Case Report: Two Unusual Cases of Human Immunodeficiency Virus–Negative Patients with *Talaromyces marneffe* Infection

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Abstract. *Talaromyces marneffe* (formerly known as *Penicillium marneffe*) is a dimorphic fungus endemic in south and southeast Asia. It is not only commonly found in human immunodeficiency virus (HIV)–infected patients, but also among HIV-negative immunocompromised patients. The infection caused by this pathogen can disseminate hematogenously to other locations. Herein, we report for the first time two cases complicated with a rare disease or involving a rare site: in the first case, *T. marneffe* infection was complicated by Langerhans cell histiocytosis, whereas the second case showed clear etiological evidence of pleural nodules and pleural effusion caused by *T. marneffe* and diagnosed by thorascopic pleural biopsy.

INTRODUCTION

Talaromyces marneffe, formerly known as *Penicillium marneffe*, is a common but serious opportunistic dimorphic fungus that can infect human immunodeficiency virus (HIV)–positive patients and is endemic in southeast Asia. However, it is increasingly observed in HIV-negative subjects without any obvious risk factors or immunocompromised condition.^{1–5} The infection can disseminate hematogenously to the bone, bone marrow, joints, lymph nodes, pericardium, liver, trachea, spleen, etc.^{6–8} Herein, we report the first case of *T. marneffe* complicated by Langerhans cell histiocytosis (LCH) and a case of pleural nodules and pleural effusion caused by *T. marneffe* using thorascopic pleural biopsy for differential diagnosis. We describe the clinical, radiographical, histopathological, and immunohistochemical features of these rare cases.

CASE REPORT

Case 1 was a 64-year-old woman who presented with a 3-year history of productive cough. Chest high-resolution computed tomography (HRCT) revealed multiple patches in the right upper lung and masses in the left lower lung. Standard antituberculous therapy (isoniazid, rifampicin, pyrazinamide, ethambutol) was given for 1 month after clinical diagnosis of tuberculosis, and her condition improved. However, after 2 months, she was referred to our hospital because of left shoulder pain. Chest HRCT scans revealed worsened bilateral lung lesions as well as arthritis in her left shoulder. Anti-tuberculous and anti-inflammatory treatments were continued; however, the condition continued to deteriorate, and the skin on her left shoulder burst and exuded pus. A weight loss of 10 kg, fever, and anemia were revealed. Multiple papules appeared on her left chest and back, skin ulcers in the right temporal region, and multiple cervical lymph nodes were swollen. Breath sounds were decreased in the left lung and a few moist rales were audible over the right lung base. White blood cell count was 11.90×10^9 cells/L, with 65.8% neutrophils, and hemoglobin was 74.00 g/L. Serum albumin was 22.0 g/L. HIV blood tests were negative. Immunologic testing showed

27.8% CD4 cells and a CD4:CD8 ratio of 0.73, which are lower than normal. Quantitative real-time polymerase chain reaction (PCR) testing for *T. marneffe* revealed 1,743 copies/μL blood. Chest HRCT showed worsened lesions and multiple fractures of the thoracic vertebrae and ribs in the right chest. Fluorodeoxyglucose positron emission tomography/CT showed significantly increased uptake in the left lower lung lobe, swollen mediastinal lymph nodes, left adrenal gland, and left chest wall, and multiple osteolytic lesions including the frontal bone, ribs, pelvic bone, sternum, scapula, thoracic vertebrae, and femur (Figure 1A–C). Suspected diagnoses of septicemia, lung cancer, lymphoma, tuberculosis, or nontuberculous mycobacteria were considered. However, antitubercular and antibiotic treatments were ineffective. Her condition continued to worsen. Sputum smear was positive for fungus but negative for acid-fast bacilli. Histopathology of a lung biopsy revealed granulomas (Figure 1D), but no tumor or pathogens, including acid-fast bacilli. On the basis of positive cultures of pus from the left shoulder using Sabouraud dextrose agar at 25°C and 37°C (Figure 1E and F), *T. marneffe* infection was diagnosed. Anti-tubercular and antibiotic treatments were stopped and intravenous amphotericin B (25 mg; 0.8 mg/kg body weight/day) was initiated for 4.5 months, and the patient was discharged.

