Clin. Lab. 2024;70:XXX-XXX

CASE REPORT

Interstitial Lesions in Both Lungs Finally Diagnosed as a Rare Coinfection of Influenza A Virus and Pneumocystis Jiroveci

Zhu Fan, Xinran Li, Jiafeng Luo, Aishuang Fu

Department of Respiratory Medicine, North China University of Science and Technology Affiliated Hospital, Tangshan, Hebei Province, China

SUMMARY

Background: **Influenza is an acute respiratory infection caused by influenza viruses, with influenza A virus (IAV) being the most common and most likely to progress to critically ill cases leading to death. Pneumocystis jiroveci is an opportunistic lung-causing fungus that occurs most often in immunocompromised individuals and can cause Pneumocystis jiroveci pneumonia (PJP). It is rare for both diseases to occur in the same patient.**
Methods: **Appropriate laboratory tests, chest computed tomography (CT), bronchoalveolar lavage fluid, second-generation macro gene sequencing, and pathogenetic tests to clarify the diagnosis.**
Results: **G test and LDH were high, and chest CT showed rapidly progressive interstitial pneumonia, which was confirmed by bronchoalveolar lavage fluid and macrogenomic second-generation sequencing (mNGS) to be a mixed infection of H. influenzae type A virus and Pneumocystis jiroveci.**
Conclusions: **In rapidly progressive interstitial pneumonia, bronchoalveolar lavage and mNGS should be done early to clarify the presence of infection with specific pathogenic organisms.**
(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240530)

Correspondence:
Aishuang Fu
North China University of Science and Technology Affiliated Hospital
73 Jianshe South Rd
Lubei District, Tangshan, Hebei Province
China
Phone: +86 13393152699
Email: maxfas@163.com

KEYWORDS

influenza A virus, pneumocystis jiroveci, rapidly progressive interstitial pneumonia, bronchoalveolar lavage fluid, macrogenomic second generation sequencing

CASE REPORT

Influenza virus belongs to the family of Orthomyxoviridae, a multi-segmented, single-stranded, negative-stranded RNA virus with an incubation period of 18 to 72 hours [1]. It can be categorized into four types, A, B, C, and D, according to the antigenicity of the nucleoprotein and matrix protein M1, of which Influenza A virus (IAV) has the strongest variability. IAV is highly contagious and spreads rapidly. Every year IAV causes global outbreaks, so we should be concerned about the possibility of IAV infection at special times. PJP is well known as a serious comorbidity in HIV-infected patients, and the incidence of PJP has decreased significantly due to the decline in the rate of HIV infection and the use of highly active antiretroviral drugs [2]. However, with the use of immunosuppressive agents in

Case Report accepted May 29, 2024

chemotherapy for hematologic neoplasms, organ and hematopoietic stem cell transplantation, and autoimmune diseases, the incidence of PJP has been increasing in non-HIV patients [3]. Moreover, compared with HIV-infected patients, PJP in non-HIV patients progresses more rapidly, predisposes to respiratory failure, and has a mortality rate of 30% - 50% [4]. The presence of pathogenic infection in the lungs of a single patient with both diseases is rare, so it increases the difficulty of diagnosis. In this paper, we report a case of a patient with rapidly progressive interstitial pneumonia on imaging, which was eventually confirmed as a mixed infection of influenza A virus and Pneumocystis jirovecii by macrogenomic next generation sequencing (mNGS). The patient was admitted to the hospital with fever for more than 1 month and dyspnea for 1 day, with temperature fluctuating at 38 - 40℃, no chills, and scattered inflammatory changes in both lungs on chest CT at the local hospital at the early stage of the disease (Figure 1A). He was successively treated with a variety of antibiotics such as moxifloxacin, Piperacillin tazobactam, meropenem, etc. The effect was not good. The patient continued to have recurrent fever and then developed dyspnea. A chest CT examination showed the progression of pneumonia and diffuse interstitial changes (Figure 1B), so he was admitted to our hospital. On admission examination, dry and wet rhonchi could be heard in both lungs, and relevant examinations were completed, with leukocytes of 5.7 x 10^9/L, neutrophils of 4.93 x 10^9/L, lymphocytes of 0.53 x 10^9/L, G test of 684 pg/mL (reference value of 0 - 70 pg/mL), and GM test of 0.27 (reference value of 0 - 0.5). The patient was treated poorly with various antibiotics in an outpatient hospital, so he underwent bronchoscopy, and the bronchial openings of each lobe segment were clear bilaterally, with no neoplastic organisms or ulceration. TBNA and bronchoalveolar lavage were performed in the posterior basal segment of the right lower lobe of the lung and the lavage fluid was sent to the pathogenetic study. TBNA of the posterior basal segment of the right lower lobe of the lung showed alveolar epithelial hyperplasia with phosphorylation and mesenchymal fibrosis (Figure 2A, B - C). mNGS revealed the presence of IVA and Pneumocystis jiroveci. He was treated with oseltamivir 75 mg orally 2/day for 10 days, SMZ 800 mg/160 mg orally 4/day for 21 days, and methylprednisolone 40 mg 2/day for 14 days. After treatment, he repeated the examination of leukocytes 7.3 x 10^9/L, neutrophils 4.87 x 10^9/L, lymphocytes 1.78 x 10^9/L, G assay 137.15 pg/mL, GM assay 0.12, and inflammatory lesions in the lungs were gradually absorbed (Figure 1C). He was discharged from the hospital with improvement.

