Author’s response to reviews

Title: Miliary tuberculosis with co-existing pulmonary cryptococcosis in non-HIV patient without underlying diseases: a case report

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Author’s response to reviews:

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Prof. T. Hassan

BMC Pulmonary Medicine

BioMed Central

Re:PULM-D-17-00339, “Miliary tuberculosis with co-existing pulmonary cryptococcosis in an immunocompetent patient: a case report” by Sawai et al.,
Dear Prof. Hassan

Thank you very much for your e-mail of November 29, 2017. We were pleased to know of your positive evaluation of the our manuscript and its potential acceptance for publication in BMC Pulmonary Medicine, subject to adequate revision raised by the referees.

Enclosed please find our revised manuscript with a figure and table.

According to the comments raised by the referees, we made some corrections. Enclosed please find our point-by-point response to the referees. We would like to take this opportunity to express our gratitude to the referee for their constructive and useful remarks. Their comments allowed us to identify areas in our manuscript that needed modification and clarification.

We would like also to thank you for giving us the opportunity to resubmit a revised version of the manuscript. We hope that the revised manuscript is now acceptable for publication in BMC Pulmonary Medicine.

Sincerely Yours,

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Encl: Point-by-point response to the comments of the reviewers.

Re: PULM-D-17-00339, “Miliary tuberculosis with co-existing pulmonary cryptococcosis in an immunocompetent patient: a case report” by Sawai et al.,

Response to comments raised by Dr. Denise Silva (REVIEWER #1):

Thank you for your helpful comments.

Discussion section

The authors should consider the possibility that the patient had only one disease, pulmonary cryptococcosis. Pulmonary cryptococcosis also causes granulomatous inflammation and chest CT can also show military pattern in these cases.

Page 10.

Line 7~8. We added the sentence as below.

Although both miliary tuberculosis and disseminated cryptococcosis present diffuse micronodules in random patterns in chest CT images,

Case presentation section and Abstract section

The positive TB PCR may indicate the presence of non-viable AFB.

As you pointed out, the positive Ziehl Neelsen stain and TB PCR may indicate the presence of non-viable AFB. However, microbiological, radiological and pathological findings can not deny miliary tuberculosis. Accordingly, antimycobacterial therapy for presumed miliary tuberculosis was initiated.

Page 7.

Line 15~17. We changed the sentence as below.

According to these findings, miliary tuberculosis was diagnosed...
→Although these microbiological findings might indicate the presence of non-viable M. tuberculosis, miliary tuberculosis was suspected...

Page 2.

Line 14. We changed the term as below.
diagnosed→suspected

Response to comments raised by Dr. Onyema Ogbuagu (REVIEWER #2):

Thank you for reviewing our manuscript. We appreciate your very kind comments concerning our submitted manuscript.

Abstract section

Page 2.

Line 7. We corrected the term as below.
patient→patient

Page 2.

Line 9. We changed the term as below.
oriental→Asian

Background section

As an example of concurrent Cryptococcal disease and disseminated/military tuberculosis in an immunocompetent host, yours is not the first case.
Most reported cases with tuberculosis and cryptococcosis co-infection involved the lung and central nervous system, respectively (Table 1). The case report which you referred to (http://casereports.bmj.com/content/2016/bcr-2015-213380.abstract), describes co-infection of cryptococcal meningitis and disseminated tuberculosis. Our case report describes co-occurrence of pulmonary cryptococcosis and disseminated tuberculosis. However, the case report is a case of concurrent infection of tuberculosis and cryptococcosis in immunocompetent patient. Accordingly, we added the case report to Table 1, and added reference 12 in our revised reference section.

Isn't being elderly an immunocompromized state?

As you pointed out, being elderly is an immunocompromised state. Therefore, we changed the title as below. In addition, we replaced the term “in an immunocompetent patient” with “in non-HIV patient without underlying diseases” in all sections.

“Miliary tuberculosis with co-existing pulmonary cryptococcosis in an immunocompetent patient: a case report”

→“Miliary tuberculosis with co-existing pulmonary cryptococcosis in non-HIV patient without underlying diseases: a case report”

Case presentation section

Mention pertinent negatives-curious about presence or absence of fevers, weight loss, night sweats.

Page 6.

Line 5~6. We added the sentence as below.

The patient had no history of cough, sputum, fever, chills, weight loss, or night sweats.

