

Metagenomics-Based Diagnosis and Monitoring Treatment of Disseminated *Talaromyces Marneffe* Infection

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Abstract

Background: The diagnosis of *T. marneffe*i infection remains challenging due to its non-specific clinical presentations and the inadequate performance of conventional diagnostic methods. Meanwhile, there is no good indicator to monitor when induction treatment should switch to consolidation treatment.

Case summary: A 53-year-old male patient presented with a one-week history of high fever on March 21, 2019, 10 years after a liver transplant. A chest CT showed multiple pulmonary nodules. Blood culture revealed *T. marneffe*i. After two weeks of intravenous treatment of voriconazole and caspofungin, the patient was discharged without fever and was given oral voriconazole on April 8. Two days later, the patient had a fever again. The patient was fever-free soon after intravenous voriconazole. However, a high fever occurred three days later. Cultures of blood and sputum were negative. Metagenomics next-generation sequencing (mNGS) detected *T. marneffe*i sequences in blood and *Enterococcus faecium* sequences in sputum. Despite the use of caspofungin and linezolid, the patient maintained a daily fever of 38.5°C. Chest CT revealed that pulmonary nodules were growing in size and number. After administering Amphotericin B, the temperature quickly returned to normal. Liposomal amphotericin B was used due to increased creatinine. When the sequence of *T. marneffe*i turned negative in the blood, the patient was discharged on May 24 and received 14-week oral voriconazole without relapse.

Conclusion: mNGS can directly detect the sequence of *T. marneffe*i in the blood. Consequently, mNGS is a powerful technique in precise diagnosis and a good monitoring technique for treating disseminated *T. marneffe*i.

Introduction

Talaromycosis is a disseminated and progressive infection caused by *Talaromyces marneffe*i (*T. marneffe*i, previously known as *Penicillium marneffe*i), a facultative intracellular pathogen and the only thermally dimorphic fungus in the genus *Talaromyces*. It is endemic in Southeast Asia (in northern Thailand, Vietnam, and Myanmar), East Asia (in southern China, Hong Kong, and Taiwan), and South Asia (in northeastern India) [1–5]. *T. marneffe*i infection is known to predominantly occur in HIV patients [1, 6], whereas it also occurs in non-HIV patients, including solid organ transplant recipients and those receiving immunosuppressive treatment, such as kidney transplantation and liver transplantation, and lung transplantation [7]. *T. marneffe*i infection occurred in liver transplant recipients is rare; only four articles about eight patients were retrieved by PubMed using the keywords 'Talaromyces', 'Penicillium', 'marneffei', 'penicilliosis', 'liver transplant', 'liver transplant recipients' on March 30, 2021[8–11].

*T. marneffe*i can invade multiple organs/systems, such as bone marrow, blood, central nervous system, lungs, and skin, presenting as disseminated infections. Common clinical features of disseminated *T. marneffe*i infection include fever, malaise, weight loss, skin, and soft tissue lesions, hepatosplenomegaly, lymphadenopathy, cough, dyspnea, and gastrointestinal abnormalities [1, 6, 12]. These signs and symptoms of disseminated *T. marneffe*i infection are non-specific and are indistinguishable from other

infectious diseases, such as disseminated tuberculosis, other systemic fungal diseases, or infections due to intracellular pathogens such as *Salmonella* species [1].

The current diagnostic methods for talaromycosis are still based on conventional microscopy, histology, and culture, with low sensitivity. The high sensitivity samples for culture are bone marrow (100%), skin biopsy (90%), and blood (76%), respectively [13]. Cultures are time-consuming, taking 3 to 14 days, resulting in diagnostic delay and raised mortality, particularly in patients without skin lesions [14].

Antigen detection (Mp1p) and PCR-based methods (5.8S rRNA and 18S rRNA genes) are promising rapid diagnostics [1, 12, 15, 16]. However, these methods require physicians to suspect the pathogen before the examination, have limited sensitivity (60%-75%) [1, 12, 15], and are not available in clinical settings of most hospitals in China, which might limit the clinical application.

Metagenomic next-generation sequencing (mNGS) is a non-target, powerful tool for detecting pathogens that can be performed directly on clinical specimens, and the results can be obtained within 24 hours. A few cases have been reported *T. marneffei* was detected in skin lesions, bone marrow, blood, cerebrospinal fluid, aqueous humor, bronchoalveolar lavage fluid (BALF), and mNGS [17–23].

