

AIDS-Associated *Cryptococcus neoformans* and *Penicillium marneffei* Coinfection: A Therapeutic Dilemma in Resource-Limited Settings

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AIDS-associated *Cryptococcus neoformans* and *Penicillium marneffei* coinfection has not been adequately studied and poses unique therapeutic challenges in resource-limited settings. Itraconazole poorly penetrates the central nervous system, whereas fluconazole has poor activity against *P. marneffei*. We prospectively report management of 1 patient and retrospectively review 7 coinfection cases from Vietnam.

Cryptococcus neoformans and *Penicillium marneffei* are among the most common opportunistic infections found in human immunodeficiency virus (HIV)-infected individuals in Southeast Asia, ranking second and third, respectively, after tuberculosis [1]. Both infections are uniformly fatal if untreated [2, 3]. Treatment is prolonged, involving induction therapy with amphotericin B, followed by 8 to 10 weeks of consolidation therapy with fluconazole for cryptococcosis or itraconazole for penicilliosis [4, 5]. Relapse is common in both infections; therefore, secondary prophylaxis is recommended until the CD4 cell count is >100 cells/ μ L for 6 to 12 months of receipt of antiretroviral therapy (ART) [6, 7]. Coinfection with *C. neoformans* and *P. marneffei* has not been adequately studied and poses unique therapeutic challenges for clinicians in resource-limited settings, where newer triazole, lipid-based polyene, and echinocandin agents are not available. We report the clinical features and management of 1 coinfecting patient and retrospectively

review 7 cases from the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam.

A 29-year-old man presented in September 2009 with 5 days of fever, headache, and vomiting. The patient was afebrile, and vital signs were normal. He was alert with Glasgow Coma Score of 15, and he had no meningismus and no focal neurological deficits. He had oral thrush. There was no rash, lymphadenopathy, or hepatosplenomegaly. The remaining physical examination was unremarkable. The risk factor for HIV was previous injection drug use. He was diagnosed with HIV in January 2009 and started ART with zidovudine, lamivudine, and nevirapine 4 days before the onset of illness. The baseline CD4 cell count was 12 cells/ μ L, and he started receiving cotrimoxazole prophylaxis. On admission the white cell count was 3770 cells/ μ L, hemoglobin was 12.3 g/dL, and platelet count was 234,000 cells/ μ L. Serum chemistry and alanine and aspartate transaminases were normal. He gave consent to enroll in a descriptive study of HIV-associated central nervous system infections. The lumbar puncture revealed cerebrospinal fluid (CSF) opening pressure of 180 mm CSF. CSF was clear with 20 nucleated cells/mL, 2 red blood cells/mL, protein 0.5 g/dL (normal, <0.45), and CSF to serum glucose (mmol/L) ratio 0.5. CSF India ink stain was positive. CSF Gram stain and Zielh-Neelsen stain were negative. Serum and CSF cryptococcal antigen were positive (CSF titer, 1:32). CSF culture grew *C. neoformans*. Blood culture grew *C. neoformans* and *P. marneffei*; isolation of each was performed in accordance with standard culture techniques [8]. Magnetic resonance imaging of the brain revealed meningeal enhancement with contrast but no parenchyma defects. CD4 cell count and HIV RNA measured on day 2 of hospitalization (day 11 of ART) were 107 cells/ μ L and 190 copies/mL, respectively. The patient was treated for 14 days with amphotericin B 1 mg/kg/day and flucytosine 100 mg/kg/day. His fever resolved after 3 days of treatment, and his headache resolved after 22 days of treatment. A repeat CSF analysis on day 7 of treatment showed 11 nucleated cells/mL, 1 red cell/mL, protein 0.277 g/dL, and CSF to serum glucose ratio 0.55. CSF India ink stain and culture were negative. CSF cryptococcal antigen titer decreased to 1:1.

The therapeutic dilemma was whether to use fluconazole or itraconazole for consolidation treatment of both *C. neoformans* and *P. marneffei*. Fluconazole has poor in vitro and in vivo activity against *P. marneffei* [3]. Conversely, itraconazole reaches negligible levels in CSF and is associated with a higher relapse rate during maintenance treatment of cryptococcal meningitis, compared with the relapse rate associated with fluconazole [4,

Received 31 May 2010; accepted 28 July 2010; electronically published 1 October 2010.
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Clinical Infectious Diseases 2010;51(9):e65–e68

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1058-4838/2010/5109-00E2\$15.00
DOI: 10.1093/cid/cir285

