IDCases 23 (2021) e01055

Contents lists available at ScienceDirect

IDCases

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Case report

Rapid diagnosis of *Talaromyces marneffei* infection assisted by metagenomic next-generation sequencing in a HIV-negative patient

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ARTICLE INFO

Article history:
Received 29 October 2020
Received in revised form 19 January 2021
Accepted 19 January 2021

Keywords:
Talaromyces marneffei
Metagenomic next-generation sequencing
Fungi infection diagnosis
Whole genome sequence

ABSTRACT

Talaromyces marneffei (T. marneffei), is an opportunistic pathogenic fungus commonly reported in southeast Asia. *T. marneffei* infection predominantly occurs in patients with immunodeficiency and can be fatal if diagnosis and treatment were delayed. Conventional diagnosis of *T. marneffei* infection relies heavily on tissue culture and histologic analysis, which is time consuming and has limited positive rate. Rapid and accurate diagnosis of *T. marneffei* remains urgent for effective therapy and prevention. This case is the first reported *T. marneffei* infection in non-HIV patients in north China diagnosed by mNGS. The successful diagnosis of *T. marneffei* infection assistant by mNGS underlies the potential of this technique in rapid etiological diagnosis.

Introduction

Talaromyces marneffei, previously named as *Penicillium marneffei*, is an opportunistic pathogenic, temperature-dependent dimorphic fungus which is commonly reported in southeast Asia [1,2]. *T. marneffei* is able to invade multiple organs/systems such as bone marrow, blood, central nervous system, lungs and skin and can be fatal for patients if not diagnosed and treated in time. *T. marneffei* infection often occurs in patients with immunosuppression, such as HIV patients and those receiving immunosuppressive treatment [3]. Conventional diagnosis methods of *T. marneffei* infection are based on the identification of fungi by histopathological staining, culture and microscopy, which has low sensitivity and is time-consuming [4]. Other techniques for *T. marneffei* detection include PCR-based molecular methods and immunological test [5,6] have low diagnostic yield rate and lack of diagnosis experience from southeast Asia. Hence, timely and accurate diagnosis of *T. marneffei* infection remains challenging.

Case report

A 29-year-old male who had been suffering from dyspnea for more than five months was admitted to PUMCH in June 2019. Previously, he sought for medical advice in a local hospital due to a continuous low-grade fever in January 2017, where he was diagnosed as pulmonary tuberculosis (TB) and 4HREZ was used as therapeutic scheme at that time. He neither took the treatment appropriately nor revisited the clinician regularly. He visited a local lung hospital when chest and back pain developed on October 2017, chest CT showed worsened infiltration with an emerging cavity in right upper lobe, and was diagnosed as secondary

Abbreviations: AIDS, acquired Immune Deficiency Syndrome; BALF, Bronchoalveolar lavage fluid; CRP, C-reactive protein; DNA, deoxyribonucleic acid; DPLD, diffuse parenchymal lung disease; HIV, human immunodeficiency virus; mNGS, metagenomic Next generation sequencing; NTM, non-tuberculosis mycobacteria; PCR, polymerase chain reaction; PCT, procalcitonin; PUMCH, Peking Union Medical College Hospital; TBLB, transbronchial lung biopsy; SNP, single nucleotide polymorphism.

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http://dx.doi.org/10.1016/j.idcr.2021.e01055

pulmonary tuberculosis. He then was received a two-month therapy of prednison (40 mg qd, reducing by 5 mg per week) as well as an anti-TB medication regimen of 3HRZEV/6HRE.

With no alleviation of chest pain, the patient was admitted to PUMCH in April 2018. Transbronchial lung biopsy (TBLB) analysis detected epithelioid granulomas and multinucleated giant cells with a positive result of weakly acid-fast stain. The patient was still highly suspected to have tuberculosis, while the possibility of non-tuberculosis mycobacteria (NTM) infection was not excluded. In this case, both TB and NTM infections treatment were prescribed. Nevertheless, after half year's treatment with HRE and clarithromycinthe, patient had not improved and developed progressive dyspnea. He revisited PUMCH on June 6, 2019 with dyspnea, since when anti-TB and anti-NTM treatment were stopped as there was no alleviation of the pulmonary process. With the pneumothorax, pleural effusion, a cavity in left upper lobe, multiple ground-glass opacity areas and nodules in both lung (Fig. 1A, B and C) shown by chest CT, the patient was considered having diffuse parenchymal lung disease (DPLD). Therefore, it was urgent to identify the etiology of this lung lesion, because the choice of treatment plan highly depended on the exact subtype of DPLD. At same time, the patient reported no significant past medical history and denied any history of chronic diseases, other infectious diseases or exposure to toxic matter and epidemic area and water. However, the patient claimed that he had eaten bamboo rat meat not long before the development of initial symptoms.