The guideline for adults and adolescents with HIV to treat *T. marneffei* [1] recommends two weeks of induction with intravenous amphotericin B, preferably liposomal amphotericin B or deoxyamphotericin B, or with intravenous voriconazole or with oral voriconazole (for patients unable to tolerate any form of amphotericin). However, there are no good indicators to monitor the exact time point when induction treatment should switch to consolidation.

Here we report the case of a 53-year-old male patient admitted ten years post-liver transplantation with recurrent fever caused by disseminated *T. marneffei*, who was initially given intravenous voriconazole and caspofungin for induction therapy for two weeks. However, after switching to oral administration only for two days, he developed a fever again. Finally, the patient was cured with the treatment of amphotericin B or liposomal amphotericin B and voriconazole, assisted by mNGS in the precise diagnosis and treatment monitoring.

Case Report

A 53-year-old male patient with a one-week history of fever with a maximum body temperature of 39.4°C, shivering with cold, followed by occasional sputum cough and no skin lesions, was admitted to our hospital on March 21, 2019.

The patient had a history of hepatitis B for over twenty years and type 2 diabetes treated with insulin. On October 9, 2008, the patient underwent orthotopic liver transplantation due to hepatitis B cirrhosis. The patient had a regular outpatient visit. Tacrolimus was given for anti-rejection therapy to maintain plasma concentrations at normal levels (reference range: 5-15ng/ml). The patient was on anti-HBV therapy with entecavir (0.5g/day.). In October 2018, the patient underwent a biopsy of the transplanted liver for

elevated alanine aminotransferase (118 U/L). Pathology showed chronic inflammatory liver injury (G2-3S3), which was considered as acute rejection. The patient was on oral methylprednisolone (8 mg/day) for over three months.

A computed tomography (CT) scan of the chest on admission showed bilateral infiltrations and scattered multiple nodules (the largest one being 14 mm in diameter) in both lungs (Fig. 1A). The blood tests performed on March 22 showed a white blood cell (WBC) count of $7.37 \times 10^9/L$, an absolute lymphocyte (LYMP) value of $1.14 \times 10^9/L$, a hemoglobin (Hb) level of 146 g/L, platelet count of $44 \times 10^9/L$, aspartate aminotransferase (AST) of 78 IU/L, ALT of 81 IU/L, total bilirubin (TBILI) of 38.1 $\mu\text{mol/L}$, direct bilirubin (DBILI) of 17.7 $\mu\text{mol/L}$ and serum creatinine of 90 $\mu\text{mol/L}$. Serum 1,3-beta-D-glucan (BDG) level was below 3.836 pg/ml. Aspergillus galactomannan (GM) was 1.593 $\mu\text{g/L}$. C-reactive protein (CRP) was 59.6 mg/L, and procalcitonin (PCT) was 0.85 ng/ml. Prothrombin time (PT) was 18.1 sec. Intravenous cefoperazone sulbactam (3.0 g every 12h) and moxifloxacin (0.4 g/day) were started for antimicrobial therapy. Intravenous voriconazole at a loading dose of 400 mg every 12h on day one and 200 mg every 12h after that was started for antifungal therapy after admission. However, the patient still had a fever of up to 39.9°C with chills. Antibacterial therapy was switched to meropenem (1 g every 8h) on March 23. Moreover, antifungal therapy with caspofungin was introduced on March 24, with a loading dose of 70 mg on day one and a maintenance dose of 50 mg daily after that. The dose of tacrolimus was gradually reduced from 1 mg bid to 1 mg q3d. After these treatments, the patient's body temperature gradually returned to normal, and the patient has been fever-free since March 25. Human immunoglobulin for intravenous administration (IVIg) was given at a dose of 5 g daily for 14 days starting March 26. On March 28, blood cultures showed *T. marneffeii*, which was performed on admission. Sputum culture was negative. On April 5, blood tests showed a WBC count of $3.04 \times 10^9/L$, LYMP of $0.38 \times 10^9/L$, Hb level of 103 g/L, platelet count of $52 \times 10^9/L$, AST of 24 IU/L, ALT of 18 IU/L, and serum creatinine of 70 $\mu\text{mol/L}$, TBILI of 20.8 $\mu\text{mol/L}$, DBILI of 8.8 $\mu\text{mol/L}$. The plasma tacrolimus concentration was 8.8 ng/ml. On April 6, a chest CT showed that the bilateral lung infiltrations were significantly absorbed, and most of the nodules in both lungs became smaller. A blood culture performed on April 5 was negative. On April 8, intravenous voriconazole, which had been used for two weeks, was switched to oral administration at a dose of 200 mg twice daily, and the patient was discharged from the hospital.

