Response to reviewers

Dear Editor:

Thank you very much for your letter and the comments about our paper submitted to World Journal of Clinical Cases, (Manuscript No: 68125). The manuscript entitled " Next-generation sequencing technology for diagnosis and efficacy evaluation of a patient with visceral leishmaniasis: A case report" has been revised according to the comments with all the edits highlighted, and we wish it to be reconsidered for publication in World Journal of Clinical Cases.

Response to reviewer 1#**:**

Thank you very much for the comments on the paper and pointing out the problems existing in our manuscript. We have revised it according to your recommendations. We would like to know if there are still somewhere need to be amended.

A list of changes and responses to your review report are listed below.

1. *It is suggested that the line number be inserted in the manuscript .*

   We inserted the line number in the manuscript， but we found the web generate full text automatically, so we can't upload the manuscript with traces of modification.

2. *Background "The incidence of VL is low in China, the clinical manifestations are complex and atypical, and the condition, which can be life-threatening, is easy to misdiagnose. Therefore, it is essential to diagnose and treat the disease quickly and accurately at an early stage. " should be rephrased as "VL has a low incidence in China, and its clinical presentation is complex and atypical. This disease is easily misdiagnosed and can be life-threatening in a short period of time. Therefore, early rapid and accurate diagnosis and treatment of the disease is essential. "*

   We have revised the sentence in the background (Line 4-7/Page 3).

3. *Core Tip:"The protozoan was finally diagnosed as Leishmania after follow-up and metagenomic next-generation sequencing (mNGS) testing." should be rephrased as "Leishmania was finally confirmed by metagenomic next-generation sequencing (mNGS) analysis of cerebrospinal fluid."*

   The mNGS analysis is from peripheral blood ,and We have revised the sentence in the core tip (Line8-11/Page 4) .

4. *Chief complaints :"A 25-year-old man was admitted to Ningbo First Hospital in Ningbo, China, with the diagnoses of pancytopenia and hepatosplenomegaly,*

lasting for 5 mo？. ” Is there some problem with the sentence? Is the expression of the month irregular?

We have revised the chief complaints(Line 4/Page 5) .As for the expression of the month, we wanted to revise it, but then we found out that the article could only be revised twice, so we didn't have a chance to revise it. But we found the expression is used in other artlcles which published in WJCC.

5. *History of present illness: “The patient did not have fever and reported having no other discomfort after discharge from the hospital. “should be rephrased as “The patient had no fever and reported no other discomfort after discharge from the hospital.”*

   We have revised the sentence in the history of present illness (Line 16-17/Page5).

6. *Final diagnosis:“Therefore, the patient was diagnosed as having VL according to the suspicious epidemiological history, clinical manifestations, and laboratory examination results. “ should be rephrased as “Therefore, the patient was diagnosed as VL according to the suspicious epidemiological history, clinical manifestations, and laboratory examination results. “ But epidemiological history had not been provided in medical history.*

   We have revised the sentence in the final diagnosis (Line 26-28/Page 7)and have added epidemiological history in personal and family history(Line 22-26/Page5) .

7. *More details are needed, such as sequencing depth, Gb per run of this sample to obtain enough data for downstream analysis, and database used for mapping the sequence.*

   The sequencing depth is 20X, to obtain enough data for downstream analysis needed about 1.5Gb of this sample, and the number of map reads are 50-75bp.The database can cover 9945 species of bacteria, 6760 species of viruses, 1551 species of fungi and 305 species of parasites. We have added in the final diagnosis (Line 22-26/Page7).

8. *To mention the limitations of NGS (based on literature reports).*

   We have added the limitations of NGS in the discussion (Line 3-5/Page 11).

Response to reviewer 2

Thank you very much for the reviewer's comments on the paper and pointing out

the problems existing in our paper. We have revised it one by one according to your valuable advice. We would like to know if there are still somewhere need to be amended.

1. *"On October 12, 2019," I suggest not putting dates in the article.*

   According to your suggestion, we have deleted dates in full text.

2. *The presentation of the case is very poor. Some data I could find in the discussion, but this has to be made clear in the case presentation. I suggest putting information that can provide tips related to the disease, such as Has the patient traveled to endemic areas? How long after did the symptoms start? Why did the patient come to his hospital? What was the patient working with? A rich epidemiological description will make a difference.*

   we have added relevant information in the Personal and family history."The patient was born in Gansu Longnan ,which is one of the endemic area of VL in China .He lived there for 18 years. But now, he has been in Ningbo for 6 years and works in a factory. The patient denies exposure to radioactive and toxic substances. His family had no similar medical history. The patient came to his hospital because he presented with pancytopenia and hepatosplenomegaly for 5 months." (Line 22-26/Page5)

3. *Explain in the text why it was not done since the likely epidemiological history + a patient with fever and splenomegaly make us think of visceral leishmaniasis.*

   The patient once lived in an endemic area, but now lives in a non-endemic area. And he attended to the hematology department due to the clinical manifestations of pancytopenia and hepatosplenomegaly. the amount of parasites in the sample and the experience of pathologists can affect sensitivity. Clinicians and pathologists lacked knowledge of Leishmania, which led to the lack of consideration of Leishmaniasis. We have revised in the discussion (Line 6-10/Page 9).

4. *Didn't amastigote forms of the parasite have been found in your pathology laboratory? Was this a mistake or was there any other technique used in Shanghai?*

This was a mistake, because clinicians and pathologists lacked knowledge of Leishmania in non-endemic areas. Pathologists in Shanghai may experience more difficult medical records ,there wasn't any other technique used in Shanghai. We have revised in the discussion . (Line 6-10/Page 9).

5. *"A retrospective review of VL cases in the United Kingdom found that the median time from first symptom to diagnosis was 6 mo[" Instead of looking for references in non-endemic areas, look for data in endemic regions that have much more experience on the subject. I suggest looking for data from Brazil.*

   We have added the data from Brazil and compared with the data in non-endemic area in the discussion (27-1/8-9).

6. *Figure 3: has writings in Chinese.*

   We have corrected the problem in figure 3.

7. *For the figures and tables, I suggest that you remove the date and put it in the number of days of illness or hospitalization.*

   We have removed the date and put it in the number of days of illness in table 1 and the number of days of hospitalization in figure 4.

8. *Would it be possible to include a photo of the patient's CT scan?*

   We have added the CT scan in figure 1.

Finally, thank you very much for your reconsidering our revised manuscript for potential publication in World Journal of Clinical Cases. I'm looking forward to hearing from you soon.

Sincerely yours,

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