Is it possible that she only had 1 week of symptoms?

→Yes, she did not have the awareness of chronic inflammatory symptoms or other symptoms more than 1 week.
Would state that she had “no KNOWN history of exposure to tuberculosis” – you cannot be overconfident about lack of exposure to tuberculosis as patients may not recall or know this definitively.

→She did not remember contact with tuberculosis patients. However, as you point out, I think that she might be exposed to tuberculosis patients. Because tuberculosis was prevalent in Japan when she was young.

How can the physical exam, including the abdominal exam, be unremarkable in a patient who complained of abdominal pain?

→Although she complained of abdominal pain, abdominal examination was unremarkable.

Page 6.

Line 10~11. We changed the sentence as below.

The physical was unremarkable.

→Respiratory, cardiac and abdominal examination were unremarkable.

Would avoid the term “shadows” when describing imaging findings, can use the term “infiltrates or opacities”, is it accurate that both chest X-ray and chest CT showed similar findings?

Page 6.

Line 11~13. As you point out, we changed the sentence as below.

Chest radiograph and chest computed tomography (CT) showed diffuse micronodular shadows in random patterns in both lung fields,

→Chest radiograph showed multiple small nodules in both lung fields and chest computed tomography (CT) showed diffuse micronodules in random patterns in both lung lobes,

Page 8.

Line 2~3.

Although INH and RFP therapy was continued, chest radiograph showed new multiple nodular shadows in the right middle lung field.
Although INH and RFP therapy was continued, chest radiograph showed new multiple nodules in the right middle lung field.

In addition, we deleted the term “shadow” in all sections.

Why was an IL-2 soluble receptor level checked- you do not mention that she had pancytopenia which would prompt concern for macrophage activation syndrome? What was the hemoglobin level and platelet.

We checked IL-2R tumor marker, because abdominal CT showed mild mucosal wall thickness on the stomach. But, abdominal CT imaging showed no abnormalities in the radiology report.

And, Hemoglobin and platelet levels were 13.1 g/dl, 22.4×10⁴ /μl, respectively. We think that leukocytopenia was caused by a serious infectious disease, because white blood cell count normalized after a 2-month treatment course.

I am surprised with her leukopenia that CD4 and CD8 counts were normal?

Page 7.

Line 1~3. We changed the sentence as below.

CD4 and CD8 lymphocyte counts were within normal ranges

→CD4/8 ratio, CD4 count and CD8 count were 1.73 (normal range 0.6-2.9), 422 /μl (normal range 344-1289) and 244 /μl (normal range 110-1066), respectively.

What were her liver function tests? You state that a biopsy showed granulomas suggesting liver involvement?

→AST and ALT were elevated 260 U/l and 117 U/l, respectively. Liver, skin and bone marrow biopsies showed epithelioid cell granuloma without caseous necrosis. We think that her liver dysfunction was caused by liver involvement of tuberculosis.
Why was a gastric aspirate performed to diagnose tuberculosis in an adult who was able to expectorate?

→Because she had no history of cough and sputum, it was difficult to obtain good quality sputum.

Why were so many biopsies performed including skin? what prompted it? Were there skin lesions- you say exam was totally unremarkable???

→According to high IL-2R and absence of lymphadenopathy, we initially suspected malignant lymphoma including intravascular lymphoma (Asian-variant intravascular lymphoma). Therefore, we performed many biopsies including at random skin biopsy. Physical examination including skin were unremarkable.

TB usually presents with caseous necrosis, all your biopsy specimens showed non caseating granulomas- could these be due to Cryptococcus rather than TB.

→As you point out, we think that noncaseating granuloma is encountered in the foci of cryptococcosis rather than tuberculosis. However, gastric aspirate culture was positive for M. tuberculosis after 1 week of culture (MGIT), and diffuse micronodules almost decreased following antimycobacterial treatment. Therefore, we think that noncaseating granuloma may be encountered as the foci of tuberculosis.

You mention a positive PCR test for TB, was this GeneXpert test, was there any genotypic resistance reported?

Page 7.

Line 14~15. We performed a PCR test for TB by using the LAMP assay. We added the term as below.

→(Loopamp; Eiken Chemical Co., Ltd. Tokyo, Japan)
Why was a three and not four drug regimen initiated in this patient (seems pyrazinamide was omitted).

→ We treated our patient with three drugs, because she was old, and her liver function deteriorated.

