Disseminated *Penicillium marneffei* Infection in a Patient without HIV Infection: A Case Report

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Penicillium marneffei infection is a fungal infection which often occurs in immunocompromised hosts, especially HIV-infected patients. Although both immunocompetent and immunocompromised patients can be infected, disseminated *Penicillium marneffei* is extremely rare in non-HIV patients. We report an HIV-negative patient who developed disseminated *Penicillium marneffei* infection that included the lung, lymph node, and bone, with the initial presentation of fever, night sweating, body weight loss and refractory pulmonary infiltrates. Grocott's Methenamine Silver stain-positive fungi were identified in specimens from transbronchial, lymph node and bone marrow biopsies. *Penicillium marneffei* was confirmed by fungal culture from sputum, bronchoalveolar lavage fluid, transbronchial lung biopsy, mediastinal lymph node biopsy, and bone marrow biopsy. After treatment with intravenous amphotericin B followed by oral itraconazole for 10 weeks, the clinical symptoms and pulmonary infiltrates resolved completely. ***(Thorac Med 2012; 27: 377-385)***

Key words: bone marrow, fever, non-HIV, *Penicillium marneffei*, pneumonia

Introduction

Penicillium marneffei (*P. marneffei*) is an emerging opportunistic fungal pathogen, and infection by this pathogen is an important cause of morbidity and mortality in human immunodeficiency virus (HIV)-infected patients, especially in Southeast Asia [1]. It also occurs occasionally in immunosuppressed patients who have had travel-related exposure to this organism [1]. *P marneffei* is endemic in Burma (Myanmar), Cambodia, Southern China, Indonesia, Laos, Malaysia, Thailand and Vietnam [2]. Although both immunocompetent and immunocompromised patients can be infected, it is extremely rare to find systemic infections in HIV-negative patients. The clinical manifestations of *P. marneffei* infection have included cough, fever, weight loss, anemia, skin lesions, lymphadenopathy, and hepatomegaly [3]. Because of its non-specific presentations, the diagnosis of *P. marneffei* infection is frequently de-

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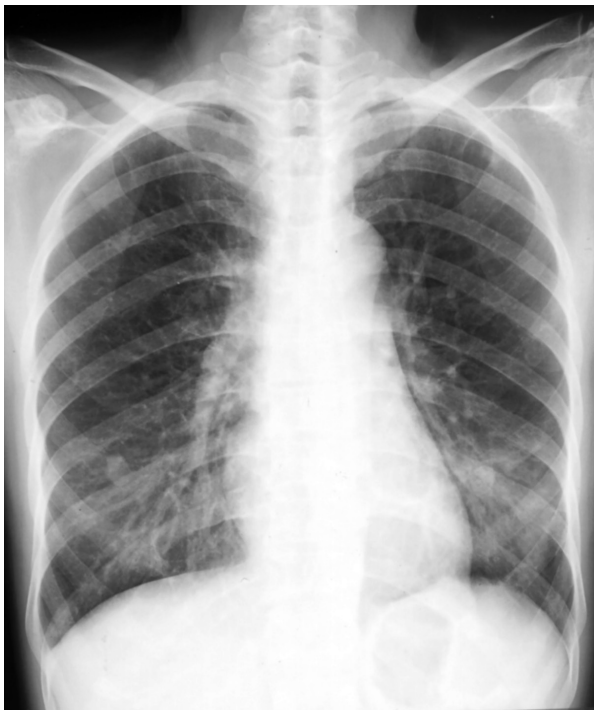
layed, especially in non-HIV-infected patients.