Two days later (on April 10), the patient suddenly developed a high fever of 39.4°C and was admitted to our hospital again. Blood tests showed a WBC count of $3.6 \times 10^9/L$, LYMP of $0.68 \times 10^9/L$, Hb level of 108 g/L, and a platelet count of $66 \times 10^9/L$. The high sensitivity C-reactive protein (hsCRP) was 49.34 mg/L. PCT was 0.179 ng/ml. Intravenous imipenem cilastatin (1.0g every 8 hours) and voriconazole (200 mg every 12 hours), and oral oseltamivir (75 mg twice daily) were initiated. Tacrolimus was discontinued. Furthermore, intravenous methylprednisolone (MP) was started at 20 mg/day for four days and then reduced to 10 mg/day for one day. The patient was fever-free from April 12 to April 14. However, on April 15, the patient became febrile again, and oseltamivir was discontinued. On April 16, the chest CT showed infiltrations of both lungs were absorbed, and some nodules in both lungs became smaller. Peripheral blood and sputum samples were collected and sent for mNGS testing by Vision Medicals CO. Ltd

(Guangzhou, China) using Illumina NextSeq 550 (USA). IVIg was given at 20 g per day for five days. mNGS detected 11 high-confidence sequence reads for *T. marneffei* in blood and 20,628 high-confidence sequence reads for *Enterococcus faecium* in sputum on April 17. The *T. marneffei* sequence reads mapped to the *T. marneffei* reference genome (NW_002196661.1), the *Enterococcus faecium* sequence reads mapped to the *Enterococcus faecium* reference genome (NC_017960.1) (Fig. 2). The patient had a fever of 39.9°C and was given linezolid (600 mg every 12 h) against *Enterococcus faecium*; imipenem/cilastatin was switched to cefoperazone/sulbactam (3.0 g every 12 h). Considering the side effects of amphotericin B, caspofungin (50 mg/day) was used synergistically as an antifungal. Although the fever peak was decreased, the patient still had a daily fever with a peak of 38.5°C. The antinuclear antibody (ANA), anti-double-stranded DNA antibody (dsDNA), anti-neutrophil cytoplasmic antibody (ANCA) series, and extractable nuclear antigen (ENA) series were all negative. GM was 5.81 µg/L. The BDG was 129.983 pg/ml. The DNA copies of CMV and EBV were less than 500 copies/ml. Hepatitis B and C viruses, and HIV were all tested negative by serology. Syphilis was tested positive by TPPA (119.58) but negative by TRUST. The plasma concentration of tacrolimus was 7.7 ng/ml. Sputum was negative for smear analysis of acid-fast bacilli. The A and B antigens of *Mycobacterium tuberculosis* were negative according to the T-SPOT blood assay. The flow cytometry of peripheral blood cells showed CD3 + CD4 + T cells/ T lymphocytes was 25.1% (34%-70%), CD3 + CD8 + T cells/T lymphocytes was 53.5% (25%-54%), and CD3 + CD4 + T cells/CD3 + CD8 + T cells was 0.47 (0.68–2.47). The blood cultures performed on April 11 and April 16 were both negative. Sputum culture was also negative. A chest CT on April 24 showed increased and larger nodules (Fig. 1B). Caspofungin was discontinued, and cefoperazone-sulbactam was switched to piperacillin-tazobactam (4.5 g every 8h). Amphotericin B was started via the central venous catheter on April 25. The dose of amphotericin B was started at 1 mg, then 3 mg, then 5 mg, and increased by 5 mg daily until the daily dose reached 35 mg. From May 3, the dose of amphotericin B was 35 mg daily. The MP was given to reduce the side effects of amphotericin B at a dose of 20 mg daily for three days, then 10mg daily. Linezolid was discontinued on April 26. Since April 27, the patient has been fever-free. On May 8, BDG was below 3.836 pg/ml, and voriconazole was discontinued, which was reduced to 200 mg per day on May 2. On May 8, IVIg was given at a dose of 15 mg per day for four days. On May 9, a chest CT showed a decrease in nodules and a smaller size. The creatinine rose to 203 µmol/L on May 13, and amphotericin B was switched to liposomal amphotericin B at a dose of 100 mg for three days on May 14, then 150 mg for seven days, then 100 mg for one day, and discontinued at discharge on May 24. Meanwhile, dexamethasone 2 mg was used daily to reduce its adverse effects. On May 17, piperacillin-tazobactam was switched to cefoperazone-sulbactam (3.0 g every 12h) and discontinued at discharge. The sequence in blood detected by mNGS turned negative on May 17. On May 24, blood tests showed a WBC count of $7.88 \times 10^9/L$, the absolute value of lymphocyte (LYMP) of $0.8 \times 10^9/L$, a hemoglobin (Hb) level of 88 g/L, a platelet count of $53 \times 10^9/L$, an AST of 18 IU/L, an ALT of 39 IU/L, serum creatinine of 120 µmol/L, TBILI of 14.6 µmol/L, DBILI of 6.9 µmol/L. The patient was discharged without fever or cough and given oral voriconazole at a dose of 200 mg every 12 h. On August 6, the sequence in the blood was detected by mNGS was negative. On August 30, a chest CT showed that the nodules had been absorbed (Fig. 1C), and oral voriconazole was discontinued after 14 weeks. The

