Disseminated *Penicillium marneffei* infection in human immunodeficiency virus-infected children

VIRAT SIRISANTHANA, MD AND THIRA SIRISANTHANA, MD

Disseminated infection with the fungus *Penicillium marneffei* is one of the most common opportunistic infection in human immunodeficiency virus (HIV) disease in northern Thailand. We report the clinical, laboratory and therapeutic features of 21 human immunodeficiency virus-infected children with disseminated *P. marneffei* who were prospectively followed. Significant clinical and laboratory features included generalized lymphadenopathy (90%), hepatomegaly (90%), body temperature $>38.5^\circ$ C (81%), papular skin lesions with central umbilication (67%), splenomegaly (67%), failure to thrive (52%), severe anemia (hemoglobin < 60 g/liter) (43%) and thrombocytopenia (platelet count $<0.5 \times 10^{11}$/liter) (21%). The response rate in patients who were treated with appropriate antifungal therapy (amphotericin B, fluconazole, or ketoconazole) was 82%. No relapse was observed in patients given ketoconazole prophylactically. Skin lesions, usually papules with central necrotic umbilication, provide the most significant clue to the diagnosis. Early diagnosis based on finding *P. marneffei* in the skin smear or lymph node provides the basis for prompt administration of antifungal therapy and improved outcome.

INTRODUCTION

*Penicillium marneffei* disease is endemic in South-east Asian countries and China. It can cause sys-

---

Accepted for publication July 19, 1995.

From the Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

Key words: *Penicillium marneffei*, human immunodeficiency virus infection, acquired immunodeficiency syndrome, opportunistic infection, fungal infection, children

Address for reprints: Virat Sirisanthana, M.D. Section of Infectious Disease, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. Fax +66-53-217144
In the past, only a few cases of \textit{P. marneffei} infection were reported even from endemic areas. Recently, the number of patients has increased dramatically\textsuperscript{1-15} and has occurred exclusively among patients infected with human immunodeficiency virus (HIV).\textsuperscript{6, 11} The clinical and laboratory features of 86 adult patients with HIV infection who had disseminated \textit{P. marneffei} infection had been reported from Chiang Mai.\textsuperscript{16} Here we report 21 children with perinatally transmitted HIV infection who had disseminated \textit{P. marneffei} infection.

**METHODS**

All HIV-infected children with disseminated \textit{P. marneffei} infection who were seen at Chiang Mai University Hospital from April 1989 to January, 1995, were included in the study. In children older than 18 months the diagnosis of HIV infection was made when the child's serum was repeatedly reactive by both enzyme-linked immunosorbent assay (Enzymun-Test\textsuperscript{®} Anti-HIV 1+2, Boehringer Mannheim GmbH Diagnostica) and particle agglutination test (Serodia\textsuperscript{®}-HIV, Fujirebio Inc., Tokyo, Japan). In children younger than 18 months old, the diagnosis of HIV infection was made when the serum was repeatedly reactive and symptoms and signs of HIV infection or HIV-related conditions were present. The diagnosis of \textit{P. marneffei} infection was made by isolation of the organism from clinical specimens, predominantly blood or specimen obtained from skin lesions. Methods of fungal isolation and mycologic characteristics of \textit{P. marneffei} have been described previously.\textsuperscript{11} In some patients, the diagnosis was made by microscopically demonstrating yeastlike organisms in the Wright's-stained skin smear. The smear was made by nicking the skin over the lesion with the lancet and scraping it; the fluid and tissue obtained were sent for culture as well as spread on a glass slide and stained. Yeastlike cells characteristic of \textit{P. marneffei} were basophilic and appeared both inside and outside the macrophage. There were spherical, oval or elliptical and varied in diameter from 3 to 8 \(\mu\)m. Some yeastlike cells were seen with central septation (Fig. 1). These findings are characteristic of \textit{P. marneffei}.\textsuperscript{16}

Patients whose diagnosis was made before death were treated with amphotericin B (AMB), fluconazole (FLZ), or ketoconazole (KTZ). Selection of an antifungal agent for therapy was based on the decision of the attending physician and infectious disease consultant. AMB was given in a dosage of 0.5 to 1 mg/kg intravenously daily for about 6-8 weeks, until the total dose of 30-35 mg/kg was reached. FLZ in a dosage of 5 mg/kg daily was given for 6 weeks either intravenously or orally. KTZ was given in a dosage of 5 mg/kg orally daily for 6 weeks. After the initial course of treatment, all patients were given KTZ, 2.5 mg/kg orally daily as long-term prophylaxis.

