

Dear Editor and reviewers

Thank you for your comments. They made our article brighter!

Below you can find our revisions

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors:

1. The imaging findings of pulmonary cryptococcosis are also very important. It is suggested whether to explain it in your journal.

AUTHORS REPLY: We added this paragraph:

According to several studies, pulmonary nodules are the most prevalent CT findings of pulmonary cryptococcosis in immunocompetent hosts, with multiple nodules being more common than solitary lesions. The majority of them are poorly defined and inhomogeneous, with air-bubble signs seen. Consolidation, ground glass opacities, and masses are also described. The halo, air bronchogram, and cavity signs can also be seen. In these individuals, the pulmonary lesions are mostly seen in the lower lung lobes and the lung periphery. In immunocompromised patients, the most common imaging findings are multiple nodules, which are usually larger than in normal hosts, pulmonary cavitations, and single or multiple consolidations. Adenopathy and pleural effusions, which are sometimes small and unilateral, are usually observed in cases of extensive lung infection.

2 "Case report of Wu et al., who described a 29 year old male case". It is suggested to describe the image and diagnosis of pleura in this case.

AUTHORS REPLY: We added the imaging findings in the first paragraph:

Chest imaging showed scattered multiple cavities in the patient's superior segment of the left lower lobe with a rough cavity wall and cavity and pleural effusion in the patient's anterior basilar segment of the left lower lobe.

Regarding the diagnosis of the pleura, it is already mentioned that neither pleural aspiration nor pleural biopsy was reported in this case.

However, the diagnosis of cryptococcal pleural effusion in the case by Wu et al^[1] was made by positive serum CrAg, positive Indian ink staining of bronchoalveolar lavage fluid and positive PAS staining for Cryptococcus of lung tissue obtained by percutaneous lung biopsy, while neither pleural aspiration nor pleural biopsy was reported^[1].

3. In recent years, scholars have applied molecular biology technology to study the latest achievements of *Cryptococcus neoformans*, including GC content determination; DNA homology hybridization; Restriction fragment length polymorphism of mitochondrial DNA; Chromosome pulse electrophoresis karyotype analysis; The cloning of *ura5* gene, *TRP1* gene and *rDNA* is helpful to study the taxonomic status, phylogenetic origin and epidemiological investigation of *Cryptococcus neoformans*. The application of specific probes can be used for early diagnosis of cryptococcosis *neoformans*. It is suggested to describe the value of molecular biology in identifying *Cryptococcus*.

AUTHORS REPLY: We added this paragraph

*In recent years, molecular identification and strain typing methods have been used to analyze *Cryptococcus*. The identification methods include DNA-DNA hybridization, nested, multiplex and real-time PCR. Regarding *Cryptococcus* typing, the following techniques have demonstrated the*

best ability to differentiate between fungal serotypes and molecular types: serotyping, random amplified polymorphic DNA, multilocus enzyme electrophoresis, restriction fragment length polymorphism, electrophoretic karyotyping, PCR-fingerprinting, amplified fragment length polymorphism, multilocus microsatellite typing, single locus and multilocus sequence typing, matrix-assisted laser desorption/ionization time of flight mass spectrometry, and whole genome sequencing. These typing methods have contributed in revealing the phylogenetic pattern, the origin of numerous lineages and their scattering patterns, the distribution of genetic variation among geographic regions and ecosystems, and precise mutations during infections. In addition, the cloning of URA5 gene, TRP1 gene and rDNA is helpful to study the taxonomic status, phylogenetic origin and epidemiological investigation of Cryptococcus neoformans.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

I am honored to review this manuscript. MAJOR COMMENTS

1. Which species of Cryptococcus referred to? Cryptococcus neoformans or Cryptococcus gatti, although most clinical laboratories will not routinely identify cryptococcus to the species level.

AUTHORS REPLY: Thank you for this comment. We actually refer to Cryptococcus neoformans as Cryptococcus gatti has not been reported as a cause of pleural infection. We specified in the text.

2. "a case report by Wu et al. who described a case of a 29-year-old male" It is recommended to describe that was the location of pulmonary nodules near the pleura in this case.