patient continues to be followed up regularly at the outpatient department of the liver transplant clinic and has not relapsed.

Discussion

Talaromycosis is endemic in southern China. During 2012–2015, *T. marneffe* co-infection was reported in 16.1% (1093/6791) of HIV/AIDS patients in South China [24]. *T. marneffe* infections are rare in solid organ transplant patients on immunosuppressive therapy [7, 8–11, 25–27]. Only eight cases in four publications of *T. marneffe* infection in liver transplant recipients. Zhou et al., from our hospital in China, reported two *T. marneffe* infections [10]. In our case, the patient had *T. marneffe* disseminated ten years after liver transplantation. This is the most extended *T. marneffe* infection-free period among the reported cases. Anti-acute rejection treatment in 2018 may cause *T. marneffe* infection. Regular prophylaxis for *T. marneffe* and other opportunistic pathogens (e.g., *Pneumocystis jirovecii*, cytomegalovirus) should be given during the anti-acute rejection period. In HIV-infected people living or traveling in endemic areas, primary prophylactic treatment with itraconazole 200 mg once daily is recommended, whether an effective antiretroviral regimen is unavailable for various reasons or treatment failure when CD4 cell counts are below 100 cells/mm³ and is discontinued in patients who are ART adherent and have a sustained CD4 count \geq 100 cells/mm³ for over six months and in patients who achieve sustained virologic suppression over six months [1]. However, the indications and duration of prophylaxis of *T. marneffe* among HIV-uninfected patients are unclear.

Skin lesions in talaromycosis have typical central-necrotic and are absent in up to 60% of patients [1]. In our case, the patient had no skin lesions. Blood culture has low sensitivity. Only the first blood culture was positive. Moreover, cultures of sputum were all negative. TB-SPOT was negative. Laboratory tests for rheumatic connective tissue disease were also negative.

Nevertheless, the patient still had recurrent fevers. What pathogens caused these fevers? Fortunately, *T. marneffe* sequences were directly detected in blood using mNGS (a non-targeted and powerful technique). Thus, mNGS can help in accurate diagnosis [17–23].

Amphotericin B is the first-line drug recommended to treat *T. marneffe* [1]. Meanwhile, voriconazole is effective against *T. marneffe* [29–33]. Huang et al. [30] reported similar response rates with voriconazole and amphotericin B as induction therapy for Talaromycosis in patients with HIV/AIDS, but voriconazole had a shorter induction time (11–13 days vs. two weeks). Induction therapy with intravenous or oral voriconazole is recommended for *T. marneffe* in patients who can not tolerate amphotericin [1, 2]. In our case, intravenous voriconazole and caspofungin were administered. Due to nephrotoxicity and hepatotoxicity of amphotericin B [28], intravenous voriconazole and caspofungin were continued after blood cultures confirmed *T. marneffe*. The patient showed significant improvement in clinical symptoms with no side effects, confirming the efficacy and safety of voriconazole and caspofungin to treat *T. marneffe*.

After two weeks of intravenous and two days of oral voriconazole, the patient developed a fever. For disseminated *T. marneffe* with HIV, two weeks of intravenous voriconazole was advised [1]. Treatment for *T. marneffe* took longer in HIV-uninfected patients than in HIV-infected patients [12]. Symptoms and disease progression must be closely monitored [34, 22]. A good monitoring indicator is urgently required to personalize the induction time. mNGS is a powerful non-targeted technique that allows rapid detection of pathogen sequences in samples, even if the blood culture was negative for *T. marneffe*. A blood mNGS test returned to negative performed before stopping intravenous amphotericin for induction therapy.

Moreover, the blood mNGS remained negative after consolidation therapy. The antifungal medication was then discontinued. Until now, the patient has not relapsed. It suggests that when mNGS in the blood return to negative, it is a good time point for patients with fungemia to switch from induction therapy to consolidation therapy for disseminated *T. marneffe*. mNGS is a good indicator for monitoring and guiding therapy in patients with disseminated *T. marneffe* infection.

