

Disseminated *Penicillium marneffei* Infection: A Report of Five Cases in Singapore

A Kurup,*^{MBBS, MRCP (UK), M Med}, Y S Leo,**^{FAMS, MRCP (UK), M Med}, A L Tan,**^{FAMS, MBBS, FRCPA},
S Y Wong,****^{FAMS, MBBS, M Med}

Abstract

Penicillium marneffei has emerged as an important opportunistic pathogen in HIV-infected patients in Southeast Asia. We report the first 5 cases of *P. marneffei* diagnosed in Singapore. All the patients were HIV infected and were either Thai nationals or had frequently travelled to Thailand. Fever, weight loss, anaemia and papular skin lesions were common clinical manifestations in our patients, all of whom had the organism isolated from blood. Skin biopsy specimens showed histological evidence of *P. marneffei* in 2 patients. In 1 patient each, the organism grew in cultures of specimens from bone marrow and respiratory secretions. Amphotericin B therapy followed by itraconazole were used in 3 of our 5 patients and was associated with good clinical response and outcome.

Ann Acad Med Singapore 1999; 28:605-9

Key words: HIV, *Penicillium marneffei*, Singapore, Therapy

Introduction

Penicillium marneffei is a dimorphic fungus that can cause infection in immunocompromised hosts.¹ Reports on infection with this organism were initially uncommon, but after the first report of disseminated *P. marneffei* infection in a HIV-infected individual from Bangkok in 1989,² the incidence has increased markedly, almost exclusively in patients with HIV infection.³⁻⁷ The disease is endemic in Southeast Asia, the Guangxi province of China and Hong Kong,^{3-5,7,8} but the ease of global travel and immigration has resulted in reports of this systemic mycosis in HIV-infected individuals from all over the world following visits to endemic regions.⁹⁻¹¹ We report the first 5 cases of *P. marneffei* infection diagnosed in Singapore.

Case Reports

Case 1

In October 1994, a 55-year-old school teacher first presented with *Pneumocystis carinii* pneumonia associated with HIV infection. He gave a history of frequent visits to Chiang Rai in Thailand where his female companion resided. His absolute CD4 lymphocyte count at that time was 5 cells/ul. In November 1994, he was

started on zidovudine and didanosine was added in May 1995.

He remained in stable condition until July 1995, when he was admitted for pyrexia of unknown origin. He underwent numerous tests including a bone marrow examination but all results were unremarkable and he was discharged after his fever responded to indomethacin. He was readmitted 2 weeks later for recurrence of fever, myalgia and loss of weight. At that time, multiple papular red lesions on the trunk and arms (Fig. 1) were noted and a skin biopsy was taken. He had anaemia of 9.0 g/dl, leukopenia of 2100/ul and thrombocytopenia of 41×10^3 /ul. His other investigations during this admission including a chest X-ray, were within normal limits. Fungal blood cultures and bone marrow cultures done in the earlier admission yielded *P. marneffei*. This was the first time that this organism was isolated from blood cultures in Singapore. Skin biopsy specimens also showed histological evidence of *P. marneffei*. With intravenous amphotericin B therapy of 40 mg/day, his fever settled on the third day and the skin lesions resolved after 7 days of treatment. He received a cumulative dose of 320 mg of amphotericin B and was then maintained on oral itraconazole 200 mg daily. With combination

* Registrar

** Consultant

**** Consultant and Head

Department of Infectious Diseases, Communicable Disease Centre

Tan Tock Seng Hospital

*** Department of Pathology

Singapore General Hospital

Address for Reprints: Dr Asok Kurup, Department of Infectious Diseases, Communicable Disease Centre, Tan Tock Seng Hospital, Moulmein Road, Singapore 308433.



