SHORT REPORT

Rapid diagnosis of *Penicillium marneffei* infection by fine needle aspiration cytology

D Lim, Y-S Lee, A R Chang

J Clin Pathol 2006;59:443–444. doi: 10.1136/jcp.2004.024976

This report describes the use of fine needle aspiration (FNA) cytology to make a rapid diagnosis of *Penicillium marneffei* infection in an HIV positive patient. *P. marneffei* is a thermally dimorphic fungus that is a mould at 25°C and a yeast at 37°C. It multiplies by fission. It can be easily mistaken for various other infections that are characterised by the presence of histiocytes with phagocytosed yeast cells. In smears the demonstration of yeast cells with a distinctive central septum confirms the diagnosis.

CASE REPORT

A 36 year old Thai woman presented with a two month history of intermittent fever and weight loss. She was in hospital two months previously with pneumonia. Clinical examination revealed generalised lymphadenopathy, hepatosplenomegaly, and a facial rash characterised by umbilicated papules. Additionally she was anaemic, had borderline thrombocytopenia, positive HIV serology, and low CD4 counts. A fine needle aspirate (FNA) was carried out on an enlarged cervical lymph node and biopsies were obtained from the nasopharynx and skin lesions.

Cytopathology

The aspirated lymph node material showed scattered lymphocytes, histiocytes, and a necrotic background. Large numbers of yeast-like organisms were present both extracellularly and within histiocytes. Those within histiocytes were spherules measuring 2–4 µm, with a central dot-like structure. The extracellular organisms were more elliptical or “sausage” shaped (fig 1A), and had a prominent central septum that was clearly seen with Gomori-methamine-silver (GMS) staining (fig 1B). These septae stained more intensely than the external cell walls. No budding forms were present, unlike in histoplasmosis. Biopsies of the nasopharynx (fig 1C) and skin showed a prominent histiocytic infiltrate that was present in the submucosa and dermis, and septated, intracytoplasmic organisms were seen. Despite treatment with amphotericin B the patient died within 72 hours of admission.

DISCUSSION

P. marneffei, the only thermally dimorphic fungus of the genus *Penicillium*, can cause a life threatening infection in humans. At 25°C on culture plates it forms a mould and at 37°C, invasive tissue phase, a yeast.

Round pathogens that have been phagocytosed by histiocytes are found in a variety of other infections. These include protozoan infections (leishmaniasis, toxoplasmosis, pneumocystis), deep mycosis (*P. marneffei*, histoplasmosis, blastomycosis, and cryptococcosis), and bacterial infections

(rhinoscleroma and granuloma inguinale). All these have to be considered in the differential diagnosis. In leishmaniasis, bar shaped kinetoplasts within amastigotes are seen under oil microscopy, whereas cells of *Toxoplasma gondii* are smaller than the yeast forms of *P. marneffei*, lack the central septum, and usually also parasitise other somatic cells. In *Pneumocystis carinii* infection, cysts measuring 4 to 7 µm in diameter are present, containing single or paired comma shaped argyrophilic foci in the walls. The presence of narrow based budding forms and prominent capsule highlighted by mucicarmine or PAS stain is characteristic of *Cryptococcus neoformans*, while broad based budding forms and thickened cell walls are seen in *Blastomyces dermatitidis*. Budding and cysts forms are absent in *P. marneffei* infection. *Calymmatobacterium granulomatis*, which is the causative agent in granuloma inguinale, presents as minute (1–2 µm) Gram negative non-motile encapsulated coccobacilli within histiocytes. In rhinoscleroma, rod shaped bacteria highlighted by Warthin-Starry silver stains or Giemsa staining are identified within large foamy histiocytes (Mukulicz cells). Different techniques can be employed to confirm *P. marneffei* infection. The fungus is readily recovered from bone marrow aspirates, blood, and skin lesions,¹ and can be cultured using Sabouraud's dextrose agar, but this can take up to three days. At 25°C, a mycelial form that produces a diffusible red pigment in culture is seen. On microscopic examination hyaline septated branching hyphae with lateral and terminal conidiophores may be seen. Immunohistochemical, serological, and polymerase chain reaction techniques have also been employed,² but all are time consuming and not widely available.

Three histological patterns of infection are recognised.³ A granulomatous or suppurative reaction is seen in immunocompetent patients, while an anergic and necrotising reaction occurs in subjects with defective immunity. The yeast form of the organism may be found both intracellularly within histiocytes, and also in the extracellular environment. The yeast cells resemble the spores of *H. capsulatum* and may be mistaken for the latter.⁴ However, the distinctive central transverse septum is unique to *P. marneffei*. A further difference is that *H. capsulatum* undergoes division by budding while *P. marneffei* uses a process of “fission” to generate a septum and produce daughter cells.⁵ *P. marneffei* is endemic in Southeast Asia and parts of southern China and causes disease in both immunocompetent and immunocompromised individuals.^{3 6 7} In northern Thailand, infection with *P. marneffei* is the third most common opportunistic infection in patients with late stage HIV infection, after tuberculosis and cryptococcal meningitis.⁶ In endemic areas *P. marneffei* infection is used as an early indicator of HIV infection. The mode of transmission of the fungus to human is still largely unknown. The organism has been isolated from the internal organs of various