AUTHORS REPLY: We added the imaging findings in the first paragraph:

Chest imaging showed scattered multiple cavities in the patient's superior segment of the left lower lobe with a rough cavity wall and cavity and pleural effusion in the patient's anterior basilar segment of the left lower lobe.

3. "Pleural involvement of cryptococcal infection is rarely observed, and is more commonly seen in immunocompromised hosts compared to immunocompetent ones" It is recommended to describe that what diseases are included in the immunocompromised hosts? For example ,newly diagnosed HIV infection; heterogeneous group of patients receiving high-dose cortico-steroids, monoclonal immunocompromised hosts. a growing and antibodies such as alemtuzumab and in-fliximab, and/or other immunosuppressive agents; and otherwise "normal" patients.

AUTHORS REPLY: We added this paragraph

Immune suppression is the major underlying mechanism that is involved in the causation of cryptococcal infection. Diseases like AIDS, diabetes, chronic liver and renal disease, prolonged use of steroids, use of monoclonal antibodies such as alemtuzumab and infliximab, and/or other immunosuppressive agents and solid organ transplantation are commonly associated with the development of cryptococcal disease.

4. Since then, approximately 50 cases of pleural effusion related to cryptococcal infection, in the context of both lung and disseminated disease, have been described. Further generalizations are recommended, such as how many cases related to disseminated Cryptococcal Disease(CNS,bones, fungemia),how many cases just in Cryptococcosis Pulmonary. Further generalization how many mild-to-moderate pulmonary cryptococcosis and severe pulmonary cryptococcosis were there? Generalized immunosuppressive risk factors , APACHE II, the location of pulmonary nodules.

AUTHORS REPLY: We added this paragraph

A total of 32 cases out of 50 had only pulmonary cryptococcosis and 18 out of 50 patients were related to disseminated disease. Eight patients experienced severe pulmonary cryptococcosis, requiring, in some cases, surgical management with decortication and lobectomy. The immunosuppressive risk factors identified in these 50 cases were solid organ transplantation, AIDS, hematological malignancies, administration of corticosteroids, diabetes mellitus, chronic obstructive pulmonary disease, bronchial asthma, liver cirrhosis, and end-stage renal disease. Interestingly, 14 patients were immunocompetent. The majority of pulmonary nodules were observed in the lower lobes and in a subpleural distribution. Of note, 26 patients had only pleural effusion on CT imaging.

Unfortunately, regarding the APACHE II score, it cannot be calculated because none of the 50 cases contains all the necessary information for the calculation of the score.

5. The detection rates of *Cryptococcus neoformans* with Gomori-methenamine silver stain and periodic acid-Schiff staining (PAS) stain are 100%. It is recommended to describe that pathological identification with histoplasmosis.

AUTHORS REPLY: We added this paragraph.

*The morphology present in tissue with *Cryptococcus neoformans* infection using GMS and PAS staining reveals arrow-based budding yeasts (4–10 μm) with a thick capsule, while the morphology present in tissue with histoplasmosis reveals small yeasts (2–4 μm) with narrow-based budding grouped in clusters inside macrophages*

6. It is recommended to describe that how long did the pleural effusion absorb after fluconazole 400mg qd or 400mg q12h?

AUTHORS REPLY: We added this paragraph

The patient in case by Wo et al. was initially treated with a daily dose of 400 mg of fluconazole , but exhibited unsatisfactory clinical outcome a week later and the therapy was modified to voriconazole 200 mg twice daily. Complete resolution of the lesions was observed after 8 weeks of therapy. In non-immunocompromized patients with pulmonary cryptococcal infection, it is recommended the administration of fluconazole 400mg daily and switching to itraconazole (200 mg twice per day orally), voriconazole (200 mg twice per day orally), or posaconazole (400 mg twice per day orally) in cases with no clinical improvement, no fluconazole availability or contraindication.

Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: I have reviewed the letter to the editor titled "Pleural involvement in cryptococcal infection". I would like to congratulate the authors for choosing a topic which gives insight about "Cryptococcal pleural infections". Scientific quality, general integrity and language quality of the manuscript is accurate. Therefore I suggest that this article to be published.