Even after continuous intravenous voriconazole and caspofungin treatment, the patient's fever persisted, and a chest CT revealed enlarging nodules. After receiving amphotericin B plus voriconazole, the patient had no fever with normal liver function. This indicates that amphotericin B plus voriconazole is a safe treatment regimen for liver transplant recipients until the therapeutic dose of amphotericin B is reached. Amphotericin B was switched to liposomal amphotericin B because it caused elevated creatinine, followed by a gradual decrease in creatinine. Then, for 14 weeks, oral voriconazole (200 mg every 12 hours) was given to consolidating the treatment.

Based on our case, anti-rejection drugs should be discontinued during the induction period until the blood mNGS test returns to negative in fungemia patients with disseminated *T. marneffe*. Meanwhile, we should be aware of voriconazole plasma concentrations and resistance. The plasma concentrations might not reach effective therapeutic concentrations when intravenous voriconazole was switched to oral voriconazole.

However, our hospital lacked voriconazole plasma concentrations and drug sensitivity tests for *T. marneffe*. Also, blood mNGS tests were not performed at the time of admission or discharge to monitor the sequence numbers of *T. marneffe*; due to the invasive nature of bronchoalveolar lavage fluid, it was not collected for culture or mNGS testing. These are the major limitations of this study.

Conclusion

Disseminated *T. marneffe* infection is rare in liver transplant recipients. Voriconazole in combination with caspofungin is effective against *T. marneffe*. Until a therapeutic dose of amphotericin B is reached, amphotericin B in conjunction with voriconazole is an effect treatment option. In cases of impaired renal function, liposomal amphotericin B is a good alternative to amphotericin B. Even if the blood culture is negative, mNGS can directly detect the sequence in the blood. The return to negative for blood sequences of *T. marneffe* detected by mNGS is a good time point for patients with fungemia to switch from

induction therapy to consolidation therapy for disseminated *T. marneffe*. mNGS is a powerful technique for accurate diagnosis and a good monitoring technique for treating disseminated *T. marneffe*.

Declarations

Acknowledgments

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Ethics Statement

This study complies with the guidelines for human research and is in accordance with the Declaration of Helsinki. This work was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University, China (No. [2021] 02-136-01). Patient consent was obtained for participation in this study and for publication of relevant data, including radiographic images. The authors confirm that the personally identifiable information of the patient data in this report is not identifiable.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Panel on Opportunistic Infections in Adults and Adolescents with HIV. Guidelines for the prevention and treatment of opportunistic infections in adults and adolescents with HIV: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed March 11, 2021.
2. Vanittanakom N, Cooper CR Jr, Fisher MC, Sirisanthana T. *Penicillium marneffe* infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbiol Rev. 2006;19(1):95–110. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/16418525>.
3. Hu Y, Zhang J, Li X, Yang Y, Zhang Y, Ma J, Xi L. *Penicillium marneffe* infection: an emerging disease in mainland China. Mycopathologia. 2013;175(1–2):57–67.
4. Le T, Wolbers M, Chi NH, Quang VM, Chinh NT, Lan NP, Lam PS, Kozal MJ, Shikuma CM, Day JN, Farrar J. Epidemiology, seasonality, and predictors of outcome of AIDS-associated *Penicillium marneffe* infection in Ho Chi Minh City, Viet Nam. Clin Infect Dis. 2011;52(7):945–52.
5. Ranjana KH, Priyokumar K, Singh TJ, Gupta ChC, Sharmila L, Singh PN, Chakrabarti A. Disseminated *Penicillium marneffe* infection among HIV-infected patients in Manipur state, India. J Infect. 2002;45(4):268–71.
6. Hu Y, Zhang J, Li X, Yang Y, Zhang Y, Ma J, Xi L. *Penicillium marneffe* infection: an emerging disease in mainland China. Mycopathologia. 2013;175(1–2):57–67.

