Clin. Lab. 2026;72:XXX-XXX

CASE REPORT

Pulmonary Cryptococcosis in an Immunocompetent Patient: Negative mNGS but Positive Antigen Test

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SUMMARY

Background: **Pulmonary cryptococcosis is common in patients with immunodeficiency, application of immunosuppressive and cytotoxic drugs. Its incidence has increased in recent years, with a greater number of patients with no underlying disease or history of exposure, and a greater number of immunocompetent patients. Its clinical presentation lacks specificity and imaging features are atypical, making it easy to misdiagnose or miss the diagnosis.**
Methods: **Diagnosis was confirmed by serum cryptococcal antigen after negative bronchoalveolar lavage fluid mNGS in an immunocompetent patient with atypical radiological findings.**
Results: **We present a 53-year-old female patient with pulmonary cryptococcosis who was immunocompetent, had atypical imaging, and was diagnosed by a negative bronchoalveolar lavage fluid mNGS test and confirmed by cryptococcal pod antigen test.**
Conclusions: **By analyzing and discussing the diagnosis and treatment process of the patient with cryptococcosis, we aim to strengthen clinicians' knowledge and diagnosis and treatment ability of pulmonary cryptococcosis in order to improve the early diagnosis and treatment rate of this disease and reduce misdiagnosis or omission.**
(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250424)

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KEYWORDS

cryptococcus neoformans, pulmonary cryptococcosis, metagenomic next-generation sequencing, diagnosis

Case Report accepted May 6, 2025

CASE REPORT

The incidence of cryptococcosis has increased in recent years, and there are two main serotypes of Cryptococcus, Cryptococcus neoformans and Cryptococcus gattii, with Cryptococcus neoformans being the most common in China [1]. Cryptococcus neoformans is a non-hereditary, budding, encapsulated yeast-like fungus found in soil contaminated with pigeon or avian feces, which readily forms aerosols, and the common route of infection in humans is respiratory inhalation, with the most frequent site of involvement being the central nervous system, followed by the lungs and the skin [2]. Cryptococcus enters the body with respiration and can remain latent in alveolar macrophages for a long period of time; when the body's immunity is lowered, cryptococcal spores multiply, causing pulmonary cryptococcosis. It can also infect other organs of the body via the blood system, most commonly the central nervous system [3]. Cryptococcal infections occur mainly in immunodeficient patients. In recent years cryptococcal disease that occurs in immunocompetent hosts has received increasing attention, especially pulmonary cryptococcosis (PC) [4]. PC is a fungal disease of the lungs with an incidence second only to pulmonary aspergillosis, with a lack of specificity in clinical presentation and atypical imaging features, making it susceptible to misdiagnosis and underdiagnosis.

This research presents a case of PC without immunodeficiency, with atypical imaging manifestations, which was finally diagnosed after a negative mNGS test by bronchoalveolar lavage fluid and a positive cryptococcal podopod antigen test. Through the study, the clinicians' knowledge of PC and diagnosis and treatment ability were improved, and misdiagnosis or omission of diagnosis were reduced.

Patient was a 53-year-old female. The main reason was the intermittent fever for more than 10 days on 2022-02-03 in North China University of Science and Technology Hospital. More than 10 days ago, the patient had fever without obvious cause. The highest temperature was 38℃. It was mostly a low-grade fever, mostly in the evening and it can decrease without medication. It was accompanied by coughing, a small amount of yellow sputum, and accompanied by fatigue, poor appetite, lethargy, occasional pain in the right lower abdomen, no chest pain, hematemesis, dyspnea, no frequency of urination, urgency of urination, and pain during urination. She had taken “cephalosporins” and “cold and flu capsules” without significant improvement. She then consulted our clinic. After taking “cephalosporin drugs and cold capsules” without significant improvement, she was admitted to our outpatient clinic, and was admitted to the hospital with “pneumonia” after a chest CT that showed patchy hyperdense shadows and solid shadows in the lower lobe of the right lung. Past history: “hypertension” for 3 months, blood pressure up to 180/90 mmHg, oral “valsartan amlodipine 1 tablet 1/day”. Her blood pressure control is still good without other underlying diseases. Physical examination: clear respiratory sounds in both lungs, no dry or wet rales detected, nothing else special. 2022-02-03 Chest CT (see Figure 1): 1. Patchy hyperdense shadow and solid shadow in the lower lobe of the right lung, possible inflammatory lesion. 2. Nodular foci in the upper lobe of the left lung. 3. Calcification of the aortic wall. 4. Pleural adhesions on both sides. After admission, relevant tests were completed: blood routine: lymphocytes $1.05*10^9$/L. Coagulation series: D-dimer 812.20 ng/mL; plasma fibrinogen 6.12 g/L; sedimentation rate 47 mm/hour. No abnormality was found in PCT, CRP, viral antibody, tuberculosis antibody, PPD test. After 5 days of administration of moxifloxacin sedation, the patient still had intermittent fever and the peak of fever did not decrease. 2022-02-08 Electronic bronchoscopy was perfected, bronchoalveolar lavage was performed in the basal segment of the right lower lobe of the lung. The lavage fluid was sent to be examined for pathogenic microorganisms. TBLB was performed in the subbranch of the right lower lobe of the right lung in the posterior basal segment. Alveolar lavage fluid macro-genomic next-generation sequencing (mNGS) results (provided by Tianjin Jinwei Medical Laboratory Co., Ltd.): Acinetobacter baumannii Sequence number 93. Alveolar lavage fluid X-pert (provided by Tianjin Jinwei Medical Laboratory Co., Ltd.): negative. TBLB pathological return: chronic inflammation of bronchial mucosa. Alveolar lavage cryptococcal antigen: positive. As the new cryptococcus is neurophilic and also often infects the central nervous system, we perfected the cranial CT, and the results were not abnormal. Pursuing the history, the patient kept parrots at home before admission. She was diagnosed with pulmonary cryptococcosis, given fluconazole 0.2 g static 2/day sequential voriconazole 0.2 g oral 2/day antifungal treatment for 2 months. 2022-04-25 the review of the chest CT shows that the right lower lobe of the lung patches of high-density shadows and solid shadows are significantly absorbed compared with the previous (see Figure 2).

