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Case Report

Metagenomic next-generation sequencing assisted in the successful treatment of pneumonia caused by *Talaromyces marneffei* in an immunocompetent patient

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Abstract

Introduction: *Talaromyces marneffei* (*T. marneffei*), a kind of endemic opportunistic pathogen, was previously thought to occur in HIV-positive individuals and non-HIV hosts with impaired immune function. However, the infection of *T. marneffei* in patient with normal immune function was rarely reported.

Case Presentation: We report a case of severe pneumonia caused by *T. marneffei* in an immunocompetent and HIV-negative patient, which was rapidly confirmed by metagenomics next-generation sequencing (mNGS) and treated successfully. The patient was a previously healthy 63-year-old male, who was admitted to hospital with fever for 11 days, cough and sputum for 1 week, and chest distress for 4 days. The infection of *T. marneffei* was quickly determined by alveolar lavage under bedside bronchoscope and mNGS test.

Results: Patient's condition improved rapidly after voriconazole treatment, and he was evaluated as a HIV-negative case of *T. marneffei* infection with normal immune function. This is a sporadic case of *T. marneffei* in non-endemic areas, and mNGS played a very important role in the treatment of the disease. The patient's immune function was relatively normal which was rare in clinical practice.

Key words: *Talaromyces marneffei*; metagenomic next-generation sequencing (mNGS); immune function.

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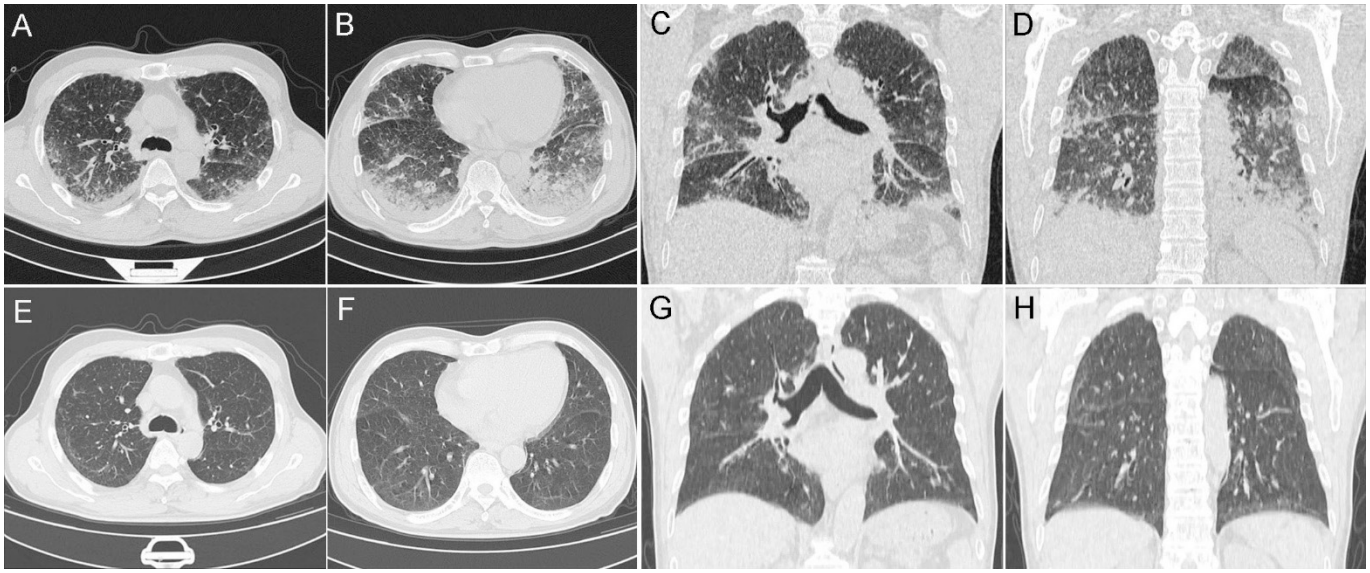
Introduction

Talaromyces marneffei (*T. marneffei*) is a highly pathogenic endemic opportunistic pathogen, which mainly infects people with low immunity in southeast Asia and southern China [1-4]. *T. marneffei* infection in acquired immune deficiency syndrome (AIDS) patients is relatively common clinically. In recent years, clinical attention has also been paid to the possibility of *T. marneffei* infection in HIV-negative patients such as malignant tumors, recipients of organ transplantation, and patients receiving long-term steroid hormone or cytotoxic drug therapy [2,3,5-7]. However, there are relatively few reports of *T. marneffei* infection in patients with normal immune function. A previous retrospective study showed that 87.7% of the patients infected with *T. marneffei* in China were HIV-positive, 12.3% were HIV-negative, and 8.5% had no underlying disease [8]. Metagenomic next-generation sequencing (mNGS), as an emerging etiological diagnostic technology, has played an increasingly important role in the rapid and accurate diagnosis and treatment of critical and difficult infections in recent years [9,10]. Compared with traditional microbiological testing methods, mNGS technology has the advantages of high sensitivity, high specificity, unbiased and rapid [11]. Here we present a case of severe pneumonia caused by *T. marneffei* in an HIV-negative patient with normal immune function, which was rapidly identified by mNGS and successfully treated in time.

