Author’s response to reviews

Title: An unusual case of acute lupus haemophagocytic syndrome: a test of diagnostic criteria

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Version: 1 Date: 28 Jan 2017

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Reviewer #1

Major points

1. As sputum culture was positive for colitis, her first presentation might be explained by hemophagocytosis related to sepsis. Her CRP was negative, so it is not strongly suspected, but at least you need to mention about this differential diagnosis in discussion. Also it is better you show the result of blood culture.

Response:

It is possible that sepsis due to respiratory tract infection due to coliforms may lead to haemophagocytosis. But it is very unlikely that it is the case in this patient due to the following reasons.

1. CRP was repeatedly normal. ESR is high, but high ESR and low CRP is well described in SLE.

2. The patient did not have any clinical sign or symptom of a respiratory tract infection. This makes sepsis secondary to a respiratory tract infection less likely (I have mentioned this in the case presentation as well as in the conclusion) I have added a more detailed discussion in the conclusion.

3. Chest X ray was normal.

4. Repeatedly sterile blood cultures.
5. Coliforms are well recognized to be isolated in sputum cultures in hospitalized patients, especially in ones receiving long term antibiotics as our patient. It is recognized that coliforms colonize the upper respiratory tracts of these patients. I have included three references to support this claim.

2. There are little information about the titre of each parameters. For example, lymphocyte counts, titres of ANA, ds-DNA antibodies, AST, ALT, triglyceride, IgG, IgM, IgA, C3, C4, CH50, anti-platelet antibody, haptoglobin, soluble IL-2 receptor, etc. It might be better you add a table that summarizes the laboratory findings of first and second admission.

Response:

In our low resource setting tests for IgG, IgM, IgA, CH50, anti-platelet antibody, haptoglobin, soluble IL-2 receptor are either expensive for an average patient or unavailable. Thus we were unable to do the above tests for this patient. In the first admission the patient did not give consent for C3 and C4 levels due to its cost. But the patient agreed to do them on the second admission and I have given the results in the case presentation segment. I have also included the titres of ANA and Ds-DNA as a revision.

Even though we were not able to do some of the above investigations, I believe that the diagnosis can be made with the investigations we have done. As we have discussed in the discussion, the diagnostic criteria for haemophagocytic lymphohistiocytosis and systemic lupus erythematosus are fulfilled (thereby confirming the diagnosis of acute lupus haemophagocytic syndrome) with the available first line investigations and important differential diagnoses such as sepsis have been excluded. Although it would have been ideal to have the above investigation results, we had to make do with what we had in our resource poor setting.

I have uploaded a table with the summary of the investigation results as an additional file.

3. It is also required to show the images of haemophagocytosis as well as that of immunofluorescence patterns of renal biopsy, otherwise we cannot see the process of diagnosis. Please add figures that represents your laboratory findings.

Response:

I have included 04 slides of the bone marrow biopsy showing haemophagocytosis along with the revised manuscript.
4. Negative finding of bone marrow cannot fully rule out malignant lymphoma (ML), which can also present positive auto-antibody. Did you examine monoclonality of immunoglobulin or genetic test for ML? If not, please explain how you made diagnosis of exclusion.

Response:

Monoclonal immunoglobulin band was excluded by serum electrophoresis which showed a polyclonal increase in gamma globulins, thus excluding the presence of a monoclonal band. I have already mentioned this in the case presentation segment. Absence of clinically detectable lymphadenopathy, a bone marrow not suggestive of lymphoma, normal chest X ray and abdominal ultrasound scan with no evidence of lymphadenopathy, absence of a monoclonal immunoglobulin band on serum electrophoresis make the diagnosis of malignant lymphoma highly unlikely. I have revised the manuscript to give this message in a clearer way.