After oral itraconazole (200 mg/day) for 6 months, she was readmitted to our hospital with a temperature of 39.5°C and a severe, persistent cough. Several enlarged lymph nodes were observed in the left cervical and inguinal regions. Chest HRCT revealed that the lesions had worsened as compared with previous scans (Figure 2A–C). She was treated with intravenous voriconazole (400 mg/day). Histopathological examination of biopsies of the left cervical and right inguinal lymph nodes revealed clusters of cells with Birbeck granules (Figure 2D and E) that were folded or grooved, representing the characteristic feature of Langerhans cells (Figure 2E). Immunohistochemistry was positive for S100 protein (Figure 2E), CD1a (Figure 2F), CD207/langerin, and CD68, confirming the final diagnosis of *T. marneffe* complicated by LCH. Intravenous voriconazole (400 mg/day) was continued; however, the patient was discharged without treatment of LCH and died after 2 months of follow-up.

Case 2 was a 57-year-old previously healthy male who had multiple lymphadenopathy with pain, dyspnea, productive cough, and pleural effusion for 3 months. A 20-year smoking history was reported, without any immunocompromising disease. After antituberculosis therapy for 1 month, he was

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FIGURE 1. Fluorodeoxyglucose positron emission tomography/computed tomography showed (A) significantly increased uptake in the lower left lung lobe, (B, C) swollen mediastinal lymph nodes, and multiple osteolytic lesions including in the sternum, scapula, and femur. (D) Histopathology of a lung biopsy revealed granulomas and abundant infiltration of chronic inflammatory cells. (E) *Talaromyces marneffei* was confirmed by positive cultures of pus from the left shoulder on Sabouraud dextrose agar at 37°C. (F) The yeast form of *T. marneffei* was demonstrated by histopathological staining of skin tissues and secretions with periodic acid-Schiff stain, which revealed a characteristic morphology, including a transverse septum.

FIGURE 2. Chest high-resolution computed tomography revealed consolidation opacities (A, B), swollen mediastinal lymph nodes (A, B), pulmonary atelectasis (A, B), pleural effusion (C), and air-filled bronchi in the right upper lung (A, B). (D) Histopathological examination of the lymph nodes showed clusters of cells with Birbeck granules that were folded or grooved, a characteristic feature of Langerhans cells (hematoxylin and eosin staining, 400×). Immunohistochemistry was positive for (E) S100 protein and (F) CD1a (200×).

referred to our hospital again because his condition worsened and multiple itchy rashes had developed. A weight loss of 5 kg and 37.8°C body temperature were revealed. Several lymph nodes were palpable at the right cervical and supraclavicular regions. Breath sounds over the whole right lung were markedly diminished. Blood examination revealed 45.10×10^9 white blood cells/L, with 87.6% neutrophils and 6.7% lymphocytes. The CD4⁺ T-lymphocyte count was 1,030 cells/ μ L. C-reactive protein was 103.87 mg/L, serum albumin was 28.1 g/L, and erythrocyte sedimentation rate was 63 mm/hour. Plasma β -D-glucan and galactomannan tested positive. Quantitative real-time PCR testing for *T. marneffei* revealed 576 copies/ μ L blood. Rheumatoid factor, antinuclear antibody, anti-DNA antibody, double-stranded DNA antibody, antineutrophil cytoplasmic antibodies, and HIV blood tests were negative. Sputum, blood, bone marrow, rash, and lymph node results were negative for pathogens. HRCT showed pleural effusion in the right thoracic cavity, enlarged mediastinal lymph nodes, and pulmonary atelectasis in the right lung field (Figure 3A). Thoracentesis was performed immediately. The pleural effusion was exudative with increased nucleated cells (680×10^6 cells/L), with 75% neutrophils and 7.1 U/L adenosine deaminase. Emission CT showed significantly increased uptake in the vertebrae and ilium (Figure 3B). Septicemia, malignancies, and lymphoma were first considered. Antibiotic treatment was ineffective. Pathological examination of the rash and lymph nodes showed atypical granulomas but