Peripheral blood examination showed a marked eosinophilia (520 cells/μL), an elevated procalcitonin (PCT) at 19 μg/L (normal range < 0.5 μg/L) and normal level of C-reactive protein (CRP) of 4.0 mg/L, white blood cell (WBC) of 5.70×10^3 cells/μL, and erythrocyte sedimentation rate (ESR, of 19 mm/h (normal range 0−25 mm/h), HIV antigens and antibodies were all negative. The patient was treated with clarithromycin 500 mg bid, but did not improve. On the 7th day of hospitalization, tracheoscopy and TBLB tests were conducted and detected chronic pulmonary inflammatory response but without explicit necrosis or focal granulomatous formation. In addition, TBLB also had negative results in acid-fast stain test and GeneXpert TB-molecular test. BALF had negative result in mycobacterium culture too. However, the patient developed fever after the tracheoscopy test, hence amikacin and amoxicillin were administrated with the dose of 400 mg q12 and 400 mg qd, respectively. The body temperature went back to normal.

BALF and TBLB samples collected on the 7th day of hospitalization showed positive culture result on the 9th day, when hyphae was discovered on fungus culture plate (28℃ culture) but without fungi identification, providing support for possible fungal infection. The patient began to received itraconazole (200 mg bid) for antifungal therapy. Meanwhile, mNGS analysis of the same BALF sample was performed on the 12th day (Supplement methods). On the 14th day of hospitalization, the mNGS analysis detected the nucleotide sequences of *T. marneffei* in the BALF, with a total of 3207 reads of *T. marneffei* covering 0.5564 % of the total genome (159393/28648375, Fig. 2). On the same day, the cultured fungi in TBLB and BALF were both identified as *T. marneffei* by Vitek MS system (bioMérieux, Marcy-l'E'toile, France). It was all sensitive to itraconazole, amphotericin B and voriconazole (MIC was 0.04, 0.32 and ≤0.02 respectively). Culture and mNGS analysis had results in accordance, confirming the possibility of *T. marneffei* infection. Hence, all the antibacterial drugs were withdrawn, only antifungal therapy with itraconazole (200 mg Bid) was continued.

After another week of anti-fungi treatment, the patient's dyspnea was relieved. On October 21, the patient paid a return visit to PUMCH and the chest CT scan revealed significant improvement of what? and alleviation in inflammation (Fig. 1D, E and F).

Whole genome sequencing of this strain

DNA of isolated *T. marneffei* strain PUMCH_TM1901 from BALF was sent for whole genome sequencing at BGI-Tianjing (Supplement methods). This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WNWX00000000. The version described in this paper is version WNWX01000000. The aligned sequences covered 96.31 % of the references genome GCA_003971505.1_ASM397150v1 (Fig. 3). Phylogenetic analysis was conducted between PUMCH_TM1901 strain and other 4 *T. marneffei* strains with assembly from NCBI (Fig. 3). The phylogenetic tree illustrated a relatively higher similarity between the isolated strain with *T. marneffei*_TM4 and *T. marneffei* _WCHTM105701strains (Table 1), which were both sequenced by institutes in South China.

