Case Report

Diagnostic Cytology and Morphometry of *Penicillium marneffei* in the Sputum of a Hypogammaglobulinemia with Hyper-IgM Patient

Chongrak Sripa BPH, CT*, Jumphol Mitchai MD*,
Warin Thongsri BPH*, Banchob Sripa PhD*

** Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*

Penicillosis caused by Penicillium marneffei is endemic in Asia and is a highly fatal disease in HIV-AIDS patients. Reports, however, in other immunocompromized diseases are scanty. This report describes the cytological diagnosis of P. marneffei infection from the sputum of a pediatric patient with hypogammaglobulinemia with hyper IgM and severe pneumonia. In this case, rapid, differential identification of the characteristic septated structure of P. marneffei in the macrophages, bronchial epithelium and also extracellularly allowed prompt and proper treatment. In addition, morphometry of P. marneffei obtained from the clinical specimen was reported. This report demonstrated the fungus was not only in the phagocytes, a phenomenon that is well recognized, but also in epithelial cells. Moreover, it also highlights the need for awareness of penicillosis in non-AIDS immunocompromized patients living in, or persons traveling to, P. marneffei-endemic areas.

Keywords: *Penicillosis, Penicillium marneffei, Cytology, Sputum, Hypogammaglobulinemia*

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Penicillium marneffei is an emerging pathogenic fungus that can cause a fatal systemic mycosis in immunocompromized patients, especially persons infected with HIV-AIDS[1,2]. After being discovered in 1956 in bamboo rats, the importance of *P. marneffei* as a human disease was recognized only when the HIV pandemic arrived in Asia. *P. marneffei* is endemic in South and Southeast Asia, particularly in Thailand, northeastern India, China, Hong Kong, Vietnam and Taiwan[1]. The prevalence of *P. marneffei* infection has increased substantially during the past few years, even in non-endemic areas[3].

P. marneffei infection in immunocompromized hosts other than HIV-AIDS patients does not seem to have been previously reported. This report presents, for the first time at Srinagarind Hospital Khon Kaen University, the cytological diagnosis as well as morphometry of *P. marneffei* in the sputum of a pediatric patient with hypogammaglobulinemia with hyper-IgM and neutropenia. Importantly, the disease is a rare but highly fatal primary immunodeficiency syndrome in children[4,5].

Correspondence to: Sripa B, Department of Pathology, Faculty of Medicine, Khon Kaen University, Mitrapap Road, Khon Kaen 40002, Thailand. Phone: 043-363-113, Fax: 043-202-024. E-mail: banchob@kku.ac.th

Case Report

A 3-year-old boy was admitted to the Cardiac Care Unit (CCU) of Srinagarind Hospital, the Faculty of Medicine, Khon Kaen University, Thailand, with a chief complaint of severe pneumonia with high fever. The boy had been diagnosed with hypogammaglobulinemia with hyper IgM and cyclic neutropenia when he was 2□. The clinical and laboratory investigations at the time implicated an X-linked hyper-IgM disease though no further cellular and molecular laboratory study supported this diagnosis[5,6]. After this diagnosis, he received immunoglobulin intravenously every 6 weeks. During the treatment period, the child experienced recurrent pneumonia and was treated for *Pneumocystis carinii*.

The physical examination on the new admission showed a high spiking fever of 39°C, a blood pressure of 120/60 mmHg, a pulse rate of 150 beats per min, and generalized crepitation in both lungs. A chest X-ray revealed bilateral pulmonary infiltration; therefore, a tracheostomy was performed and ventilation supplied. The boy was clinically diagnosed with severe pneumonia and sputum was sent to the Cytology Unit,

Department of Pathology to rule out a *P. carinii* infection.

Cytology and morphometry

The sputum was smeared and fixed in 95% ethanol for Papanicolaou staining. Air dried slides were stained for Gomori's methenamine silver (GMS), periodic acid-Schiff (PAS) with diastase, acid-fast bacilli and mucicarmine. The Papanicolaou-stained smears showed yeast-like organisms both extracellularly and within epithelial cells and histocytes (Fig. 1A-C). The GMS stain clearly revealed round- to oval-shaped organisms, with an occasional septal structure at the middle (Fig. 1D). The septae were deeply stained compared to the external cell walls. Some organisms were more elongated and elliptical or "banana" -shaped. A small amount of necrotic material mixed with bronchial epithelium was also found in the specimen. No typical silver-stained, round-shaped bodies usually seen in the cyst wall of *P. carinii* were seen. The acid-fast bacilli and mucicarmine staining, for *Mycobacterium tuberculosis* and *Cryptococcus neoforman,* respectively, was also negative. All of these findings are compatible with a *P. marneffei* infection.