AUTHORS REPLY: Dear Reviewer, we really thank you for your comments.

Reviewer #4:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: I read with a great interest the letter to the editor titled "Pleural involvement in cryptococcal infection" by Georgakopoulou VE et colleagues. It is based on "Pleural effusion in an immunocompetent host with cryptococcal pneumonia: A case report." by Fang SY. I have some revisions to suggest.

1. The authors mentioned the significance of the ADA level to discriminate the etiology of the effusions. Most of the fungal effusion including cryptococcosis are lymphocytic effusions and tuberculous effusions are lymphocytic effusions, too. The authors have reviewed the literature, and they mentioned some studies reported elevated level of ADA was rarely found in nontuberculous lymphocytic pleural effusions. What is the most recent interpretation of the ADA level? ADA level check is still valuable?

AUTHORS REPLY: We added this.

ADA test has high negative predictive value and is an excellent test to rule out tuberculosis. Some studies demonstrate that an ADA level >45 to 60 units/L has a sensitivity of 100% and a specificity up to 97 % for tuberculous pleural effusion.

2. Yoshino et al. described a case of cryptococcal pleuritis, diagnosed by the isolation of *Cryptococcus neoformans* in the culture of the pleural effusion, containing a high level of ADA in a patient with AIDS. Wee et al. also reported a case of a patient with acute myeloid leukaemia and a cryptococcal pleural effusion with increased pleural fluid ADA level[12]. ---> Put the proper reference numbers to the sentences 'Yoshino et al. described~~~' and 'Wee et al. also~~~', respectively.

AUTHORS REPLY: We corrected.

3. Correct the typographical errors; immonocompromised, cerebrospinal fluid

AUTHORS REPLY: We corrected.

4. Check the sentence in Abstract: 'Pleural involvement in cryptococcal infections includes pleural infection without or without pleural effusion.'

AUTHORS REPLY: We modified as follows:

Pleural involvement in cryptococcal infections can manifest without or without pleural effusion.

5. periodic acid-Schiff staining (PAS) stain ---> periodic acid-Schiff (PAS) stain

AUTHORS REPLY: We corrected.

6. unify the following words: Cr Ag vs. CrAg, India ink vs. Indian ink

AUTHORS REPLY: We corrected.

(1) Science editor:

This Letter to the Editor focused on the Pleural involvement in cryptococcal infection, which is based on "Pleural effusion in an immunocompetent host with cryptococcal pneumonia: A case report." by Fang SY. This manuscript is interesting and of some significance to the clinical field, attracting the attention of readers.

However, some issues have to be addressed. A more in-depth discussion of *Cryptococcus* is necessary. There are some writing errors that need to be paid attention to. Please check the full text carefully.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade C (Good)

AUTHORS REPLY:

Dear Editor thank you for your comments.

By adding this paragraphs:

Cryptococcus is an invasive fungus that causes cryptococcosis, a disease that is common in immunocompromised people and rare in healthy individuals. Cryptococcus neoformans and Cryptococcus gatti are the two Cryptococcus species most frequently associated with human cryptococcal infections. The organism is widespread in several parts of the world. The most

prevalent types of exposure involve a history of exposure to soil containing bird droppings. The capsule of the fungus comprises polysaccharides glucuronoxylomannan and glucuronoxylomannogalactan which are the main factors that contribute to the virulence of fungus.

Immune suppression is the major underlying mechanism involved in the development of cryptococcal infection. Diseases like AIDS, diabetes, chronic liver and renal disease, prolonged use of steroids, use of monoclonal antibodies such as alemtuzumab and infliximab, and/or other immunosuppressive agents and solid organ transplantation are commonly associated with the development of cryptococcal disease.

Cryptococcus species spread by inhalation and despite the fact that the virus most commonly enters the body through the lungs, meningoencephalitis is the most prevalent clinical manifestation of the illness.

along with the discussion about the molecular methods for detecting *Cryptococcus* we provide a more in depth analysis for this fungus