Disseminated *Penicillium marneffei* infection contracted in China

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Abstract

Penicillium marneffei **infection is a rare fungal disease that cause significant disease in immunosuppressed patients. The geographical distribution of this dimorphic fungus is restricted to Asia, Southeast and Far East, where the disease is considered as an indicator of acquired immunodeficiency syndrome (AIDS).**

Case report. A 42-year old Congolese woman living in Lubumbashi was admitted at the university hospital of Liège for exploration of a general status impairment. She experienced for three months spiking fever, weight loss, productive cough with bloody expectorations and progressive dyspnoea. She reported also to have non-bloody mild diarrhoea with abdominal pain.

The HIV antibody status was positive with a low CD4 T lymphocytes count (28/µl). Pulmonary infiltrates were visualized on chest radiography and the computed tomography revealed the presence of a severe pneumopathy characterised by bilateral micronodular lesions. Mediastinal polyadenopathies associated with hepato- and splenomegaly were also highlighted.

Bronchoscopy was performed and bronchial aspirations revealed the presence of numerous leucocytes with the presence of intracellular Gram positive organisms suggestive of yeasts. Ziehl, Giemsa and Gomori-Grocott staining were also performed. Ziehl staining was negative. The morphological aspect given by Giemsa staining excluded infection and the PCR specific for *T. gondii* B1 gene was negative. However, Gomori-Grocott staining revealed the presence of intracellular oval, elongated, sausage-shaped cells with a single transverse septum (3 to 5 µm). *Penicillium marneffei* was isolated from blood culture and respiratory samples.

Intraveinous amphotericin B treatment was administrated during 15 days followed by itraconazole oral administration (200 mg/j). The antimycotic treatment improved the patient condition and despite other clinical troubles she was prematurely discharged because of financial problems.

Conclusion. Opportunistic agents involved in HIV-infected patients differ in Africa and Asia and it is important to be able to make a rapid diagnosis with the aid of an experienced laboratory.

Background

Penicillium marneffei is a dimorphic fungus which was first isolated from the liver of Chinese bamboo rats by Capponi et coll. in 1956 (1). The fungus was identified by Segretain in 1959 at the Pasteur institute as a pathogenic agent invading the reticular-endothelial system of these rats.

P. marneffei is a rare fungal pathogen in humans that is responsible for disseminated infection in immuno-suppressed patients living in endemic area. The geographical distribution of this fungus is restricted to Asia, Southeast and Far East, where the disease is considered as an indicator of acquired immunodeficiency syndrome (AIDS).

The first case of human infection was reported in 1973 in an American minister suffering from Hodgkin disease and reporting a past travel in Asia one year before. From the years1988-89 there is an increasing number of *P. marneffei* infections among the HIV population who come from or who have visited endemic areas of South-east Asia.

The mode of contamination is until now not fully clear.

Rhizomys sinensis

Endemic areas: south-eastern Asia: Indonesia, Laos, Hong-Kong, Singapore, Thailand, Myanmar, Malaysia, Vietnam, Taiwan and the Guanxi province of China.

Objectives

- To point out the importance to know the travel history of a patient
- To suggest a systematic detection of *P. marneffei* in AIDS patients with pulmonary disease

Patient

Admission. A 42-year old congolese female was admitted at the University Hospital of Liège on 25th July 2006 because of a general status impairment despite several courses of antibiotics. She was first admitted at the University Hospital of Lubumbashi (RDC) where she is living and she was suspected to have tuberculosis.

Clinical signs. She experienced for 3 months asthenia, anorexia, spiking fever, productive cough with bloody expectorations, progressive dyspnea, dysphagia, abdominal pain and tenderness. She reported also weight loss.

Physical examination revealed hypoventilation of lungs bases and hepatosplenomegaly.

Biology. RBC and WBC counts were respectively: 4.22 10^6 and 8.15 10^3 /mm³ (with 85% granulocytes) . The CD4+ T lymphocytes number was 18 /mm³.

