Author’s response to reviews

Title: Bone pathologic fracture revealing an unusual association: Coexistence of Langerhans cell histiocytosis with Rosai-Dorfman disease Case report and a literature review

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Author’s response to reviews:

Dear Editor, dear reviewers,

We would like to thank both reviewers for their pertinent assessment of our manuscript. In fact, through our case we have reported a very complex and unresolved issue, consisted of the combination of 2 histiocytic disorders (LCH (Langerhans cell histiocytosis) and Rosai Dorfman disease (RDD)). The issue requires a thorough review of the related literature, and we thank again reviewers for their valuable efforts, for taking their time to do this effort. Below, are our responses and comments about reviewers suggestions.
Reviewer 1:

1. There is a problem with the diagnosis of "combined" histiocytic lesions. It is generally thought that…….could be substituted (given appropriate controls).

Response: Here, the reviewer raises the issue of the diagnosis of combined LCH and RDD, especially the histological combination pattern of these 2 entities and the immunohistochemical staining by S-100 protein that was weak in our case.

- We agree with the reviewer that emperipolesis itself is not specific to RDD. In our case, there were some areas where some histiocytes with abundant cytoplasm and rounded-nuclei with nucleoli, are found (especially in figure 2, as noticed later by the reviewer, but we think these RDD-elements are more visible in figure 3, that we have removed as suggested by the reviewer). Another fact is that, to the best of our knowledge, LCH is not associated with proeminent emperipolesis figures. But, recently, Chougule et al. claimed to report the first case of LCH with prominent histiocytois (Chougule A, et al. Nodal Langerhans cell histiocytois with prominent eosinophilic emperipolesis. Diagn Cytopathol. 2015;43(12):1000-2.). Some giant cells or osteoclast-like cells associated with LCH have been previously reported, but without evident emperipolesis (intact engulfed cells surrounded by a halo, within the histiocyts cytoplasm) (da Costa CE et al. Presence of osteoclast-like multinucleated giant cells in the bone and nonostotic lesions of Langerhanscell histiocytois. J Exp Med. 2005;201(5):687-93.). About S-100 immunohistochemical staining, we agree with the reviewer that it is expected to be strongly positive in RDD. For that, we have reviewed some of our initial slides, and effectively we found some areas where it shows strong staining (we have added a new figure to show that staining). However, we think that there was a background staining that affect the quality of the image. Another comment of the reviewer, is that we blamed the decalcification process for that. In fact, our laboratory technician have repeated S-100 immunohistochemistry for this specimen several times as often he encountered technical problems with tissue fragments of this case. Decalcification process is to blame as we have used a strong acid (nitric acid) as a decalcifiyer and the specimen lasted days in it (with alternance, formalin-acid). Also, in the literature, there were many contradictory reports about the effect of decalcification process on immunohistochemistry (Gruchy JR et al. CytoLyt® fixation and decalcification pretreatments alter antigenicity in normal tissues compared with standard formalin fixation. Appl Immunohistochem Mol Morphol. 2015;23(4):297-302., Schrijver WA, et al. Influence of decalcification procedures on immunohistochemistry and molecular pathology in breast cancer. Mod Pathol. 2016;29(12):1460-1470., ...etc.). Also, unfortunately we have not Fascin in our service.

Another reviewer’s comment we would like to respond, is the pattern of histological combination of LCH-RDD, when they are found in the same organ synchronously. This issue is in fact very complex and controversial. O’Malley DP et al. have tried to microdissect their 9 cases of LCH-
RDD in order to separate the 2 entities, but have failed to do it (as they stated in the ‘materials and method’ section). This shows that these 2 entities are histologically very intricate, as some cases reported by these authors have only patchy areas of RDD or LCH. Only immunohistochemistry can highlights well their pattern of combination that can show sometimes admixture of LCH-RDD elements with ‘transitional cells’ (cells that resemble RDD-histiocytes but with LCH immunophenotype, CD1a+). We have found these transitional elements in our case, that we would like to show in the now-figure 3B (but the reviewer said that ‘the figure does little for the case’ and we don’t think so!).

- ‘In the Discussion the authors reference O’Malley (4) and Cohen-Barak (5), .....but still worth the discussion’. Here, the reviewer raised the issue of clinical (anatomical) combination of RDD-LCH and suggested to notify this fact in our discussion section. We think that, in almost an entire paragraph (the first paragraph of the discussion section), along with the table 1, we have clearly discussed about that issue. The pattern of clinical combination of RDD-LCH is complex : it can be either synchronous (in one organ) or ‘metachronous’ (initial LCH, and recurrence as RDD !). The No.7 case of O’Malley illustrated a case of ‘metachronous combination’ of RDD-LCH, with initial LCH and a recurrence as RDD. Also cases reported by Cohen-Barak et al., and Katty SA et al. showed the same type of combination. The remaining cases showed synchronous combination in a single organ.

2) ‘The radiologic image...to including it’.

- Response : we have included the radiologic image as suggested.

3) ‘In the Background, the authors refer to LCH as representing abnormal Langerhans cells. There is abundant contemporary literature...dimension (See Beres ML et al, J Exp Med 2014;211:669-83)’. We agree with the reviewer that LCH cells are bone-marrow derived myeloid cells, and there is no contradiction with our definition of the LCH. Our statement was not gratuitous as we provided sources that define LCH by these words : almost similarly, these words were used by Cohen-Barak and O’Malley et al. (ref.4, 5). Also, the last WHO book of classification of tumours of soft tissue and bone, has defined LCH as ‘’a clonal and likely neoplastic proliferation of pathological Langerhans cells’’, p356.

- ‘A little more detail on treatment and follow-up’
Response: Very pertinent remark! In fact surgeons have done curetage and osteosynthesis. Since, the patient recovered well with no signs of the disease. (We have added these details in the manuscript, as suggested).

Images: As suggested, we have included the radiologic image and withdrawn the figure 1. We have withdrawn the figure 3, as the figure 2 illustrates the diagnostic issue of our case, as suggested by the reviewer. However, we maintained the figure 4B (now 3B), as our purpose is to show large histiocytes suggestive of RDD-elements but that have LCH-immunophenotype (CD1a+), this type of cells have been termed as ‘transitional cells’ by O’Malley et al., and we find that issue should be pointed out for future research, as to date, it remains controversial. The figure 5 (CD68) has been withdrawn as we agree with the reviewer that it doesn’t make any contribution to the diagnosis. We have changed the figure 6 by another we thought shows more strong S-100 staining, although the quality is not very good.

Reviewer 2:

The reviewer raises the question of the diagnosis of the LCH in our case.

Response: we totally agree with the reviewer that it would be ideal to use CD207, molecular studies and electron microscopy. Unfortunately we don’t have any of these tools in our laboratory. Based on clinical, radiological and histopathological features of the patient, we think that the diagnosis of LCH can be made: Tumor cells with CD1a+, CD68+, S-100+.

Language and images: we have extensively reviewed our manuscript to improve the quality of the images and the english language. We thank the reviewer for his valuable comments, and every improvement in english language or image quality, is welcome, as suggested by the reviewer.