The GMS stained slides were further examined morphometrically, using a microscope equipped with the AxioVision LE image analysis software (Carl Zeiss, German). Positive *P. marneffei* appeared as a unicellular organism with round to elongated oval cells. The maximal width and length of 100 randomly selected *P. marneffei* cells, taken both extracellularly and within epithelial cells and histiocytes, were measured. The

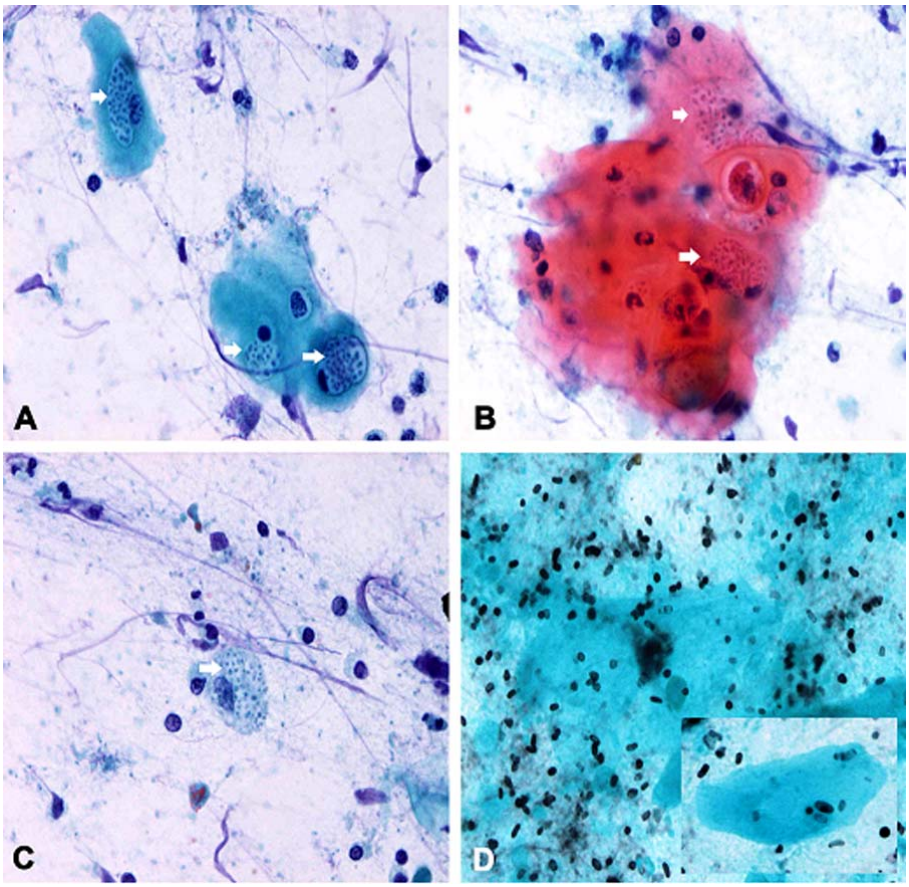

Fig. 1 Photomicrograph of Papanicolaou (A-C) and Gomori-methenamine-silver (D) stained sputum smears. Intracellular round to elongated yeast forms (arrows) are found in the epithelial cells (AB) and histiocyte (C). Extracellular and intracellular round to elongated organisms are clearly observed by GMS staining (D). Prominent binary fission is noted (D, inset). Original magnification, A-C (x 200), D (x 400).

average width and length of the fungus were 0.89 ± 0.16 (0.53-1.39) micrometers and 1.66 ± 0.33 (1.16-2.8) micrometers, respectively.

The patient was treated with itraconazole until he recovered before being discharged from the hospital.

Discussion

Immunodeficiency with hyper-IgM is a rare, highly morbid and fatal primary immunodeficiency syndrome with an estimated minimal incidence of ~1/ 1,030,000 live births[4,5]. It was first described in 1960 and was known to have genetic alterations of the CD40 ligand (CD154) [Hyper IgM Type I (HIGM1), Hyper Ig Type III (HIGM3)] or activation-induced cytidine deaminase (AID) [Hyper IgM Type II (HIGM2)] gene[6]. The immunodeficiency classically presents with elevated or normal IgM levels and low IgA, IgG and IgE concentrations. Neutropenia is frequently coincidental[4,5]. Clinical manifestations of HIGM1 include recurrent upper and lower respiratory tract infection, interstitial pneumonia, chronic diarrhea, oral ulcers, sclerosing cholangitis and hepatitis[4,5]. Patients with HIGM1 are uniquely susceptible to interstitial pneumonia caused by *P. neumocyctis carinii* and often a cause of death in children[4]. In this case, the patient had a past history of *P. carinii* infection. With the most recent hospital admission, the clinician believed that it was a pneumocystis pneumonia similar to that of the previous admission a year prior. But, according to the cytological diagnosis, the boy had been infected with the fungus *P. marneffei*. This appears to be the first case report of *P. marneffei* infection in a patient with hypogammaglobulinemia with hyper-IgM.