Evaluation of clinical improvement was performed at regular intervals. Blood culture was taken intermittently every 1-2 weeks until sterile or until the end of treatment. Response to treatment was defined as resolution of fever, skin lesions, and other symptoms and signs of \textit{P. marneffei} infection and absence of fungal growth in the follow up blood cultures. Failure was defined as no clinical improvement or deterioration of symptoms and signs of \textit{P. marneffei} infection and persistent of fungemia during the course of treatment.

**RESULTS**

Between April 1989, to January 1995, 23 children with disseminated \textit{P. marneffei} infection were diagnosed at Chiang Mai University Hospital. Prospective data collection in all cases. One patient was an 8.5-year-old non-HIV-infected girl and a second was a 14-year-old thalassemic patient who acquired HIV through blood transfusion. The remaining 21 children had acquired HIV infection perinatally. In 16 of these 21 patients, \textit{P. marneffei} infection was diagnosed by isolation of the fungus from the blood or from the blood and skin lesion (Table 1). \textit{P. marneffei} was cultured from specimens obtained from the skin lesion only in 2 patients. In the remaining 3 patients the diagnosis was made microscopically by demonstrating yeastlike organisms characteristic of \textit{P. marneffei} in the Wright's-stained skin smear (2 cases) or in the skin biopsy (1 case). During this same period 362 children with symptomatic HIV infection, diagnosed according to the Centers for Disease Control case definition\textsuperscript{17} were seen at Chiang Mai University Hospital.

**Clinical features.** The clinical features of the 21 perinatally HIV-infected children with \textit{P. marneffei} infection are shown in table 1. Some of the clinical data from the first 5 of these 21 patients have been shown in the following table.
Table 1. Clinical features of 21 perinatal HIV-infected children with disseminated *P. marneffei* infection.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Male : female</th>
<th>Age (median, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 : 5</td>
<td>32 months, 4 months-5.5 years</td>
</tr>
</tbody>
</table>

Classifications of HIV infection (no. of patients)

- Clinical category A: 2
- Clinical category B: 8
- Clinical category C: 11

Documentation of *Penicillium marneffei* infection

| Blood and skin culture | 9/21 (43)* |
| Blood culture only * | 7/21 (33) |
| Skin culture only | 2/21 (10) |
| Skin histopathology | 3/21 (14) |

Presenting symptoms (no. of patients)

- Fever: 21 (100)
- Cough: 2 (10)
- Abdominal pain: 1 (5)
- Gastrointestinal bleeding: 1 (5)

Signs

- Body temperature >38.5°C: 17 (81)
- Failure to thrive < 5 percentile (NCHS): 20 (95)
- <10 percentile (growth curve for Northern Thai children): 15 (71)
- <3 percentile (growth curve for Northern Thai children): 11 (52)
- Generalized lymphadenopathy: 19 (90)
- Hepatomegaly: 19 (90)
- Oral candidiasis: 15 (71)
- Splenomegaly: 14 (67)
- Skin lesions: 14 (67)
- Swollen fingers and/or toes: 3 (14)
- Meningeal signs: 1 (5)

* Number of parentheses, percent.
† In 4 cases there were other sites of documentation of *Penicillium marneffei* infections, namely: lung necropsy histopathology (1 case), positive cerebrospinal fluid culture (1), positive cerebrospinal fluid culture and lung necropsy histopathology (1), positive bone marrow aspirate culture and paraaortic node histopathology (1).

NCHS, National Center for Health Statistics.

reported.¹⁹ Eighteen patients lived in Chiang Mai province and 3 came from other neighboring provinces. All 21 mothers acquired HIV infection heterosexually either by working as a prostitute or from husbands who had visited prostitutes. The median age at the time of presentation of disseminated *P. marneffei* infection was 32 months (range 4 months to 5.5 years). At the time of diagnosis of *P. marneffei* infection, all 21 patients had clinical manifestations of HIV infection. Eleven patients could be classified as Category C, according to the new Centers for Disease Control classification of HIV infection in children¹⁸. These include wasting syndrome (5 patients), recurrent bacterial septicemia (3), cryptosporidiosis (2), brain atrophy (1), isosporiasis (1), *Pneumocystis carinii* pneumonia (1), disseminated cytomegalovirus infection (1), chronic diarrhea (1), and chronic fever (1). In 4 patients there had been 2 or more episodes of these conditions.