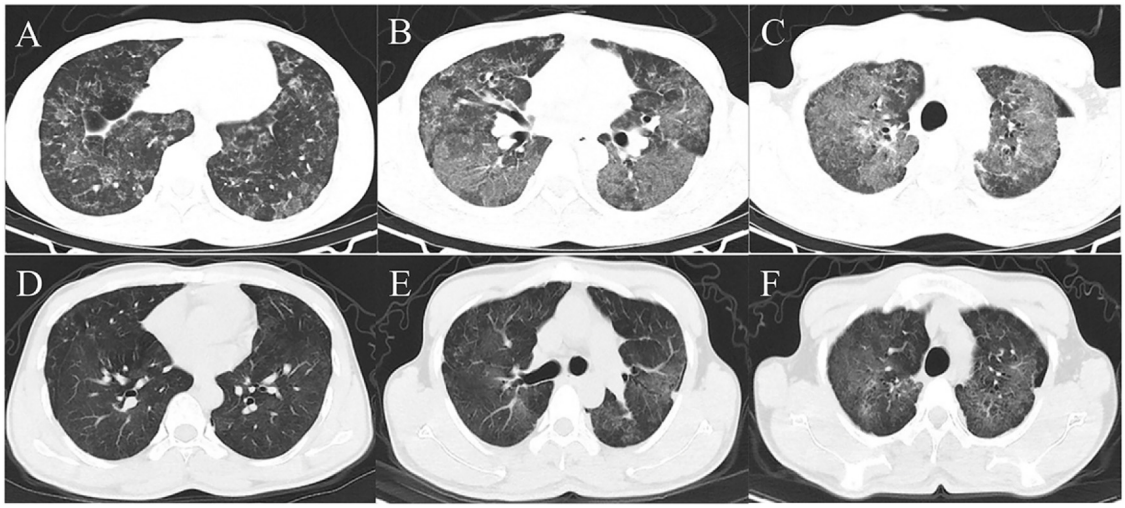

Fig. 1. Imagine examination of the lung. A, B and C. Chest CT result on June 10. Pneumothorax and small volume of pleural effusion were detected, with cavity in left upper lobe, multiple ground-glass opacity and nodules in both lungs. D, E and F. Chest CT result on October. Significant improvement in lung imageology and alleviation in inflammation.

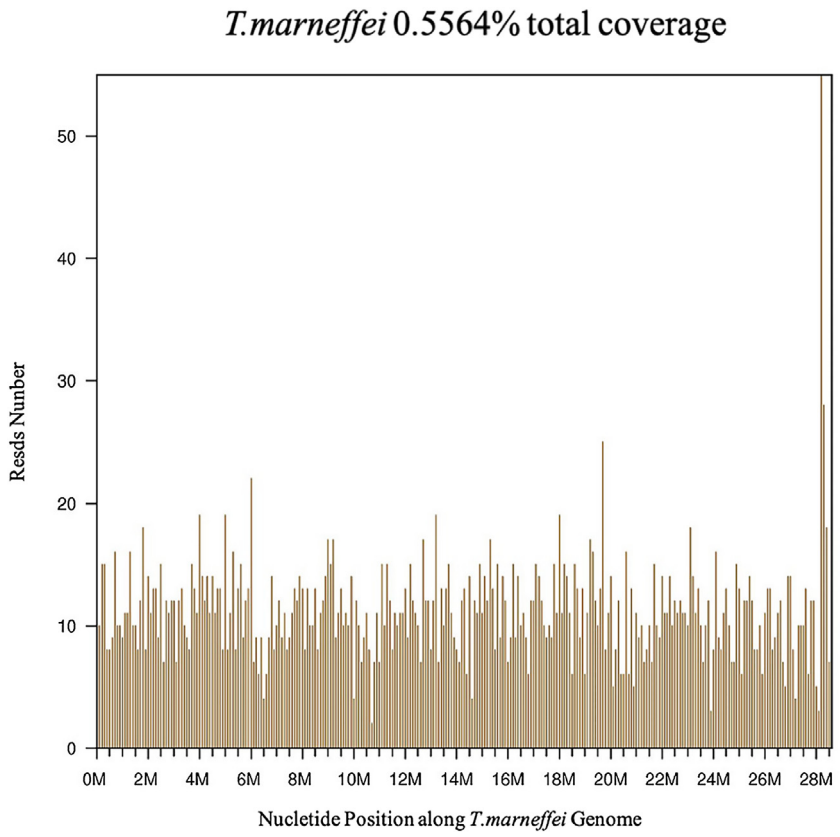

Fig. 2. The genome coverage of detected *T. marneffei* sequences. A total of 3207 reads mapped to *T. marneffei* were detected in BALF, conducted a total coverage of 0.5564 % of the whole genome.

Fig. 3. Phylogenetic tree of strain PUMCH_TM1901 and 4 published *T. marneffei* strains.

Discussion and conclusions

This is the first case report of diagnosing *T. marneffei* infection in a HIV-negative patient with the assistance of mNGS in North China. In this case, mNGS detected and identified *T. marneffei* in the BALF within 48 h, contributing to prompt treatment and quick relieve of the disease which was formerly diagnosis as tuberculosis for nearly two years. The sequencing data, which was in consistent with the patient's clinical symptoms features and culture result, finally assisted clinical physicians in achieving the diagnosis of *T. marneffei* infection.

T. marneffei infection is frequently reported in Southeast Asia, including several provinces and regions in south China such as Guangdong, Guangxi, Yunnan, Hong Kong and Taiwan [1,2]. *T. marneffei* is widely distributed in environment and can be separated from four types of bamboo rats (*Rhizomys sinensis, Rhizomys pruinosus, Rhizomys sumatrensis* and *Cannomys badius*) [7] and the soil near their burrows [8]. *T. marneffei* infection is often

Table 1
Strains and assembly used in phylogenetic analysis.