Fig. 1. A close-up view of the umbilicated papular skin lesions of Case 1.

antiretroviral therapy, the absolute CD4 lymphocyte count rose from 5 cells/ul in July 1995 to 220 cells/ul in June 1998 with a HIV RNA viral load that was undetectable (less than 20 copies/ml plasma). Current antiretrovirals are zidovudine, lamivudine and indinavir. At his clinic review in June 1998, 3 years after the diagnosis of *P. marneffeii* infection, he continues to be in stable health with a Karnofsky score of 90.

Case 2

In January 1997, a 25-year-old female ex-commercial sex worker from Thailand was admitted for an acute abdomen for which emergency laparotomy performed showed a ruptured Graafian follicle. She was diagnosed to be HIV positive following a sharp injury incident involving a healthcare worker in the postoperative period. Further history revealed fever and weight loss in the preceding month.

Physical examination revealed her to be febrile with multiple cervical lymphadenopathy and oral hairy leukoplakia. Chest X-ray was suggestive of miliary tuberculosis. She responded to treatment for disseminated tuberculosis and remained in stable health until May 1997, when she was re-admitted for fever of 4 weeks duration and unproductive cough. Physical examination revealed fever and pallor. Her blood tests showed bicytopenia (haemoglobin 9.0 g/dl; leukocyte count 2300/ul) and an absolute CD4 lymphocyte count of 5 cells/ul. The chest X-ray showed resolution of the miliary shadows. All other initial investigations were unremarkable and she went on to have a computed tomographic (CT) scan of the abdomen which revealed hepatosplenomegaly with enlarged mesenteric lymphadenopathy. The fever persisted despite a course of empiric antibiotics followed by indomethacin. Two weeks after admission, fungal blood cultures yielded *P. marneffeii*. Her fever resolved on the third day of amphotericin B treatment. After a cumulative dose of 315 mg was

reached, she was started on itraconazole 200 mg twice daily. Thereafter, her care was transferred back to her home country and she has not been reviewed in Singapore since May 1997.

Case 3

A 25-year-old-Thai woman, who was an ex-commercial sex worker from Bangkok, was admitted for complaints of dyspepsia and weight loss in December 1997. Physical examination revealed pallor and oral thrush. In the preceding 2 years, she had similar complaints but had repeatedly declined HIV screening tests.

Her blood test confirmed HIV seropositivity with an absolute CD4 lymphocyte count of 34 cells/ul. There was also anaemia of 9.4 g/dl. Chest X-ray revealed bilateral lower zone infiltrates and *Pneumocystis carinii* cysts were seen on cytological examination of bronchoalveolar lavage fluid. She was discharged after responding well to co-trimoxazole and prednisolone.

On clinic review a month after discharge, both fungal blood cultures and fluid from the bronchoalveolar lavage, which were done in the previous admission, yielded *P. marneffeii*. She was re-admitted for intravenous amphotericin B treatment. On physical examination, she was febrile and there were some papular skin lesions on her left face and back. The skin lesions were not biopsied. Her fever settled after 2 days of amphotericin B treatment and she was discharged. She received a cumulative dose of 471 mg of amphotericin B followed by oral itraconazole 200 mg twice daily. Lamivudine and stavudine were commenced in February 1998. Although she subsequently defaulted follow-up and medication for 2 months, there was no relapse of *P. marneffeii* infection. Thereafter, she was resumed on lamivudine, stavudine and itraconazole and at her clinic review in June 1998, she was in stable health with a Karnofsky score of 80 and an absolute CD4 lymphocyte count of 196 cells/ul.

Case 4

A 40-year-old-Malaysian Chinese businessman was admitted for midazolam overdose in September 1997. Clinical history revealed fever, weight loss and unproductive cough in the preceding month. He admitted to multiple unprotected heterosexual exposure in Thailand. Apart from fever and pallor, clinical examination also revealed oral thrush, hepatosplenomegaly and generalised maculopapular umbilicated skin lesions.