5. It is not just the difference of diagnosis criteria that delayed the diagnosis of this case. It is really rare that a patient showed negative ANA at the time of first clinical symptom of SLE, because HLH usually occur due to hyper-cytokine status due to autoimmune reaction. What is your speculation of negativity of ANA on her first admission? Is there any other trigger for HLH, or you think HLH might have triggered production of autoantibody? You described some in the first paragraph of Discussion section, but there is no explanation about how you made exclusion diagnoses. I strongly recommend you to discuss the differential diagnoses of haemophagocytic syndrome first, then explain how you excluded each disease, and discuss why you think this is the manifestation of SLE in discussion.

Response:

I have given our hypothesis for this patient having negative ANA on presentation in the 7th and 9th paragraphs (in the revised manuscript) of the conclusions. The main reasons we have outlined are the rarer form of cytokine mediated pathogenesis of ALHS in this patient and early stage of autoimmune disease in the initial presentation.

Bone marrow aspiration was carried out in this patient due to the persistent and progressive pancytopenia. The diagnosis of HLH was rather a straightforward one after the bone marrow results which revealed haemophagocytic activity. The combination of suggestive bone marrow results, elevated serum triglycerides and ferritin, fever and pancytopenia completed the diagnostic criteria for HLH. I had initially not mentioned the serum triglyceride value in this patient by mistake and I included it as revision in the revised manuscript.

The main differential diagnoses as the underlying causes for HLH were autoimmunity, infection and malignancy. I have already discussed how we excluded infection as a cause and this discussion is given in the fourth paragraph of the conclusions. I have discussed above how we
excluded a diagnosis of malignant melanoma and have dedicated the 6th paragraph of the revised manuscript for exclusion of malignancy as an aetiology. I have discussed how we confirmed autoimmunity as the underlying cause of HLH in the 3rd, 4th, 7th, 8th and 9th paragraph in the conclusions.

I have added to the conclusions segment and have discussed these differentials in the revised manuscript.

Minor points

1. Human immunodeficiency virus also present nephritis and positive autoantibodies, which resemble type IV lupus nephritis. So I am interested how you ruled out HIV. What was the 'screen of hepatitis B, C, and HIV’? Did you measure HIV-antibody, or did you conduct Western blot? Please clarify it.

Response:

We checked hepatitis B surface antigen, hepatitis C IgM and IgG antibodies and HIV 1 and 2 antibodies in this patient. They were all negative.

2. Also, ruling out infection is important in such a case, because hemophagocytic syndrome due to viral infection may also show temporal remission by treatment with corticosteroid. Which kind of EBV antibody did you measure? Did you measure serum EB-DNA? Also, which antibody did you measure to exclude CMV infection? And usually, CMV antigenemia needs to be measured to exclude CMV infection. Please clarify the methods you used to exclude these infections.

Response:

Ig M antibody to EBV Viral capsid antigen and Ig M antibody to EBV nuclear antigen was tested and both were negative. CMV Ig M antibody was done and it was also negative. We did not do CMV antigen levels as it is not available for us.

3. The patient presented alopecia on first admission. Did you conduct skin biopsy? If not, do you think this is dermatological symptom of SLE? If it is so, there must be sign of inflammation, such as rash. If not, what is your diagnosis about this alopecia?

Response:
The patient presented with non-scarring alopecia which is well recognized in SLE. We did not do a skin biopsy. The patient did not have a rash. Alopecia without a rash can occur in SLE. As non-scarring alopecia is included in the 2012 SLICC diagnostic criteria for SLE, we believe that it is fair to assume that the alopecia in this patient is due to SLE itself.

4. Also, her first symptom was confusional episodes. Do you think this is the symptom of NP-SLE? What is your diagnosis? Please explain.

Response:

The patient was not confused during the hospital stay. She had had confusional episodes while at home. The confusional episodes are probably neuro psychiatric manifestations of SLE. Acute confusional states have been recognized as a neuro-psychiatric manifestation of SLE by ACR. Most other metabolic and infective causes were excluded during investigations.

5. Page 4, paragraph 3: Please add the unit of platelet counts (/mm3)

Response:

Correction made.

6. Page 4, paragraph 3: please spell out ABST and IV.

Response:

Correction made.

