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CASE SERIES

Pneumocystis Jirovecii Pneumonia in Two Immunosuppressed Non-HIV Infected Patients: A Clinical and Therapeutic Analysis

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Abstract: Pneumocystis jirovecii pneumonia (PJP) is an opportunistic fungal infection that often occurs secondary to human immunodeficiency virus (HIV) infection. However, for non-HIV immunocompromised patients, such as those undergoing novel immunosuppressive treatments to manage malignancies, organ transplants, or connective tissue diseases, PJP is emerging as an increasing threat. The clinical manifestations of PJP in HIV-infected and non-HIV-infected patients differ significantly. In non-HIV-infected patients, PJP progresses rapidly and is challenging to diagnose, resulting in severe respiratory failure and a poor prognosis. We describe lymphocytopenia in two women who were recently treated with methotrexate, tacrolimus, and corticosteroids for immunosuppressive therapy following adjuvant chemotherapy for breast cancer and kidney transplantation. The initial examination included a high-resolution chest CT indicating atypical pneumonia, and treatment was initiated with trimethoprim - sulfamethoxazole and oxygen support. Subsequently, bronchoscopy and bronchoalveolar lavage with mNGS detected Pneumocystis jirovecii. After 3 weeks of treatment with cotrimoxazole, the two patients recovered significantly and their condition was stable.

Keywords: pneumocystis jirovecii Pneumonia, non-HIV-Infected, malignancy, organ transplantation, immunosuppression

PJP is an interstitial plasmacytic pneumonia caused by pneumocystis. It is an opportunistic infection and is typically observed in immunosuppressed patients. With the progress of tumor chemoradiotherapy technology, the extensive development of organ transplantation technology, and the wide application of various immunosuppressive drugs, the number of PJP cases in non-HIV-infected individuals with low immune status has risen. PJP can be severe, with a fatality rate of approximately 5–15% in HIV-infected patients and 30–60% in non-HIV-infected PJP patients,[1] which is higher than that in HIV-infected PJP patients.[2] Studies have discovered that the use of glucocorticoids and/or cytotoxic drugs and low lymphocyte levels may raise the risk of PJP in non-HIV infected individuals.[3] Long-term immunosuppression can lead to immune deficiency, thereby increasing the host's susceptibility to various opportunistic pathogens. Recent advancements in high-throughput sequencing technologies have enhanced their application in infectious disease diagnosis, enabling rapid and accurate identification of pathogens. In particular, metagenomic Next generation sequencing (mNGS) enables direct sequencing of infectious samples, thereby capturing and preserving a large amount of pathogen data. This method provides the basis for rapid and accurate diagnosis of complex infections following the use of corticosteroids and immunosuppressants.[4] The drug usage of this group of people is complex, there are interactions among drugs, and they are also prone to adverse reactions, so the drug treatment of this group of people is the focus of monitoring. The key to the successful treatment of PJP is early detection and treatment. For such patients, individualized pharmaceutical care should be provided, as reported below.

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Case Reports

Case 1

A 36-year-old female patient was admitted to the hospital on May 30, 2024, due to an intermittent cough with frothy sputum for one week, accompanied by wheezing and chest tightness for two days. One week before admission, the patient developed a cough and sputum production after catching a cold. Two days before admission, the symptoms above worsened, with coughing and a small amount of frothy sputum that was difficult to expectorate. The patient experienced chest tightness, shortness of breath, and exertional dyspnea, with limited exercise tolerance, experiencing dyspnea and chest tightness after walking only 50 meters. She also reported fatigue and myalgia, as well as fever with a self-reported temperature of 37.2°C. A chest CT scan performed at an outside hospital revealed ground-glass opacities in both upper lungs. The patient had undergone a left breast-conserving surgery (BCS) under general anesthesia on February 29, 2024, for triple-negative breast cancer. The postoperative course was uneventful, and she had received three cycles of adjuvant chemotherapy with doxorubicin and cyclophosphamide-(DC). A complete blood count conducted at an outside hospital on May 6, 2024, after the completion of chemotherapy, revealed a leukocyte count of 2.69×10^9/L. Treatment with pegfilgrastim was initiated to elevate the leukocyte count.

On physical examination: T: 36.4°C, P: 122 beats/min, R: 22 breaths/min, BP: 101/70 mmHg, SpO2: 88% (without oxygen supplementation). Coarse breath sounds with scattered moist rales were auscultated bilaterally. The patient was admitted with a diagnosis of Interstitial pneumonia, etiology to be determined; Triple-negative breast cancer, post breast-conserving surgery; Leukopenia.