In addition to HIV seropositivity and an absolute CD4 lymphocyte count of 1 cell/ul, pancytopenia was also present. Chest X-ray revealed a left upper zone cavitating lesion. Smears from the sputum for acid-fast bacilli were negative on two separate occasions. A CT scan of the abdomen showed hepatosplenomegaly with para-aortic lymph node enlargement.

Amphotericin B treatment was started because the serum cryptococcal antigen was positive at 1:512. Although cryptococcal lung infection was a consideration, concomitant pulmonary tuberculosis could not be excluded despite the negative smear examination. Therefore, empiric anti-tuberculosis (anti-TB) treatment was also commenced. Before any response to treatment could be ascertained, he requested for his care to be transferred to a Malaysian hospital, where he died soon thereafter. Significantly, blood cultures done in Singapore before transfer isolated *P. marneffeii* instead of *Cryptococcus neoformans*. Furthermore, histology of the skin biopsy revealed the presence of intracellular yeasts which was consistent with *P. marneffeii*.

Case 5

A 35-year-old Chinese bartender, with multiple unprotected heterosexual exposure in Thailand, first presented in March 1996, complaining of fever, cough and dyspnoea during the preceding 3 months. In addition to HIV seropositivity and an absolute CD4 lymphocyte count of 31 cells/ul, he was hypoxic with a PaO₂ of 30% in room air. The chest X-ray revealed perihilar infiltrates. Although bronchoalveolar lavage staining was negative for *Pneumocystis carinii*, he responded to treatment for presumptive *Pneumocystis carinii* pneumonia. During the course of treatment and further investigation, he manifested features of a personality disorder and insisted on discharge against medical advice.

A month later, fluid from the bronchoalveolar lavage grew *Mycobacterium tuberculosis* complex which was sensitive to all first line agents. Despite counselling and persuasion, he failed to comply with treatment. Thereafter, he had seven admissions for non-specific complaints with progressive deterioration of his general health. In one of these admissions in March 1997, he also complained of fever, cough and loss of weight. Physical examination revealed him to be febrile with severe oral thrush. He was noted to have anaemia of 11 g/dl. He demanded discharge shortly after admission. Fungal blood cultures yielded *P. marneffeii* 3 weeks after discharge. Unfortunately, he refused treatment with antifungals.

He was last admitted in July 1997 with severe oral thrush, cytomegalovirus proctitis and retinitis. He eventually succumbed to *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Despite refusing specific anti-fungal treatment for *P. marneffeii* infection, he survived 4 months after being diagnosed with this disease.

Discussion

A dimorphic fungi of the genus *Penicillium*, *P. marneffeii* grows as a mould at 25°C. The colonies of the mycelial form on Sabouraud dextrose agar are greyish white, turning to greyish red with maturity as a soluble red

pigment diffuses into the agar. When incubated at 37°C on brain heart infusion agar, the mould converts to yeast form. It takes about 2 to 3 weeks before it can be formally identified by culture.

Very little is known about the natural reservoirs or animal hosts of *P. marneffeii*. It had been isolated from bamboo rats in Thailand^{1*} but no definite route of transmission has been documented. History of exposure to or consumption of bamboo rats was not a risk factor for infection.¹³ Several reports had postulated that inhalation of conidia from a contaminated reservoir in the environment may cause subsequent disease in immunocompromised hosts.^{4,8,14} Suitable conditions for growth during rainy months could perhaps explain why more cases are seen during this season.¹⁴ There has been no documented person to person transmission.

In our cohort, the 2 females were Thai nationals and all 3 males (2 Singaporeans and 1 Malaysian) had travelled to Thailand, an endemic region for *P. marneffeii*. The 2 Singaporeans in our cohort represent 1.4% of the 144 Singaporeans with AIDS diagnosed from 1985 until March 1998.¹⁵ This is in contrast to the data from Thailand where it is reported to be the third most common opportunistic infection in HIV-infected patients, following extrapulmonary tuberculosis and cryptococcosis.⁷ We postulate that the infection was acquired from Thailand rather than from Singapore.