Infectious serology. HIV, HBV and HCV serology were positives.

Imaging examination. CT scan of chest and abdomen showed enlarged mediastinal and mesenteric lymph nodes.

Treatment at admission. Antiretroviral therapy (Triomune 40 2/d), fluconazole 400 mg 1/d, Bactrim 100mg 3/d, Acyclovir 400 mg 3/J, Dexamethazone 12mg 3/D. Antituberculous treatment including rifampicin, ethambutol, isoniazid and pyrazinamide was also prescribed during the first hospitalisation in Lubumbashi.

Results

Laboratory findings

•Bronchoalveolar lavage fluid (BAL)

Microscopic examination of the BAL fluid performed after Giemsa staining and Gram staining reported the presence of numerous intracellular microorganisms. Gomori methenamine staining was also performed to identify the agent (Figures 2-4)

Bacteriological and fungal cultures. Fungal cultures performed on Sabouraud agar medium showed after 48hs at 28°C the development of a filamentous fungus and the apparition of a red pigment within the medium after 4 days. The fungus was identified as *Penicillium marneffei* (Figure 5)

Mycobacterial cultures. Microscopic examination of 3 respiratory samples and cultures were negative.

•Blood cultures

Blood cultures performed on Bact/Alert (BioMerieux, France) and incubated at 35°C were positive after 48h. The microscopic examination of a Gram stained smear revealed the presence of septate filaments. The subculture performed on Sabouraud agar medium showed the development of *P. marneffei*

Figure 1. Intracellular yeast-like cells in bronchoalveolar lavage fluid (Giemsa stain, x1000)

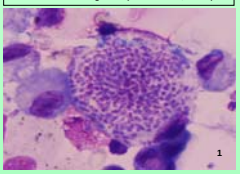
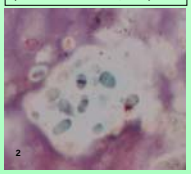


Figure 2. Intracellular yeasts in BAL fluid (Gomori methenamin stain, x1000)



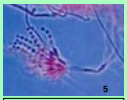
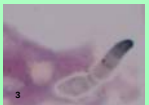


Figure 5. Microscopical examination of P. marneffei in BAL **culture.** Lactophenol blue stain preparation (x400)



Figures 3. Extra-cellular short filaments in broncho-alveolar lavage fluid (Gomori methenamine stain, x 2000)

Figures 4. Extra-cellular short filaments in broncho-alveolar lavage fluid (Gomori methenamine stain, x 2000)

•Travel history. The patient who was questioned again after the first laboratory findings reported one month travel in South China.

•Treatment. Amphotericin B was administrated (0.6 mg/kg/d) for 2 weeks followed by oral Itraconazole (200 mg/d). Dramatic improvement (clinical and biological) was observed after 2 weeks of Amphotericin B. Abdominal and thoracic scan indicated regression of enlarged lymph nodes. She was discharged from the hospital under maintenance therapy of 200 mg/d Itraconazole.

Conclusions

- *Penicillium marneffei* infection was suggested on the first microscopic examination of the BAL fluid. Giemsa staining did not permit the differentiation of intracellular microorganisms like *Histoplasma*, *Leishmania* and *Toxoplasma.* The use of Gomori methenamine silver stain was necessary to assess the fungal nature of the microorganisms and to show the specific yeast-like cells with transverse septa.
- The patient had no cutaneous involvement and the clinical presentation was similar to tuberculosis. It is quite interesting to emphasize that blood infection was also detected by an automated blood culture system.
- Since there is a case of PM infection in an African patient living in Germany with no history of travel in endemic area (2), it could be hypothesized than the contamination could occur in other areas than Southeast Asia.

References

1. Capponi M. et al. 1956. Penicillosis from *Rhizomis sinensis*. Bull. Soc. Pathol. Ex. Filiales. 49:418-421.

2. Lo Y. et al. 2000. Disseminated *Penicillium marneffei* infection in an African AIDS patient. Trans. R. Soc. Trop. Med. Hyg. 94:187.