Case presentation

A 63-year-old male patient was admitted to our hospital due to fever for 11 days, cough and expectoration for 1 week, and chest tightness for 4 days on December 30, 2021. The patient was hospitalized at a local hospital (Xianju People's Hospital, Zhejiang Province, China) for treatment before admission, and the lung lesions significantly progressed after receiving anti-infection and other treatments. On December 29, 2021, chest computed tomography (CT) performed in

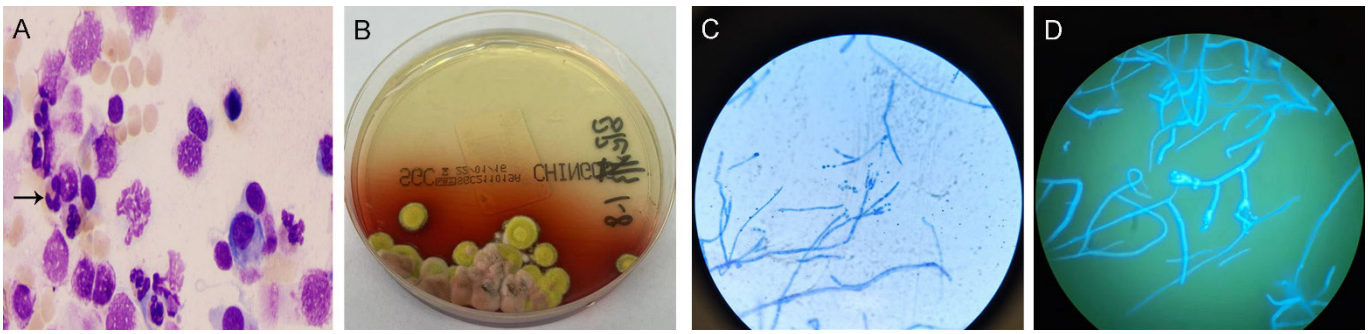
Figure 1. Chest computed tomography (CT) findings (A, B, C, D) were observed before admission to the local hospital, and both lung lesions were obviously absorbed after 12 weeks of voriconazole treatment (E, F, G, H).



the local hospital showed exudation and consolidation in both lungs near the outer zone of the lungs, mainly in the lower lobe and the bottom of the lung (Figure 1). The patient was transferred to our hospital due to deterioration of oxygenation compared with before, with an oxygen saturation of 88% (no oxygen intake). The emergency blood gas analysis indicated that arterial partial pressure of oxygen (PaO_2) was 56 mmHg (partial pressure of oxygen/fraction of inspiration O_2 193.1 mmHg) and arterial partial pressure of carbon dioxide ($PaCO_2$) was 32 mmHg with PH 7.46. He was admitted to the ward with the diagnosis of severe pneumonia, type I respiratory failure, and interstitial lung disease to be ruled out. The patient had a history of catheter radiofrequency ablation but denied a medical history of special drugs, drug abuse, HIV, organ transplantation, tumor chemoradiotherapy, diabetes, etc. He had a smoking history of over 40 years, with an average of 10 cigarettes per day, and denied alcoholism.

On admission, his temperature was 36.3 °C, pulse rate was 82 beats per minute, respiratory rate was 28 breaths per minute, and blood pressure was 127/73 mmHg. He had no clubbing of fingers, icterus, or generalized lymphadenopathy. Clinical examination of cardiovascular, gastrointestinal, and nervous systems was normal but some crackles were found in both lungs during the lung auscultation. He was admitted to the respiratory intensive care unit (RICU) and treated with high-flow nasal cannula oxygen therapy (HFNC), empirical anti-infection, and glucocorticoid anti-inflammatory therapy. Bedside bronchoscopic examination was performed immediately on the second day of admission, and related etiological tests were carried out by alveolar lavage in the posterior basal segment of the left lower lobe. The classification of cells in alveolar lavage fluid suggested that neutrophils accounted for 51%, lymphocytes for 23%, and phagocytes for 24% (Figure 2A). The overall condition of the patient was not significantly improved, and the

Figure 2. Cell classification of bronchoalveolar lavage fluid (BALF) suggested a high proportion of neutrophils (× 1000, black arrow) (A). *Talaromyces marneffei* growth was seen on day 7 of BALF culture (B). Typical fungal hyphae (× 100) were seen by lactophenol cotton blue staining (C) and fluorescence staining (D).