HIV-infected patients with disseminated *P. marneffeii* often present with non-specific symptoms and signs. Common clinical manifestations include fever, weight loss, and anaemia^{5,7} and these were also seen in all our patients (Table I). In addition, leukopenia featured in 3 of our patients. Papular skin lesions were present in 3 of our patients, 1 of whom had the classically described papules with central necrotic umbilication seen in approximately 70% of patients in various reports from Thailand.^{2,4,7} The skin biopsy specimen of Case 1 showed histological evidence of *P. marneffeii*. The yeasts seen in the skin biopsy of Case 4 were initially identified as *Histoplasma capsulatum* but on subsequent review, this was thought to be consistent with *P. marneffeii*. *Penicillium marneffeii* and *H. capsulatum* are both intracellular pathogens with similar morphological resemblance and may be mistaken for each other. Differentiation of these 2 organisms can be made if budding is seen as the yeast cell of *H. capsulatum* divides by budding, not fission.

Cough was a prominent symptom for 3 of our patients but was likely to be due to concomitant pulmonary infections. Abnormalities on chest radiography had been reported in the literature to occur in 21% to 30% of patients,^{2,5,7} manifesting as reticulonodular or alveolar infiltrates, effusion, hilar lymphadenopathy or cavities. Although bronchoalveolar lavage fluid from Case 2 isolated *P. marneffeii*, the bibasal infiltrates on the chest X-ray could be explained by concomitant *Pneumocystis*

TABLE I: SUMMARY OF THE CLINICAL FEATURES OF OUR PATIENTS WITH *P. marneffei* INFECTION

Clinical features	Case 1	Case 2	Case 3	Case 4	Case 5
Sex	male	female	female	male	male
Age	55	25	25	40	35
Risk behavior	heterosexual sex	heterosexual sex	heterosexual sex	heterosexual sex	heterosexual sex
Site of isolation	blood, bone marrow, skin	blood	blood, bronchial washings	blood, skin	blood
Absolute CD4 count	*NA	5	34	1	31
Loss of weight	+	+	+		
Fever	+	+	+	+	+
Anaemia	+	+	+	+	+
Leukopenia		+			
Cough		+		+	+
Skin lesions				+	
Lymphadenopathy		+			
Concomitant opportunistic infections	nil	**TB	***PCP	probable TB and cryptococcosis	TB, ****CMV proctitis and retinitis
Hepatosplenomegaly	-			+	
Outcome	survived	survived	survived	died	died

* not applicable

** tuberculosis

*** *Pneumocystis carinii* pneumonia

**** Cytomegalovirus

carinii pneumonia. Similarly, concurrent cryptococcal lung infection or pulmonary tuberculosis may explain the cavitating lesion on the chest radiography of Case 4.

Concomitant infections with 2 or more pathogens are not uncommon in patients with advanced HIV infection. In this series, all except Case 1 had an additional pathogen isolated at the time of diagnosis of *P. marneffei* infection (Table I). Consistent with the occurrence of this disease late in the course of HIV infection,⁷ all our patients had absolute CD4 lymphocyte count that was less than 60 cells/ul at the time of diagnosis of *P. marneffei* infection.

Hepatosplenomegaly was seen in 2 of our patients. In a review by Tuan⁵ of 155 patients infected with *P. marneffei*, the incidence of hepatomegaly, splenomegaly and lymphadenopathy was 43.9%, 13.5% and 52.3%, respectively. Only Case 2 had evidence of lymphadenopathy. As she had already received 5 months of TB treatment by the time mesenteric lymphadenopathy was detected, we speculate that this may be due to *P. marneffei* infection.