no evidence of tumor and pathogens. Thoracoscopy revealed fibrous pleural adhesions (Figure 3C) and multiple nodules on the pleura (Figure 3D). The final diagnosis was based on isolation of *T. marneffei* from cultured pleural effusion and pleural nodules (Figure 3E and F). The patient's condition improved after oral voriconazole (200 mg every 12 hours) for 1 month, though two relapses occurred in the second month. Thus, we changed the treatment to intravenous liposomal amphotericin B (1 mg/kg/day), which led to significant improvement in 2 weeks. Then, the patient took oral itraconazole 200 mg twice daily as a maintenance treatment of 4 months. There was no relapse of penicilliosis during 4-month follow-up.

DISCUSSION

Talaromyces marneffei is increasingly observed in non-HIV-infected immunocompromised patients, such as transplant recipients, patients with hematologic malignancies, patients with diabetes, and patients receiving corticosteroids or immunosuppressive agents, even in the immunocompetent.^{2-5,7} However, *T. marneffei* complicated by LCH has not been reported. LCH is a proliferation of Langerhans cells mixed with inflammatory cells, particularly eosinophils, characterized by the expression of CD1a and S100 protein, and the presence of Birbeck granules on ultrastructural examination. It manifests as a unisystem or multisystem disease, and pathogenesis may be related to immune dysfunction.^{9,10} Bones, skin, and lymph

FIGURE 3. (A) Chest high-resolution computed tomography (HRCT) showed pleural effusion in the right thoracic cavity, swollen mediastinal lymph nodes, and pulmonary atelectasis in right lung field. (B) Emission CT showed significantly increased uptake in the thoracic vertebra, and ilium. (C) Thoracoscopy showed yellow pleural effusion and adhesions, and (D) multiple small nodules in the pleura. (E) At 25°C, a mold from cultured pleural nodules produced a red pigment on Sabouraud dextrose agar. (F) Staining with lactophenol cotton blue revealed smooth conidiophores with three to five metulae, each with several phialides, producing chains of smooth, spherical conidia (400 $\times$).

nodes are most commonly affected.^{9,11} The clinical symptoms of *T. marneffei* infection in HIV-negative patients (enlarged lymph nodes, osteolytic lesions, and pulmonary lesions) and pathological findings (suppuration and granuloma formation) are similar to those of LCH, tuberculosis or nontuberculous mycobacterial infection, histoplasmosis, and allergic granulomatous vasculitis, leading to a risk of missed diagnosis or misdiagnosis, especially by inexperienced clinicians, when both diseases coexist.

In case 1, *T. marneffei* was isolated from the culture of pus from the left shoulder, and diagnosis was further based upon histopathological examination of the lymph nodes and lungs. The patient's condition exacerbated during the 10 months of regular antifungal maintenance treatment with itraconazole most likely because of failure to detect the underlying disease of LCH and consequent untimely treatment. Clinicians should pay close attention to the results of microbial, pathological, and immunohistochemical analyses to identify other diseases associated with immune dysfunction. Pathological examination may reveal granulomas in both *T. marneffei* infection and LCH; however, LCH granulomas are intermixed with inflammatory cells, particularly eosinophils and Langerhans cells,¹² whereas *T. marneffei* granulomas particularly include neutrophils.¹³ Specific stains, such as periodic acid–Schiff and Wright's stain for *T. marneffei*, acid-fast stain for tuberculosis, and hematoxylin and eosin for LCH are important for making a differential diagnosis. LCH should be immunohistochemically confirmed by characteristic Birbeck granules (X bodies) and positivity for S100 protein and CD1a.¹⁰