DISCUSSION

PC is a common opportunistic fungal infection caused by Cryptococcus neoformans or Cryptococcus gattii [5]. It usually occurs in immunocompromised patients, such as human immunodeficiency virus infection, solid organ transplantation, autoimmune diseases, and the use of glucocorticoids and other immunosuppressive agents [6]; however, in recent years, the prevalence of PC has increased rapidly in immunocompetent hosts [4]. Its clinical manifestations lack specificity, imaging features are atypical, and it is easy to misdiagnose or miss the diagnosis. The clinical manifestations of Cryptococcus pneumoniae infection are diverse, mainly manifested as cough, small amount of mucus sputum or bloody sputum, accompanied by fever, and some patients may present with chest pain, hemoptysis, malaise, night

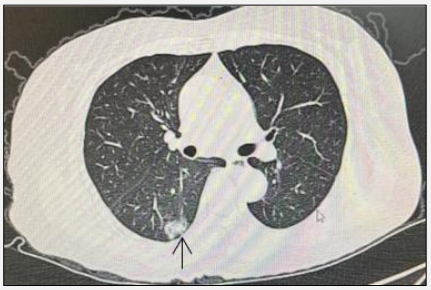

Figure 1. 2022-02-03 chest CT suggests patchy hyperdense and solid shadows in the lower lobe of the right lung.

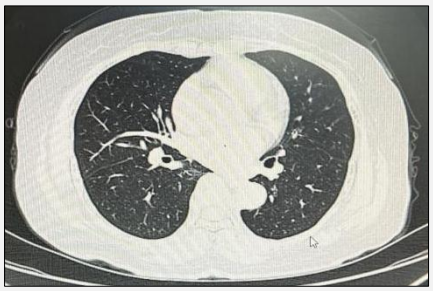

Figure 2. 2022-04-25 repeat chest CT showed patchy hyperdense shadows and solid shadows in the lower lobes of the right lung that were significantly more absorbed than before.

sweats, etc. Clinically asymptomatic patients with chronic insidious onset of the disease are also common [7]. Different immune statuses may lead to a variety of CT manifestations in patients with cryptococcal lung infections, which are usually categorized into three types: single or multiple nodular massy shadows, lamellar infiltrative shadows, and diffuse mixed lesions [8]. Pulmonary cryptococcosis has no obvious favorable site, all lobes of both lungs can be involved, but generally it is seen in the lower pleural end, immunocompetent patients with pulmonary cryptococcosis have limited and mixed lesions on chest CT, without any specific clinical manifestations and imaging manifestations. It is difficult to differentiate from lung cancer, tuberculosis or pneumonia [9]. Therefore, cryptococcal infection still shows a certain degree of underdiagnosis and misdiagnosis in the clinic. Confirmation of the diagnosis mainly relies on histopathological examination and pathogenetic smear and culture of pus puncture specimens within the lesion, usually taken from sterile sites such as percutaneous lung tissue puncture biopsy specimens, such as fungal smear and positive culture, have a definite diagnostic significance; sputum, pharyngeal swabs, or bronchoalveolar lavage specimens taken from sputum or culture positive, as well as serum cryptococcal podococcal polysaccharide antigen latex agglutination test is positive have a clinical suspicion for the diagnostic value [10]. The clinical symptoms of this patient manifested as fever, with cough and sputum, accompanied by fatigue, poor appetite, and emaciation. The chest CT suggested that the lower lobe of the right lung had patchy hyperdense shadows and solid shadows. The patient's clinical symptoms were atypical, the bronchoalveolar lavage fluid mNGS test was negative, the alveolar lavage fluid cryptococcal antigen was positive, and the patient was asked about her medical history. The fact that she kept parrots in her home was considered to be the most probable reason for her infection with cryptococcus.

