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A Case Report of *Talaromyces marneffei* Infection Repeatedly Misdiagnosed as Tuberculosis in an HIV-Negative Patient

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Abstract: Talaromycosis is the most common opportunistic fungal infection in AIDS (acquired immunodeficiency syndrome) patients and its causative agent, *Talaromyces marneffei*, is endemic in Southeast Asia and southern China. However, in recent years, there has been an increasing number of reports of *Talaromyces marneffei* infection in non-endemic areas around the world, and incidences of infection in HIV (human immunodeficiency virus)-negative patients have also seen an upward trend. *Mycobacterium tuberculosis*, which causes human tuberculosis, not only invades the lungs but can travel with cells and lymph, and subsequently invade the whole body. Tuberculosis and *Talaromyces marneffei* share similar clinical manifestations, making them prone to being overlooked or misdiagnosed. This article reports a case of an HIV-negative patient who was repeatedly misdiagnosed with cervical lymph node tuberculosis and pulmonary tuberculosis. The symptoms did not improve after several months of treatment. After a series of diagnostic methods such as matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, galactomannan (GM) test, fungal microscopy, and fungal culture, the patient was finally diagnosed with *Talaromyces marneffei* infection. This case report is intended to serve as a reference for clinicians to reduce missed diagnoses and misdiagnoses.

Keywords: *Talaromyces marneffei*; HIV-negative; Misdiagnosis; Anti-gamma interferon; MALDI-TOF

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1. Introduction

Talaromycosis, caused by the thermally dimorphic fungus *Talaromyces marneffei* (*T. marneffei*), is regionally endemic in Southeast Asia and southern China, especially Guangxi and Guangdong [1]. However, in recent years, there has been an increasing number of reports of *T. marneffei* infection in non-endemic areas around the world. In October 2022, the World Health Organization (WHO) issued the first warning list of deadly fungi including *T. marneffei*, highlighting the threat of this pathogenic fungus to humans [2]. *T. marneffei* infection is more likely to occur in immunocompromised individuals, 90% of whom are HIV (human immunodeficiency virus)-infected, and is the most common opportunistic fungal infection in AIDS (acquired immunodeficiency syndrome) patients in endemic areas [1]. *T. marneffei* enters the mold phase and yeast phase respectively at 25°C and 37°C. After infection, it mainly invades the monocyte-macrophage system in the body and can induce systemic disseminated infection [3] that often involves multiple organs, and the disease is dangerous with a high mortality rate [4]. It is the third leading cause of death among HIV patients, second only to tuberculosis and new cryptococcosis [5]. There is a complex interaction between HIV and *Mycobacterium tuberculosis*, with each promoting the progression of their respective diseases [6]. Studies have found that AIDS patients are complicated by *Mycobacterium tuberculosis* and *T. marneffei* infections. The clinical manifestations and symptoms of the two are similar and atypical, resulting in difficult identification [7]. However, there are few reports of non-AIDS patients with tuberculosis. This article reports a case that was misdiagnosed as pulmonary tuberculosis and cervical lymph node tuberculosis. After several months of anti-tuberculosis treatment, the symptoms did not improve. Fungal infection was subsequently considered, and *T. marneffei* infection was diagnosed with a series of methods such as matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, galactomannan (GM) test, fungal microscopy, and fungal culture. This case report aims to increase microbiology personnel and clinicians' understanding of *Talaromyces marneffei*, so that the infection can be diagnosed promptly and treated effectively.

2. Case presentation

A 77-year-old male presented with a 10-month history of scattered lumps on both sides of the neck, which were initially considered cervical lymph node tuberculosis and were treated with anti-tuberculosis drugs in an infectious disease hospital, including isoniazid, rifampicin, and ethambutol (HRE regimen). However, the masses on the neck gradually increased, and the patient developed coughing. A computed tomography (CT) examination of the neck and chest showed secondary tuberculosis and mediastinal lymph node enlargement and calcification. Needle aspiration of cervical lymph nodes revealed reactive proliferation of lymphoid tissue with plasma cell infiltration. Despite continued anti-tuberculosis treatment, the neck lumps worsened significantly one month ago, accompanied by local swelling, pain, ulceration, pus, and bleeding. The patient also had skin manifestations such as erythema, papules, and itching all over the body. The patient was transferred to the dermatology department for further treatment. Based on the patient's clinical manifestations and examination results, *T. marneffei* infection was suspected, and a series of tests such as MALDI-TOF mass spectrometry, GM test, fungal microscopy, and fungal culture were performed to confirm the diagnosis.

