Penicilliosis is a disseminated and progressive infection that is mainly found in immunocompromised individuals, especially those with human immunodeficiency virus (HIV) infection. Because of the high mortality of patients with disseminated Penicillium marneffei infection, rapid diagnosis and early treatment are required. Diagnosis is traditionally made by biopsy and/or culture of blood or any involved organ. Cytology offers several advantages over biopsy, including more rapid diagnosis and greater resolution of cytomorphologic details of organisms, allowing rapid initiation of treatment. Here, we describe a case of penicilliosis in an HIV-positive patient with emphasis on the morphological characteristics of the organism in cytologic specimens, as well as a comparison of bronchial washing and biopsy findings.

Key words: Bronchial washing cytology; HIV; penicilliosis

Penicillium marneffei infection in a HIV-Positive patient: A comparison of bronchial washing cytology and biopsy

ABSTRACT

Penicilliosis is a disseminated and progressive infection that is mainly found in immunocompromised individuals, especially those with human immunodeficiency virus (HIV) infection. Because of the high mortality of patients with disseminated Penicillium marneffei infection, rapid diagnosis and early treatment are required. Diagnosis is traditionally made by biopsy and/or culture of blood or any involved organ. Cytology offers several advantages over biopsy, including more rapid diagnosis and greater resolution of cytomorphologic details of organisms, allowing rapid initiation of treatment. Here, we describe a case of penicilliosis in an HIV-positive patient with emphasis on the morphological characteristics of the organism in cytologic specimens, as well as a comparison of bronchial washing and biopsy findings.

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Introduction

Penicilliosis, a disseminated and progressive infection caused by *Penicillium marneffei*, is endemic in Southeast Asia, including Thailand, Southern China, Hong Kong, Vietnam, Indonesia, and Laos. The organism was first reported in 1956 and the first human infection was described in 1973 in an American who lived in Southeast Asia and was diagnosed with Hodgkin lymphoma. In 1988, the first human immunodeficiency virus (HIV)-associated case was reported, and, since then, penicilliosis has been regarded as an hAIDS-indicating disease.\[1\] Because of nonspecific clinical manifestations, penicilliosis in HIV-infected patients can be easily misdiagnosed as other infections, such as tuberculosis, histoplasmosis, and cryptococcosis, and malignancy. Penicilliosis is associated with a high mortality rate, which has been reported to be 13.5% (7/52) in HIV-infected patients and 60% (6/10) in noninfected patients.\[2,3\]

Here, we report a case of penicilliosis in an HIV-positive patient who was diagnosed after bronchial washing and biopsy, with emphasis on the morphological characteristics of the organism in a cytologic specimen.

Case Report

A 42-year-old male patient visited the emergency room of our hospital in November 2013 with fever, sore throat, and diarrhea. He was a known seropositive patient for HIV and syphilis and a frequent traveler to Guangxi, China, and Hong Kong. Laboratory testing on admission showed a white blood cell count of 9600/µl (8764/µl, neutrophils, 470/µl lymphocytes), 7.0 g/dL hemoglobin, and 86 K/µl platelets. The peripheral CD4+ T lymphocyte count was 25 cells/µp. Computed tomography (CT) scans revealed bilateral septal thickening and micronodules of both the lungs, severe hepatosplenomegaly, and multiple enlarged lymph nodes in the chest and abdominal cavity, with tuberculosis or malignancy, especially lymphoma, suspected. Bronchoscopy revealed multiple micronodules in the wall of the right bronchi. Bronchoscopic biopsy and washing cytology with concurrent supraclavicular lymph node biopsy were performed.

The bronchial washing cytology was diffuse and cellular smear contained mixed inflammatory infiltrate, including numerous histiocytes, lymphocytes, some eosinophils, and neutrophils. Most histiocytes were packed with many yeast-like organisms measuring 3 mm in diameter that stained dark brown on methenamine silver (GMS) stain [Figure 1]. Focusing up and down through histiocytes, the organisms appeared to have a central dot-like structure. Bronchoscopic biopsy revealed anergic tissue reactions with histiocytic infiltration. In Hematoxylin and eosin (H and E)-stained sections, the organisms were seen as nonbudding round-to-oval spherules within the histiocytes in the submucosa of bronchi [Figure 2]. These organisms were also observed extracellularly but not in bronchial epithelial cells. In the lymph node, there was lymphoid depletion with diffuse proliferation of histiocytes engorged with many organisms.

On the basis of cytologic and histologic findings, the initial cytopathologic interpretation suggested histoplasmosis. Two days after admission, the mycelial form presented in an interim report on a fungal culture obtained from the blood. Given its dimorphic feature and the patient’s history of travel to endemic areas, penicilliosis was suspected and a polymerase chain reaction assay was positive for *P. marneffei*. We reviewed the bronchial washing and biopsy findings. In the cytologic specimen, while intracellular organisms appeared to be uniform yeasts of 3 µs in size, extracellular

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Figure 1: Bronchial washing cytology shows bronchial epithelial cells and mixed acute and chronic inflammatory infiltrate, including numerous histiocytes. Most histiocytes were packed with many yeast-like organisms (Pap, ×400) with inset showing oval to oblong in shape with central transverse septum (GMS, ×1000)

Figure 2: Bronchoscopic biopsy shows diffuse histiocytic proliferation with organisms in submucosa. (H&E, ×400)
fungi were elongated and up to 8 μi in length (sausage form). The most characteristic feature was a central transverse septum that was transparent with Papanicolaou stain but highlighted by GMS stain [Figure 1]. The size, shape, and distribution of the organisms were similar in both biopsy and cytologic specimens, except that central dots and septa were more readily identified on cytologic smears.

