Penicilliosis marneffei – West meets East

Penicillium marneffei causes one of the most important opportunist infections in South-east Asia. Clinicians in other parts of the world are often not well acquainted with the disease, sometimes resulting in delayed diagnosis and treatment. Interestingly, the first documented human case was a laboratory-acquired infection in France in 1959 [1]. In 1973 a natural infection was reported in an American suffering from Hodgkin’s disease who had a history of travel to South-east Asia [2]. Since then, cases have been reported in several countries in this area. With the advent of the AIDS pandemic there was a dramatic increase in the number of penicilliosis marneffei patients, and the epidemiology, pathology and clinical management of the disease have been clarified.

Mycology

P. marneffei is a dimorphic fungus with a restricted distribution. Most infections are seen in indigenous inhabitants of the south-western part of China (e.g., the Guangxi Province), Vietnam, Thailand and Hong Kong, or in those with a history of travel to these areas. Although the fungus was initially isolated from bamboo rats [3], the role of the rodents in the natural history of the fungus has not been established. It is postulated that P. marneffei exists as a saprophyte in soil, and that human beings, as well as bamboo rats, become infected through inhalation of the conidiophores [4]. However, attempts at isolation of P. marneffei from soil have not been very successful.

P. marneffei is unique among over 400 Penicillium species in being the only one that demonstrates thermal dimorphism [5]. The fungus grows well on Sabouraud dextrose agar. When grown at 25°C, the fresh culture appears similar to other Penicillium spp.: rapidly growing greenish-yellow mycelial colonies. The most characteristic feature is a soluble brick-red pigment that diffuses into the medium. Few Penicillium spp. produce such a pigment. For a definitive mycological identification, demonstration of thermal dimorphism is essential. At 37°C the fungus assumes a yeast form, in culture and in vivo. Colonies are glabrous, beige-coloured and do not produce red pigment.

Microscopically, the mycelial form resembles other Penicillium spp. with smooth-walled conidiophores borne on terminal verticils of aerial hyphae. The yeast forms are ovoid or elongated measuring 2–3 μm × 26.5 μm. Similar forms are observed within macrophages or extracellularly in tissue samples from patients. In contrast to the yeasts of other fungi, those of P. marneffei divide not by budding, but by fission, so that a transverse septum is often seen in dividing cells. This helps to differentiate P. marneffei from other dimorphic fungi, especially Histoplasma capsulatum.

Clinical features and diagnosis

Penicilliosis marneffei is characterised by systemic infiltration of the cells of the reticulo-endothelial system, resulting in a progressive febrile illness that is almost always fatal if untreated. Common presentations included fever (up to 98% of patients), anaemia (75%), weight loss (72%), skin lesions (70%) and lymphadenopathy (52%) [6]. The skin lesions are particularly distinctive: molluscum contagiosum-like papules, commonly on the forehead, trunk, upper extremities and abdomen, that may be skin-coloured or pigmented with central umbilication [7]. Occasionally the lesions may appear vesicular, necrotic, or pustular, and may appear as ulcers on the skin or mucosal surfaces. Microscopy of the lesions reveals numerous yeast cells both intra- and extra-cellularly. Pulmonary involvement can result in cough, infiltration, abscess, or cavitation. Outside the lungs and the skin, disease may be found in the bones and joints, lymph nodes, pericardium, liver, and even the central nervous system [6].

HIV infection is the single most important underlying disease for penicilliosis marneffei [8]. In Thailand, for example, >90% of the documented cases were HIV-infected [9]. Indeed, among HIV-infected patients, penicilliosis marneffei is the third commonest opportunistic infection in parts of South-east Asia after tuberculosis and cryptococcosis [9]. Some patients may have other underlying diseases or may be receiving corticosteroids; a few have no recognisable condition that impairs their immune status. The relative predominance of different types of patients can vary in different centres: in our hospital only 31% of patients with penicilliosis marneffei have AIDS, while 54% have other underlying diseases, including systemic lupus erythematosus and haematological malignancy,
or were on corticosteroids; 15% were not obviously immunocompromised.

Clinical diagnosis in endemic areas is not usually difficult, especially in AIDS patients with characteristic skin lesions. Elsewhere, diagnosis may not be immediately obvious as there are few, if any, pathognomonic signs and symptoms. The only clue may be a history of travel to endemic areas, which should arouse clinical suspicion, especially in immunocompromised patients. Most patients present with pyrexia of unknown origin, and an initial diagnosis of typhoid fever, tuberculosis, melioidosis or autoimmune disease is often entertained, reflecting the local incidences of these diseases. In c. 80% of our patients, initial coverage with broad-spectrum antibiotics, including β-lactam antibiotics and the newer quinolones, was given; 60% of patients were given empirical anti-tuberculosis treatment, as tuberculosis remains endemic in much of South-east Asia. Laboratory confirmation of the diagnosis is therefore essential.

**Laboratory diagnosis**

The only way to make a definitive diagnosis is conventional fungal culture of a good clinical specimen. *P. marneffei* can be readily differentiated from other *Penicillium* spp. by the production of the diffusible red pigment and by demonstration of thermal dimorphism. *P. marneffei* is isolated most frequently from skin biopsies and peripheral blood, followed by bone marrow, lymph nodes and other tissue biopsies [6]. It may be found rarely on direct examination of a peripheral blood smear in those with heavy, disseminated infections [10]. However, 24–45% of patients may not have a positive blood culture, especially if the disease is localised, and the time for a positive culture to appear varies from 2 days to 4 weeks. In patients without fungaemia, broncho-alveolar lavage, biopsies and fine needle aspiration, or even pericardial aspiration, are necessary to obtain tissues from the suspected sites of infection.

