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Case Report: Pneumocystis Pneumonia Following Liver Transplantation Identified by High-Throughput Second-Generation Gene Sequencing Technology

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Abstract: Pneumocystis, a fungus species, has emerged as one of the predominant infectious pathogens in immunocompromised patients, particularly in liver transplant recipients who require immunosuppressants. The pathogen colonizes the recipient's lower respiratory tract and induces a pulmonary infection. Respiratory failure is one of the leading causes of mortality in organ transplant recipients, as the disease progresses swiftly, its diagnosis is difficult, and it is simple for it to occur. This report details the case of a patient admitted to Calmette Hospital, affiliated with Kunming Medical University, diagnosed with pneumocystis pulmonary infection post liver transplantation. The diagnosis and successful treatment of the patient were facilitated by high-throughput second-generation gene sequencing technology based on metagenomics, enlightening future clinical diagnoses and treatments.

Keywords: Liver Transplantation; Pneumocystis; Metagenomics

1. Introduction

Pneumocystis pneumonia(PCP) is an opportunistic infection caused by pneumocystis pneumonia, which predominantly accumulates in the airways. Due to the disease's insidious onset and rapid progression, the lack of rapid and specific diagnostic methods in the past poses a grave threat to the lives of patients. This paper describes the successful treatment of pneumocystis pneumonia in a liver transplant recipient using Metagenomic next generation sequencing (mNGS) technology.

2. Case Presentation

A 28-year-old male was admitted to the hospital on February 17, 2021 with the chief complaint of "fever for six days, six months after liver transplantation." Six days prior to admittance, the patient experienced high fever (up to 41°C), cough, white and thin sputum due to a cold, along with chest discomfort, shortness of breath after activities, and fatigue. The physical examination of the patient revealed low vitality, minor coarse breath sounds in both lungs, and no evident rales. There were no positive abdominal indications. The patient underwent allograft orthotopic liver transplantation on September 20, 2020 at a different hospital due to acute liver failure and chronic viral hepatitis B. He recovered well and was discharged. The patient was given Tacrolimus sustained release capsule 4 mg/day, Sirolimus 1 mg/day, Prednisone acetate 5 mg/day, and Tenofovir alafenamide Fumarate tablets 25 mg/day after being discharged. Mycophenol sodium enteric-coated tablet 360 mg, twice daily; Ursodeoxycholic acid capsule 250 mg, twice daily. Regular follow-up in other facilities and discontinuation of hormone therapy.The patient was diagnosed with acute rejection following liver transplantation after undergoing a liver biopsy on January 6, 2021 due to irregular use of anti-rejection medications. In addition to Methylprednisolone pulse therapy, the patient received Tacrolimus sustained release capsule 7 mg/day, Sirolimus 1 mg/day, and Propofol tenofovir fumarate tablets 25 mg/day. Ursodeoxycholic acid capsule 250 mg/time, Mycophenolate sodium enteric-coated tablets 540 mg/time, and Kadsura japonica L. soft capsule 0.6 g/time, all three administered twice daily. The patient's condition improved following treatment, and he was discharged from the hospital.At the time of admittance, the patient was taking Tacrolimus sustained-release capsules 4 mg per day, Sirolimus 1 mg per day, Prednisone acetate 5 mg per day, and Tenofovir propofol fumarate tablets 25 mg per day. Ursodeoxycholic acid 250 mg twice daily and Mycophenolate sodium enteric-coated 360 mg twice daily.White blood cell count was $14.28*10^9$/L, neutrophil percentage was 75.7%, C-reactive protein was 118.77 mg/L, procalcitonin was 0.995 mcg/L, and fungal 1, 3-β-D-glucan (G test) was 235.73 pg/ml. The ratio between $CD4^+$ T cells and $CD8^+$ T cells was 1.11, and the concentra-