Hospital Course: Upon admission, the patient was started on empirical therapy with 0.4g of moxifloxacin once daily for infection control. Acetylcysteine and ambroxol nebulization were administered for expectoration, doxofylline for broncho-dilation therapy, and terbutaline nebulization for the management of bronchospasm. Laboratory investigations revealed: Interleukin-6: 12.103 pg/mL, high-sensitivity C-reactive protein (hs-CRP) >5.0 mg/L, CBC: leukocyte count 13.03×10^9/L, lymphocyte count 3.8%, neutrophil count 88.2%, Antinuclear antibody (ANA) titer was 1:1000 with a homogeneous nuclear granular pattern. On May 31, 2024, the patient developed a fever with a maximum temperature of 38.1°C, heart rate ranging from 114 to 128 beats/min, respiratory rate of 21 breaths/min, and blood pressure of 109/59 mmHg. Oxygen saturation was 95%. An electrocardiogram showed sinus tachycardia. External cooling measures were implemented, and 10 mL of ibuprofen suspension was administered orally. After physical cooling, the patient's temperature returned to normal, and her heart rate fluctuated between 76–88 beats/min. Methylprednisolone sodium succinate 40 mg once daily was initiated for anti-inflammatory therapy, misoprostol for prophylaxis of gastric mucosal injury, and Tanreqing injection 20 mL intravenously once daily for 3 consecutive days. On June 1, 2024, the patient continued to cough and experience shortness of breath and dyspnea upon exertion. Bronchoscopy was performed, and bronchoalveolar lavage fluid (BALF) was sent for metagenomic next-generation sequencing (mNGS), bacterial culture, acid-fast bacilli (AFB) smear and culture, and Xpert MTB/RIF assay. Sputum cultures were also obtained. Laboratory investigations revealed: Bronchoscopy revealed inflammatory changes in the bronchial mucosa and local submucosal uplift at the opening of the left lower lobe basal segment. The patient's asthma improved on June 3, 2024, but she still felt general fatigue. Laboratory investigations revealed: The Grocott's methenamine silver (GMS) stain of the BALF was positive, Epstein-Barr virus (EBV) DNA viral load was 1.21×103 copies/mL, Liver function tests showed alanine aminotransferase (ALT) 80 U/L and alkaline phosphatase (ALP) 142 U/L. BALF cell count differential revealed neutrophils 14%, lymphocytes 6%, and macrophages 80%. (mNGS results can be seen in Table 1).

Table 1 Key Pathogens for Detection

Classification	Genus	Species	Normalized Sequence Count	Reference Concentration (Copies/mL)
Bacteria	Staphylococcus	Staphylococcus aureus	4180	21240
Mycobacteria	Pneumocystis	Pneumocystis jirovecii	3357	17,058

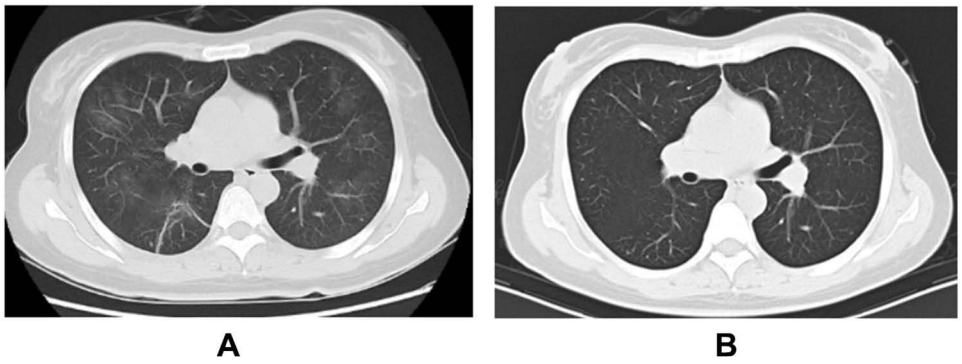

Figure 1 Case 1: Comparison of imaging before and after treatment.
Notes: Chest computed tomography (CT) images of case 1 at the diagnosis and the end of a series of treatments. (**A)** Diffuse distribution of patchy ground-glass opacities in both lungs; minimal consolidation, infiltration, and linear opacities in the basal segments of the lower lobes of both lungs. (**B)** Significant improvement in bilateral pneumonia with resolution of inflammation after treatments.