aerobic concentration was gradually increased to 60-70%. Relevant laboratory tests indicated that anti-nuclear antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), anti-myositis antibodies, and HIV antibody were negative. The T lymphocyte subsets showed that the absolute value of CD4 was 351/uL (normal range: 414-1123/uL). The alveolar lavage fluid underwent a series of procedures, such as freezing storage (-20 °C refrigerator), DNA extraction, library construction, sequencing, data analysis, etc. [12,13]. On the afternoon of the fourth day of admission, mNGS reported that the sequence of *T. marneffei* was 9494 with a total coverage of 1.58% of the whole genome (Figure 3). Therefore, we adjusted the treatment plan to voriconazole injection and stopped glucocorticoid, and the patient's condition improved rapidly. On January 8, 2022, a small amount of *T. marneffei* growth was observed in the culture of alveolar lavage fluid (25 °C culture of Sabouraud's medium) (Figure 2B). Thus, the diagnosis of severe pneumonia of *T. marneffei* was clear. In order to find out whether the patient had immune deficiency caused by other factors, we further tested for γ-interferon (γ-IFN) antibody and the result was negative (Kindstar Globalgene Technology, Inc. Wuhan, China). The whole exome sequencing of peripheral blood was tested two times (Dian Diagnostics Group Co., Ltd. Hangzhou, China and BGI Genomics, Shenzhen, China) and no abnormal genes related to immune deficiency were found. Therefore, we considered the patient as an HIV-negative case of *T. marneffei* infection with relatively normal immune function. After the targeted treatment, the patient's

Figure 3. The genome coverage of detected *Talaromyces marneffei* sequences. A total of 9494 reads mapped to *T. marneffei* were detected in BALF, conducted a total coverage of 1.58% of the whole genome.

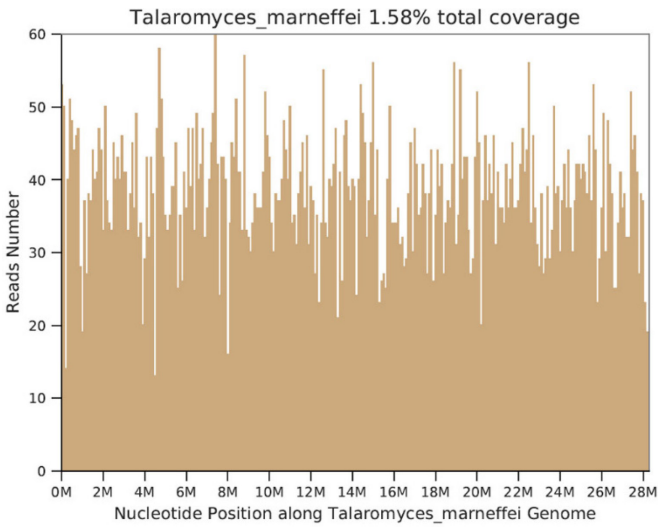
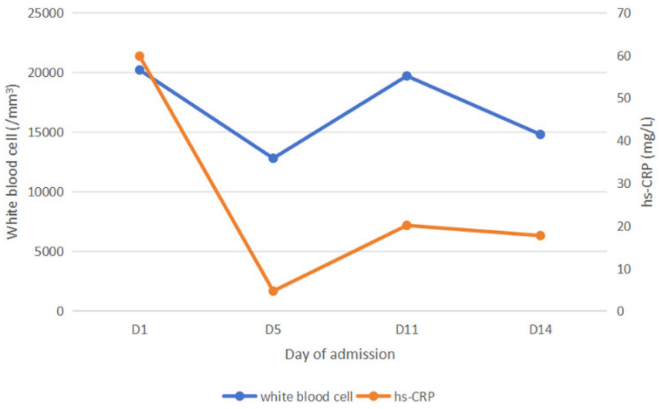


Figure 4. Patient's laboratory parameters throughout the hospitalization period.



condition recovered quickly. His white blood cells and inflammatory indicators gradually decreased (Figure 4). On January 10, 2022, the patient was transferred out of the RICU and into the general ward for treatment. After CT review of absorption of both pulmonary lesions, the patient was discharged from the hospital on January 13, 2022. Anti-fungal therapy with voriconazole tablets (200mg q12h) was continued after discharge and stopped at 16 weeks, with good image absorption (Figure 1).