tion of FK506 in the blood was 6.2 ng/ml. alanine aminotransferase was 199 unit/L, aspartate aminotransferase was 161 unit/L, γ-glutamyl transpeptidase was 1160 unit/L, and alkaline phosphatase was 278 unit/L. The serum creatinine concentration was 60.3 umol/L. Negative IgM serum antibodies were detected against respiratory syncytial virus, adenovirus, influenza A virus, influenza B virus, parainfluenza virus, Chlamydia pneumoniae, Legionella pneumophila bacteria, and Mycoplasma pneumoniae. Negative results were obtained for the Aspergillus galactomannan test (GM test), sputum bacterial culture, fungal culture, and blood culture. Chest radiograph revealed radiographic evidence of pneumonia in both lungs, specifically pulmonary infection (Figure 1A). Chest computed tomography (CT) revealed extensive infectious exudative lesions in both lungs (Figure 1B). The patient was diagnosed with pulmonary infection and received antibacterial therapy with Cefoperazone sodium and sulbactam sodium 3 g every 8 h, as well as the initial dose of Caspofungin 70 mg, followed by 50 mg daily. Prednisone acetate was discontinued, Sirolimus was reduced to 0.5 mg once daily, Mycophenolate sodium enteric-coated tablets were reduced to 180 mg twice daily, and all other oral medications were continued at the original dose. However, the patient continued to exhibit elevated fever and worsening dyspnea. Analysis of arterial blood gases revealed that PaO^2 was less than 70 mmHg. The medication was altered on February 24 to include Imipenem and cilastatin sodium 0.5 g every 8 h, Micafungin 100 mg once daily, and Compound Sulfamethoxazole 0.96 g four times daily. The reexamination of the chest X-ray on February 25 revealed signs of bilateral pneumonia, including pulmonary infection and dense local lesions, and bilateral pneumonia that was more severe than before (Figure 1C). To prevent pulmonary fibrosis and rejection, intravenous Methylprednisolone 40 mg was administered daily. Imipenem was discontinued immediately, while Micafunzin and Compound Sulfamethoxazole were continued for two and three weeks, respectively. Simultaneously, Methylprednisolone was gradually discontinued. A reexamination of the patient's chest computed tomography(CT) on March 12 revealed extensive infectious exudative lesions in both lungs that were largely absorbed (Figure 1D). The blood concentration of FK506 was 4.9 ng/ml. Total bilirubin was 16.46 mol/L, direct bilirubin was 10.07 umol/L, and indirect bilirubin was 6.39 umol/L. Additionally, alanine aminotransferase was 77 unit/L, aspartate aminotransferase was 64 unit/L, -glutamyl transpeptidase was 527 unit/L, and alkaline phosphatase was 67 unit/L. The levels of serum creatinine, 1, 3--D-glucan (G test), and procalcitonin were 50umol/L, 46.48 pg/ml, and 0.119 mcg/L, respectively. Immunosuppressive therapy included oral Tacrolimus sustained release capsule 4 mg/day and Sirolimus 1 mg/day. Mycophenolate sodium enteric-coated tablets, 180 mg twice per day. Close post-discharge monitoring revealed that the patient did not experience chest tightness, shortness of breath, or abnormal physical activity.

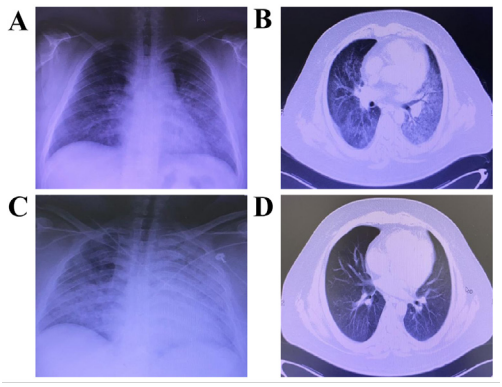

Figure 1 Chest X-ray on 17 February (A) , Chest computed tomography on 17 February (B), Chest X-ray on 25 February (C) ,Chest computed tomography on 12 March(D)

3. Discussion

Pneumocystis pneumonia (PCP), now referred to as Pneumocystis jirovecii pneumonia, is a fungal infection that typically affects immune function. This includes those with acquired immunodeficiency syndrome, organ transplant recipients, and individuals undergoing chemotherapy for malignant tumors or with autoimmune diseases. It also carries a high morbidity and mortality rate [1-2]. Due to the use of a large number of immunosuppressants in the early phase of anti-rejection after liver transplantation, the recipient's immunosuppression is severe, and the majority of cases develop within 6-9 months, more than 70% of cases develop within 12 months after liver transplantation, but

the development can occur as early as 1-3 weeks after liver transplantation [3-4]. The incidence rate among organ transplant recipients ranges from 5 to 15%, and the mortality rate can reach as high as 38% due to atypical clinical manifestations, rapid disease progression, critical illness, and difficulties in diagnosis and treatment, [5-6]. Therefore, rapid and accurate identification of pathogenic microorganisms and early clinical interventions are crucial for reducing mortality and enhancing the prognosis of PCP patients.

