Dear Editors:

Thank you for your comments concerning our manuscript entitled "Next-generation sequencing technology for the diagnosis of *Pneumocystis* pneumonia in an immunocompetent female: A case report" (84892). Those comments are all valuable and very helpful for revising and improving our paper. We have read the comments carefully and made point to point revises. We hope this article can be accepted for publication after the corrections. The responds to the reviewer's comments are given in the blue text as flowing:

Reviewer 1#

1. The review's comment: " However, it is not clear is this female patient had HIV infection."

The authors' answer: Thank you for clearly pointing this out. This female patient had no human immunodeficiency virus (HIV) infection. No personal or family history of infectious disease, genetic disease, or other particular disease was noted in this case. The initial screening test result for HIV antigens/antibodies of this patient was negative during hospitalization. According to your suggestion, we have supplemented the above contents in the revised manuscript and shown them in bold and underlined on page 4, lines 24 to 25 and page 5, lines 11 to 12.

2. The review's comment: "There is little description about the process of NGS in material and method, and the data about NGS is too simple to make the connection between the gene detection and other test parameters."

The authors' answer: We sincerely appreciate the valuable comment. This female patient was admitted due to fever and cough, and rapidly developed acute respiratory failure upon admission. Based on the positive results of the (1-3)-beta-D-glucan test (G test), increased inflammatory indicators, and elevated lactate dehydrogenase (LDH) levels, we preliminarily considered that the patient might have fungal infection. However, we could not determine the cause of respiratory failure and the specific source of infection with routine testing. Treatment with broad-spectrum antibiotics and

ventilators could not prevent disease progression. To diagnose and save the patient more quickly and accurately, we performed next-generation sequencing (NGS) of bronchoalveolar lavage fluid (BALF), which offers greater sensitivity and specificity than traditional laboratory techniques in clarifying the etiology. NGS used the PMseq pathogen high-throughput sequencing platform MGISEQ-2000 for metagenomic sequencing of RNA pathogenic microorganisms at Complete Genomics (BGI, Shenzhen, China). Based on your suggestions, we added the above contents to the revised manuscript and shown them in bold and underlined on page 5, lines 14 to 25.

3. The review's comment: "I suggest to have a section of result to fully state the result of NGS, and how it can help the diagnosis of PCP. It looks like the descriptino of NGS was only in the Discussion section."

The authors' answer: Thank you very much for your valuable feedback; it's a good suggestion for us. Based on your suggestion, we replenished the NGS results and their significance in bold and underlining on page 5, lines 14 to 27 and page 6, lines 4 to 7. In this case, the patient developed bilateral interstitial pneumonia and rapidly aggravated respiratory failure. In addition to a positive G test, increased inflammatory indicators and elevated levels of LDH, we did not obtain more effective laboratory positive test results. The pathogen causing pulmonary infection and respiratory failure has not been found, resulting in poor effects of antibiotics and respiratory support treatments. The results of BALF-NGS identified one fungus, *Pneumocystis* jirovecii (Table 3), with a sequencing depth of 31 M. The number of sequences was 12152. Based on the patient's clinical manifestations, imaging, and laboratory results, we determined that *Pneumocystis* jirovecii was the causative pathogenic microorganism and diagnosed the patient with *Pneumocystis* pneumonia (PCP).

Reviewer 2#

1. The review's comment: "It would be interesting to understand the rationale

behind the suspicion of Pneumocystis infection and how the choice to perform an NGS analysis came about."

The authors' answer: Thank you for reading our article carefully. PCP in people with normal immune function is rare and difficult to clearly diagnose by permanent routine methods. In this case, this female patient was admitted due to fever and cough, and rapidly developed acute respiratory failure upon admission. Based on the positive results of the G-test, increased inflammatory indicators and elevated levels of LDH, we preliminarily considered that the patient might have fungal infection. We did not consider PCP infection at the beginning because the patient was immunocompetent. However, because the pathogen causing pulmonary infection and acute respiratory failure had not been found, treatment with broad-spectrum antibiotics and ventilators could not prevent disease progression. To diagnose and save the patient more quickly and accurately, we performed BALF-NGS. BALF-NGS has emerged as a new diagnostic technique that is used in clinical settings for its high sensitivity and specificity in recent years, particularly for the detection of special pathogen infections and mixed infections in immunosuppressed and immunocompetent patients. The results of BALF-NGS identified one fungus, *Pneumocystis jirovecii*, which was confirmed to be the a pathogenic microorganism. After been diagnosed of PCP and given anti-PCP treatment, the patient recovered and had a good prognosis.

(The modified parts could be found on page 5, lines 14 to 27 and page 9, lines 15 to 17 of the revised manuscript and were indicated in bold and underlined.)

2. The review's comment: "The authors should also specify the kit and instrument used to perform the NGS analysis. The Kit included, for instance, a multi-target plate including many pathogen microorganisms; nevertheless, was a Pneumocystis-specific analysis performed?"

The authors' answer: We sincerely thank you for bringing up this point. Based on your suggestion, we have supplemented the contents on page 5,

lines 17 to 27, page 6, lines 4 to 6 and page 9, lines 18 to 19 of the revised manuscript and indicated them in bold and underlined. In this case, we could not determine the cause of respiratory failure and the specific source of infection with routine testing. Treatment with broad-spectrum antibiotics and ventilators could not prevent disease progression. To diagnose and save the patient more quickly and accurately, we conducted bronchoscopy examination and collected BALF specimens for NGS with the PMseq pathogen high-throughput sequencing platform MGISEQ-2000 for metagenomic sequencing of RNA pathogenic microorganisms at Complete Genomics (BGI, Shenzhen, China). With a sequencing depth of 31 M, BALF-NGS identified one fungus, *Pneumocystis jirovecii*. The number of sequences was 12152. According to the patient's clinical manifestations, imaging, and laboratory results, we determined that *Pneumocystis jirovecii* was the causative pathogenic microorganism. No pneumocystis-specific analysis was performed for our case, which might be a limitation.

We tried our best to improve the manuscript by making some changes, which were shown in bold and underlined in the revised manuscript.

We appreciate the reviewers' earnest work , and hope that the corrections will meet with approval. Once again, thank you very much for your comments and suggestions.

Yours sincerely,

Jing Wu

Intensive Care Unit, Guizhou Provincial People's Hospital, No. 83 Zhongshan East Road, Guiyang 550000, Guizhou Province, China

Telephone: +86-851-13984376424

E-mail: saidiny1120@qq.com