Discussion

The most common problem faced by respiratory doctors in clinical practice is whether the patient's disease is infectious or non-infectious, which is also observed in this case. The patient's oxygenation worsened and lung imaging progressed after treatment with anti-infectious and anti-inflammatory drugs in the local hospital. CT revealed symmetrical exudation and consolidation in both lungs, mainly in the middle and lower lobes of the lung. It was very difficult to distinguish between pulmonary infectious and non-infectious lesions, so 'severe pneumonia' was considered as the first diagnosis but 'rapid-progressed interstitial lung disease (RP-ILD)' caused by connective tissue diseases were not excluded. We chose a broad spectrum of antibiotics plus a high dose of intravenous glucocorticoids as the initial empiric treatment. Meanwhile, we quickly performed an etiological evaluation of alveolar lavage with HFNC-supported bedside bronchoscopy. *T. marneffei* was confirmed by mNGS within 48 hours after alveolar lavage. At the same time, the patient's other laboratory tests did not suggest evidence of collagenous vascular or connective tissue disease, so the possibility of pulmonary infectious lesions was considered to be high clinically and his symptoms improved rapidly after

precise treatment for *T. marneffei*. In this case, we kept in close communication with the laboratory department. Due to the slow growth of *T. marneffei*, no bacterial growth was observed in the alveolar lavage fluid cultured for 2 days, but small yellowish-brown yeasty colonies were observed after 4 days of 25 °C culture of Sabouraud's medium, and typical soluble wine red pigment was observed to spread into the agar after 7 days culture. Technology of mNGS rapidly and accurately locked suspected pathogens (less than 48 hours from bronchoscopic sampling to obtaining results), guiding the direction of clinical diagnosis and treatment, making up for the shortcomings of traditional laboratory examination with low sensitivity and delayed culture time, and playing a decisive role in the reversal of patient's disease. The gold standard for the diagnosis of talaromycosis is to isolate and culture *T. marneffei* from various clinical specimens, including blood, skin, bone marrow, lymph nodes, and sputum [14]. The successful isolation and culture of *T. marneffei* from the alveolar lavage fluid of this patient confirmed the results of mNGS. In this case, the close communication between clinical and laboratory was exemplary, and the diagnosis and treatment of etiology formed a very good closed loop.

According to the existing literature reports, talaromycosis occurs mostly in southeast Asia and southern China, such as Thailand, Vietnam, Taiwan, Guangdong province, Guangxi province and other places [1]. This patient was located in eastern China (Xianju County, Zhejiang Province), and had no history of travel to overseas or epidemic areas, nor host factors with impaired immune function. Why did he develop *T. marneffei* infection? We performed an adequate autoimmune deficiency-related assessment with negative γ-IFN antibody, no potential immunodeficiency-causing gene detected by two peripheral blood whole exon sequencing, routine negative HIV antibody, and CD4 cells slightly below the lower limit of normal (presumably related to systemic glucocorticoid use). No evidence of impaired immune function was found in this patient. Therefore, this patient was considered to be infected with *T. marneffei* with relatively normal immune function, and t was a sporadic case in non-endemic areas in eastern China, which was extremely rare clinically. Wang *et al.* [2] reported a case of acute disseminated talaromycosis in a 59-year-old male patient with normal immune function in 2017. The patient presented multiple subcutaneous masses on the forehead, neck and chest wall, and enlarged mediastinal lymph nodes, which was confirmed as *T. marneffei* infection by puncture fluid culture of subcutaneous mass on the forehead and marrow culture. After voriconazole treatment, acute respiratory distress syndrome (ARDS) was complicated, but later recovery was good. Shi *et al.* [5] described another case of *T. marneffei* pneumonia diagnosed by mNGS and sputum culture in 2021. The patient was a 79-year-old male with diabetes but had no other underlying diseases, and recovered well after treatment with voriconazole. Interestingly, similar to our case, these two cases were also from Zhejiang Province, China (the former from Hangzhou, the latter from Ningbo), however, no in-depth assessment of basic immune function was performed in these two cases. However, this reminds us that despite being in an area where *T. marneffei* is not endemic, it is necessary to consider the possibility of infection of special pathogens including *T. marneffei* when the clinical treatment is tortuous and the effect of conventional therapy is not good.

There are also some limitations in this case. First, although the patient's immune function was evaluated in detail in this case, the patient's living environment was not investigated in detail, and it was unclear whether the patient's living environment was related to the infection of *T. marneffei*. Second, this is only a case report, and large-scale retrospective case studies or prospective case studies are needed to evaluate or explore the infection status and treatment recommendations of *T. marneffei* in HIV-negative patients in non-endemic areas.

In conclusion, this is a case of severe pneumonia caused by *T. marneffei* from eastern China (non-endemic area). In terms of pathogen diagnosis, the emerging diagnostic technology (mNGS) and the traditional laboratory diagnostic technology (microbial culture) corroborate each other, and the rapid diagnosis of the pathogen by mNGS which was produced six days earlier than traditional microbial culture reversed the disease. We have fully evaluated the immune function of the patient, and the patient belongs to the HIV-negative host with relatively normal immune function. The above three points are worthy of clinical reference.

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