Page 7, line 15- should be "...had increased..." not "was increased"

Page 8.
Line 5. As you point out, we changed the term as below.
...was increased and new multiple nodules...
→..., had increased and new multiple nodules...

Evidence for cryptococcal disease is still weak- PAS stain is not specific to Cryptococcus (other fungi take up the stain) and a positive CrAG test while signifying exposure to Cryptococcus is not definitive evidence of disease. If organisms were present in tissue, why didn't it grow in culture (Cryptococcus is not a difficult organism to grow if present)? Moreover BAL studies were negative.

→ As you point out, we think that evidence for cryptococcal disease is weak. Unfortunately, we could not detect Cryptococcus in transbronchial lung biopsy specomens, and culture of the bronchial lavage fluid was negative. However, the diagnosis of cryptocpccosis was made because of a positive cryptococcal antigen test, Periodic acid-Schiff-positive bodies in cytology specimens and treatment effect of FLCZ. Occasionally, we experience that Cryptococcus is not isolated although histpathology show Cryptococcus-like bodies. However, the cause is not clear. In our laboratory, microorganisms in the bronchial lavage fluid were harvested by centrifugation, the sediment was cultured on a YPD agar and a Bird seed agar.

Page 10, line 7 - what do you mean by "blood diseases and kidney diseases?"

Page 9.
Line 5. We changed the term as below.
...kidney diseases, blood diseases...
Page 11, I would be cautious with attributing a focus of infection arbitrarily to a single nodule, if solitary nodules are rare as you say for pulmonary cryptococcosis, why are you stating confidently that it represent the disease focus

→We think that it is difficult to diagnose pulmonary cryptococcosis presenting with nodules less than 5 mm on chest CT. Because there was an inappropriate sentence, we deleted the sentence as below.

Page 10.

Line 16-18.

Generally, patients with pulmonary cryptococcosis presenting with a small nodule (approximately 5 mm in diameter) on chest CT are relative rare.

It is speculative to state that a cryptococcal antigen test (which wasn't checked) would have been negative on admission. Why advocate for Quantiferon test for diagnosing active TB? works best to define latent disease

→Because tuberculosis was prevalent in Japan when she was young, it is difficult to distinguish between active TB and old TB by using QuantiFERON test.

Response to comments raised by Dr. Ferry Hagen (REVIEWER #3):

Thank you for reviewing our manuscript and your helpful comments.

The main question here is "what is immunocompetent"? The patient is an apparently healthy 84-year old subject who seems to have developed 'spontaneously' tuberculosis followed by cryptococcosis.

→As you pointed out, being elderly is an immunocompromised state. Therefore, we changed the title as below. In addition, we replaced the term “in an immunocompetent patient” with ”in non-HIV patient without underlying diseases” in all sections.
“Miliary tuberculosis with co-existing pulmonary cryptococcosis in an immunocompetent patient: a case report”

→“Miliary tuberculosis with co-existing pulmonary cryptococcosis in non-HIV patient without underlying diseases: a case report”

Some immunological markers were tested, among them are IL2, CD4 and CD8, but can be stated from that the patient was immunocompetent? At least, in my opinion, we need to speak about an apparently immunocompetent subject.

→CD4/8 ratio, CD4 count and CD8 count were within normal range. Although sIL-2R was elevated, she did not have lymphoma or malignancies. And, sIL-2R level gradually decreased under antimycobacterial treatment.

A wide range of tests were performed, although the case report itself is informative, the background information about the specific tests is missing: what are the normal ranges for the given clinical chemistry markers? (and the observed values?)

Page 6.
Line 15~19.
We added the normal ranges of clinical chemistry markers (CRP, PCT, CEA, CA19-9 and sIL-2R).

what are the normal ranges for the CD4 and CD8 lymphocyte counts? (and the observed values?)

Page 7.
Line 1~3. We changed the sentence as below.
CD4 and CD8 lymphocyte counts were within normal ranges

→CD4/8 ratio, CD4 count and CD8 count were 1.73 (normal range 0.6-2.9), 422 /μl (normal range 344-1289) and 244 /μl (normal range 110-1066), respectively.
Antigen testing was performed for Streptococcus pneumoniae and Legionella pneumophila, but which tests were used for that?

Page 7.

Line 7. We added the term as below.