P. marneffei is the only dimorphic fungus member of the genus *Penicillium* and is able to infect both immunocompromized and healthy hosts. The unique feature of *P. marneffei* relative to other penicillia is its thermal dimorphism[1]. At 25 to 30°C, *P. marneffei* grows as a mycelium but the fungus develops as a pathogenic yeast-like cell that divides by fission and appears as a cell with a centrally-located transverse septum at 37°C. This feature allows *P. marneffei* to be differentiated from other dimorphic fungi, especially *Histoplasma capsulatum*. The size of the *P. marneffei* collected from the clinical specimen (1.6-2.8 x 0.53-1.39 micrometers) is slightly longer than previous reports of the fungal culture (1.3-2 x 0.7-1.6 micrometers) from a Thai isolate[7].

The differential diagnosis of *P. marneffei* from other round intracellular pathogens has been documented[8]. For upper and lower respiratory tract infections, some common infections are noted as follows: In *P. carinii* infection, foamy cells (by Papanicolaou staining) or cysts (by GMS staining) measuring 4 to 7 mm in diameter are present, containing single or paired comma-shaped argyrophilic foci in the walls. The presence of narrow-based budding forms and prominent capsules, highlighted by mucicarmine or PAS staining, is a 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* infections. Laboratory diagnosis, such as fungal culture from lesions, immunocytochemistry, serodiagnosis and molecular assay-based diagnostic methods, have been employed but all are time-consuming and not widely available[1]. Morphological-based diagnosis by conventional cytology, with special staining as described above, is more rapid, reliable and practicable.

More importantly, one should be aware that *P. marneffei* is not recognized as a health problem of AIDS patients only but also in other immunocompromized diseases such as hypogammaglobulinemia, as in this pediatric case. This connection may be life-saving in areas endemic for *Penicillosis marneffei*. Without rapid and appropriate antifungal treatment (amphotericin B or its derivatives)[9,10], the patients, especially in severe pediatric cases, may undergo serious complications and die.

Conclusion

This report highlights a rapid cytological diagnosis of *P. marneffei* infection. Since this fungal infection in immunocompromized children is highly fatal, rapid diagnosis is necessary.

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การวินิจฉัยทางเซลล์วิทยาและขนาดทางสัณฐานวิทยาของเชื้อ Penicillium marneffei ในเสมหะผู้ป่วย hypogammaglobulinemia with hyper-IgM

จงรักษ์ ศรีภา, จุมพล มิตรชัย, วาริน ทองศรี, บรรจบ ศรีภา

ภูมิหลัง: *โรคติดเชื้อราเพ็นนิซิโลซิสเกิดจากการติดเชื้อ Penicillium marneffei พบได้บ่อยในเอเชีย และเป็นสาเหตุสำคัญที่ทำให้เสียชีวิตในผู้ป่วยโรคเอดส์ ส่วนรายงานในผู้ป่วยโรคภูมิคุ้มกันบกพร่องชนิดอื่นๆ มีน้อย*
รายงานผู้ป่วย: *รายงานผู้ป่วยนี้ได้นำเสนอการวินิจฉัยทางเซลล์วิทยาในกรณีผู้ป่วยเด็กชายซึ่งเป็นโรค hypogammaglobulinemia with hyper-IgM มาด้วยอาการปอดอักเสบอย่างรุนแรง แพทย์ได้ส่งเสมหะเพื่อตรวจทางเซลล์วิทยา ผลการตรวจพบว่ามีสิ่งมีชีวิตลักษณะคล้ายยีสต์มี septated hyphae ที่เด่นชัดตรงกลาง ซึ่งจำเพาะสำหรับ Penicillium marneffei ในเซลล์เยื่อบุท่อทางเดินหายใจ ฮิสติโอซัยท์ และ อยู่ภายนอกเซลล์ นอกจากนี้ยังวัดขนาดของเชื้อเพื่อเป็นข้อมูลพื้นฐานทางสัณฐานวิทยาจากสิ่งส่งตรวจจากผู้ป่วยเพื่อช่วยในการวินิจฉัยด้วย*
สรุป: *รายงานนี้นอกจากได้แสดงให้เห็นผลการตรวจเชื้อราทั้งในเซลล์อักเสบและในเซลล์เยื่อบุผิวแล้ว ยังย้ำเตือนให้แพทย์ผู้ทำการรักษาได้ตระหนักถึงโรคราเพ็นนิซิโลซิสในผู้ป่วยโรคภูมิคุ้มกันบกพร่องชนิดอื่นๆ ที่ไม่ใช่โรคเอดส์ที่อาศัยหรือเดินทางเข้ามาในพื้นที่ระบาดของโรคนี้*