Case Report
Pathological diagnosis of a rare intestinal *Penicillium marneffei* infection in an acquired immunodeficiency syndrome patient: a case report and literature review

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Abstract: *Penicillium marneffei* infection is a common opportunistic infection in human immunodeficiency virus (HIV)-infected patients, but intestinal *Penicilliosis marneffei* has rarely been reported. In this report, we presented a rare case of an HIV-infected Chinese patient with intestinal *Penicilliosis marneffei*, who was characterized by an insidious onset, initial symptoms with a mild pain in the lower left abdomen, and negative results of repetitive fungal culture. The histological findings of colonic biopsy (non-budding yeast cells with central transverse septum and extracellular elongated or sausage-shaped cells) gave a clue to the correct diagnosis and guided effective clinical therapy. In addition to the above analysis, the various clinical manifestations and outcomes of intestinal *Penicilliosis* in the literatures are also summarized.

Keywords: AIDS, *Penicillium marneffei*, intestinal, pathological

Introduction

*Penicillium marneffei*, an emerging opportunistic pathogen, can cause systemic mycosis in either immunocompromised or previously healthy subjects [1]. There has been a marked increase in the number of reports of opportunistic infections by *Penicillium marneffei* among acquired immunodeficiency syndrome (AIDS) patients in recent years [2]. These reports have been mainly from southeast Asia, especially in Thailand, northeastern India, southern China, and Vietnam, where this infection is considered an endemic disease [2-4]. *Penicillium marneffei* infection is not an uncommon AIDS-related opportunistic infection in mainland China, and it is even an endemic in the southern provinces such as Guangxi, Guangdong, Fujian, Yunnan, and Hunan [4, 6]. *Penicilliosis* can be fatal if the diagnosis is delayed or it is untreated [7]. Appropriate recognition, early diagnosis and properly administered antifungal therapy are important for a good prognosis [8]. Prompt diagnosis can often be established through careful pathomorphological observation of clinical specimens. We herein report the case of a rare intestinal *Penicillium marneffei* infection in an AIDS patient diagnosed through pathology.

Case report

A 52-year-old previously healthy Chinese male was admitted because of mild pain in the lower left abdomen for eight months. He presented with mild lower-left abdominal pain radiating to the back, associated with anorexia and progressive weight loss of 20 kg in eight months, without fever, chill, vomiting or diarrhea. He was born in Yiwu city, Zhejiang province and had lived for 10 years in Yunnan province, China. The patient was heterosexual and an intravenous drug abuser.

Physical examination revealed a pale and emaciated patient with mild tenderness in the lower left abdomen but without rebound tenderness. Neither liver nor spleen was enlarged. He developed no fever (temperature 37.5°C), no lymphadenopathy and no skin lesions.
A blood examination revealed hemoglobin 78 g/l, white blood cell count 4.9×10⁹/l, with 82.5% neutrophils and 9.2% lymphocytes, and platelet count of 259×10⁹/l. Results of routine biochemical tests were normal except for serum albumin of 31.4 g/l. A test for antibodies to HIV was positive and CD4 lymphocyte count was 28 cells/mm³, while C-reactive protein and erythrocyte sedimentation rate were 41.9 mg/l and 113 mm/h respectively. Stool examinations revealed positive for occult blood. He was negative for the T-SPOT.TB test, cerebrospinal fluid (CSF) cryptococcus neoformans smears, and serologic tests to cytomegalovirus, Epstein-Barr virus and herpes simplex virus. Numerous cultures of blood, stool, sputum, urine and CSF showed no growth of bacteria, fungi, or acid-fast bacilli.

On the third day of admission he presented with fever (temperature 38.9°C), and a computed tomographic (CT) scan of the chest revealed multiple nodules disseminated throughout both lung fields. Tuberculosis was considered as a clinical diagnosis according to the characteristic imaging findings and clinical manifestations. Anti-tuberculous treatment was started (rifampicin + isoniazid + pyrazinamide + ethambutol); however, his temperature remained high. On the fifth day of admission he developed severe diarrhea (three to four watery stools per day), with fever and abdominal pain. An enhanced CT scan of the abdomen showed mesenteric lymphadenopathy and marked edematous expansion of the right side of the colon. Persistent fever of unknown origin and abdominal pains may have indicated that the patient suffered from a fungal infection, despite the negative hemoculture, sputum smear, sputum culture, and CSF culture. Colonoscopy revealed multiple solitary shallow ulcers of varying sizes with elevated margins in the transverse colon, descending colon, sigmoid colon and rectum recta. The mucosa of the rest of the colon appeared normal as shown in Figure 1.