DISCUSSION

IAV, as a subtype of influenza virus, is susceptible to antigenic mutation. It is transmitted mainly by droplet and contact, and also by aerosol in crowded and airless environments [5]. The virus can survive in the air for about half an hour, which increases the likelihood of infection. IAV is the major cause of most human respiratory infections. It typically presents mild or subclinical symptoms in most populations, with severe cases often presenting with severe pneumonia of influenza A, characterized by persistent high fever, altered mental status, and severe vomiting. In addition, IAV infection can cause extrapulmonary damage, such as acute encephalopathy, which can lead to death or severe neurologic deficits. In summary, IAV is characterized by wide spread, easy mutation and severe clinical symptoms, which deserves the attention of clinical workers.

Pneumocystis carinii is a fungus that parasitizes human lungs and is transmitted through airborne droplets. It has two forms: trophozoite and encapsulated. When host immunity decreases, Pneumocystis carinii infects and invades the alveolar lumen or interstitial space of the lungs, and it can reproduce abundantly in alveoli, and the immune system is unable to clear the pathogen, thus damaging alveolar or interstitial tissues [6], leading to the occurrence of PJP. In recent years, the incidence of PJP has increased in non-HIV patients, such as non-HIV immunocompromised patients with autoimmune diseases, malignant tumors, antitumor therapy, use of immunosuppressants, and use of glucocorticoids. It has been shown [7] that non-HIV/AIDS immunosuppressed patients have a more rapid onset of disease and can enter respiratory failure more rapidly than AIDS-combined PJP patients, with a mean incubation period of 2 weeks. Patients with non-HIV/AIDS combined PJP have a severe condition, rapid disease progression, and extremely aggressive condition. If not treated in time, the imaging manifestations and condition will be linearly aggravated, and the condition of severe cases can rapidly deteriorate within 24 hours, resulting in acute respiratory distress syndrome (ARDS), and the lethality rate can be as high as 100%.

These two pathogens belong to two systems and are rarely present in the same patient at the same time, resulting in a double infection with different pathogens. In our case, the patient started with fever, was poorly treated with various antibiotics and presented with symptoms of dyspnea. Imaging showed a rapidly progressive interstitial lesion. To clarify the diagnosis, we perfected bronchial lavage fluid combined with mNGS, which returned a mixed infection of IAV and Pneumocystis japonicus.

The rapid detection of influenza virus antigen can be done by colloidal gold and immunofluorescence, which is simple and fast for clinical diagnosis and treatment, but with low sensitivity and poor specificity. Detection of Pneumocystis carinii can utilize the (1,3)-β-D glucan (BG) test method. BG is the main component that constitutes the cell wall of Pneumocystis carinii, but since glucan is not a specific component of the cell wall of Pneumocystis carinii, the specificity of plasma BG test is poor. Sputum Pneumocystis tests have a low positive rate because of the low amount of loaded bacteria in the