Microscopic examination of the sample: Microscopic examination revealed a small number of leukocytes, a large number of macrophages and ciliated columnar epithelial cells. Targeted mNGS identified Staphylococcus aureus and Pneumocystis jirovecii. Trimethoprim/sulfamethoxazole (TMP/SMX) 0.48 g three times daily was initiated to treat PJP. A high-flow nasal cannula (HFNC) oxygen therapy was initiated (FiO2 35%, flow rate 30 L/min, temperature 35°C), maintaining oxygen saturation between 90% and 95%. Intravenous immunoglobulin (IVIG) 20 g was administered daily for 3 consecutive days, along with oral Yishanfu 228mg thrice daily. The dose of methylprednisolone sodium succinate was adjusted to 20 mg once daily. On June 5, 2024, The patient's cough, wheezing, and fatigue significantly improved; however, she reported occasional palpitations and insomnia. Laboratory investigations revealed: CBC: leukocyte count 14.99×10^9/L, lymphocyte count 6.3%, neutrophil count 84.0%. Follow-up chest CT: minimal consolidation, exudation, and streaking in the basal segments of both lower lobes; multiple solid nodules in the remaining bilateral lungs, the largest measuring approximately 6×5 mm located in the anterior segment of the right upper lobe (Figure 1A). A consultation with the Department of Traditional Chinese Medicine was requested for the potential use of traditional Chinese medicine to invigorate the spleen, resolve dampness, broaden the chest, and regulate qi. The patient was transitioned to alternating low-flow nasal cannula (oxygen flow 2 L/min) and HFNC oxygen therapy, and blood oxygen saturation fluctuated between 95%-98%. The patient's clinical status improved, and her vital signs remained stable. Moxifloxacin, TMP/SMX 0.48 g three times daily, and IVIG 10 g daily for 3 consecutive days were continued.

On June 8, 2024, Follow-up liver function test showed ALT 66 U/L, ALP 135 U/L, CBC: leukocyte count 10.94×10^9/L, lymphocyte count 8.1%, neutrophil count 84.6%. Corticosteroid therapy was discontinued, and symptomatic and supportive care, including expectorants, bronchodilators, antimicrobial treatment, liver-protective agents, and oxygen therapy, was continued. On June 12, 2024, the patient was asymptomatic. Clear breath sounds without rales were auscultated bilaterally. Repeat chest CT: Significant improvement in bilateral pneumonia compared to the previous study; multiple solid nodules in the remaining bilateral lungs, stable in size compared to the previous study; resolution of the small pericardial effusion compared to the previous study (Figure 1B).On June 13, 2024, the patient developed a fever with a temperature of 38°C. Her vital signs were: heart rate 102 beats/min, respiratory rate 21 breaths/min, blood pressure 110/62 mmHg, oxygen saturation 95%. Physical cooling measures and 10 mL of oral ibuprofen suspension were administered. After physical cooling, the patient's temperature returned to normal. Urgent CBC revealed a leukocyte count of 2.83×109/L. Liver function tests revealed ALT 260 U/L, ALP 97 U/L, and GGT 74 U/L. The erythrocyte sedimentation rate (ESR) was 40 mm/h. (See Table 2 for detailed data).The patient was discharged in stable condition and continued an entire 21-day course of TMP/SMX to treat PJP outside the hospital.

Case 2

A 72-year-old female patient presented to the hospital on June 12, 2024, with a one-week history of chest tightness and dyspnea. The patient was two years post allogeneic renal transplantation. One week before admission, she experienced

Table 2 Results of Patient's Laboratory Examination was Modified to Laboratory Test Results from the Patient in Case 1

Index	Date				Reference range
	5.30	6.3	6.8	6.13	
WBC/($\times10^9\cdot L^{-1}$)	13.03	14.99	10.94	2.83	3.5–9.5
NEUT/($\times10^9\cdot L^{-1}$)	11.50	12.59	9.25	1.83	1.8–6.3
LYM/($\times10^9\cdot L^{-1}$)	0.49	1.38	0.89	0.59	1.1–3.2
CR/($\mu mol\cdot L^{-1}$)	60	56	70	62	41–73
ALT/($U\cdot L^{-1}$)	78	80	66	260	7–40
IL-6/(pg·mL)	12.103	-	-	<1.50	<7.00
Blood culture	-	-	-	-	-
BALF smear and culture	-	-	-	-	-
BALF metagenomic sequencing	-	Pneumocystis jirovecii Staphylococcus aureus	-	-	-
BALF G test	-	+	-	-	-

Abbreviations: WBC, White blood cell count; NEUT, Neutrophil count; LYM, Lymphocyte count; CR, creatinine; ALT, Alanine aminotransferase; IL-6, Interleukin 6.

chest tightness, dyspnea, fatigue, and melena. A chest CT scan performed at an outside hospital revealed scattered exudates in both lungs and interstitial fibrosis in the right lower lobe (Figure 2A).The patient underwent allogeneic renal transplantation two years prior due to uremia and had been maintained on a post-transplant immunosuppressive regimen of mycophenolate mofetil 0.5 g twice daily (9 AM and 9 PM), tacrolimus 1 mg twice daily (9 AM and 9 PM), and methylprednisolone 4 mg once daily after breakfast.