Specimens from the mucous membrane of the transverse colon were taken, for pathological examination. Tissue sections from the colon biopsy were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Grocott methenamine silver (GMS) stain and fast antacid stain. H&E-stained sections showed mucosa hyperemia; swelling and additional exudates; mucosal ulceration and necrotizing histiocytic granuloma formation with infiltration of the lamina propria and submucosa by lymphocytes; and markedly distended histiocytes laden with ingested microorganisms. High magnification revealed numerous small basophilic spherules, round to oval, measuring 2-5 μm in diameter. Some of the thin-walled yeast cells appeared to have a central or eccentric dot with central septation, were inside histiocytes or had infiltrated into the lamina propria (Figure 2). Yeast forms were well stained by the GMS preparations, round fungal bodies were well demonstrated, and elongated and septate forms were often better seen outside the histiocytes (Figure 3). PAS preparation revealed a few septate yeast forms inside the histiocytes within a granuloma, as suggested in Figure 4. Acid-fast bacilli were not demonstrable by antacid coloration. The appearance of the fungus was consistent with that of Penicillium marneffei, and the diagnosis of disseminated Penicillium marneffei infection was made, with these credible clues for clinical diagnosis. Consistent with the histopathologic diagnosis, the patient was treated with intravenous itraconazole at a dosage of 250 mg Qd for a week, followed by itraconazole capsules at a dosage of 200 mg twice daily orally. He responded well to the treatment, and his fever, diarrhea, and abdominal pains gradually resolved.

Discussion

Disseminated Penicillium marneffei infection is a common opportunistic infection in HIV-
Intestinal penicilliosis in AIDS

infected patients. The first description of this disease in an HIV-infected patient was made in 1988 [9]. *Penicillium marneffei* is the only known *Penicillium* species that exhibits temperatur-dependent dimorphic fungus, which grows at 37°C as yeast-like forms, and appears as a mycelial form at 25°C, producing fluffy gray-white colonies with a characteristic diffus-

Figure 2. Histologic section of transverse colon biopsy showing histiocytic granuloma formation and numerous yeast cells scattered in tissues or were phagocytosed within distended histocytes. (H&E stain, magnification, A: ×100, B: ×200, C: ×400, D: ×1000).

Figure 3. GMS staining of tissues revealed some intracellular and extracellular yeasts, elongated (white arrow) and septate forms (black arrow) (magnification, ×1000).

Figure 4. PAS staining of tissues revealed some intracellular and extracellular yeasts, elongated (white arrow) and septate forms (black arrow) (magnification, ×1000).
### Table 1. Clinical data for 11 patients with intestinal *Penicilliosis marneffei*: literature and present report review

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Region and year reported</th>
<th>Age (yrs)/gender</th>
<th>Underlying conditions</th>
<th>Clinical symptoms</th>
<th>Physical signs</th>
<th>Method for diagnosis</th>
<th>CD4 cell count (cells/mm³)</th>
<th>Treatments</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hong Kong China 1988 [12]</td>
<td>58/M</td>
<td>Hemolytic anemia</td>
<td>Fever anemia</td>
<td>Severe abdominal pain, peritonitis</td>
<td>Sputum, urine, blood, CSF(-)</td>
<td>ND</td>
<td>Amphoteric-inB</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>Guangxi, China 1988 [13]</td>
<td>4 months/M</td>
<td>NM</td>
<td>Fever, diarrhea, anemia</td>
<td>Steady deterioration</td>
<td>ND</td>
<td>Lymph nodes, liver, lung, bone marrow, spleen, bowel, kidney (A+H)</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Hong Kong China 1992 [14]</td>
<td>72/M</td>
<td>AIDS</td>
<td>Anorexia, dysphagia, weight loss</td>
<td>NM</td>
<td>Small intestine (B+C+H), mesenteric lymph node, liver (A+H)</td>
<td>ND</td>
<td>NM</td>
<td>Died</td>
</tr>
<tr>
<td>5</td>
<td>Taiwan China 1999 [16]</td>
<td>33/M</td>
<td>Renal transplant</td>
<td>Tarry stool fever</td>
<td>Septic shock</td>
<td>Blood (+)</td>
<td>ND</td>
<td>None</td>
<td>Died</td>
</tr>
<tr>
<td>6</td>
<td>Taiwan China 1999 [16]</td>
<td>52/M</td>
<td>AIDS</td>
<td>Fever, watery diarrhea, abdominal pain</td>
<td>Improvement after treatment</td>
<td>Blood, skin, bone marrow (+)</td>
<td>20</td>
<td>Amphoteric-inB itraconazole</td>
<td>Survived</td>
</tr>
<tr>
<td>7</td>
<td>Taiwan China 1999 [16]</td>
<td>30/M</td>
<td>AIDS</td>
<td>Dyspepsia, intermittent abdominal cramping pain, watery diarrhea</td>
<td>Fever, bloody stool, weight loss</td>
<td>Blood (+) bone marrow (-)</td>
<td>ND</td>
<td>Amphoteric-inB itraconazole</td>
<td>Survived</td>
</tr>
<tr>
<td>9</td>
<td>Hong Kong China 2010 [18]</td>
<td>39/M</td>
<td>AIDS</td>
<td>Watery diarrhea, weight loss, fever sore throat</td>
<td>Improved after treatment</td>
<td>Sputum, urine, blood, stool (-)</td>
<td>11</td>
<td>Amphoteric-inB itraconazole</td>
<td>Survived</td>
</tr>
<tr>
<td>10</td>
<td>India 2014 [19]</td>
<td>28/M</td>
<td>AIDS</td>
<td>Abdominal pain, fever and rigor, weight loss</td>
<td>Preiror Umbilicated lesions</td>
<td>Blood (+)</td>
<td>8</td>
<td>Amphoteric-inB itraconazole</td>
<td>Survived</td>
</tr>
<tr>
<td>11</td>
<td>Present report</td>
<td>52/M</td>
<td>AIDS</td>
<td>Mild pain in the lower left abdomen, anorexia, weight loss</td>
<td>Fever diarrhea</td>
<td>Sputum, urine, blood, stool CSF (-)</td>
<td>28</td>
<td>Itraconazole</td>
<td>Survived</td>
</tr>
</tbody>
</table>