When the patient came to our dermatology department, he had obvious neck pain. Further observation found that the right side of the patient's face and neck was flushed and swollen, and felt hard to touch. The local skin temperature was high and tender, with visible pustules and rice grains. They are about the size of soybeans, with some densely packed into sheets. There were two ulcers of about 4×1 cm^2 and 2×3 cm^2 on the right side and middle of the neck respectively, with granulation hyperplasia on the surface and yellow-white purulent

secretions. A swollen lymph node, the size of a peanut, could be palpated in the left side of the neck. Scattered miliary-sized pus spots could be seen on both upper limbs, and verrucous hyperplasia, scattered erythema, and papules with unclear boundaries could be seen on the back of the right hand (**Figure 1**).

Auxiliary examination: Routine blood test revealed white blood cell count of 23.11×10^9/L, red blood cell count of 3.80×10^{12}/L, hemoglobin of 108.70 g/L, platelet count of 376.10×10^9/L, neutrophil absolute value of 18.33×10^9/L, eosinophils absolute value of 1.13×10^9/L; Liver function test yielded direct bilirubin 12.90 μmol/L, indirect bilirubin 2.00 μmol/L, albumin 32.0 g/L, high-sensitivity C-reactive protein (full process) 141.56 mg/L. Serum ferritin was 784.20 ng/ml. He had no special discomfort, no fever, cough, chest tightness, etc. He had poor energy, appetite, and sleep, with normal bowel movements. The breath sounds in both lungs were clear, and dry and wet rales and pleural friction were not heard.

