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Disseminated *Penicillium marneffei* infection in an HIV infected patient returning from Vietnam

N. Prim^a, G. López^b, N. Domínguez^d, O. Torres^{b,e}, V. Pomar^{b,c,e}, F. Sánchez-Reus^{a,e,*}

^a Servicio de Microbiología, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

^b Servicio de Medicina Interna, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

^c Unidad de Enfermedades Infecciosas, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

^d Servicio de Anatomía Patológica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

^e Universidad Autónoma de Barcelona, Barcelona, Spain

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PALABRAS CLAVE

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Abstract A 33-year-old Spanish man presented with fever, expectoration, weight loss and lung cavitary lesions. HIV-positive serology, the lack of clinical improvement under anti-tuberculosis treatment, and the teamwork carried by clinicians, microbiologists and pathologists led to the diagnosis of *Penicillium marneffei* infection. This case supports the importance of the correct evaluation of the epidemiological history of the patients.

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Infección diseminada por *Penicillium marneffei* en paciente infectado por VIH que vuelve de Vietnam

Resumen Presentamos el caso de un paciente con fiebre, síndrome tóxico y lesiones pulmonares cavitadas. La positividad de la serología de VIH, la ausencia de mejoría con tratamiento tuberculostático, y el trabajo conjunto de clínicos, microbiólogos y patólogos permitió llegar al diagnóstico de infección por *Penicillium marneffei*. Además, el caso refuerza la importancia de una correcta evaluación de los antecedentes epidemiológicos.

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Penicillium marneffei (*P. marneffei*) is a dimorphic fungus endemic in a wide area of Southeast Asia. Although the

primary source of this soil saprophyte remains unknown, it has been found in bamboo rats in this area.^{1,2} The infection is mainly acquired by inhalation of conidia, causing respiratory symptoms, lung infiltrates and cavitations. Fever, weight loss, malaise and generalized lymphadenopathies can be accompanied by cutaneous lesions resembling *molluscum*

* Corresponding author.

E-mail address: fsanchezr@santpau.cat (F. Sánchez-Reus).

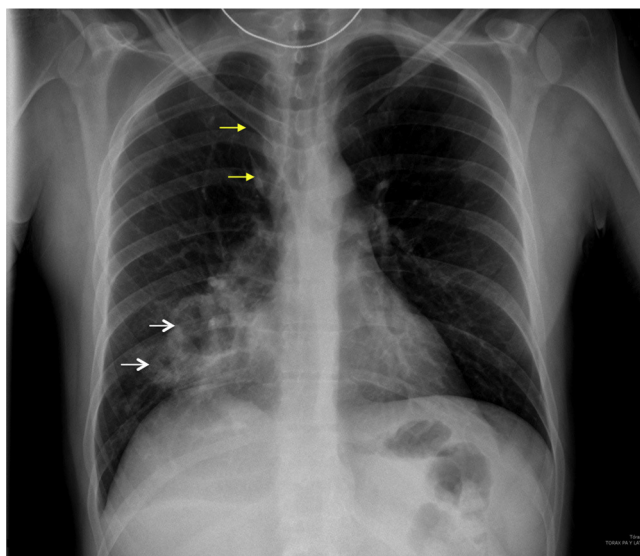


Figure 1 Chest X-ray showing two nodular cavitary images (white arrows) and an azygos lobe (yellow arrows).

contagiosum.^{3,4} The present case reports the first imported penicilliosis in a Spanish HIV-infected traveller.

Clinical observation

A 33-year-old Spanish man presented with fever, expectoration, and weight loss of 10 kg in the last three months. Cachexia and fine basal crackles stood out in the physical examination. Abnormal laboratory values included C-reactive protein of 125 mg/L and elevated transaminases (AST 93 U/L, ALT 137 U/L, GGT 137 U/L). Chest X-ray showed two nodular cavitary images in right middle and lower lobes (Fig. 1). Chest computer tomography (CT) revealed a 65-mm cavitary mass in the right middle lobe, peripheral consolidated images in the right middle and lower lobes, and subcarinal, paratracheal and hilar adenopathies. Three serial sputum samples and a fibrobronchoscopy were performed. A bronchial biopsy and a bronchoalveolar lavage (BAL) were taken for histological and microbiological analysis. Two sets of blood cultures (FAN bottles, bioMérieux) were also taken. Serological tests for cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus were requested. Serology for HIV was positive. The patient had a count of 25 CD4⁺/mm³ and 479,815 viral copies/ml. Rest of serologies were negative except for chronic CMV infection. Antiretroviral therapy was started, four first-line antituberculosis drugs and empirical treatment with cotrimoxazole against *Pneumocystis jirovecii* were added, but fever and malaise continued.