Case Report

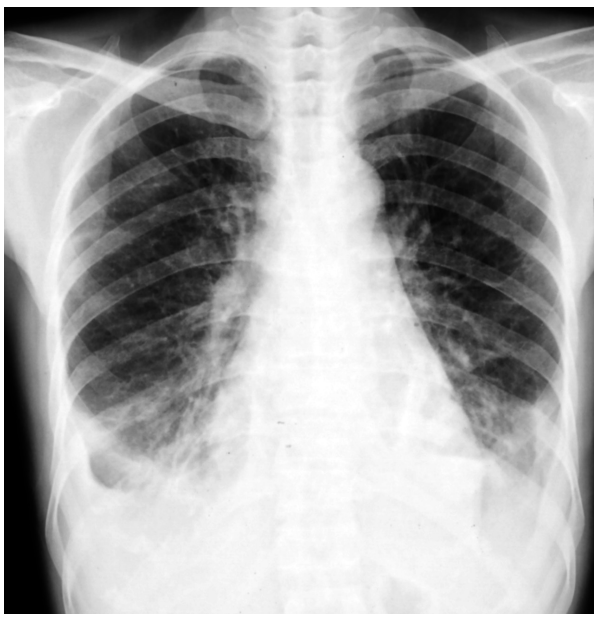
A 48-year-old woman presented to our hospital with low-grade fever and night sweating for more than 1 month. She also complained of body weight loss of approximately 3 kilograms within 1 month. The patient did not smoke and denied recent travel. She also denied having a prior history of systemic diseases, and had had an active lifestyle until 1 year ago. She suffered from 2 episodes of community-acquired pneumonia about 1 year and 6 months, respectively, prior to this admission. Due to the symptoms/signs as described above, she was admitted to another hospital about 3 weeks before coming to our institution, under the impression of atypical pneumonia. She received parenteral antibiotics, but her symptoms persisted. She was then transferred to our hospital and admitted for further evaluation.

Upon arrival, the patient appeared pale and ill-looking. Her vital signs were as follows: heart rate, 115/min; respiratory rate, 20/min; blood pressure, 106/54 mmHg; body temperature, 38.3°C. Physical examination revealed pale conjunctiva and fine crackles in the left lower lung fields. No skin lesions could be found. The laboratory test results were: WBC, 17,200/cumm; hemoglobin, 9.1 mg/dL; platelet, 554,000/cumm; C-reactive protein level, 9.36 mg/dL. Chest radiograph revealed increased infiltrates in the bilateral lower lung fields (Figure 1A). Chest computed tomography (CT) showed localized alveolar infiltration in the left lower lobe with multiple confluent mediastinal lymphadenopathy with rim enhancement (Figure 2). The patient received amoxicillin/clavulanic acid and azithromycin initially as empirical treatment, but the symptoms of intermittent fever persisted. Serology tests for atypical pathogens, including *Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Chlamydophylla pneumoniae*, were all negative. Acid-fast bacilli were not found in the sputum, and sputum culture showed no growth of bacteria. The ELISA screening test for HIV was checked twice, and also revealed negative results. Serum titers of autoantibodies were not elevated and the analyses of lymphocyte subpopulations also showed normal distributions. Parenteral levofloxacin was administered later, but the chest radiographs revealed that infiltrates in the bilateral lower lung fields were in progression, with an accumulation of pleural effusion (Figure 1B).

The patient then underwent bronchoalveolar lavage (BAL) and transbronchial lung biopsy of the left lower lobe. Mediastinoscopy was arranged for mediastinal lymph nodes biopsy. Cytological examination of the BAL fluid (BALF) disclosed Grocott's Methenamine Silver (GMS) stain-positive non-budding bisected fungus spores (Figure 3). The histopathologic examination of specimens from transbronchial lung biopsy and lymph node biopsy demonstrated granulation tissue with the presence of numerous GMS-positive yeast forms of microorganisms within the cytoplasm of macrophages and extracellular tissue. A centrally located transverse septum, which was consistent with *P. marneffei*, could also be identified (Figure 4). Fungal cultures of the sputum, BALF, and tissue specimens from transbronchial lung biopsy and mediastinal lymph node biopsy showed growth of *P. marneffei* (Figure 5). In order to exclude occult hematological malignancy, bone marrow biopsy was also performed. GMS stain-positive yeast was found in the bone marrow biopsy specimens and *P. marneffei* was isolated