7. In the abstract you described 'coombs test', but in main manuscript you mentioned 'direct antiglobulin test'. It might be better the description is consistent.

Response:

Correction made. Both termed as direct antiglobulin test.
Reviewer #2:

1. ALL definitions should be defined at first use.
   Response:
   Correction made.

2. The authors should avoid using abbreviations in the abstract as much as possible.
   Response:
   Revision done so as to minimize the use of abbreviations.

3. Background section of main text
   a) P1,L4: Avoid starting a sentence with an abbreviation, please correct through.
   Response:
   Correction made.

   b) P3,L2: Delete the comma before 'liver function abnormalities'
   Response:
   Correction made.

   c) P3,L3: Do well to list the clinical manifestations of HLH before the paraclinicals. Hence, let 'weight loss' come before 'liver function abnormality'
   Response:
   Correction made.

4) Case presentation section of the main text
   a) P1,L2: Replace "history of on and off fever of three months with loss of appetite and loss of weight..." with "three months history of intermittent fever, anorexia and weight loss"
Response:

Revision made.

b) P1, L3: Replace "Initially the fever had been there for around two days every week but it had progressively worsened and by the time of admission, she had daily fever spikes." with "A worsening of the fever characterized by daily episodes with spikes prompted admission."

Response:

Revision made.

c) P1, L13-18: should read "She complained of significant hair loss and on and off confusional episodes over the last two weeks, but denied symptoms of focal infections including respiratory symptoms. Also, she disaffirmed any history of arthritis and features of autoimmune diseases such as photosensitive rashes, oral ulcers or symptoms of Raynaud's phenomenon."

Response:

Revision made.

d) P1, L9: Please give the patient's vital parameters.

Response:

Revision made.

e) P2L1: Did you have a tentative diagnosis and differentials before requesting for paraclinical exams? If yes, please list them.

List of differential diagnoses added in to the 02nd paragraph of the case presentation.

f) P3, L3: What do you mean by "infective screen"? FBC, ESR and CRP also screen for infections. Also, you said she denied any respiratory symptom. That means cough included. How did you obtain sputum sample for culture?
I have meant the cultures (blood, urine, sputum) by the term “infective screen”. In the revision I have omitted the term.

The patient did not have cough. Thus the patient’s “sputum” sample probably was a poor quality one consisting of saliva and upper respiratory tract secretions. This may have contributed to the sputum yielding a growth of coliforms as these organisms are known to colonize the upper respiratory tracts of hospitalized patients, especially in ones receiving long term antibiotics. I have discussed this point in the 5th paragraph of the discussion.

g) P3, L4: Delete "... and there was no sign of infection". If the Chest X-ray was normal, then automatically, there was no sign of infection on chest x-ray.

Response: Revision made.

h) P4,L1: Dimensional not dimensiona. Correct accordingly.

Response: Revision made.

i) P6,L1: Negative not negaive. Please correct that.

Response: Revision made.

j) P7, L3: At what frequency did she receive prednisolone? Aside prednisolone, did she receive any other treatment worth mentioning?

She received prednisolone 60 mg daily and it was tapered off. She also received CaCO3 500 mg + vitamin D 400 IU tablets twice daily for protection against steroid induced osteoporosis.

k) P8, L1: Please endeavour to write numbers less than 10 in words.

Response:
Revision made.

l) P8, L2: Replace "and readmitted" with "which motivated a second admission."

Response:
Revision made.

m) P9: Was there any follow up visits after the treatment with oral prednisolone and mycophenolate mofetil?

Response:
On follow up she was clinically improved and remained asymptomatic while on immunosuppressive treatment. The prednisolone dose was gradually reduced and maintained at a dose of 7.5 mg daily. Mycophenolate Mofetil was continued. I have mentioned this in the revised manuscript in the 9th paragraph of the discussion.

5) Conclusion section of the main text

a) You are discussing and not concluding. Please separate the discussion from the conclusion section.

Response:
Revision made.

b) P5, L4: Correct "has" to have.

Response:
Revision made.