Physical examination revealed a temperature of 36.8°C, heart rate of 78 bpm, respiratory rate of 21 bpm, blood pressure of 120/75 mmHg, and oxygen saturation of 90% on room air. Bilateral lung auscultation revealed slightly coarse breath sounds without wheezes, rales, or rhonchi. A 15 cm well-healed surgical scar was noted in the right iliac fossa. The remainder of the physical examination was unremarkable.

Admission diagnoses included: Status post allogeneic renal transplantation, nephrolithiasis in the allograft, pulmonary infection, anemia, right lower lobe interstitial fibrosis, and multiple gastric ulcers (A2 type).

Treatment course: Upon admission, the patient was empirically started on moxifloxacin 0.4g daily for the presumed pulmonary infection, nebulized acetylcysteine, doxofylline for bronchodilation, and isosorbide mononitrate 10mg daily for vasodilation. Initial laboratory results were notable for a leukocytosis of 23.13×109/L with a neutrophilia of 93.6% and lymphopenia of 3.2%, hemoglobin of 84g/L, N-terminal pro-brain natriuretic peptide (NT-proBNP) of 1848 pg/mL, procalcitonin of 0.29 ng/mL, D-dimer of 4.8 mg/mL, hypoalbuminemia with an albumin level of 29.1 g/L, and positive fecal occult blood. On June 13, 2024, the patient underwent bedside bronchoscopy with bronchoalveolar lavage, which was sent for mNGS. Laboratory results on this day showed a decrease in leukocyte count to 8.48×109/L with a neutrophil

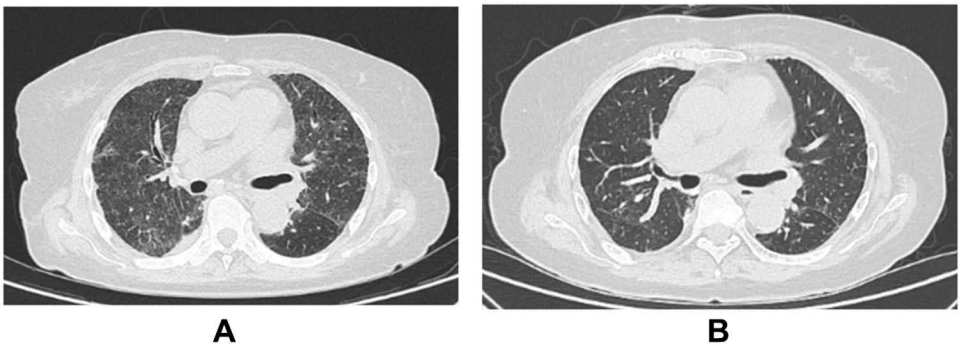

Figure 2 Case 2: Comparison of imaging before and after treatment.
Notes: CT images of case 2 at the diagnosis and the end of a series of treatments. (**A)** Multiple interstitial changes in both lungs at the onset of interstitial lung disease. (**B)** Significant improvement in bilateral pneumonia with resolution of inflammation after treatments.

Table 3 Results of Patient's Laboratory Examination was Modified to Laboratory Test Results from the Patient in Case 2

Index	Date						Reference range
	6.12	6.13	6.15	6.17	6.18	6.20	
WBC/($\times10^9\cdot L^{-1}$)	23.13	11.03	8.48	12.96	13.03	15.07	3.5–9.5
NEUT/($\times10^9\cdot L^{-1}$)	21.66	8.69	6.7	10.49	10.37	14.01	1.8–6.3
NEUT/%	93.6	78.7	79.0	81.0	79.6	93.0	40–75
LYM/($\times10^9\cdot L^{-1}$)	0.73	1.16	1.22	1.17	1.55	0.92	1.1–3.2
CRP/ (mg·L)	-	136	-	30	66.7	-	0–6
PCT/(ng·mL)	0.29	0.38	0.17	0.11	-	0.08	<0.10
NT-proBNP/(pg·mL)	1848	2582	4316	1396	-	2736	<900
CR/($\mu mol\cdot L^{-1}$)	85	88	77	97	89	100	41–81
ALT/($U\cdot L^{-1}$)	10	9	8	29	31	27	7–40
Blood culture	-	-	-	-	-	-	-
BALF smear and culture	-	-	-	-	-	-	-
BALF metagenomic sequencing	-	-	Pneumocystis jirovecii	-	-	-	-
BALF G test	-	-	+	-	-	-	-