(A)



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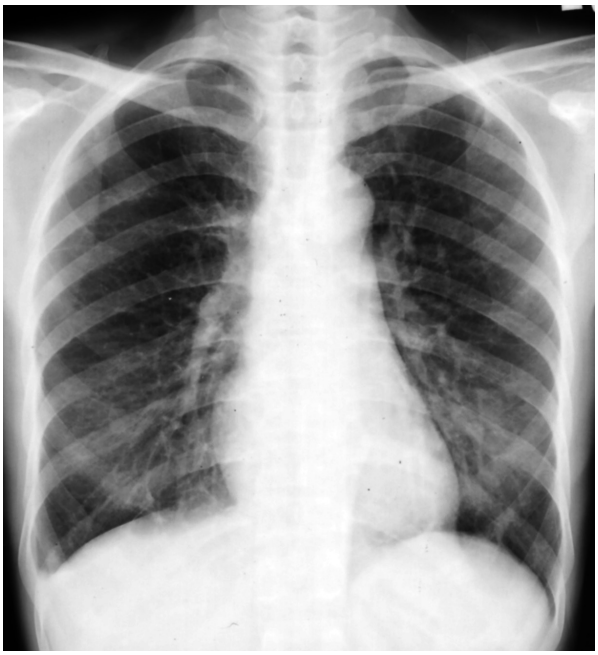


(C)

Fig. 1. (A) Chest radiograph on the day of admission showed localized infiltrates in the bilateral lower lung fields. (B) Chest radiograph on the 10th day showed progressive change with accumulation of pleural effusion. (C) Chest radiograph after completion of intravenous amphotericin B followed by oral itraconazole for 10 weeks showed total resolution of pulmonary infiltrates.

from cultures of bone marrow aspirates.

With a diagnosis of disseminated *P. marneffei* infection with lung, lymph node and bone marrow involvement, the patient received intravenous amphotericin B (0.6 mg/kg) for 2 weeks, followed by oral itraconazole 400 mg daily for 10 weeks. The pulmonary infiltrates on chest radiographs improved completely (Figure 1C). The clinical symptoms of intermittent fever, night sweating, and body weight loss also subsided.

Discussion

P. marneffei was first isolated from the bamboo rat in Vietnam in 1956, and the first case of human infection was described in a man with Hodgkin's lymphoma in 1973. *P. marneffei* can cause infection in immunocompetent patients, but prefers immunocompromised hosts and has