Fever was a major presenting symptom. Two of four patients who did not have raised body temperature were in a terminal condition at the time of presentation. Fourteen patients (67%) presented with papular skin lesions, the majority of which had central necrotic umbilication (Fig. 2). They were predominantly found on the face and extremities.

Significant laboratory findings of these patients are shown in table 2. Chest roentgenography revealed abnormal findings in 10 patients. Three children had clinical and roentgenographic findings compatible with lymphoid interstitial pneumonitis. One patient had physical examination and roentgenographic findings diagnostic of ventricular septal defect. Lung necropsies were done in 2 patients who died with severe pneumonia. In one *P. marneffei* was the sole pathogen. In the other, *P. marneffei, Pneumocystis carinii*, and cytomegalovirus were seen in the lung tissue. Three patients presented with clinical and roentgenographic evidence of osteomyelitis. The bone lesions healed after treatment with antifungal drug(s).

Concurrent HIV-related opportunistic infections. Nine patients also had other HIV-related opportunistic infections at the same time the diagnosis of *P. marneffei* infection was made. These included nontyphoid *Salmonella* bacteremia (4 cases), cryptosporidi-
Table 2. Significant laboratory findings of 21 perinatal HIV-infected children with disseminated *Penicillium marneffei* infection

<table>
<thead>
<tr>
<th>Hematological findings</th>
<th>No.</th>
<th>Response</th>
<th>Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (average and range)</td>
<td>72 (33-109) g/liter</td>
<td>2/21 (57%)</td>
<td>0/21 (0%)</td>
</tr>
<tr>
<td>Hemoglobin &lt; 80 g/liter</td>
<td>9/21 (43)</td>
<td>0/9 (0)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin &lt; 60 g/liter</td>
<td>1/21 (5)</td>
<td>0/1 (0)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin &lt; 40 g/liter</td>
<td>1/21 (5)</td>
<td>0/1 (0)</td>
<td></td>
</tr>
<tr>
<td>White blood cell count (average and range)</td>
<td>8.9 (2.0-25.3) x 10^9/liter</td>
<td>1/21 (5)</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte count (average and range)</td>
<td>4.2 (0.6-19.9) x 10^9/liter</td>
<td>0/21 (0)</td>
<td></td>
</tr>
<tr>
<td>Platelet count (average and range)</td>
<td>1.7 (0.8-5.2) x 10^11/liter</td>
<td>1/21 (5)</td>
<td></td>
</tr>
<tr>
<td>Chest roentgenography</td>
<td>Not done</td>
<td>3/21 (14)</td>
<td></td>
</tr>
<tr>
<td>With in normal range</td>
<td>8/21 (38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>10/21 (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe pneumonia</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patchy infiltrates</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral fibronodular</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased vascular marking (VSD)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone roentgenography</td>
<td>Osteomyelitis of phalanges</td>
<td>2/21 (10)</td>
<td></td>
</tr>
<tr>
<td>Osteomyelitis of phalanges and ribs</td>
<td>1/21 (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percent.

VSD, ventricular septal defect.

Fungal therapy, 9 were diagnosed based on the finding of *P. marneffei* in the Wright’s - stained skin smear (8 patients) or Wright’s - stained lymph-node biopsy smear and treated with antifungal drug several days before the culture results became available. All 7 culture-proven patients who did not receive antifungal therapy died. Six of these patients did not have skin lesions and a correct diagnosis had not been made antemortem. One other patient whose diagnosis was made from the skin smear died during the first hour of admission.

Nine culture-proven cases and 3 cases diagnosed histopathologically responded to initial antifungal treatment. All were given KTZ prophylaxis. Two patients died of other causes and 2 were lost to follow-up within 1 month of initial antifungal treatment. In the remaining 8 patients whose mean follow-up was 6.8 months, no relapse of *P. marneffei* infection was found.