7. Chan JF, Lau SK, Yuen KY, Woo PC. *Talaromyces (Penicillium) marneffeii* infection in non-HIV-infected patients. *Emerg Microbes Infect.* 2016;9(3):e19. 5).
8. Seo JY, Ma YE, Lee JH, Lee ST, Ki CS, Lee NY. A case of disseminated *Penicillium marneffeii* infection in a liver transplant recipient. *Korean J Lab Med.* 2010;30(4):400–5. Korean.
9. Karadag N, Demirtas G, Selimoglu A, Yilmaz S. Intracerebral Hemorrhage Related With *Penicillium* Species Following Deceased-Donor Liver Transplant. *Exp Clin Transplant.* 2021;19(1):83–7.
10. Zhou F, Bi X, Zou X, Xu Z, Zhang T. Retrospective analysis of 15 cases of *Penicilliosis marneffeii* in a southern China hospital. *Mycopathologia.* 2014;177(5–6):271–9.
11. Nakamura A, Kotera E, Moriguchi Y, Matsushima Y, Nishioka J, Morishita Y, Sakurai H, Yabu Y, Wada H, Uemoto S, Nobori T. Early and differential diagnosis for fungal infection by PCR in case of living donor liver transplantation. *Rinsho Byori.* 2005;53(8):685–91. Japanese.
12. Cao C, Xi L, Chaturvedi V. Talaromycosis (Penicilliosis) Due to *Talaromyces (Penicillium) marneffeii*: Insights into the Clinical Trends of a Major Fungal Disease 60 Years After the Discovery of the Pathogen. *Mycopathologia.* 2019;184(6):709–20.
13. Supparatpinyo K, Khamwan C, Baosoung V, Sirisanthana T, Nelson KE. Disseminated *Penicillium marneffeii* infection in southeast Asia. *Lancet.* 1994;344(8915):110–3.
14. Le T, Wolbers M, Chi NH, Quang VM, Chinh NT, Lan NP, Lam PS, Kozal MJ, Shikuma CM, Day JN, Farrar J. Epidemiology, seasonality, and predictors of outcome of AIDS-associated *Penicillium marneffeii* infection in Ho Chi Minh City, Viet Nam. *Clin Infect Dis.* 2011;52(7):945–52.
15. Wang YF, Cai JP, Wang YD, Dong H, Hao W, Jiang LX, Long J, Chan C, Woo PC, Lau SK, Yuen KY, Che XY. Immunoassays based on *Penicillium marneffeii* Mp1p derived from *Pichia pastoris* expression system for diagnosis of penicilliosis. *PLoS ONE.* 2011;6(12):e28796.
16. Wang YF, Xu HF, Han ZG, Zeng L, Liang CY, Chen XJ, Chen YJ, Cai JP, Hao W, Chan JF, Wang M, Fu N, Che XY. Serological surveillance for *Penicillium marneffeii* infection in HIV-infected patients during 2004–2011 in Guangzhou, China. *Clin Microbiol Infect.* 2015;21(5):484–9.
17. Zhu YM, Ai JW, Xu B, Cui P, Cheng Q, Wu H, Qian YY, Zhang HC, Zhou X, Xing L, Wu R, Li Y, Zhang WH. Rapid and precise diagnosis of disseminated *T. marneffeii* infection assisted by high-throughput sequencing of multifarious specimens in a HIV-negative patient: a case report. *BMC Infect Dis.* 2018;18(1):379.
18. Shi T, Wu L, Cai J, Chen H. An Iris Tumor Secondary to *Talaromyces Marneffeii* Infection in a Patient with AIDS and Syphilis. *Ocul Immunol Inflamm.* 2021;1–4.
19. Wang DM, Ma HL, Tan MQ, Wu YM, Wang SN. Next-generation sequencing confirmed the diagnosis of isolated central nervous system infection caused by *Talaromyces marneffeii* in an immunocompetent patient. *Chin Med J (Engl).* 2020;133(3):374–6.
20. Zhang W, Ye J, Qiu C, Wang L, Jin W, Jiang C, Xu L, Xu J, Li Y, Wang L, Jin H. Rapid and precise diagnosis of *T. marneffeii* pulmonary infection in a HIV-negative patient with autosomal-dominant STAT3 mutation: a case report. *Ther Adv Respir Dis.* 2020;14:1753466620929225.