→(Binax NOW; Binax, Inc., Portland, ME)

"polymerase chain reaction was positive for Mycobacterium tuberculosis", which assay was used (commercial, in-house?)?

→We performed a PCR test for TB by using the LAMP assay (Loopamp; Eiken Chemical Co., Ltd. Tokyo, Japan) in our laboratory.

Page 7.

Line 14~15. We added the term as below.

→(Loopamp; Eiken Chemical Co., Ltd. Tokyo, Japan)

"Microbiological testing" was performed, what kind of tests?

→We performed bacterial, mycobacterial and fungal cultures.

The cryptococcal antigen testing was positive, which (commercial) test was used? It is important to know, as the diagnosis of cryptococcal infection was made based on the outcome of this test (maybe a less well-performing test was used that has cross-reactivity with other fungi/yeasts).

→We performed a cryptococcal antigen test by using the Serodirect “EIKEN” Cryptococcus (Eiken Chemical Co., Ltd. Tokyo, Japan) in our laboratory.

Page 8.

Line 12. We added the term as below.

→(Serodirect “EIKEN” Cryptococcus; Eiken Chemical Co., Ltd. Tokyo, Japan)
In the Discussion section "cryptococcosis is caused by Cryptococcus neoformans", indeed this is the major cause of cryptococcosis, but there are at least 7 species within the C. neoformans/C. gattii species complexes... and they all cause cryptococcosis.

→As you pointed out, Cryptococcus species except C. neoformans cause cryptococcosis.

Page 9.

Line 3~4. Therefore, we changed the sentence as below.

Both tuberculosis (caused by Mycobacterium tuberculosis) and cryptococcosis (caused by Cryptococcus neoformans) have a wide range of clinical presentations,

→Both tuberculosis and cryptococcosis have a wide range of clinical presentations,

"we found no evidence of immunodeficiency in our patient", can that be reliably concluded based on three immunological markers?

→Our patient did not have a history of malignancy, diabetes mellitus, cytotoxic therapy or corticosteroid use. CD4/8 ratio, CD4 and CD8 lymphocyte counts were within normal ranges. Testing for human immunodeficiency virus infection was negative. According to these results, we considered that our patient did not have evidence of immunodeficiency.

Page 7.

Line 3~5. We added the sentence as below.

→IgG, IgA and IgM were 998 mg/dl (normal range 870-1700), 128 mg/dl (normal range 110-410), 59 mg/dl (normal range 46-260), respectively.

The sentence thereafter "Therefore, our case appears to be the first English article immune competent patient" is a statement that cannot be made. Better to put it like "In conclusion, the described patient was diagnosed with miliary tuberculosis with a co-existing pulmonary cryptococcal infection".

Page 10.

Line 1~4. As you pointed out, we changed the sentence as below.
Therefore, our case appears to be the first English article about miliary tuberculosis with co-existing pulmonary cryptococcosis in an immunocompetent patient.

→In conclusion, the described patient was diagnosed with miliary tuberculosis with a co-existing pulmonary cryptococcal infection.

How sure are you that the culprit of disease was Cryptococcus neoformans? Was there any molecular identification performed (not mentioned)? It might be very well that one of the members within the Cryptococcus gattii species complex was the cause of cryptococcal infection.

→As you pointed out, causative Cryptococcus species was not identified, because Cryptococcus species was not isolated. Although Cryptococcal gattii infection are recognized as an imported infectious disease, only several cases have been reported in Japan since 2001. Accordingly, there is no commercial laboratory test to distinguish between C. neoformans and C. gattii in Japan. And, C. gattii infections may have been misdiagnosed as C. neoformans.

"The results of all immunological markers" > provide the immunological markers instead of "all"

Page 11.

Line 12~13. As you pointed out, we changed the sentence as below.
the results of all immunological studies in the present case were normal.

→the results of CD4 and CD8 counts and immunoglobulins in the present case were normal.

Replace "we decribed the first" by "we described a"

Page 13.

Line 1. We changed the sentence as below.
...we described the first case of miliary tuberculosis with co-existing pulmonary cryptococcosis...

→...we described a case of miliary tuberculosis with co-existing pulmonary cryptococcosis...

Provide a list with the used abbreviations in Table 1 and what they mean.
As per your suggestion, we added a list of abbreviations in Table 1.

In the abstract correct "patinet" into "patients"

Page 2.

Line 7. We corrected the term as below.

patinet→patient