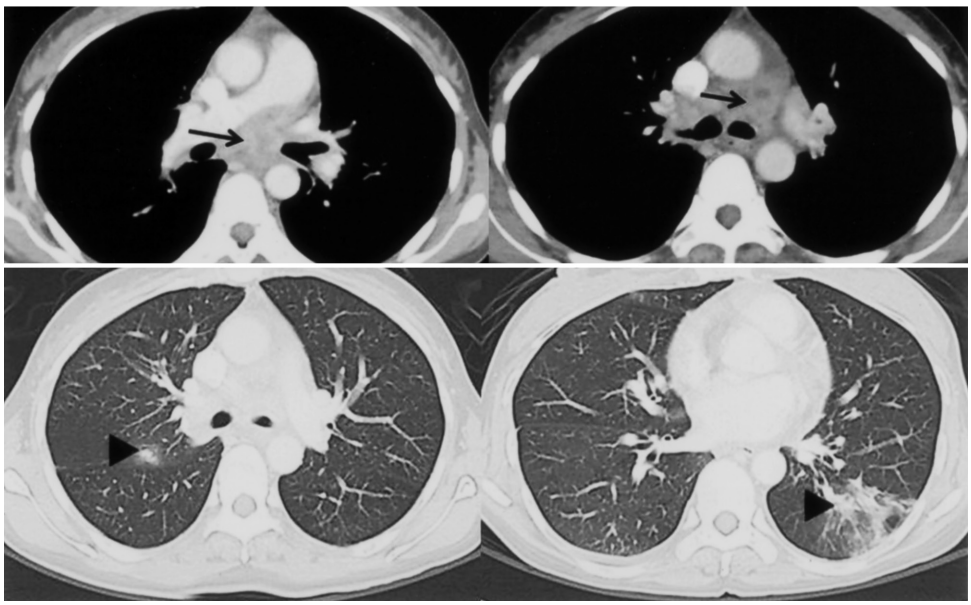
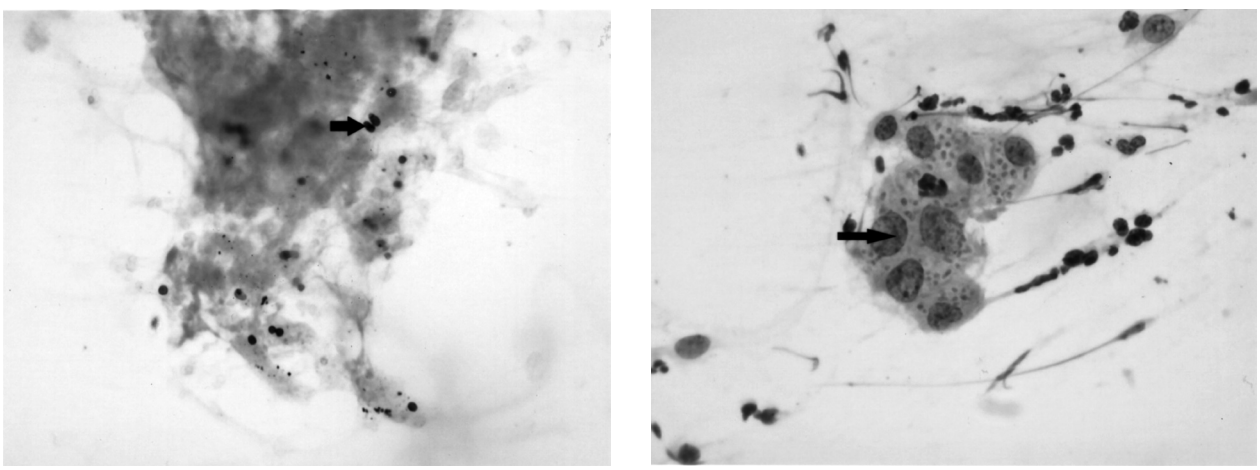


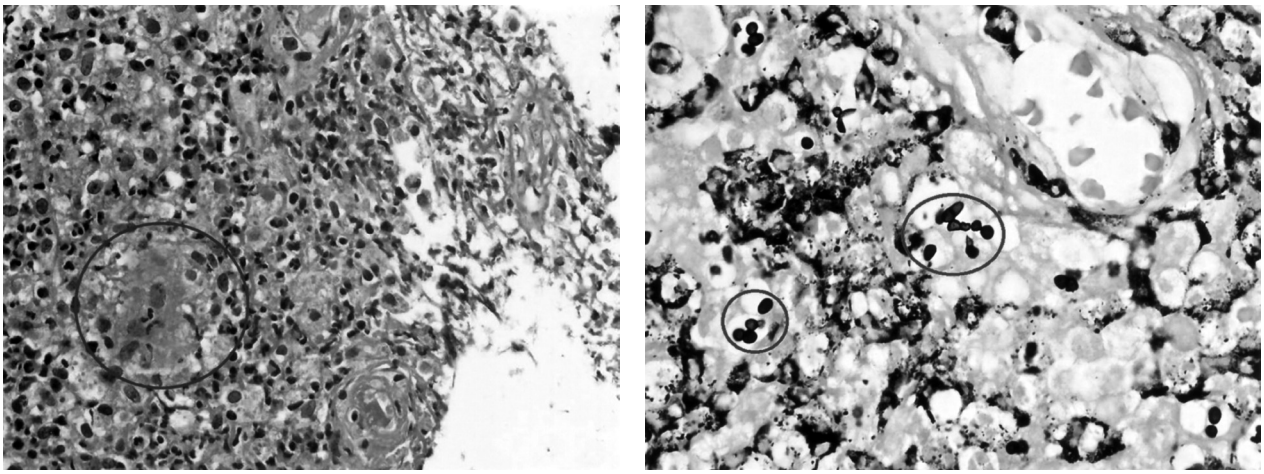
Fig. 2. Chest computed tomography on the day of admission showed confluent mediastinal lymphadenopathy with rim enhancement (arrow) and localized alveolar infiltrates in the left lower lobe (arrow head).