21. Zhang J, Zhang D, Du J, Zhou Y, Cai Y, Sun R, Zhou J, Tian J, Wu H, Lu M, Xu Y, Yang Q. Rapid diagnosis of *Talaromyces marneffe* infection assisted by metagenomic next-generation sequencing in a HIV-negative patient. *IDCases*. 2021;23:e01055.
22. Zhang HC, Zhang QR, Ai JW, Cui P, Wu HL, Zhang WH, Wang T. The role of bone marrow metagenomics next-generation sequencing to differential diagnosis among visceral leishmaniasis, histoplasmosis, and *talaromycosis marneffe*. *Int J Lab Hematol*. 2020;42(2):e52–4.
23. Chen D, Chang C, Chen M, Zhang Y, Zhao X, Zhang T, Wang Z, Yan J, Zhu H, Zheng L, Zhao K. Unusual disseminated *Talaromyces marneffe* infection mimicking lymphoma in a non-immunosuppressed patient in East China: a case report and review of the literature. *BMC Infect Dis*. 2020;20(1):800.
24. Jiang J, Meng S, Huang S, Ruan Y, Lu X, Li J, et al. Effects of *Talaromyces marneffe* infection on mortality of HIV/AIDS patients in southern China: a retrospective cohort study. *Clin Microbiol Infect*. 2019;25(2):233–41.
25. Stathakis A, Lim KP, Boan P, Lavender M, Wrobel J, Musk M, Heath CH. *Penicillium marneffe* infection in a lung transplant recipient. *Transpl Infect Dis*. 2015;17(3):429–34.
26. Chang A, Musk M, Lavender M, Wrobel J, Yaw MC, Lawrence S, Chirayath S, Boan P. Epidemiology of invasive fungal infections in lung transplant recipients in Western Australia. *Transpl Infect Dis*. 2019;21(3):e13085.
27. Hermans F, Ombelet S, Degezelle K, Testelmans D, Van Raemdonck DE, Verleden GM, Verbeken EK, Van Bleyenbergh P, Lagrou K, Vos R. First-in-man observation of *Talaromyces marneffe* transmission by organ transplantation. *Mycoses*. 2017;60(3):213–7.
28. Botero Aguirre JP, Restrepo Hamid AM. Amphotericin B deoxycholate versus liposomal amphotericin B: effects on kidney function. *Cochrane Database Syst Rev*. 2015; (11):CD010481.
29. Ouyang Y, Cai S, Liang H, Cao C. Administration of Voriconazole in Disseminated *Talaromyces (Penicillium) Marneffe* Infection: A Retrospective Study. *Mycopathologia*. 2017;182(5–6):569–75.
30. Huang W, Li T, Zhou C, Wei F, Cao C, Jiang J. Voriconazole Versus Amphotericin B as Induction Therapy for Talaromycosis in HIV/AIDS Patients: A Retrospective Study. *Mycopathologia*. 2021.
31. Supparatpinyo K, Schlamm HT. Voriconazole as therapy for systemic *Penicillium marneffe* infections in AIDS patients. *Am J Trop Med Hyg*. 2007;77(2):350–3.
32. Luo H, Pan KS, Luo XL, Zheng DY, Andrianopoulos A, Wen LM, Zheng YQ, Guo J, Huang CY, Li XY, Hu R, Li YJ, Li TM, Joseph J, Cao CW, Liang G. In Vitro Susceptibility of Berberine Combined with Antifungal Agents Against the Yeast Form of *Talaromyces marneffe*. *Mycopathologia*. 2019;184(2):295–301.
33. Zheng YQ, Pan KS, Latgé JP, Andrianopoulos A, Luo H, Yan RF, Wei JY, Huang CY, Cao CW. Calcineurin A Is Essential in the Regulation of Asexual Development, Stress Responses and Pathogenesis in *Talaromyces marneffe*. *Front Microbiol*. 2020;10:3094.
34. Masur H, Brooks JT, Benson CA, Holmes K, Pau A, Kaplan JE. Prevention and treatment of opportunistic infections in HIV-infected adults and adolescents—updated guidelines from CDC, NIH,