Abbreviations: WBC, White blood cell count; NEUT, Neutrophil count; LYM, Lymphocyte count; CRP, C-reactive protein; PCT, Procalcitonin; NT-proBNP, N-terminal forebrain natriuretic peptide; CR, creatinine; ALT, Alanine aminotransferase; IL-6, Interleukin 6.

count of 6.70×109/L, a further decline in hemoglobin to 66 g/L, hypoalbuminemia with an albumin level of 26.3 g/L, glucose of 10.3 mmol/L, procalcitonin of 0.17 ng/L, NT-proBNP of 2852 pg/mL, and a tacrolimus level of 2.9 ng/L. All returned pathogen tests were negative. The patient received three consecutive days of human albumin 20 g infusions for hypoalbuminemia and two units of washed-packed red blood cells for moderate anemia. The morning tacrolimus dose was increased to 1.5 mg due to the slightly low blood concentration. On June 15, 2024, laboratory results revealed an albumin level of 29.1 g/L, C-reactive protein of 64.80 mg/L, hemoglobin of 90 g/L, strongly positive fecal occult blood, procalcitonin of 0.10 ng/L, and NT-proBNP of 4316 pg/mL. (See Table 3 for detailed data). Renal ultrasound demonstrated the renal allograft in the right iliac fossa measuring approximately 122×44×48 mm with a parenchymal thickness of 19 mm. The morphology was unremarkable, with an intact renal capsule, clear corticomedullary differentiation, and good overall structure. The patient's anemia significantly improved after the transfusion of two units of washed packed red blood cells. Her overall clinical condition also improved, and the aforementioned treatment was continued. With comprehensive treatment, the patient's chest tightness and dyspnea resolved. Follow-up chest CT demonstrated significant improvement in bilateral pneumonia with resolution of inflammation. Multiple follow-up visits after discharge revealed no recurrence of respiratory symptoms, and chest CT scans showed continued resolution of the bilateral pulmonary lesions (Figure 2B).

Discussion

Treatment of Pneumocystis Jirovecii Pneumonia (PJP) in Non-HIV Patients With Malignancy

Pneumocystis jirovecii is an opportunistic fungal pathogen whose colonization is associated with many chronic lung diseases, including chronic obstructive pulmonary disease,[5] severe asthma,[6] and cystic fibrosis.[7] In the past few years, research on the life cycle of Pneumocystis jirovecii has been a considerable challenge. Pneumocystis jirovecii has a tropism for alveolar epithelial cells. Once inhaled by the human body, Pneumocystis jirovecii utilizes extracellular matrix proteins, primarily fibronectin and vitronectin, to bind to type I alveolar epithelial cells. Despite the initiation of anti-PJP therapy, mortality remains substantial in non-HIV-infected individuals (30–60%). Currently, the population with the highest incidence of PJP is those receiving immunosuppressive therapy after allogeneic bone marrow or organ transplantation, solid and hematological malignancies, and autoimmune diseases. In particular, some chemotherapeutic agents used to treat these malignancies can significantly increase the risk of

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PJP infection, including methotrexate, vincristine, cytarabine, fludarabine, rituximab, and long-term use of corticosteroids.[8]

PJP in non-HIV patients is characterized by the classic clinical triad of cough, fever, and dyspnea. Lung auscultation findings are often minimal (lacking positive findings or revealing only a few scattered moist rales), which is disproportionate to the severity of symptoms and imaging findings.