PCP is believed to be transmitted from person to person via the airborne route, asymptomatic colonization of the lungs may occur in individuals with healthy immune systems, and these individuals may unwittingly serve as reservoirs for Pneumocystis transmission to immunocompromised individuals [7].The clinical manifestations of PCP are fever, cough, chest tightness, dyspnea, etc., and the positive signs of specialist physical examination are usually less, dyspnea gradually appears within 1-2 weeks, and respiratory failure will occur in serious cases. The pulmonary signs are often not proportional to the severity of respiratory distress.In the typical imaging examination for PCP, chest X-rays demonstrate bilateral perihilar diffuse infiltrates, which become progressively more severe as the disease progresses. The computed tomography(CT) examination revealed shadows of ground glass density or cystic lesions. The primary diagnostic criterion for PCP still necessitates the detection of respiratory bronchoalveolar lavage fluid or sputum samples, but the vast majority of patients are critically ill and cannot tolerate it, thereby impeding clinical operations. If a pathogen infection is caused by a complex pathogen, deficiencies such as a lengthy cycle and limited precision can lead to diagnostic delay or neglect [7]. Due to the time-consuming and low positive detection rate of conventional detection methods, there is an imperative need for pathogen detection and identification techniques with greater diagnostic efficacy.

The rapid development of mNGS technology in recent years has made it a potent tool in medical microbiology, particularly for the detection of uncommon or emerging pathogens. mNGS has greater diagnostic advantages than conventional methods and provides a theoretical foundation for the diagnosis of human pathogens [8]. As a novel DNA/RNA sequencing technique, mNGS can directly extract the sample's nucleic acid sequence for detection, and then acquire pathogen data via bioinformatics analysis, which can theoretically identify all microorganisms [9]. Compared to the sequencing technology of the first generation, mNGS has thediagnostic advantages of high efficiency, high throughput, high sensitivity, and low cost [10]. It has been demonstrated that mNGS has a high clinical application value in the etiological detection and identification of respiratory tract infection [11].

This patient's primary clinical symptoms were fever, cough, expectoration, chest constriction, and shortness of breath. Specialized physical examination and supplementary examination revealed no evidence for the diagnosis of PCP.Due to the patient's severe hypoxemia and inability to cooperate with the operation, bronchoalveolar lavage was not performed at the time of admission. The symptoms were initially relieved by empirical broad-spectrum antibiotic therapy, but they progressively deteriorated until dyspnea developed. The results of a routine blood test revealed that the white blood cell and neutrophil counts were lower than in the past, indicating the absence of a bacterial infection. The result of G test was increased, and the possibility of fungal infection with rare pathogens was considered. The diagnosis was confirmed using mNGS technology, the treatment plan was actively modified, and the patient's condition improved progressively. In our experience, PCP should be considered and mNGS should be performed when pulmonary infection occurs in liver transplant recipients whose pulmonary signs are not proportional to the severity of respiratory distress, when traditional etiological tests are repeatedly negative, and when conventional anti-infective therapy is ineffective. After diagnosis, Compound Sulfamethoxazole tablets and Micafungin combination therapy is advised. The combination therapy was effective in treating this case, and it is deserving of applicability in PCP cases.

In conclusion, organ transplant recipients have a compromised immune system, mNGS technology should be utilized when mixed pathogens and rare pathogens are suspected in clinical diagnosis and treatment, particularly in the case of severely ill patients who who require prompt diagnosis to reduce the duration of bacterial and fungal infections and promote targeted antimicrobial treatment. The use of mNGS technology provides significant benefits in terms of improving the recipients' prognosis.

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