9]. Thus, the optimal azole therapy for one infection is suboptimal for the other. Prolonged amphotericin B use is unaffordable in resource-poor settings, inconvenient, and associated with increased toxicity. The newer triazoles are expensive and not available in Vietnam. This therapeutic dilemma may be better addressed in resource-rich settings with the availability of newer antifungal drugs. For instance, voriconazole has good CSF levels and has been used to treat penicilliosis and central nervous system fungal infections, including cryptococcal meningitis [10, 11]. Posaconazole has structure and antifungal activity similar to itraconazole [12], is theoretically effective against *P. marneffei*, and has been used to treat cryptococcal meningitis [13]. Because mortality from cryptococcal meningitis in our hospital is higher than mortality from penicilliosis, the patient received 8-week consolidation therapy with fluconazole 400 mg/day, followed by 200 mg/day for maintenance therapy along with frequent monitoring. At months 3, 6, 8, and 10 of follow-up, he was well and able to return to his previous work. Repeat blood cultures at follow-up visits showed no growth. Routine hematology and liver function tests were normal. He continued to take antiretroviral medications, and his CD4 cell count at month 8 was 173 cells/ μ L.

The Hospital for Tropical Diseases is the primary and referral center for infectious diseases and HIV in southern Vietnam, with 2600 HIV-infected patients admitted yearly. *C. neoformans* and *P. marneffei* are the pathogens most commonly isolated from blood cultures at the Hospital for Tropical Diseases, ranking first and second. From January 2004 through December 2009, 677 incident cases of penicilliosis were identified by culture of skin scrapings, blood, lymph node, bone marrow, or other body tissue culture. Seven of these 677 patients had concurrent cryptococcosis, isolated from CSF ($n = 6$) and/or blood culture ($n = 7$). The clinical features and hospital outcome of these 7 patients (patients 1 to 7) and the case patient (patient 8) are reported in Table 1. All 8 patients had HIV infection with a median absolute lymphocyte count of 233 cells/ μ L (interquartile range [IQR], 143–405 cells/ μ L). The median age was 25.5 years (IQR, 23–28 years); 7 of the 8 patients were men. Most patients presented with a subacute febrile illness (median duration, 5.5 days; IQR, 4.5–14 days), and headache and/or vomiting were features in 6 of the 8. Seven of 8 patients were anemic; 6 had thrombocytopenia. *C. neoformans* and *P. marneffei* were isolated from blood culture in all 8 patients. One patient had concurrent *Salmonella* species isolated from blood. *C. neoformans* grew in all 7 CSF specimens cultured, and the India ink stain was positive in 5 of 6 CSF samples examined. One patient had concurrent *P. marneffei* isolated from CSF. Two patients died during acute hospitalization, 1 within 24 hours of hospitalization, the other after 10 days of amphotericin B 0.7 mg/kg/day. Six patients survived and were discharged home with symptom resolution after being treated

with amphotericin B for 14 days, followed by fluconazole 450 mg to 600 mg in 4 patients and itraconazole 400 mg in 1 patient. Additional follow-up data could not be obtained for these patients because of incomplete or incorrect contact information.

This case series demonstrates that although HIV-associated coinfection with *C. neoformans* and *P. marneffei* is uncommon, coinfection occurs in severely immunocompromised people and poses a unique therapeutic dilemma that needs to be addressed in underresourced settings where disease is endemic. Itraconazole is the drug of choice for consolidation treatment of penicilliosis. Although it has fungicidal activity similar to that of fluconazole in the consolidation phase, it has a higher relapse rate in the maintenance treatment of cryptococcal meningitis [4]. Likely, this is because of its variable absorption and low CSF levels [9]. Fluconazole is the preferred consolidation treatment for cryptococcosis and has good oral bioavailability and CSF penetration [4, 14]. However, it is associated with a high treatment failure rate (in 7 of 13 cases) against *P. marneffei* infection at 400 mg/day [3]. In our hospital, fluconazole is the preferred choice for coinfection, because of concerns regarding the higher relapse rate in patients with cryptococcal meningitis who were treated with itraconazole. To our knowledge, there has never been a randomized controlled trial of induction and/or consolidation therapy for penicilliosis, and the optimal treatment of this infection is not well defined. In an early case series of penicilliosis from Thailand, 4 of 13 patients who received fluconazole 400 mg/day had good clinical and microbiological responses during the acute hospitalization [3]. Whether this response rate could be improved by higher fluconazole dosage is an important question. After 2 weeks of amphotericin B and flucytosine, our case patient was treated with fluconazole 400 mg/day for consolidation and 200 mg/day for maintenance therapy, and he had a good treatment outcome after 10 months of follow-up. His CD4 cell count continued to increase on ART. The patient's good response to fluconazole, despite possibly suboptimal treatment for his penicilliosis, may have been a result of his excellent acute response to ART, manifested by the dramatic rise in CD4 cell count (from 12 to 107 cells/ μ L after 11 days of ART). The temporal association between starting ART 4 days before the onset of illness, the low baseline CD4 cell count, and the rapid immunological response to ART in this patient suggest the possibility of an immune reconstitution inflammatory syndrome unmasking subclinical *C. neoformans* and *P. marneffei* coinfection. Fungal burden is strongly associated with outcome in cryptococcal disease, and this is likely to be true in penicilliosis. Subclinical disease with lower fungal burden is likely to respond better to treatment. A higher dosage of fluconazole (800 to 1200 mg) has been shown to be safe and fungicidal in a dose-response manner during induction therapy for cryptococcal meningitis [15, 16] and is likely to be more effective in *C. neoformans* and *P. marneffei* coinfection.