(A) (B)

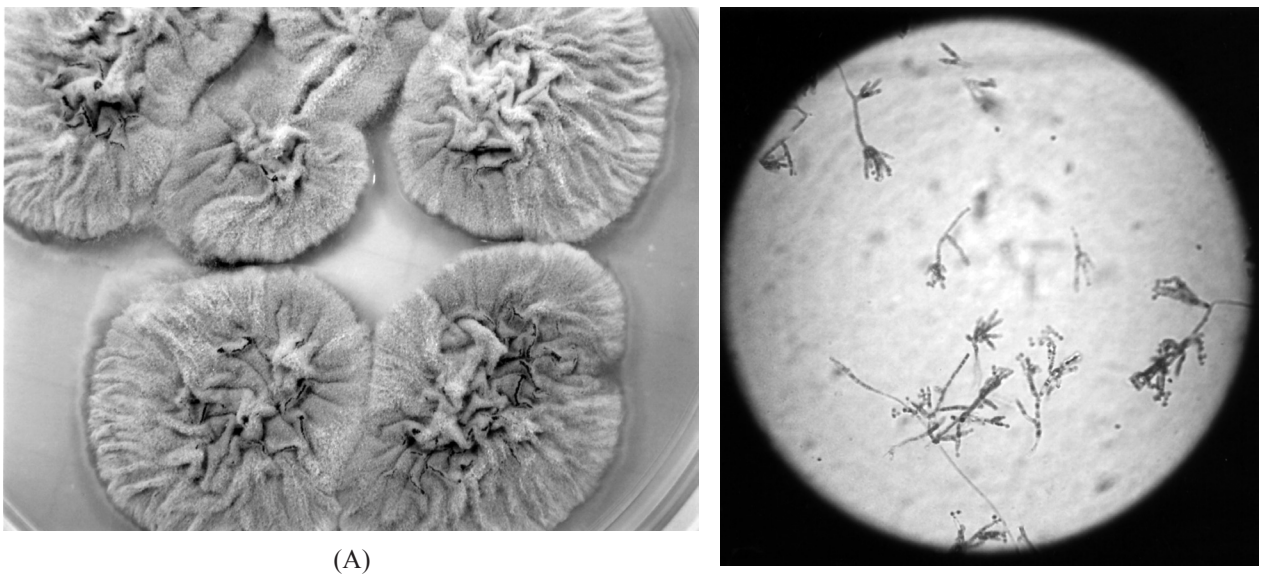
Fig. 3. Cytological examination of bronchoalveolar lavage fluid. (A) Grocott's methenamine silver (GMS) stain showed non-budding bisected fungus spores (arrow). (x400) (B) Papanicolaou stain showed centrally located transverse septum within the yeast (arrow). (X400)

become an important opportunistic pathogen in HIV-positive individuals in Asian countries [3-4]. As the HIV/AIDS epidemic has spread, the prevalence of *P. marneffei* infection also has increased in countries in the region, including Vietnam, India, Taiwan, Malaysia, Cambodia, and the provinces of Guangxi and Guangdong in China [5-7]. Reports of *P. marneffei* infection in Taiwan are relatively scarce. Hsueh *et al*. described 24 patients with *P. marneffei* infection from 1987 through 1998. Of these 24 patients, 16 had AIDS and 20 had disseminated *P.*