Non-HIV patients might have a lower fungal load, leading to fewer symptoms and a delayed diagnosis.[9] How we can diagnose these patients more rapidly and at an earlier stage is particularly significant for them. The traditional diagnostic approaches for pneumocystis pneumonia include special staining (such as silver hexamine staining, immunofluorescence staining, etc.) and visualization of samples (primarily alveolar lavage fluid samples under bronchoscopy). Although these methods have high specificity, they all have the drawbacks of poor sensitivity and a long detection cycle.[10] Therefore, timely and effective assistance is often not provided in clinical practice for the rapidly progressing disease course of non-HIV-associated PJP patients. This is one of the reasons for the application of molecular biology techniques in PJP to develop novel etiological detection methods. Currently, polymerase chain reaction (PCR), second-generation sequencing (NGS), loop-mediated isothermal amplification (LAMP), flow cytometry, antibody/antigen detection, and so forth have been utilized in the clinic to a certain extent.[11] However, these technologies also have certain issues. Taking PCR and NGS as examples, although these detection technologies are highly sensitive, they are not widely employed in many hospitals in many regions of the world, and there is a lack of specific detection conditions, which may impose a greater economic burden on patients. In all the cases reported, we ultimately considered that the etiological diagnosis of PJP could only be achieved through histological examination of the specimen, as Pneumocystis yersini cannot be cultured in vitro and visual evidence of the pathogen cannot be obtained through traditional microbial culture techniques.[12] Neither of the two cases had a positive result for Pneumocystis yersini through conventional microbiological testing. At the molecular biology level, PCR has also been partially affirmed in the detection of Pneumocystis yersini in recent years. However, PCR detection requires specific primers. There are many genotypes of Pneumocystis yersini, and different genotypes correspond to different primers, significantly increasing the workload and difficulty of detection.[13] Additionally, laboratory tests for PJP only yield non-specific results such as elevated inflammatory markers, decreased lymphocyte counts, and elevated lactate dehydrogenase, which do not play a decisive role in the diagnosis of PJP. Moreover, samples from the site of respiratory infection will enhance the accuracy of the results. Therefore, we finally decided to detect the mNGS of alveolar lavage fluid to overcome the above limitations and achieve the early diagnosis of PJP in non-HIV patients. In addition, the sequence number of pathogens in the mNGS test results may be related to the severity of the patient's disease and prognosis. A case report indicated that the sequence number of Pneumocystis yerbii in two of the deceased patients was > 10000, which was much larger than that of the patients with a better prognosis.[14] Therefore, mNGS can not only achieve the early diagnosis of PJP but also predict the subsequent development and outcome of the disease to a certain extent. In our case 1, the number of Pneumocystis jirovecii cases was lower than the reference level. The low sequence number of pathogens detected by mNGS is not clearly defined. Possible contamination has been strictly avoided during testing, and reliable specimens for lower respiratory tract infections have been used as much as possible. When the following pathogens with a low sequence number are detected in the respiratory tract specimen, it is more likely to consider the pathogenic microorganisms. However, in the case of patients with severely suppressed immune function, even if low-virulence pathogens are detected, it is still necessary to consider the possibility of pathogenic microorganisms. In this case, the BALF fungus 1-3-β-D glucan (G test) was also positive, with the result > 1000pg/mL (reference range < 60). The determination of disease-causing microorganisms should be combined with a comprehensive analysis of the patient's host factors, blood physicochemical indexes and imaging characteristics, anti-infective drug use history and treatment response, and traditional microbial detection results. According to Miao et al,[15] the positive rate of mNGS was higher than that of traditional microbial cultures in patients who were tested for pathogens after antibiotics had been used. This indicates that in clinical practice, empirical treatment can be carried out simultaneously or even before using mNGS for pathogen infection detection, which will not affect the final pathogen detection results, and strong etiological evidence can still be obtained. In addition, it is worth discussing in case 1 that the results of mNGS in BALF suggest the presence of Staphylococcus aureus. The clinical manifestations of the patient are mainly consistent with the characteristics of pneumocystis yerbii pneumonia, and the patient has a good response to the

corresponding treatment, so we consider that it may not be necessary to treat Staphylococcus aureus specifically. There was also no imaging evidence of infection or complications caused by Staphylococcus aureus (empyema, cavity formation, acute exacerbation, etc).[16] Given the risk of multi-drug resistance and unnecessary antibiotic use, additional treatment of Staphylococcus aureus was avoided in the absence of clear evidence of its involvement in active infections.

In recent years, there have been no reports of PJP in adults with normal immune function, and it almost only occurs in patients with immune dysfunction for various reasons. Rishma[17] et al analyzed the data of England from 2000 to 2010, and the incidence of PJP per million population was ranked from high to low: Hematological malignancies, malignancies outside the hematological system, blood diseases, any transplants, lung diseases (tuberculosis, COPD, cystic fibrosis, bronchiectasis, asthma, interstitial lung disease), kidney failure/dialysis. In addition to the above overall analysis, there are also studies on the occurrence of PJP in patients with certain diseases. For example, Alsayed et al[18] conducted a study on whether PJP occurred in asthmatic patients after COVID-19 infection. They used PCR assay to detect P. jirovecii in sputum samples. Of the 31 patients included, 3 (9.7%) were positive. Consider that all three patients had been on oral corticosteroids (OCS) in the past two months and had received OCS for COVID-19 due to severe asthma. Finally, it is concluded that chronic lung disease may be a risk factor for the colonization of Pseudomonas yersini, possibly due to the immunosuppression of corticosteroids. In another study, Lang Q et al developed a nomography with good diagnostic capability for PJP diagnosis in pneumonia patients receiving oral glucocorticoid therapy, which may help promote timely treatment of PJP and thereby reduce mortality in these patients.[19] Because routine PCR and next generation sequencing techniques can detect the DNA of P. yersii in a variety of samples, and provide a rapid diagnosis of PJP. However, because the threshold has not yet been established, it is difficult to distinguish between PJP and pneumospora colonization, even by real-time PCR. These molecular techniques have not been sufficiently standardized. So emerging technologies such as this column chart may be able to solve this problem in the future.