**DISCUSSION**

*P. marneffei*, the only *Penicillium* species that is dimorphic, was first reported by Capponi et al., as a disease agent of the bamboo rat (*Rhizomys sinensis*) in 1956. The first report of natural human infection with *P. marneffei* was in 1973 in a 61-year-old missionary with Hodgkin's disease who had had a splenectomy; he had been living in Southeast Asia. Since then 125 patients with *P. marneffei* infection have been reported in the English literature. Almost all were residents of Thailand, the Guangxi province of China, or Hong Kong or were visitors to these areas. Supparatpinyo et al. reported 86 adult patients with HIV and disseminated *P. marneffei* infection from Chiang Mai. Similar to that report our 21 pediatric patients with HIV and disseminated *P. marneffei* infection were diagnosed during a relatively short period from April 1989 to January 1995. No case had been diagnosed in Thailand before 1989. This recent increase in the number of patients coincides with the explosive HIV/acquired immunodeficiency syndrome (AIDS) epidemic in northern Thailand. There are only 6 reported cases of *P. marneffei* infection in children outside the Chiang Mai area. Only 1 of these 6 cases, an 11-year-old hemophiliac from Hong Kong, was also infected by HIV.

It has been suggested that *P. marneffei* infection be regarded as another AIDS-defining illness. Our finding that 19 (90%) of our patients were classified as Center for Disease Control Category B or C and that 9 patients had concurrent HIV-related opportunistic infections supported this suggestion. In our study the median age at the time of diagnosis of *P. marneffei* infection was 32 months. In a similar group of vertical HIV-infected children seen during the same period, the median age at the time of diagnosis of other opportunistic infection was 7 months. Thus *P. marneffei*...
infection occurred late in the course of HIV infection. This is consistent with the finding by Supparatpinyo et al.\textsuperscript{16} that the average CD4 count in HIV-infected adult patients with disseminated \textit{P. marneffei} infection was 9/\mu L.

Many of the clinical features in our patients were not specific for \textit{P. marneffei} infection and could be caused by HIV or other HIV-related opportunistic infection. A characteristic clinical finding was the skin lesion, seen in two-thirds of our patients, most commonly occurred on the face and extremities. The lesions were usually papules with central necrotic umbilication. The septate yeastlike organisms seen in the skin smear from these lesions is diagnostic of \textit{P. marneffei}.\textsuperscript{11} Fifty-seven percent of our patients had low hemoglobin values and one-third had thrombocytopenia, both of which were uncommon in our other symptomatic HIV-infected children and might also be clues to the diagnosis of systemic \textit{P. marneffei} infection. \textit{P. marneffei} has been isolated from the bone marrow of all 26 adult patients in whom aspiration were performed.\textsuperscript{16}

The case fatality rate of patients with \textit{P. marneffei} infection is very high.\textsuperscript{1, 2, 12, 23} This is mostly because of failure to make a timely diagnosis. There was often a delay in starting antifungal therapy. In our study, 6 patients who presented with nonspecific symptoms and signs without skin lesions died without antifungal treatment. The immediate mortality rate in those given appropriate antifungal therapy was 18 \%. In 9 of these 11 patients, the diagnosis was based on finding \textit{P. marneffei} in the skin smear or lymph node biopsy smear, and antifungal drug was given before the culture result was available. Thus it is clear that early diagnosis and treatment improve survival. In the report of treatment and outcome of adult patients with HIV and \textit{P. marneffei} infection, many who responded to initial therapy relapsed within 6 months.\textsuperscript{21} In our study all patients who responded to initial therapy were given KTZ as long term prophylaxis. There was no relapse in any of the patients after a mean follow-up period of 6.8 months.

Studies of animal and human infection with \textit{P. marneffei} indicated that the fungus is endemic in Thailand,\textsuperscript{4, 6, 10, 11} China,\textsuperscript{2, 23} Hong Kong,\textsuperscript{5, 8, 9, 12} Vietnam,\textsuperscript{25-30} and Indonesia.\textsuperscript{31} Thailand has one of the most explosive HIV/AIDS epidemic in the world. It is estimated that 6000-7200 HIV-infected children are born in Thailand every year.\textsuperscript{22} Many of these children will develop systemic \textit{P. marneffei} infection. As the HIV/AIDS pandemic spreads across Southeast Asia and China, infection with \textit{P. marneffei} will become an important public health issue. Medical practitioners in these areas should be aware of the clinical and laboratory features of this disseminated fungal infection.

REFERENCES