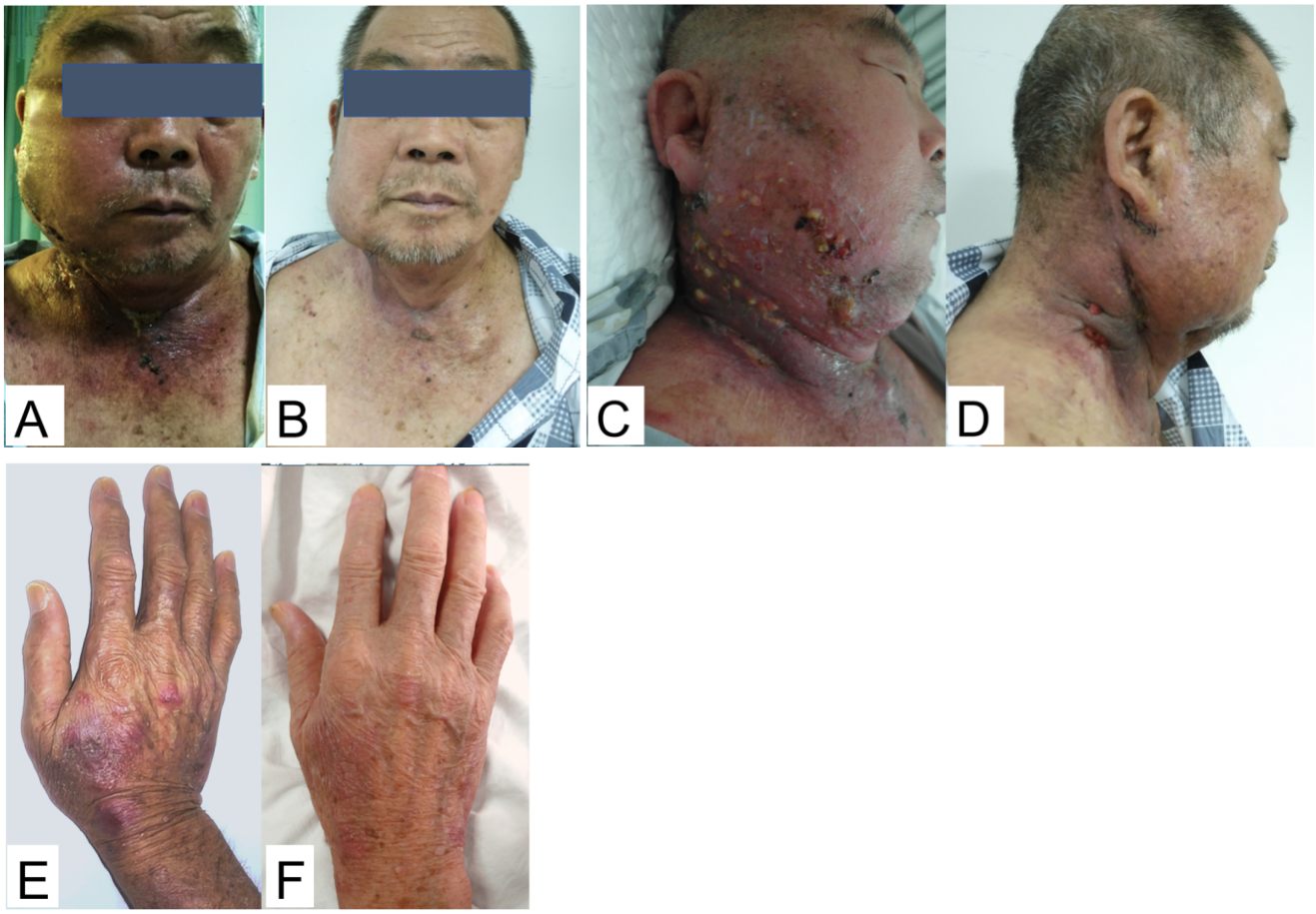

Figure 1. (A, C) Before antifungal treatment: The right side of the face and neck was flushed and swollen, with visible pustules and rice grains, about the size of soybeans, and some were densely packed into sheets. There were ulcers of about 4×1 cm^2 and 2×3 cm^2 on the right side and the middle of the neck respectively, with granulation hyperplasia on the surface and yellow-white purulent secretions. (B, D) After antifungal treatment: Facial flushing and swelling subsided significantly, and pustules were eliminated. (E) Before antifungal treatment: Verrucous hyperplasia was visible on the back of the right hand, with scattered miliary-sized pus spots, scattered erythema, and papules with unclear boundaries. (F) After antifungal treatment: The erythema and papules subsided significantly, and the pus spots disappeared.

As the patient's neck lumps did not improve after 10 months of antituberculosis treatment, we suspected presence of other infections. Another biopsy of the neck skin lumps was performed and sent for bacterial culture, fungal culture, (1,3)-β-D glucan (G test) or GM test (ERA Biology Co., Ltd, Tianjin), and anti-interferon gamma autoantibody test. The result indicates an elevated optical density of G test 108.69 pg/mL.

The GM test was positive, with an increased optical density value of 3.8 pg/mL. The anti-interferon gamma autoantibody test showed a strong positive at a dilution of 1:2500. Microscopic observation demonstrated sausage-shaped yeast cells. SDA (Sabouraud Dextrose Agar) medium was used to culture the biopsy of the neck skin lumps for 2 days. Tubes cultured at 25°C yielded pale yellow mold-like colonies with diffusing wine-red pigment; microscopic examination of the colonies showed broom-like branch structures. Tubes cultured at 37°C grew yeast-like colonies, and a large number of yeast cells were visible under the microscope (**Figure 2**). Subsequently, the microbial mass spectrometer Autof MS 1000 (Autobio Diagnostics Co., Ltd., Zhengzhou, China) was used to obtain the protein fingerprint of cultures, which was suggested to be *T. marneffei* (**Figure 3**). Additionally, ITS (Internal Transcribed Spacer) was used for molecular identification, and the result identified *T. marneffei*. The ITS primers were ITS1 Forward: TCCGTAGGTGAACCTGCGG; ITS4 Reverse: TCCTCCGCTTATTGATATGC.