In Case 1, the patient had received three cycles of adjuvant DC chemotherapy for triple-negative breast cancer. Both agents are profoundly immunosuppressive. One study demonstrated that DC significantly and persistently suppresses B lymphocytes. After one cycle of chemotherapy, the level of B lymphocytes in patients decreased to 5.4% of the median before chemotherapy. After 9 months, B cells and CD4+ T cells partially recovered, but their levels remained significantly lower than before chemotherapy.[20] Other studies have confirmed that B lymphocyte loss exceeds 90% after using DC in patients with triple-negative breast cancer. Natural killer cells and CD4+ T lymphocytes are depleted by approximately 50%, while CD8+ T cell counts are relatively preserved.[21] Furthermore, studies suggest that tumor-specific factors may promote or induce infection, and the use of targeted therapies in patients with malignancies can also increase the risk of opportunistic infections. A study by Narita Y et al[22] showed that 36 patients (81.8%) received TMP-SMZ as prophylactic treatment for pneumonia because Pneumocystis jirovecii pneumonia was observed in patients treated with ibrutinib, and the risk of opportunistic infections may be increased due to ibrutinib's interference with B cell function. Del Castillo M et al[23] reported that 3 out of 740 melanoma patients treated with immune checkpoint inhibitors developed PJP, with an incidence rate of 0.4%.

Guidelines[24] recommend TMP/SMZ as the first-line treatment for PJP in non-HIV-infected individuals, at a dosage of 15–20 mg/kg/day of TMP and 75–100 mg/kg/day of SMZ, administered orally or intravenously in 3–4 divided doses for 21 days. Alternative therapies include intravenous pentamidine (4 mg/kg/day), primaquine/clindamycin (30 mg/day plus 600 mg every 8 hours), and atovaquone (750 mg every 8–12 hours). Based on the patient's symptoms and signs: cough, mainly dry cough; fever; dyspnea on minimal exertion; resting arterial oxygen saturation maintained between 90–95%; and chest CT showing diffuse interstitial shadows in both lungs, the patient's Pneumocystis jirovecii pneumonia was classified as moderate in severity. The patient received a therapeutic dose of TMP/SMZ (0.48 g three times daily) for the treatment of PJP.

Treatment of Pneumocystis Jirovecii Pneumonia (PJP) in Non-HIV Organ Transplant Recipients

PJP has emerged as a significant concern in non-HIV patients, particularly solid organ transplant recipients. This is due to the potential for PJP to manifest as severe pneumonia in post-transplant patients and contribute to nosocomial

infections.[25] Among organ transplant recipients, kidney transplant recipients are at particularly high risk for PJP.[26] PJP frequently occurs within the first 6 months after kidney transplantation,[27] a period of profound immunosuppression. The use of potent immunosuppressants, including tacrolimus, mycophenolate mofetil, corticosteroids, and anti-thymocyte globulin (ATG), along with factors such as history of acute rejection, frequent anti-rejection treatments, cytomegalovirus (CMV) infection, and pre-transplant desensitization, contribute to the persistently high prevalence of PJP in transplant recipients.[28] For example, in a study of patients with CKD, the PJP group was significantly more likely to receive cyclophosphamide than the control group.[29] In contrast, PJP is less common in liver transplant recipients, with an incidence of 1%-11% and a mortality rate of 7%-88%.[30] In liver transplant patients, everolimus was used significantly more frequently in PJP patients than in the control group, and blood levels of everolimus were higher in PJP patients 180 days before diagnosis than in the control group, while there was no difference between tacrolimus groups.[31]

In Case 2, this kidney transplant recipient received long-term maintenance immunosuppression using the calcineurin inhibitor tacrolimus as the primary drug, in conjunction with mycophenolate mofetil and corticosteroids. Tacrolimus, a calcineurin inhibitor, exerts its immunosuppressive effects by inhibiting T cell activation and proliferation and reducing interleukin-2 production via calcineurin inhibition.[32] Lymphopenia is a known risk factor for late-onset PJP.[33] Acute rejection following kidney transplantation indicates an activated immune system, whereas PJP typically occurs in immunocompromised individuals. Thus, acute rejection typically precedes PJP. Anti-rejection therapy suppresses the immune system, resulting in lymphopenia, particularly a decline in CD4+ T cell counts.[34] Although there are differences among different studies, in general, these iatrogenic immunosuppressions have been widely shown to be risk factors for PJP. However, clinicians usually only focus on the use of corticosteroids, but cell-toxic drugs and biological therapies should also be given equal attention.Individualized risk assessment and tailored prophylaxis duration could be a more effective strategy for PJP prevention, reducing incidence, healthcare costs, and mortality. A study of 1469 kidney transplant recipients, of whom 81.21% received prophylactic TMP/SMX (20 mg TMP/100 mg SMX), found that both daily and every-other-day regimens were effective in preventing PJP and well-tolerated, with low rates of adverse drug reactions such as liver and kidney dysfunction, bone marrow suppression, rash, and Stevens-Johnson syndrome.[35] Based on these findings, the Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend universal PJP prophylaxis with daily TMP/SMZ for 3–6 months after kidney transplantation.[36]