(A) (B)

Fig. 4. Histopathological examination of transbronchial lung biopsy. (A) Hematoxylin and eosin (H&E) stain showed acute inflammation with granulation tissue (circle). (x100) (B) Grocott's methenamine silver (GMS) stain showed numerous yeast forms of microorganisms within the cytoplasm of macrophages and extracellular tissue (circle). (X200)



(A) (B)

Fig. 5. Culture from transbronchial lung biopsy revealed growth of *P. marneffei*. (A) *P. marneffei* grows as mold and exhibits diffusible red pigment when growing on Sabouraud's glucose agar medium at 25°C. (B) Conidiophores of *P. marneffei* bearing phialides and chains of conidia at 37°C.

marneffei infection. In addition, 17 of 24 cases were diagnosed from 1996 through 1998, which indicated an increasing trend of *P. marneffei* infection in Taiwan [8].

Patients with *P. marneffei* infection commonly present with symptoms and signs of infection involving the reticuloendothelial system, including generalized lymphadenopathy,

hepatomegaly, and splenomegaly [9-10]. Anemia is the most common laboratory finding, and was also found in our patient. Non-specific respiratory symptoms, including cough, dyspnea, and chest pain may be present as well. Approximately 1/3 of patients may exhibit gastrointestinal symptoms, such as diarrhea [11-12]. The skin lesions of *P. marneffei*, which occur on the face, upper trunk, and extremities, may manifest as papules, pustules, abscesses, nodules, or ulcers. In HIV-infected individuals, the skin lesions typically are umbilicated papules that resemble those of molluscum contagiosum [13], whereas in individuals not infected with HIV, abscesses are a more common manifestation of cutaneous involvement.

Radiographic features of pulmonary *P. marneffei* infection are diverse and non-specific. In a series of 30 cases reported by Supparatpinyo *et al*., the abnormal chest radiographic findings included diffuse reticulonodular infiltration (13 cases), localized alveolar infiltration (12 cases), diffuse alveolar infiltration (3 cases), and localized interstitial infiltration (1 case) [2]. Localized reticular infiltration and cavitary lesion were also reported by Deesomchok and colleagues [12]. In these reports, diffuse reticulonodular infiltration seems to be the most common presentation in chest radiographs. In our case, the initial chest radiograph showed localized alveolar infiltration in the left lower lobe of the lung. The nonspecific findings in the chest radiograph again highlight the importance of histopathological examination and taking cultures to make an accurate diagnosis and exclude the possibility of other microorganisms, such as mycobacterium or other fungal infections.

Diagnosis of *P. marneffei* infection is confirmed by the identification of the organism from smear, culture, or histopathological sections. Supparatpinyo reported that bone marrow culture was the most sensitive (100%) in diagnosing *P. marneffei* infection, followed by skin biopsy cultures (90%) and blood cultures (76%) [2,11]. *P. marneffei* can be seen in histopathological sections stained with hematoxylin and eosin (H&E), GMS, or periodic acid-Schiff stain (PAS). The mold-to-yeast conversion is a diagnostic characteristic of *P. marneffei*. In our case, the presence of GMS-positive fungus with typical pathological features of *P. marneffei* within macrophages in specimens from both BALF and mediastinal lymph node biopsy, transbronchial lung biopsy, and bone marrow biopsy confirmed the diagnosis. The centrally located transverse septum can be clearly identified and differentiated from *Histoplasma capsulatum*. The fungal cultures of sputum, transbronchial lung biopsy, BAL fluid, and bone marrow aspirate also revealed growth of *P. marneffei*.