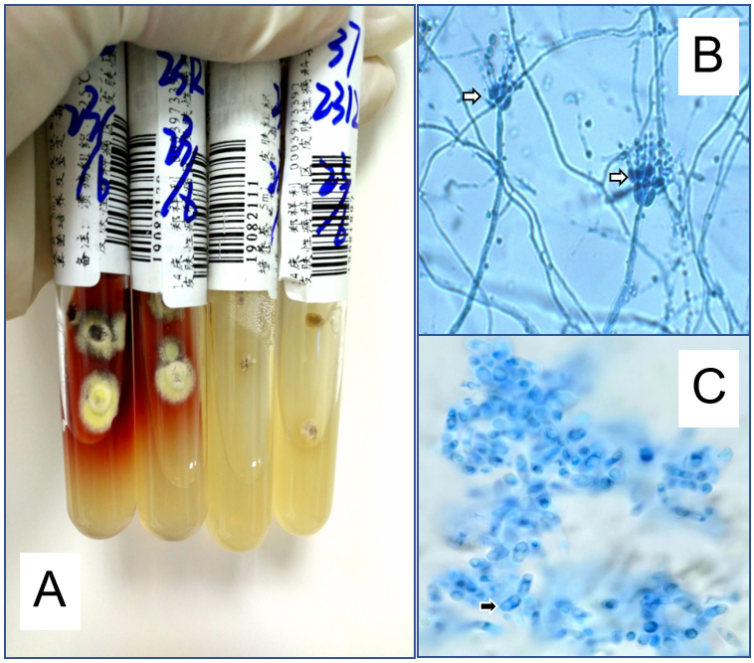

Figure 2. (A) The SDA medium was cultured for 2 days, the two test tubes on the left were cultured at 25°C. Filamentous fungal colonies with a downy texture were visible. A soluble wine-red pigment could be seen diffusing into the agar. The two test tubes on the right were cultured at 37°C. The colonies were arranged in a gyrus shape, with a smooth surface and no pigment produced. (B) A broom-like branch structure could be seen under the microscope. (C) Sausage-shaped yeast cells with transverse septa were visible under the microscope.