Ziehl–Neelsen stain and tuberculosis nucleic acid detection on BAL were negative. Giemsa staining on this specimen ruled out *P. jirovecii* infection. BAL smears stained with Papanicolaou showed a background of inflammatory cells composed of 40% of macrophages, 35% of lymphocytes and 25% of polymorphonuclear leucocytes. Multiple small round-to-oval yeast-like organisms with no budding were seen both inside the cytoplasm of macrophages and extracellularly. The biopsy showed fragments of bronchial epithelium with

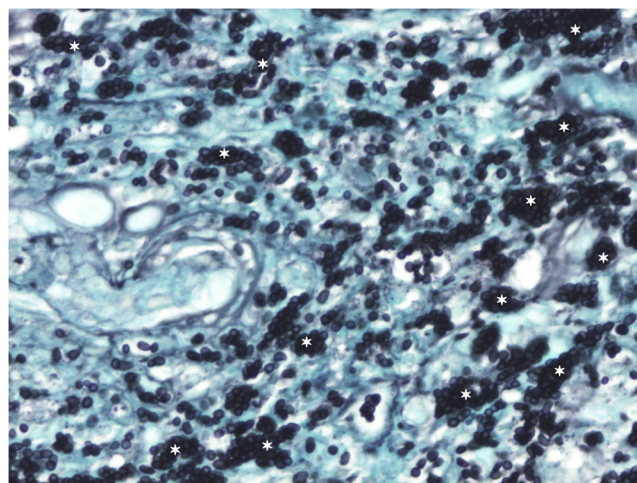


Figure 2 Yeast-like cells observed on the bronchial biopsy (methenamine silver stain) (white stars).

a subepithelial granulomatous process composed mainly of macrophages with the same intracytoplasmic organisms. Periodic acid-Schiff (PAS) and methenamine silver stains were performed on both respiratory samples confirming the presence of fungal organisms (Fig. 2). Gram and calcofluor white stains performed on both respiratory samples showed yeast-like cells (Fig. 3). No bacterial pathogens were detected on these specimens. After a five-day period of incubation at 28 °C, velvety greyish colonies grew on Sabouraud agar plates (Bio-Rad, Marnes-La-Coquette, France) showing an appearance of filamentous fungi rather than yeast-like forms. Galactomannan (GM) antigen detection on BAL (BioRad Platelia™ *Aspergillus* EIA) was positive with an index > 10. A dimorphic fungus was suspected due to the morphological discrepancy between typical fungal filamentous growth and direct microscopic observation of yeasts.

Clinicians re-interviewed the patient concerning possible travels to regions endemic areas of histoplasmosis or other dimorphic fungal infections. It was noted that he had

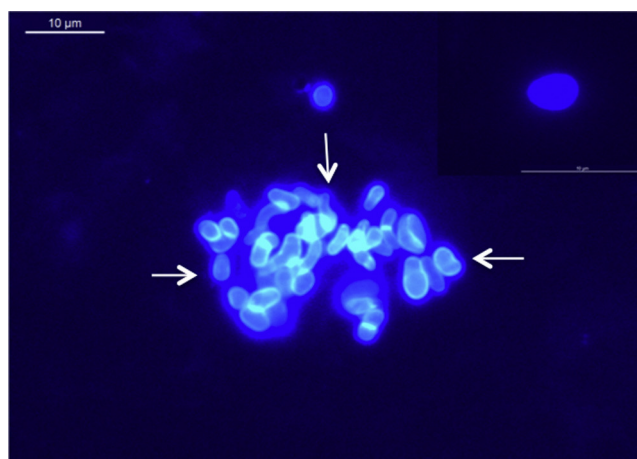


Figure 3 A composite image of calcofluor white stain performed on BAL, showing non-budding yeast-like cells (white arrows). The central transverse septum is revealed at higher magnification. Both bars correspond to 10 µm.

travelled to Vietnam during the previous year and to the Dominican Republic four months before consulting.