Patients with *P. marneffei* infection always suffer high mortality, especially those who are misdiagnosed at an early stage or left untreated. Previous studies demonstrated the high susceptibility of *P. marneffei* to miconazole, itraconazole, ketoconazole, and flucytosine, and its intermediate susceptibility to amphotericin B. Treatment with amphotericin B (0.6 mg/kg) for 2 weeks followed by oral itraconazole 400 mg daily for 10 weeks resulted in excellent responsiveness [13-14]. In our case, the patient was treated by intravenous amphotericin B for 2 weeks followed by oral itraconazole for 10 weeks. The clinical symptoms and pulmonary lesions resolved completely, which suggested that such a therapy is effective for disseminated *P. marneffei* infection.

Although *P. marneffei* infection was most frequently found in HIV-positive individuals, it may infect non-HIV individuals as well. In pre-

vious reports, *P. marneffei* infections were found in patients with lymphoma, renal transplant recipients, systemic lupus erythematosus patients, and those receiving corticosteroid therapy or undergoing allogeneic bone marrow transplantation [10,15-17]. The authors believed that the infection was related to a cell-mediated immunity defect in these patients [10,15,18]. In our case, the ELISA screening test for HIV was checked twice with negative results. Autoimmune diseases were unlikely as no characteristic clinical presentation was identified and serum titers of autoantibodies were not elevated. Occult hematological malignancy was also excluded by mediastinal lymph node biopsy and bone marrow biopsy. The analyses of lymphocyte subpopulations also showed normal distributions. Given the fact that the patient suffered from disseminated *P. marneffei* infections combined with 2 episodes of community acquired pneumonia within 1 year prior to this admission, certain unidentifiable defects in cellular immunity remained highly possible.

Conclusions

P. marneffei infection is an important cause of morbidity and mortality in HIV-infected patients in Southeast Asia, and is occasionally found in immunosuppressed patients who have had travel-related exposure to this organism. It may also occur in patients without definite underlying diseases that are associated with immune defects. The diagnosis of *P. marneffei* infection depends on characteristic histopathological findings with fungus cultures, and timely antifungal therapy is crucial to reduce the morbidity and mortality. However, a proper diagnosis is frequently delayed, especially in non-HIV-infected patients. The case presented herein demonstrates the importance of clinical suspicion in making an early diagnosis. The possibility of *P. marneffei* infection should be kept in mind in endemic areas, even in patients with competent immune functions.

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非愛滋病毒感染全身性馬爾尼菲青黴菌者之個案報告：一病例報告

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馬爾尼菲青黴菌是一種常發生在免疫不全宿主的黴菌感染，特別好發在 HIV 陽性的患者。雖然在免疫健全或是免疫不全的人都可能造成感染，全身性馬爾尼菲青黴菌卻很少發生在 HIV 陰性的病人。東南亞地區，包括台灣、泰國、印尼與大陸地區都屬於馬爾尼菲青黴菌的流行區。我們報告一位 HIV 陰性且無旅遊史的病人受到全身性馬爾尼菲青黴菌的感染，受影響部位包括肺部、淋巴結、和骨髓。一開始的症狀包括發燒、夜間盜汗、體重減輕和持續的肺部浸潤。我們從支氣管穿刺切片、支氣管肺泡沖洗液、淋巴結切片及骨髓切片可以發現 Grocott's Methenamine Slive（GMS）染色呈現陽性的酵母菌樣病原菌；此外從病患的痰液、支氣管肺泡沖洗液、經支氣管穿刺切片、縱膈腔淋巴切片及骨髓切片的黴菌培養均可培養出馬爾尼菲青黴菌。經針劑 amphotericin B 治療二星期及口服 itraconazole 十週治療後，病患的臨床症狀及肺部浸潤均有顯著改善。*(胸腔醫學 **2012; 27: 377-385**)*

關鍵詞：骨髓，發燒，HIV 陰性，馬爾尼菲青黴菌，肺炎

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