After amphotericin B administration, the patient exhibited clinical and radiologic improvement. Amphotericin B was changed to oral itraconazole 400 mg/day after 2 weeks. Toxoplasma IgG, influenza, and pneumococcal-conjugated (PCV 13) vaccines were also prescribed to prevent opportunistic infections. The patient continues to visit the outpatient clinic without any specific problems.

Discussion

Penicilliosis, an emerging opportunistic infection in the Southeast Asian region, especially among patients with HIV infection, is the third most common “ost commonis, an emergi,” following tuberculosis and cryptococcosis in frequency. Diagnosis is difficult due to nonspecific clinical manifestations that match those of other generalized infections, such as tuberculosis, histoplasmosis, and cryptococcosis, or malignancy. Identification of a characteristic thermal dimorphism on fungal culture is diagnostic for penicilliosis, however, it is a time-consuming method for proper management of critically ill patients.

Diagnosis can be quicker using biopsy or cytology specimens when pathologists are aware of the epidemiology and morphology of *P. marneffei* infection. The histopathology of penicilliosis can lead to three distinct tissue reactions depending on the host immunity. A granulomatous or supplicative reaction occurs in immunocompetent patients, whereas an anergic and necrotizing reaction is often observed in patients with compromised immunity. In infected organs, *P. marneffei* can be observed both extracellularly and intracellularly, mainly within histiocytes. Our case showed diffuse submucosal histiocytic infiltrate with numerous intracellular granular organisms. In contrast to the report of Chongrak *et al.*, no *P. marneffei* were detected within bronchial epithelial cells in either surgical biopsy or cytologic specimens.

Cytologic specimens are less invasively and more easily obtained than tissue biopsy. Although the primary goal of cytology is to screen for malignancy, inflammation, and reparative changes, other abnormalities may also be detected during specimen examination. The morphologic diagnosis of fungal infection is another important function of cytology. A key observation in our current case is that morphologic evidence of *P. marneffei* was more apparent in cytologic specimens than that in biopsy. The main advantage of cytologic preparations in *P. marneffei* infection is that the Papanicolaou stain (a routine stain in cytology) specifically highlights the central transverse septum, unlike H and E stain (a routine stain in histology). This accounts for greater resolution of the cytomorphologic details of routine cytology for identifying *P. marneffei* because cytology lacks formalin-fixation artifacts and has less crush artifacts than biopsy. Another advantage of cytology over histology is that noncohesive histiocytes engorged with *P. marneffei* may be better visualized. Thus, cytology may be superior to biopsy for the detection of *P. marneffei*.

In addition to the morphologic comparison, another important consideration is diagnostic yield. Because the present case was diagnosed with both modalities, the comparative diagnostic yield of biopsy versus cytology cannot be addressed. *P. marneffei* infection has been diagnosed by fine-needle aspiration of lymph nodes, sputum cytology, touch preparation from skin scrape, and bronchoalveolar lavage. A review of the literature reveals variable findings of lung cytology. The differences may be due to the method adopted, which ranges from bronchial washing, sputum, percutaneous lung aspirate, and bronchoalveolar lavage. Although the diagnostic yield of bronchial washing cytology for *P. marneffei* has been reported as zero, bronchial washing from our case showed diagnostic features on review. Thus, we believe that bronchial washing cytology is comparable to that of other lung specimens.

Other fungi with cytomorphologic findings similar to *P. marneffei* include *Histoplasma capsulatum* and *Pneumocystis jirovecii*. Histoplasmosis is most commonly confused with penicilliosis, as in our case. *H. capsulatum* yeast divides by budding, not fission, and shows a more uniformly round shape, not an oval or sausage form. An epidemiologic link to an area of endemicity of the two fungi can also help in the diagnosis. *P. jirovecii* is mainly recognized in an extracellular location and in crescent forms; the organisms lack septation.

Conclusion

*P. marneffei* causes a disseminated infection and can be the initial manifestation in HIV-infected patients. Because the number of HIV-positive patients is increasing around the world, we need to be aware of *P. marneffei* as a potential pathogen in immunocompromised patients. Mortality in patients with disseminated *P. marneffei* infection is high, regardless of HIV infection status, hence, rapid diagnosis and early treatment are required. In cytologic specimens, the diagnosis is made by linking the detection of oval or sausage-shaped yeast-like
organisms with characteristic central transverse septum to a clinical history of visits to endemic areas.

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Conflicts of interest
There are no conflicts of interest.

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