How to manage the prognosis of PJP in patients on long-term immunosuppressive therapy is particularly important. Factors associated with poor prognosis of PJP include: aging; Past episodes of PJP; Cytomegalovirus was present in bronchoalveolar lavage fluid. Serum lactate dehydrogenase concentration increased; CD4+T cell count was low.[37] PJP as an opportunistic fungal infection, that is, the occurrence of disease is based on the reduction or destruction of immunity, and lymphocytes are the core cellular components of the body's immune response. Therefore, the decrease of lymphocytes, especially CD4+T cells, due to various reasons has been identified as a risk factor.[38] In such studies, the reduction of lymphocytes and CD4+T cells was generally limited to < 750 / mm3 and < 200 / mm3. In addition, in a mouse experiment, CD8+T cells were found to help protect the mice from the development of PJP in individuals with significantly reduced CD4+T cells.[39] Therefore, although the number of CD8+T cells is influenced by CD4+T cells, based on such studies and CD8+T cell function, it should be considered that not only CD4+T cell reduction is a risk factor for PJP development, but also CD8+T cell reduction is associated with PJP development. When both are reduced, more attention should be paid to the patient's likelihood of developing PJP. The prognosis of interstitial pneumonia is closely related to whether timely targeted treatment is given. Once diagnosed, immediate and adequate treatment is required, including: (1) anti-pathogenic agent therapy; (2) Active oxygen therapy, including nasal catheter oxygen, mask oxygen, non-invasive/invasive ventilator oxygen, etc.; (3) Reduce oxygen consumption, such as braking, control gastrointestinal food intake, etc.; (4) symptomatic treatment, supportive treatment and prevention of complications. For patients with immunosuppressive state, due to the decreased ability of the body to clear PJP, the lung damage is more serious, it is necessary to actively reduce the dose of immunosuppressive agents or even stop using them, and gradually resume the use after the condition is significantly improved. In addition, further discussion is still needed on the dosage of TMP-SMZ, which is the first-line drug for the prevention and treatment of PJP. TMP [15 to 20 mg/(kg·d)] and SMZ [75 to 100 mg/(kg·d)], for a total course of 21 days, are the standard treatment protocols for HIV-infected PJP patients, but there are no guidelines for therapeutic dosages for non-HIV-infected PJP patients, especially those in the non-

immunosuppressed state.[40] Some scholars have suggested that the recommended duration of treatment for non-HIV-infected PJP patients is 14 days, and can be extended as appropriate.[27] In addition, the study found that treating non-HIV-infected PJP patients with the same dose of TMP-SMZ may lead to an increased incidence of side effects such as hyponatremia, renal insufficiency, and even affect prognosis.[41] At present, the feasibility of low dose TMP-SMZ [trimethopril <15 mg/(kg·d)] has been confirmed. Compared with high-dose regimens, low-dose regimens do not increase the risk of death and have advantages in reducing discontinuation due to drug-related side effects. Therefore, the specific treatment options for PJP patients who are not HIV-infected still need to be further validated by more studies.

Conclusion

The above two cases of patients with Pneumocystis jirovecii pneumonia combined with various pathogens infection present a complex condition and require a wide variety of drugs. During the process of diagnosis and treatment, we should fully utilize the high diagnostic value of mNGS for non-HIV infected patients, and it is particularly crucial to customize individual treatment plans based on the immune status. In the course of diagnosis and treatment, we need to pay timely attention to the progress of patients' lung diseases, the therapeutic effect and adverse reactions after medication, and also focus on the control of patients' underlying diseases and complications after discharge, so as to enhance the quality of life of patients.

Ethics Approval and Informed Consent

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Ethical approval for this study was Provided by the Ethics Committee of the Medical Faculty of Qinghai University, Qinghai Province, China(P-SL2023-035). The patients themselves in both cases provided written informed consent, which included consent to the publication of details of the case and any accompanying images. The Ethics Committee of Qinghai University agreed to publish all the details of the two cases in this paper.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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