Figures

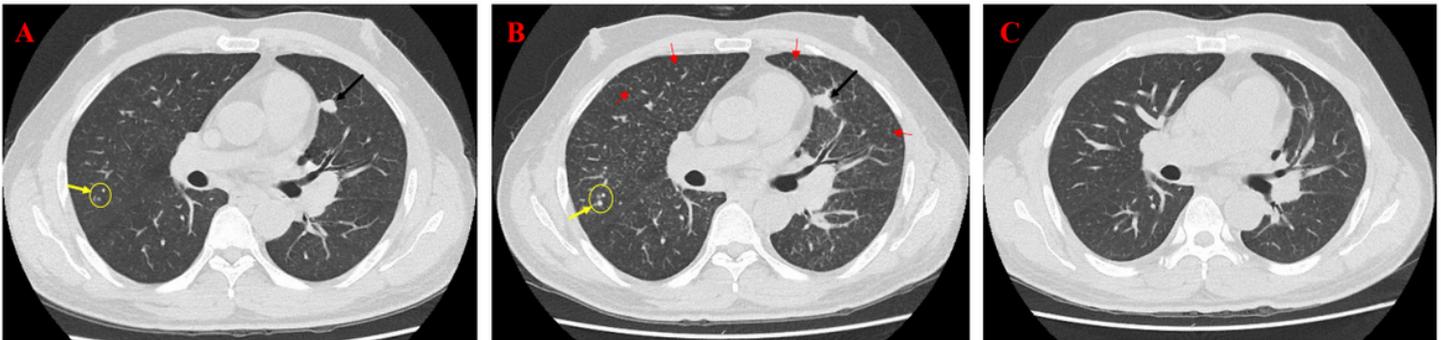


Figure 1

A: Chest CT on March 21 showing the largest nodule (black arrow) and two small nodules (yellow arrow).
 B: Chest CT on April 24 showing the largest nodule getting larger (black arrow), two small nodules increasing to three nodules and getting larger (yellow arrow), and new sporadic small nodules (red arrow).
 C: Chest CT on August 30 showing that the nodules have all been absorbed.

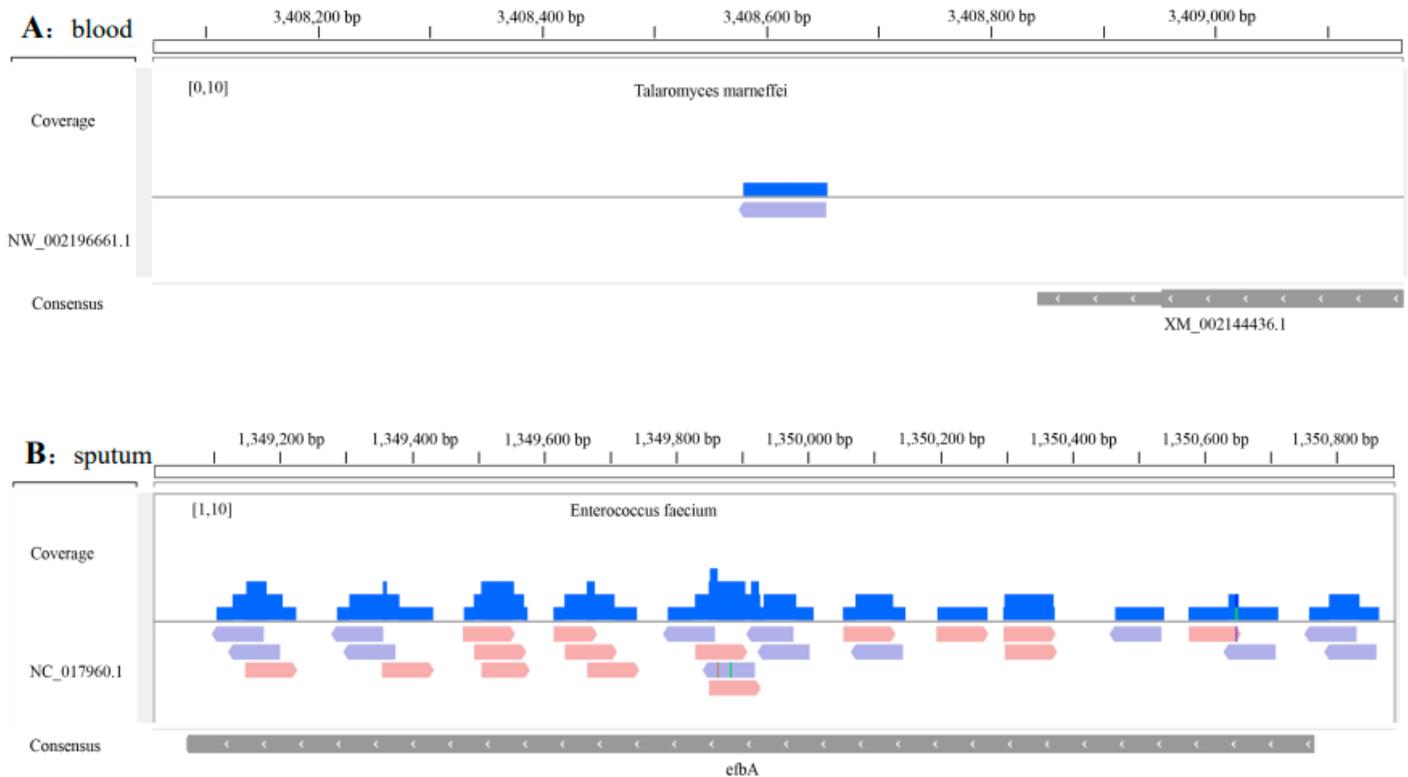


Figure 2

A: *T. marneffei* sequence reads mapped to the *T. marneffei* reference genome (NW_002196661.1), which was detected in the blood. B: *Enterococcus faecalis* sequence reads mapped to the *Enterococcus faecalis* reference genome (NC_017960.1), which was detected in sputum.