope software that authors use has its limitation providing the scale-bar data; authors encouraged to purchase the product similar to https://www.tedpella.com/calibration_html/Light-Microscopy-Calibration-Standards.htm and take images of the physical scale at the same magnification as the samples. If such products are not available, authors can use hemocytometer grid, which is 250 um squares in the corner quadrants. Taking the image of the grid at the same magnification as H&E and IHC images will allow authors to produce the scale. Authors can use any imaging software to determine size of the 250 um in pixels and then recalculating number of pixels for any desired scale. i.e. 250 um pixel number/5=50 um in pixels for the given magnification. This is not mandatory for this project, but certainly something authors might to consider for their future work.

Response:
Many thanks for your comments. In the revised manuscript, we add the scale-bar.