ND, not done; NM, not mentioned. Methods for diagnosis of *Penicillium marneffei* were autopsy (A), biopsy (B), culture (C), histopathology (H), and fine-needle aspiration cytology (F).
ible red pigment on Sabouraud's glucose agar [10]. The patient we reported presented negative results of repetitive fungal culture (blood, urine, stool, sputum and CSF), and finally the diagnosis of *penicilliosis* was confirmed by the histological findings of colonic biopsy. *Penicillium marneffei* diagnosed by pathological examination mainly relies on microscopic histological features, which can quickly confirm *Penicillium marneffei* infection. A number of microorganisms have to be differentiated from *Penicillium marneffei*, such as histoplasma capsulatum, cryptococcus, tubercle bacillus, and so on. Histologically, *Penicillium marneffei* tends to be misdiagnosed as histoplasma, because both of them are thermally dimorphic, proliferate within histiocytes and are of similar size (about 2-5 μm), but *Penicillium marneffei* differs by its greater variation in size, the presence of septate and elongated yeast forms, and the absence of buds attached by a narrow neck, since the yeast form multiplies by schizony and not by budding. Cryptococcus is differentiated by a generally larger size, greater variation in size (5-20 μm), and the presence of teardrop-shaped buds and mucoid capsules. Tubercle bacillus can be distinguished by acid-fast stain.

Studies have demonstrated high sensitivity from fungal culture, but it takes a long time to grow and yields false positives or negatives in a few cases. Pathological features would give a presumptive diagnosis, which can provide a reliable clue for clinical diagnosis.

Diarrhea is the most common gastrointestinal manifestation of AIDS; more than 60% of patients with AIDS have diarrhea [11]. The most common infectious organisms of AIDS-related diarrhea are cytomegalovirus, parasites, bacteria and so on. However, a fungal infection of the gastrointestinal tract is uncommon in AIDS. Intestinal mycoses may be caused by Histoplasma capsulatum and Cryptococcus neoformans. Although *penicilliosis* has become an important opportunistic infection in HIV-infected patients, intestinal *penicilliosis marneffei* has rarely been reported. As far as we know, including the case we report, only 11 patients with intestinal *penicilliosis* had been reported in English-language publications, 7 cases of which were HIV-infected, and most of these had diarrhea or acute abdominal pain (Table 1) [12-19].

The most common non-specific symptoms of disseminated *penicilliosis marneffei* in patients with AIDS are fever (98%), anemia (74.8%), weight loss (71.6%), skin lesions (69.7%), lymphadenopathy (52.3%) and hepatomegaly (51%) [20]. Diarrhea was reported in 31% of patients with AIDS and disseminated *penicilliosis marneffei* in a series of 92 patients from Thailand [6], and in 23.2% of cases in another review of 155 HIV- and non-HIV-infected patients [20]. The morbidity of intestinal *penicilliosis marneffei* with AIDS may be higher than the cases reported, because gastrointestinal infection often accompanies systemic fungal infection. Intestinal *penicilliosis marneffei* may have been underdiagnosed because of the diagnostic yield of cultures of blood, bone marrow, and skin rather than endoscopic biopsy, or patients may have died untreated. *Penicillium marneffei* is highly sensitive to itraconazole, ketoconazole, voriconazole, miconazole and amphotericin B, and less sensitive to fluconazole. Our patient was an exception because the onset of symptoms was insidious and non-specific, such as mild pain in the abdomen, anemia and weight loss. As far as we know, this case is the first report of an AIDS patient complicated with intestinal *penicilliosis marneffei* in a non-endemic province (Zhejiang province) of China.

In conclusion, intestinal *penicilliosis marneffei* with AIDS is probably more common than the small number of reported cases would indicate, because the clinical manifestations are diverse and non-specific. Early colonoscopy and pathological examination can lead to an early diagnosis and a reduction in mortality. Intestinal *penicilliosis marneffei* is an important diagnostic consideration in HIV-positive patients (or in homosexuals, drug abusers and others with predisposing factors) with fever and gastrointestinal symptoms, especially in patients who have travelled to or lived in endemic areas.

**Disclosure of conflict of interest**

None.

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