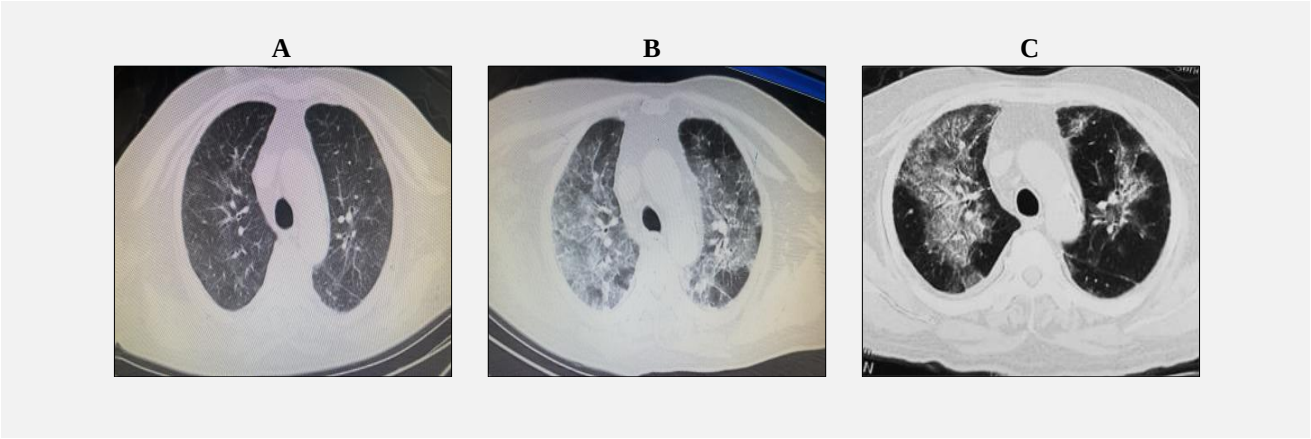

Figure 1A. Chest CT showed a few scattered inflammatory lesions in both lungs.
Figure 1B. The patient's imaging shows worsened condition after various antibiotic treatments in the outside hospital.
Figure 1C. The patient's imaging showed improvement after complete bronchoscopy in our hospital and comprehensive treatments such as antiviral and suppression of inflammatory response to regulate immunity were given.

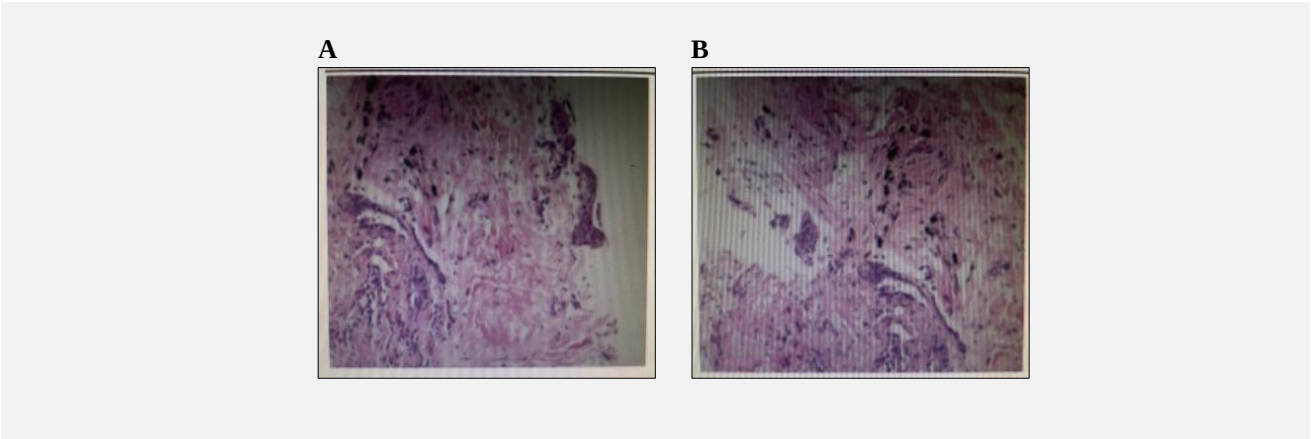

Figure 2A - B. Alveolar epithelial hyperplasia with phosphorylation and interstitial fibrosis in TBNA tissue of the posterior basal segment of the lower lobe of the right lung.