In recent years, with the development of emerging molecular biology detection technologies, there has been a gradual increase in the number of research reports on

the detection of clinically pathogenic microorganisms by mNGS. mNGS can detect low abundance pathogens that lack a culture system, such as Pneumocystis jirovecii, viruses, etc., and it can recognize co-infections, which is potentially valuable for the diagnosis of infectious diseases, including cryptococcal infection, and it has seen growing application in clinical practice [11]. mNGS is a technique for determining the internal base sequences of nucleic acid molecules. When a patient is infected by exogenous pathogenic microorganisms, the specimen collected from the infected site contains both human nucleic acids and exogenous pathogenic microbial nucleic acids. mNGS technology can be utilized to obtain the sequence information of human nucleic acids and exogenous pathogenic microbial nucleic acid sequences in the specimen in parallel at the same time by utilizing the high throughput characteristics of the mNGS technology. The sequences can be annotated by matching the sequences with the human genome sequence libraries and the pathogenic microorganisms' genome sequences libraries, so as to realize the identification of pathogenic microorganisms [12]. Currently, the cost of second-generation sequencing is still high, and commercialized second-generation sequencing assays are less sensitive to different types of respiratory clinical samples than traditional molecular assays, such as fluorescent PCR, based on cost considerations and their relatively fixed data coverage [12]. Therefore, it cannot replace traditional detection methods, unless the sequencing coverage is increased so that its sensitivity is greater than that of traditional molecular detection methods, but should be used as a supplement when traditional methods are unable to clarify the infectious agent. For common respiratory tract infections, traditional methods should be perfected first; if they cannot be clarified and the patient's condition is prolonged, second-generation sequencing can be used as the first choice of detection [13]. For immunosuppressed patients presenting with respiratory infections or in critical condition, respiratory specimens should be tested by second-generation sequencing as soon as possible along with the traditional pathogenetic testing methods sent for early clarification of rare pathogens or mixed infections [14]. It has been suggested that the mNGS test has clinical significance in confirming the diagnosis of cryptococcal infections, both in cerebrospinal fluid and alveolar lavage fluid. It is able to identify cryptococcal infections at an early stage and recognize the species type [13]. However, in this case, the negative mNGS test result of the patient's bronchoalveolar lavage fluid may have been due to: 1) mNGS detection threshold: there is still no standardized criterion for a positive mNGS threshold to identify cryptococcal infections, i.e., it is possible that the infection itself is a cryptococcal infection, and that cryptococci were detected by the mNGS but were excluded on the basis of contaminated background bacteria because of their poor detection sequence exclusion; 2) Specimen: Cryptococcus itself has a thick cell wall, it is difficult to break the wall, and since the removal of the specimen, it needs to undergo a longer period of time before detection. This process also undergoes a number of freezing and thawing cycles, the formation of damage to the specimen, coupled with the interference of the host cells, which can reduce the detection rate. Ji et al. found that utilizing 0.025% saponin to selectively deplete human DNA increased the detection rate of cryptococcal infection [15]; 3) Application of antifungal drugs before mNGS testing: In some cases, as in the above study, antifungal drugs had been applied for a long period of time before specimens were taken. In the clinic, if the patient has clinical manifestations such as fever, vomiting, headache, meningeal irritation signs, or chronic cough, sputum and chest imaging manifestations of fungal infections, and there is a history of contact with poultry and birds, and cryptococcal infections are suspected, mNGS can be used to identify the patients; however, in view of the low sensitivity of the mNGS for cryptococcal detection, it should not be relied on completely.

CONCLUSION

When infectious lesions are considered in patients with fever and lung shadows, early pathogenetic testing is important for the diagnosis and prognosis of the disease, but the results of a single test should not be relied upon, and a detailed history may sometimes provide new diagnostic ideas. In addition, novel cryptococci often lead to central nervous system infections, but can also cause systemic infections at multiple sites, and we should be on the lookout for non-central nervous system cryptogenic infections. We also need to be alert to clinical inertia and the possibility of other diseases to avoid misdiagnosis and underdiagnosis.

Acknowledgment:
We would like to thank other members of the Department of Respiratory Medicine, Affiliated Hospital of the North China University of Technology for the constructive criticism.

Source of Support:
This work was supported by the Medical Applicable Technology Tracking Project of Hebei Provincial Health Commission (GZ20250080).

Ethical Approval:
This study was approved by the ethics committee of North China University of Science and Technology Affiliated Hospital. All procedures performed in studies were in accordance with the ethical standards. Informed consent was obtained.

Declaration of Interest:
No conflicts of interest.

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