The aerobic FAN bottles of both blood cultures became positive after being incubated in an automated BactAlert® system (bioMérieux) during 6 and 11 days, respectively. Highly septate hyphal cells were observed on the direct blood gram stain. Colonies resembling those previously observed in respiratory samples grew on Sabouraud agar plates within four days of incubation at 28 °C. Lactophenol cotton blue wet mount preparation showed the typical *Penicillium* terminal verticils with three and five metulae, each containing several phialides with conidia in chains. Fungal dimorphism was confirmed by culturing the fungus on 5% sheep blood agar plates at 28 °C and 37 °C. Mycelium converted to the yeast form after one week at 37 °C. The isolate was identified as *P. marneffei* according to thermal dimorphism, microscopy observation of both forms, and the presence of a red diffusible pigment around the filamentous colonies.

Treatment with liposomal amphotericin B (4 mg/kg/day) was started and fever disappeared five days later. After 18 days the initial treatment was changed to oral itraconazole (400 mg/day). The patient was discharged from the hospital one month later and continued with this treatment uneventfully for 10 weeks, followed by a dose of 200 mg/day to prevent recurrences.

Discussion

Lung cavitory lesions are relatively frequent in HIV patients due to both infectious and non-infectious pathological processes. Differential diagnosis poses further difficulties in these patients and should consider not only their state of immunosuppression but also other risk and epidemiological factors. The infectious aetiology of this kind of lesions greatly depends on the populations under study and endemicity of certain diseases.⁵ In Europe, these lesions are mainly caused by bacteria and mycobacteria.⁶ *P. jirovecii* is the most frequent fungus involved although fungal aetiologies are not prevalent in our area.⁶ In contrast, the main cause of cavitory lesions in Southeast Asia is fungal, particularly due to *P. marneffei*.⁵ This fungal infection is increasingly reported in immunocompromised patients returning from this endemic area.^{7,8} However, clinical diagnosis may be hindered because *P. marneffei* infection is rarely seen in our environment and may be easily confused with infections such as tuberculosis.

Penicilliosis especially affects patients with strong T-cell immunosuppression in whom the disseminated disease may be fatal. This is the case of HIV-infected patients under 100 CD4+/mm³. The prevalence of penicilliosis has risen markedly since the HIV-pandemics in the aforementioned endemic area, where it has become a common acquired immune deficiency syndrome (AIDS)-defining illness in HIV patients.³

P. marneffei is the only thermal dimorphic fungus included in the genus *Penicillium*. The typical mycelial structures of this genus develop when incubated at 25–30 °C whereas oval yeast-like cells eventually form at 35–37 °C and in tissues.² Cell division occurs by fission with some cells showing a characteristic central transverse septum.² These

non-budding forms are not considered as true yeasts strictly speaking. As in the present case, diagnosis of penicilliosis may be histologically suspected on cytological specimens or biopsies upon observation of small yeast-like cells located either extracellularly or intracellularly in histiocytes.⁹ Some other microorganisms can easily be misidentified on these specimens, the most common being *Histoplasma capsulatum* and *Toxoplasma gondii*.⁹ Demonstration of septate forms on respiratory specimens instead of budding cells would support the diagnosis of penicilliosis rather than histoplasmosis. However, microbiological isolation from clinical specimens remains the gold standard for diagnosis of *P. marneffei* infection.³ Clinical specimens yielding the best isolation rates are bone marrow, skin biopsies and blood.³ In the present case, the fungal isolate was obtained from respiratory and blood specimens. The observation of hyphae on blood culture smears after being incubated at 37 °C can sometimes be misleading since the expected yeast-like forms appear only after hyphal fragmentation with time.³ In our case histological and microbiological studies converged in time to give the initial alert of a dimorphic fungus. Based on its thermal dimorphism, the isolate was identified as *P. marneffei* both macroscopically and microscopically.

Galactomannan (GM) is a heat-stable heteropolysaccharide present in the cell walls of most *Aspergillus* and *Penicillium* species.¹⁰ However, cross-reaction with GM detection has also been reported for other invasive fungal infections such as histoplasmosis and cryptococcosis.^{11,12} GM detection on serum samples is used as a screening test in newly diagnosed HIV patients in endemic areas because it facilitates early diagnosis of penicilliosis.^{3,10} Although there are no cut-off values for *P. marneffei* detection, patients with fungemia have significantly higher GM levels than those with penicilliosis without fungemia.¹⁰ In the present case, GM detection on serum was not performed but the initial high GM index on BAL suggested a possible invasive fungal infection.

As migratory movement is increasing worldwide, unusual microorganisms in a certain geographic area should be included in the diagnosis of cavitory lung lesions in HIV-infected travellers. *P. marneffei* infection should be considered in HIV-infected patients who have travelled to the endemic area.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgements

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