Abbreviation: FNA, fine needle aspiration

Figure 1 (A) Elliptical or "sausage" shaped extracellular organisms. (B) The prominent central septum is clearly seen with Gomori-methamine-silver staining. (C) Prominent histiocytic infiltrate present in the submucosa and dermis of the skin.

species of bamboo rats and also from the soil around the burrows of the animals. However, studies have shown that bamboo rats may not be directly involved in the transmission of the organism to humans, but rather both humans and rats are infected from a common unidentified source.⁸ Other postulated modes of transmissions are through the respiratory and percutaneous routes. The signs and symptoms of patients infected with *P. marneffei* are often non-specific and co-infections with other opportunistic pathogens have been reported. Common clinical features include fever, weight loss, and anaemia.⁶ Disseminated disease is also characterised by skin lesions that resemble the umbilicated papules seen in molluscum contagiosum. In patients with AIDS, lymph nodes, skin, bone, and the bone marrow are the most commonly involved organs, whereas in non-AIDS patients, lymph nodes, liver, lung, and kidney are affected. FNA cytology on lymph node material allows a diagnosis to be rapidly reached.

P. marneffei is recognised as a health problem among HIV infected patient in Southeast Asia. However, reported cases of *P. marneffei* infection in other parts of the world have also been increasing, a result of international travel and the AIDS epidemic.⁷⁻⁹ Mortality in patients with disseminated *P. marneffei* infection is high, regardless of their HIV status, but with early treatment with amphotericin B and itraconazole survival is enhanced.¹⁰ Thus a rapid and confident diagnosis of the infection is essential and this can be made by aspiration cytology.¹¹⁻¹³

Authors' affiliations

D Lim, Department of Pathology, National University Hospital, Singapore

Y-S Lee, Department of Pathology, National University of Singapore

A R Chang, Department of Pathology, National University Hospital, Singapore

Correspondence to: Dr D Lim, Department of Pathology, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074; patv3@nus.edu.sg

Accepted for publication 22 June 2005

Take home messages

- FNA cytology is a simple, rapid, and reliable method for diagnosing *P. marneffei* infection, so that appropriate life saving treatment can be instituted. The use of GMS and PAS stains allows the characteristic septated organisms to be seen.
- Though this infection was once seen mainly in Asia, a combination of the AIDS epidemic and increased international air travel will give it a more global distribution, which pathologists outside Asia need to be aware of.

REFERENCES

- 1 **Cooper CR**, McGinnis MR. Pathology of *Penicillium marneffei*. An emerging acquired immunodeficiency syndrome-related pathogen. *Arch Pathol Lab Med* 1997;**121**:798-804.
- 2 **LoBuglio KF**, Taylor JW. Phylogeny and PCR identification of the human pathogenic fungus *Penicillium marneffei*. *J Clin Microbiol* 1995;**33**:85-9.
- 3 **Deng Z**, Ribas JL, Gibson DW, *et al*. Infection caused by *P. marneffei* in China and Southeast Asia: review of eighteen published cases and report of four more Chinese cases. *Rev Infect Dis* 1988;**10**:640-52.
- 4 **Jayaram G**, Chew MT. Fine needle aspiration cytology of lymph nodes in HIV-infected individuals. *Acta Cytol* 2000;**44**:960-6.
- 5 **Deng ZL**, Connor DH. Progressive disseminated penicilliosis caused by *Penicillium marneffei*. Report of eight cases and differentiation of the causative organism from *Histoplasma capsulatum*. *Am J Clin Pathol* 1985;**84**:323-7.
- 6 **Suppraratpinyo K**, Khanwan C, Baosoung V, *et al*. Disseminated *Penicillium marneffei* infection in Southeast Asia. *Lancet* 1994;**344**:110-13.
- 7 **Remadi S**, Lotfi C, Finci V, *et al*. *Penicillium marneffei* infection in patients infected with the human immunodeficiency virus. A report of two cases. *Acta Cytol* 1995;**39**:798-802.
- 8 **Chariyalertak S**, Sirisanthana T, Suppraratpinyo K, *et al*. Case-control study of risk factors for *Penicillium marneffei* infection in Human immunodeficiency virus - infected patients in northern Thailand. *Clin Infect Dis* 1997;**24**:1080-6.
- 9 **Piehl MR**, Kaplan RL, Haber MH. Disseminated penicilliosis in a patient with acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 1988;**112**:1262-4.
- 10 **Sirisanthana T**. Infection due to *Penicillium marneffei*. *Ann Acad Med Singapore* 1997;**26**:701-4.
- 11 **Chaiwun B**, Khunamornpong S, Sirivanichai C, *et al*. Lymphadenopathy due to *Penicillium marneffei* infection: diagnosis by fine needle aspiration cytology. *Mod Pathol* 2002;**15**:939-43.
- 12 **Chan JK**, Tsang DN, Wong DK. *Penicillium marneffei* in bronchoalveolar lavage fluid. *Acta Cytol* 1989;**33**:523-6.
- 13 **Ma KF**, Tsui MS, Tsang NC. Fine needle aspiration diagnosis of *Penicillium marneffei* infection. *Acta Cytol* 1991;**35**:557-9.