Talaromyces marneffei associated with pleural effusion has been reported in HIV-negative individuals; however, microbial or pathological evidence for its causative role was lacking, and multiple nodules on the pleura caused by *T. marneffei* have not been reported. The most common causes for pleural effusion in adults are heart failure, malignancy, pneumonia, tuberculosis, and pulmonary embolism. In case 2, *T. marneffei* was isolated from pleural effusion and pleural nodule cultures, providing the first direct evidence of *T. marneffei* infection in the thoracic cavity diagnosed by thoracoscopic pleural biopsy. The reasons why *T. marneffei* infected this adult without immunocompromise and caused pleura nodules and pleural effusion in the thoracic cavity remain unknown. This patient had no HIV or other underlying diseases. Living in an endemic area was the only risk factor for infection. Previous work has suggested that deficiency of CD4⁺ T-cell-mediated immunity plays a key pathogenic role in HIV-positive patients and may also be involved in immunocompromised HIV-negative patients, potentially leading to opportunistic infections including *T. marneffei* infection.^{2,3} However, our patient had essentially normal numbers of CD4⁺ T cells. Meanwhile, anticytokine autoantibodies are increasingly recognized to play a role in the pathogenesis of infectious disease and to be associated with susceptibility to infection.² Browne and others reported that anti-interferon- γ autoantibodies were correlated with disseminated opportunistic infections.^{3,7} Thus, humoral immunologic defect involving anti-interferon- γ autoantibodies or heterozygous mis-sense genetic mutations may provide plausible explanations.

Talaromyces marneffei is associated with high mortality, especially in misdiagnosed or untreated patients. Systemic intravenous amphotericin B and itraconazole are the main effective treatment options.^{1–5} However, in case 1, after 10 months of regular and adequate antifungal treatments, but without treat-

ment of LCH, the patient ultimately died. This outcome suggested that it is essential to treat not only the secondary infection, but also the underlying disease such as connective tissue diseases, etc. The optimal treatment of *T. marneffei* complicated by LCH is unclear. Recommended treatments for adult LCH include surgical curettage or resection, irradiation, local or systemic (e.g., prednisone) chemotherapy, which may have deleterious effects on immune function.^{10,14,15} However, systemic chemotherapy may lead to spreading of the infection, hampering its control.⁸ The use of chemotherapy thus remains controversial. As for *T. marneffei* in patients with systemic lupus erythematosus,¹⁶ the use of antifungals, together with prednisone for LCH, may be beneficial. As this is the first report of *T. marneffei* complicated by LCH, clinical experience is limited. More data are needed to establish the best treatment options for this combined condition and for similar diseases that affect host immune function.