The yeast form of *P. marneffei* may be stained by methenamine silver or periodic acid-Schiff stains, but this may not differentiate it from other dimorphic fungi such as *H. capsulatum* in histological sections. A monoclonal antibody to galactomannan (EB-A1) may be used to detect *P. marneffei* (and *Aspergillus* spp.) in formalin-fixed, paraffin-embedded tissues [11]. Various serodiagnostic tests have been developed for the detection of antibodies or antigens in the serum and body fluids of infected patients. In the early studies culture filtrates or whole cell extracts were used as antigens. *P. marneffei* was cultured in liquid media, and the culture filtrate, or anti-*P. marneffei* sera raised in rabbits, was incorporated in an immunodiffusion test to detect antibody or antigens respectively.

We have used an indirect immunofluorescent antibody test for serodiagnosis, with yeast hyphae (representing the tissue multiplication phase) or germinating conidia (representing the initial tissue invasion phase) as antigens [12]. Much higher IgG titres were found in all eight patients with culture-positive penicilliosis marneffei (including three with HIV), than in 78 healthy controls. Cross-reactivity to other dimorphic fungi was not tested, because of the lack of such cases locally, but sera from two patients with cryptococcosis and two with candidaemia showed IgG titres similar to those of healthy controls. In our experience, an IgG titre >80 is indicative of penicilliosis marneffei, and a thorough investigation of the patient is warranted. However, this test may be less useful for diagnosis in patients living in endemic areas.

A latex agglutination test, with polystyrene beads coated with rabbit anti-*P. marneffei* globulin, has also been developed; antigenaemia was detected in 13 of 17 *P. marneffei* culture-positive HIV patients [13]. Similarly, purified hyperimmune IgG, from rabbits immunised with yeast cells, has been used in an enzyme-linked immunosorbent assay (ELISA) to quantify *P. marneffei* yeast antigens in urine samples. Urine samples from all of 33 *P. marneffei* culture-positive HIV patients yielded high titres [14].

There have been several attempts to characterise *P. marneffei* antigens. The gene encoding one of them, the Mplp mannoprotein, has been sequenced, and the protein has been expressed and used for diagnosis [15]. Among 17 *P. marneffei* culture-positive HIV patients and two culture-positive HIV-negative patients, 82% had antibodies against Mplp. However, 71% of 24 *P. marneffei* culture-positive HIV-positive patients and neither of the two culture-positive HIV-negative patients had Mplp mannoprotein in their sera [16]. It is likely that antibody and antigen detection tests are required for diagnosis in non-immunosuppressed and immunocompromised patients, respectively.

Although encouraging results have been obtained with tests to detect antigen or antibody in blood and body fluids, the sensitivity of these tests, and their cross-reactivity in other fungal, mycobacterial and bacterial infections must be evaluated further. Test kits must be available commercially, before wide acceptance and common use is possible.

The detection of the *P. marneffei* genomic DNA in clinical specimens has also been reported. Primers to amplify a 347-bp fragment of the internal transcribed spacer region between 18S rDNA and 5.8S rDNA have been described [17]. A PCR-Southern hybridisation format, with amplification of a 631-bp fragment of the 18S rDNA, followed by hybridisation with a *P. marneffei*-specific 15-oligonucleotide probe has also been used [18]. We are presently testing the possibility of using a one-tube nested PCR to detect the Mpl gene.
Treatment

Penicilliosis is very amenable to antifungal treatment. *P. marneffei* is susceptible to itraconazole and amphoteracin B in vitro, although the susceptibility to fluconazole and 5-fluorocytosine is less uniform [19]. There have been few well-controlled clinical trials comparing the efficacy of different antifungal regimens. Theoretically, patients with disseminated disease and fungaemia should benefit from an initial course of amphoterin B, as it is fungicidal. If there is a high fungal load, with or without impairment of host immune response, this is a definite advantage. In one non-randomised study, intravenous amphoteracin B (0.6 mg/kg daily) for 2 weeks, followed by oral itraconazole (400 mg/day) for 10 weeks, resulted in clinical and microbiological cure in 97.3% of the patients [20]. This seems a reasonable regimen in most cases, based also on previous clinical experience and in-vitro susceptibility results. In the only double-blind, placebo-controlled randomised trial published [21], the efficacy of secondary prophylaxis with itraconazole was demonstrated in HIV-positive patients with penicilliosis marneffei: after an initial 2-week regimen of intravenous amphoteracin B, 0.6 mg/kg daily, oral itraconazole was given at a dose of 200 mg twice daily for 10 weeks. The relapse rate in the placebo group within 1 year was 57%, with no relapse in the treatment group.

S. S. Y. WONG, H. SIAU, K. Y. YUEN
Department of Microbiology,
University of Hong Kong,
Queen Mary Hospital,
Hong Kong
Corresponding author: Professor K. Y. Yuen
(e-mail: kyyuen@hkueh.hku.hk)

References

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