It is unlikely that the serum cryptococcal antigen of Case 4 was a false positive result and concomitant cryptococcosis may be a more plausible explanation. Blood cultures isolated *P. marneffei* from all of our patients. In 1 patient each, the organism grew in cultures of specimens from bone marrow and respiratory secretions. It took 2 to 3 weeks before the organism could be isolated and confirmed on morphological examination. In a study by Supparatpinyo et al,⁷ bone marrow culture was the

most sensitive method (100%) followed by culture of skin biopsy (90%) and blood culture (76 %). *P. marneffei* has also been cultured from stool,¹⁶ urine,¹⁷ bronchial washings,¹⁷ fluid from knee joints¹⁸ and cerebrospinal fluid.¹⁹ There have been attempts at serodiagnosis of *P. marneffei*^{20,21} but the clinical utility of serological tests has not yet been clearly defined. In reports from Thailand,^{4,7} presumptive diagnosis may be made by microscopic examination of the Wright's-stained samples of bone-marrow aspirate, and/or touch smears of the skin and lymph node biopsy specimens.

There were 2 deaths among our 5 patients. Case 4 had advanced AIDS with multiple concurrent infections which led to his rapid deterioration and demise. There were numerous studies that reported a high mortality with untreated disseminated disease.^{7,8,18} In their study of 80 patients with *P. marneffei* infection, Supparatpinyo et al⁷ reported that there was a delay in diagnosis in 12 patients and the mortality rate in this group was 75%. Case 5 refused specific anti-fungal treatment but despite this, he survived for 4 months from the time of diagnosis before succumbing to *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

In vitro, this fungus is sensitive to amphotericin B, itraconazole and ketoconazole.²² A recent study by Sirisanthana et al²³ evaluated the efficacy and safety of treatment of 74 HIV-infected patients with disseminated *P. marneffei* infection with amphotericin B at a dosage of 0.6 mg/kg/day intravenously for 2 weeks, followed by a 400 mg daily dosage of oral itraconazole for 10 weeks. The study showed a 97.3% response to

treatment with no serious adverse drug effects. A report by Supparatpinyo et al²² also revealed that 12 out of 40 patients who responded to initial therapy relapsed within 6 months without maintenance suppression therapy and recommended itraconazole 200 mg/day orally as secondary prophylaxis for life.

Treatment with the above regimen in 3 of our patients resulted in rapid defervescence and resolution of skin lesions. Two of the 3 patients who received treatment returned for subsequent review as outpatients. In both these patients, there was no relapse of *P. marneffeii* infection as of July 1998. After initial treatment for disseminated *P. marneffeii* infection, Case 1 remained in stable health and showed good immunological recovery with highly active anti-retroviral therapy.

The recent recognition of *P. marneffeii* infection in Singapore highlights numerous learning points. Local clinicians should be aware of this condition in individuals who present with fever, weight loss, anaemia, and papular skin lesions; especially if they had lived in or travelled to endemic regions such as Thailand. Underlying HIV infection should also be excluded in all cases of disseminated *P. marneffeii* infection. Early recognition of this syndrome is important because it is an infection that responds favourably to treatment whereas delay in diagnosis and treatment would lead to increased morbidity and mortality.

REFERENCES

- Disalvo A F, Fickling A M, Ajello L. Infection caused by *P. marneffeii*: A description of first natural infection in man. *Am J Clin Pathol* 1973; 60:259-63.
- Sathapatayavongs B, Damrongkitchaiporn S, Saengolatha P, Kiatbonsri S, Jayanetra P. Disseminated penicilliosis associated with HIV infection [letter]. *J Infect Dis* 1989; 19:84-5.
- Supparatpinyo K C, Chiewchanvit S, Hirunsri P, Uthammachai C, Nelson K E, Sirisanthana T. *P. marneffeii* infection in patients infected with HIV. *Clin Infect Dis* 1992; 14:871-4.
- Sirisanthana T. Infection due to *Penicillium marneffeii*. *Ann Acad Med*