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REFERENCES

1. Zeng W, Qiu Y, Lu D, Zhang J, Zhong X, Liu G, 2015. A retrospective analysis of 7 human immunodeficiency virus-negative infants infected by *Penicillium marneffei*. *Medicine (Baltimore)* 94: e1439.
2. Lee PP, Chan KW, Lee TL, Ho MH, Chen XY, Li CH, Chu KM, Zeng HS, Lau YL, 2012. Penicilliosis in children without HIV infection: are they immunodeficient? *Clin Infect Dis* 54: e8–e19.
3. Browne SK, Burbelo PD, Chetchotisakd P, Suputtamongkol Y, Kiertiburanakul S, Shaw PA, Kirk JL, Jutivorakool K, Zaman R, Ding L, Hsu AP, Patel SY, Olivier KN, Lulitanond V, Moosikapun P, Anunnatsiri S, Angkasekwinai N, Sathapatayavongs B, Hsueh PR, Shieh CC, Brown MR, Thongnoppakhun W, Claypool R, Sampaio EP, Thepthai C, Waywa D, Dacombe C, Reizes Y, Zelazny AM, Saleeb P, Rosen LB, Mo A, Iadarola M, Holland SM, 2012. Adult-onset immunodeficiency in Thailand and Taiwan. *N Engl J Med* 367: 725–734.
4. Qiu Y, Liao H, Zhang J, Zhong X, Tan C, Lu D, 2015. Differences in clinical characteristics and prognosis of penicilliosis among HIV-negative patients with or without underlying disease in southern China: a retrospective study. *BMC Infect Dis* 15: 525.
5. Kawila R, Chaiwarith R, Supparatpinyo K, 2013. Clinical and laboratory characteristics of penicilliosis marneffei among patients with and without HIV infection in northern Thailand: a retrospective study. *BMC Infect Dis* 13: 464.
6. Le T, Wolbers M, Chi NH, Quang VM, Chinh NT, Lan NP, Lam PS, Kozal MJ, Shikuma CM, Day JN, Farrar J, 2011. Epidemiology, seasonality, and predictors of outcome of AIDS-associated *Penicillium marneffei* infection in Ho Chi Minh City, Viet Nam. *Clin Infect Dis* 52: 945–952.

7. Chitasombat M, Supparatpinyo K, 2013. *Penicillium marneffei* infection in immunocompromised host. *Curr Fungal Infect Rep* 7: 44–50.
8. Qiu Y, Zhang J, Liu G, Zhong X, Deng J, He Z, Jing B, 2015. Retrospective analysis of 14 cases of disseminated *Penicillium marneffei* infection with osteolytic lesions. *BMC Infect Dis* 15: 47.
9. Lin JN, Lin HH, Lai CH, Wang JL, Yu TJ, 2010. Renal transplant recipient infected with *Penicillium marneffei*. *Lancet Infect Dis* 10: 138.
10. Herwig MC, Wojno T, Zhang Q, Grossniklaus HE, 2013. Langerhans cell histiocytosis of the orbit: five clinicopathologic cases and review of the literature. *Surv Ophthalmol* 58: 330–340.
11. Babeto LT, de Oliveira BM, de Castro LP, Campos MK, Valadares MT, Viana MB, 2011. Langerhans cell histiocytosis: 37 cases in a single Brazilian institution. *Rev Bras Hematol Hemoter* 33: 353–357.
12. Girschikofsky M, Arico M, Castillo D, Chu A, Doberauer C, Fichter J, Haroche J, Kaltsas GA, Makras P, Marzano AV, de Menthon M, Micke O, Passoni E, Seegenschmiedt HM, Tazi A, McClain KL, 2013. Management of adult patients with Langerhans cell histiocytosis: recommendations from an expert panel on behalf of Euro-Histio-Net. *Orphanet J Rare Dis* 8: 72.
13. Yousukh A, Jutavijittum P, Pisetpongsa P, Chitapanarux T, Thongsawat S, Senba M, Toriyama K, 2004. Clinicopathologic study of hepatic *Penicillium marneffei* in northern Thailand. *Arch Pathol Lab Med* 128: 191–194.
14. Zhang JQ, Yang ML, Zhong XN, He ZY, Liu GN, Deng JM, Li MH, 2008. A comparative analysis of the clinical and laboratory characteristics in disseminated penicilliosis marneffei in patients with and without human immunodeficiency virus infection. *Zhonghua Jie He He Hu Xi Za Zhi* 31: 740–746.
15. Donadieu J, Chalard F, Jeziorski E, 2012. Medical management of Langerhans cell histiocytosis from diagnosis to treatment. *Expert Opin Pharmacother* 13: 1309–1322.
16. Chong YB, Tan LP, Robinson S, Lim SK, Ng KP, Keng TC, Kamarulzaman A, 2012. Penicilliosis in lupus patients presenting with unresolved fever: a report of 2 cases and literature review. *Trop Biomed* 29: 270–276.