Strain	Assembly ID	Institute	Year
PM1-1	GCA_000227055.2_ASM22705v2	The university of Hong Kong	2011
PM1-2	GCA_000750115.1_ASM75011v1	Texas A&M University	2014
TM4(ref)	GCA_003971505.1_ASM397150v1	The First Affiliated Hospital of Guangxi Medical University	2018
WCHTM105701	GCA_006111635.1_ASM611163v1	West China Hospital, Sichuan University	2019
ATCC_18224	GCF_000001985.1_JCVI-PMFA1-2.0	J. Craig Venter Institute	2008
PUMCH_TM1901		Peking Union Medical Collage Hospital	2019

developed in patients with human immunodeficiency virus (HIV), and is an indication of AIDS. It is reported that 4.4 %–11.0 % of *T. marneffei* infection was developed in HIV-infected individuals in Vietnam between 2007 and 2008 [9], 30 % in Thailand,4.8 % in Hubei province of China between 2006–2010, and 16.1 % in southern China which involving 6,791 HIV/AIDS patients during January 2012 and December 2015 [2]. Nonetheless, non-HIV *T. marneffei* infection has been reported in patients receiving immunosuppressive treatment or with autoimmune diseases [1]. It was reported that 8.1%–16% of patients receiving lung transplant developed *T. marneffei* infection in Western Australia between 2004–2017 [10].

The asexual spores may enter the patient through inhalation or diseased skin. *T. marneffei* could invade multiple organ systems including blood, marrow, lung and skin, and can be fatal when diagnosis and treatment delays [11]. Typical symptoms of *T. marneffei* infection includes fever, weight loss, anemia, and hepatosplenomegaly. Lymph node enlargement, diarrhea, and necrotizing rash are more likely to occur in HIV-positive patients, and dyspnea is more likely to occur in the HIV-negative group.

The traditional gold standard for infection diagnosis relies on the culture and isolation of pathogen, which suffers from limited positive rate due to the difficulty to cultivate slow-growing and fastidious microbes. Tradition methods for *T. marneffei* detection often include stained histopathological sections, culture and microscopy examination [4]. However, such a procedure is relatively time-consuming, which may delay prompt treatment. Due to the high mortality caused by *T. marneffei* infection, various diagnostic methods have emerged in recent years, including serological test [6], PCR-based molecular methods [6,12]. However, these methods require the physician to raise hypothesis prior to examination. *T. marneffei* is rare pathogen in non-HIV patients, especially in region out of Southeast Asia where there might be limited experience of diagnosis and management of *T. marneffei* infection. Under these circumstances, the implementation of unbiased mNGS enabled a fast and accurate detection without predefined suspicious pathogens, which provides obvious advantage in diagnosis uncommon infections such as *T. marneffei* infection.

After two years anti-tuberculosis treatment without disease alleviation, the patient sought medical advice in PUMCH located in north China. This case is a valuable exploration of the potential and possibility of mNGS assisting the rapid clinical diagnosis of *T. marneffei* and possibly other uncommon fungi from respiratory samples. As a complement to the traditional laboratory culture and imaging tests, mNGS may thus facilitate the precise diagnosis and the efficacious antimicrobial treatment in management of fungi infection.

Ethics approval and consent to participate

The acquisition of the sample and performance of the study was approved by the ethics review committee of Peking Union Medical Collage Hospital (PUMCH, Approval No. : S-K746). Because this was a retrospective study, and the patients' privacy will not be revealed so the hospital's ethics review committee agreed the waiver of informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The data is available upon request. Please contact the corresponding author Qiwen Yang

Funding

This work was supported by National Natural Science Foundation of China (No. 82072318), National Key Research and Development Program of China (No. 2018YFC1200100, No. 2018YFC1200105), National Science and Technology Major Project of China (No. 2018ZX10305409-001-001), Beijing Key Clinical Specialty for Laboratory Medicine - Excellent Project (No. ZK201000). The funders had no role in the study design, collection, and analysis of data, interpretation of results, or preparation of the manuscript.

Authors' contributions

QY and YX collected the sample and designed the study. JZ, DZ, JD and YZ analyzed medical data of the patient, drafting and revised the manuscript. JZ performed the metagenomic next generation sequencing and data analysis. YZ, YC, RS, HW and JZ performed the whole genome sequencing and phylogenetic analysis. JT and ML interpreted and revising the clinical data results. QY and YX made a critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

We acknowledge the professionalism and compassion of all the healthcare workers involved in the patient's care. Special thanks to Dr. Xiaoling Xu for technical support.

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