- Singapore 1997; 26:701-4.
5. Tuan A D. *P. marneffeii*, an emerging pathogen: Review of 155 reported cases. *Clin Infect Dis* 1996; 23:125-30.
6. Rokiah I, Ng K P, Soo-Hoo T S. *P. marneffeii* infection in an Aids patient-A first case report from Malaysia. *Med J Malaysia* 1995; 50:101-4.
7. Supparatpinyo K, Khamwan C, Baosoung V, Nelson K E, Sirisanthana T. Disseminated *P. marneffeii* infection in SEA. *Lancet* 1994; 344:110-3.
8. Deng Z L, Ribas J L, Gibson D W, Connor D H. Infections caused by *P. marneffeii* in China and SEA; Review of 18 published cases and report of 4 more Chinese cases. *Rev Infect Dis* 1988; 10:640-52.
9. Piehl M R, Kaplan R L, Haber M H. Disseminated penicilliosis in a patient with acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 1988; 112:1262-4.
10. Jones P D, See J. *Penicillium marneffeii* infection in patients infected with human immunodeficiency virus: late presentation in an area of nonendemicity [letter]. *Clin Infect Dis* 1992; 15:744.
11. Peto T E A, Bull R, Millard P R. Systemic mycosis due to *P. marneffeii* in a patient with antibody to HIV. *J Infect Dis* 1988; 16:285-90.
12. Capponi M, Sureau P, Segretain G. Penicilliose de *Rhizomys sinensis*. *Bull Soc Pathol Exot* 1956; 49:418-21.
13. Chariyalertsak S, Sirisanthana T, Supparatpinyo K, Praparattanapan J, Nelson K E. Case-control study of risk factors for *P. marneffeii* infection in HIV-infected patients in Northern Thailand. *Clin Infect Dis* 1997; 24:1080-6.
14. Chariyalertak S, Sirisanthana T, Supparatpinyo K, Nelson K E. Seasonal variation of disseminated *P. marneffeii* infection in Northern Thailand; a clue to the reservoir? *J Infect Dis* 1996; 173:1490-3.
15. The National AIDS Control Programme in Singapore [press release]. Singapore: Ministry of Health, 30 May 1998.
16. Li P C K, Tsui M S, Ma K F. *P. marneffeii*: Indicator disease for Aids in SEA [letter]. *AIDS* 1992; 6:240-1.
17. Sekhon A S, Stein L, Garg A K, Black W A, Glezos J D, Wang C. Pulmonary *Penicilliosis marneffeii*: Report of first imported case in Canada. *Mycopathologia* 1994; 128:3-7.
18. Jayanetra P, Nityanant I, Ajello L. *P. marneffeii* in Thailand: A report of 5 human cases. *Am J Trop Med Hyg* 1984; 33:637-44.
19. Louthrenoo W, Thamprasert K, Sirisanthana T. Osteoarticular *P. marneffeii*: A report of 8 cases and review of the literature. *Br J Rheumatol* 1994; 33:1145-50.
20. Yuen K Y, Wong S S, Tsang D N, Chau P Y. Serodiagnosis for *P. marneffeii*. *Lancet* 1994; 344:444-5.
21. Kauffmann L, Standard P G, Jalbert M, Kantipang I, Limpakarnjanarat K, Mastro T D. Diagnostic antigenaemia tests for *P. marneffeii*. *J Clin Microbiol* 1996; 10:2503-5.
22. Supparatpinyo K, Nelson K E, Merz W G, Breslin B J, Cooper C R, Kamwan C, et al. Response to antifungal therapy by HIV-infected patients with disseminated *P. marneffeii* infections and *in vitro* susceptibilities of isolates from clinical specimens. *Antimicrob Agents Chemother* 1993; 37:2407-11.
23. Sirisanthana T, Supparatpinyo K, Perriens J, Nelson K E. Amphotericin B and itraconazole for treatment of disseminated *Penicillium marneffeii* infection in human immunodeficiency virus-infected patients. *Clin Infect Dis* 1998; 26:1107-10.