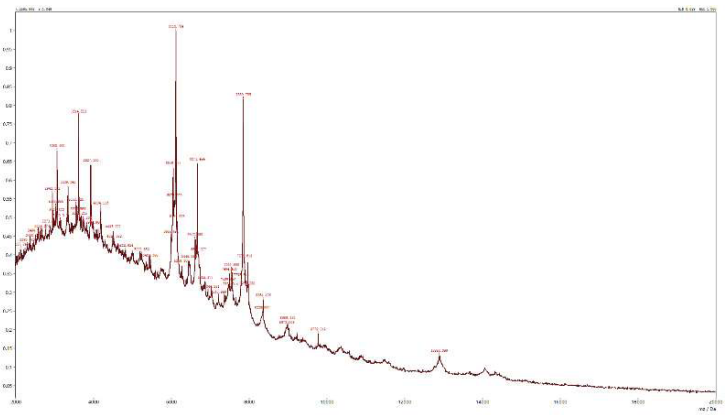

Figure 3. Autof MS 1000 mass spectrometry of *Talaromyces marneffei* MN151898

Consultation by the respiratory department concluded unlikely tuberculosis infection, as the patient's conditions gradually worsened even with anti-tuberculosis treatment for 10 months. The patient has now been diagnosed with *Talaromyces marneffei* infection. Pulmonary and tissue infections caused by *T. marneffei* can easily be misdiagnosed as *Mycobacterium tuberculosis* infection in the early stage. In this case report, *T. marneffei* has been cultured from tissues, thus the possibility of tuberculosis is ruled out. Anti-tuberculosis treatment should be discontinued and replaced with anti-fungal treatment. Infection with *T. marneffei* can be treated with amphotericin B and voriconazole for induction treatment. However, considering the older age of the patient and the serious side effects of amphotericin B, voriconazole treatment is recommended. After communication with the patient and family, the antifungal treatment plan initiated with intravenous infusion of 0.3 g voriconazole every 12 hours for 7 days, followed by 0.25 g voriconazole tablets taken orally every 12 hours for 3 days, and finalized with 0.2 g voriconazole taken orally every 12 hours for 7 days. After the above treatment, the patient's facial edema and neck swelling subsided significantly and the papules on the back of his right hand reduced (**Figure 1**). At the same time, a review of lung CT showed that the lung lesions were significantly improved. After discharge, treatment of 0.2 g voriconazole taken orally every 12 hours was continued for half a year. The patient is currently being followed up by phone and is in good condition.

3. Discussion

Talaromycosis is considered an endemic fungal disease. Southern regions such as Guangxi, Guangdong, and Yunnan in China, as well as Thailand, Vietnam, and other regions in Southeast Asia are considered to be the natural habitats of *T. marneffei* [8]. It is generally believed that people with low immunity become ill by inhaling *T. marneffei* spores from the environment [9]. HIV is an important predisposing factor for talaromycosis, and international guidelines have listed talaromycosis as an AIDS-defining opportunistic infection. It is challenging to identify and diagnose talaromycosis, and their mortality rate is extremely high [10,11]. It has been previously reported that among HIV-negative talaromycosis patients, most adults suffer from delayed immunodeficiency syndrome caused by neutralizing anti-interferon gamma autoantibodies (nAIGA) [12]. IFN-γ (interferon gamma) produced in patients can be neutralized by this autoantibody, resulting in severe impairment of Th1 immune response. Anti-interferon gamma antibody (AIGA) syndrome is a late-onset immunodeficiency disease [13], which tends to occur in immunocompetent individuals [14]. Studies have shown that AIGA is closely related to multiple opportunistic infections [15-17], infection dissemination [18], disease activity [19], and poor prognosis. In a study related to talaromycosis, 20.41% of non-HIV adult patients with talaromycosis had AIGA [20]; 94.8% of immunocompetent patients without underlying diseases had nAIGA [12], suggesting that nAIGA has become the most common predisposing factor for talaromycosis in non-HIV patients. The case reported in this article is an HIV-negative patient in the advanced stage of *T. marneffei* infection. The AIGA test showed strong positive and can still be detected after the serum was diluted 2500 times.

At present, the gold standard for the diagnosis of *T. marneffei* infection is fungal culture, which can be confirmed not only through blood culture but also sputum culture, lavage fluid culture, or lung biopsy through bronchoscopy. In recent years, serological G test and GM test have provided the basis for the early diagnosis of deep fungal infection. Studies have shown that the GM test is significant for the early diagnosis of *T. marneffei* infection [21,22]. The patient's G test (108.69 pg/mL) and GM test (3.8 pg/mL) were both elevated, indicating the possibility of deep fungal infections. In addition, MALDI-TOF, metagenomic next-generation sequencing (mNGS), reverse transcription-polymerase chain reaction (RT-PCR), and other methods have also been used clinically to identify *T. marneffei* infection [23]. In this case report, the MALDI-TOF Autof MS 1000 was used

for detection and identification of *T. marneffei*. Combined detection with multiple methods is the key to rapid and accurate diagnosis of diseases.

4. Conclusion

The most notable feature of this case is the misdiagnosis of tuberculosis by multiple departments in various hospitals, with the fungal infection being overlooked, causing the patient to undergo unnecessary procedures such as receiving incorrect treatment, experiencing disease aggravation, and incurring financial loss. When a patient presents with symptoms suggestive of tuberculosis, clinicians should place increased emphasis on investigating fungal and other microbial infections. A thorough examination for fungal infections can lead to a more rapid and accurate diagnosis, enabling timely and appropriate treatment.

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Disclosure statement

The authors declare no conflict of interest.

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