lungs of patients with non-AIDS combined PJP, and the sputum volume is small and not easy to cough up. The traditional diagnosis of PJP can also be confirmed by bronchoalveolar lavage fluid smear staining microscopy, which is infrequently used because of its low detection rate and limited clinical application. Also, known as high-throughput or massively parallel sequencing, mNGS can identify a wide range of known and unexpected pathogens while recognizing all potential infection factors in the sample, avoiding the need to predetermine diagnostic targets. Most mNGS platforms have an average typical turnaround time (tat) of 48 hours from sample receipt to final results [8,9], which effectively improves the analytical sensitivity for identifying picky microorganisms and diagnosing pulmonary co-infections, and shortens the time to diagnosis. Compared to histopathologic methods [10,11], mNGS achieved 100% specificity in assessing fungi in lung biopsy tissue. In contrast to culture-dependent methods [12], the results of mNGS are not affected by prior antibiotic exposure and provide a comprehensive view of the pathogens in a given sample, thus enabling the detection of new and rare pathogenic pathogens in the diagnosis of pneumonia of unknown origin. Therefore, the examination using mNGS has a good clinical application for both early diagnosis and targeted treatment of the disease.

CONCLUSION

Pneumocystis jiroveci co-infections with influenza virus are rare in patients with pulmonary infections. As a useful technique for detecting novel or rare microorganisms, mNGS has clear advantages in the diagnosis of lung infections of unknown origin and co-infections. In patients with rapidly progressive interstitial pneumonia, we should be alert to the possibility of mixed infections with uncommon pathogens. Early implementation of bronchoalveolar lavage and mNGS allows rapid and accurate identification of pathogens and enables targeted therapy.

Acknowledgment:
We would like to thank the 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 Doctoral Project Fund of North China University of Science and Technology and the Medical Science Research Program of Hebei Province [bs2110] [20201246].

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

Declaration of Interest:
No conflicts of interest declared.

References:

1. Li X, Gu M, Zheng Q, Gao R, Liu X. Packaging signal of influenza A virus. Virol J 2021 Feb 17;18(1):36. (PMID: 33596956)
2. Chen YH, Fang XY, Li YT, et al. Characterization of Pneumocystis jirovecii pneumonia at three tertiary comprehensive hospitals in southern China. Braz J Microbiol 2020 Sep;51(3):1061-9. (PMID: 32363569)
3. Cilloniz C, Dominedo C, Alvarez-Martinez MJ, et al. Pneumocystis pneumonia in the twenty-first century: HIV-infected versus HIV-uninfected patients. Expert Rev Anti Infect Ther 2019 Oct; 17(10):787-801. (PMID: 31550942)
4. Schmidt JJ, Lueck C, Ziesing S, et al. Clinical course, treatment and outcome of Pneumocystis pneumonia in immunocompromised adults: a retrospective analysis over 17 years. Crit Care 2018 Nov 19;22(1):307. (PMID: 30454031)
5. Cowling BJ, Ip DK, Fang VJ, et al. Aerosol transmission is an important mode of influenza A virus spread. Nat Commun 2013; 4:1935. (PMID: 23736803)
6. Ojuawo O, Htwe T, Thein OS, Sahal A. Pneumocystis pneumonia causing cavitating lung nodules in an immunocompetent individual. BMJ Case Rep 2021 Apr 27;14(4):e241061. (PMID: 33906876)
7. Roblot F, Le Moal G, Kauffmann-Lacroix C, et al. Pneumocystis jirovecii pneumonia in HIV-negative patients: a prospective study with focus on immunosuppressive drugs and markers of immune impairment. Scand J Infect Dis 2014 Mar;46(3):210-4. (PMID: 24450839)
8. Wang J, Han Y, Feng J. Metagenomic next-generation sequencing for mixed pulmonary infection diagnosis. BMC Pulm Med 2019 Dec 19;19(1):252. (PMID: 31856779)
9. Miao Q, Ma Y, Wang Q, et al Microbiological Diagnostic Performance of Metagenomic Next-generation Sequencing When Applied to Clinical Practice. Clin Infect Dis 2018 Nov 13;67 (suppl_2):S231-S240. (PMID: 30423048)
10. Li H, Gao H, Meng H, et al. Detection of Pulmonary Infectious Pathogens From Lung Biopsy Tissues by Metagenomic Next-Generation Sequencing. Front Cell Infect Microbiol 2018 Jun 25; 8:205. (PMID: 29988504)
11. Chen X, Cao K, Wei Y, et al. Metagenomic next-generation sequencing in the diagnosis of severe pneumonias caused by Chlamydia psittaci. Infectio 2020 Aug;48(4):535-42. (PMID: 32314307)
12. GBD Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis 2017 Sep;17(9):909-48. (PMID: 28579426)