Table 1. Clinical and Laboratory Features of 8 Human Immunodeficiency Virus–Infected Patients with *Penicillium marneffe* and *Cryptococcus neoformans* Coinfection

Identifier	Age in year, sex	Days of illness	Clinical characteristics		Laboratory and radiographic characteristics						
			Presenting symptoms	Presenting signs	Blood cell count and transaminases	CSF analysis and culture	CXR	Other	Treatment	LOS	Acute hospital outcome
1	22, M	3	Headache, diarrhea, abdominal pain	T 37°C, alert and FC, thin, hepato-splenomegaly	ASL 663, Hb 4.9, Plt 71, AST 21, ALT 41	W 1640 (94%N), R 12, P 0.4, G 2/3.5, Indian ink +/- <i>P. marneffe</i> and <i>C. neoformans</i>	Normal	U/S: liver 16 cm, spleen 14 cm, abdominal LAD	AmB × 14 d, then fluconazole 450 mg	21 d	Improved at d/c with fluconazole
2	28, F	14	Malaise, anorexia, cough, headache, vomiting	T 39°C, alert and FC, wasting, thrush, nuchal rigidity	ASL 104, Hb 8, Plt 135	W 0, R 0, P 0.4, G 1.5/3.9 <i>C. neoformans</i>	Bilateral reticulonodular infiltrate	...	AmB × 13 d, then itraconazole 400 mg	16 d	Improved at d/c with itraconazole
3 ^a	23, M	15	Malaise, anorexia, cough	T 38°C, alert and FC, wasting, thrush, skin lesions on face, cervical LAD	ASL 156, Hb 10, Plt 90	W 1, R 1, P 0.44, G 2/7.8 Indian ink +/- <i>C. neoformans</i>	Enlarged hilar lymph nodes	...	AmB × 10 d, then itraconazole 400 mg	14 d	Died of refractory encephalopathy
4	23, M	3	Cough, headache	T 38°C, alert and FC, thin	ASL 74, Hb 8, Plt 69, AST 59, ALT 72	W 0, R 0, P 0.4, G 3.5/3.8 Indian ink +/- <i>C. neoformans</i>	Bronchial cuffing	U/S: liver 14 cm, spleen 12 cm	AmB × 14 d, then fluconazole 600 mg	25 d	Improved at d/c with fluconazole
5	27, M	5	Anorexia, cough, headache, vomiting	T 38°C, alert and FC, thin	ASL 256, Hb 6, Plt 267, AST 90, ALT 56	<i>C. neoformans</i>	Infiltrate	U/S: liver 13.4 cm, abdominal LAD	AmB × 13 d	13 d	Improved at d/c with unknown oral antifungal
6	24, M	6	Headache, vomiting, mental status change, malaise	T 39°C, ↓ GCS, wasting, nuchal rigidity	ASL 352, Hb 11, Plt 25	W 560 (24%N, 76%L), R 0, P 0.5, G 1.1/2.5 Indian ink +/- <i>C. neoformans</i>	Normal	...	AmB × 1 d	...	Died 24 h after admission
7	28, M	4	Malaise, anorexia, abdominal pain	T 37°C, alert and FC, wasting	ASL 210, Hb 11, Plt 94, AST 118, ALT 129	CSF was not obtained	Normal	...	AmB × 14 d, then fluconazole 600 mg	23 d	Improved at d/c with fluconazole
8	29, M	5	Headache, vomiting	T 37°C, GCS 15, thrush	ASL 565, Hb 12, Plt 234, AST 16, ALT 15, crypto Ag +	W 20, R 2, P 0.5, G 2.6/5.2 Indian ink +, crypto Ag +/- <i>C. neoformans</i>	Enlarged hilar lymph nodes	Head MRI: contrast-enhanced meninges	AmB × 14 d, then fluconazole 400 mg × 8 wk	22 d	Improved at d/c; resolved at months 6, 8, and 10

NOTE. All patients presented with fever, and all patients had *Penicillium marneffe* and *Cryptococcus neoformans* recovered from blood culture. Ag, antigen; ALT, aspartate transaminase in U/L; AmB, amphotericin B; ASL, absolute lymphocyte count in cells/ μ L; AST, alanine transaminase in U/L; CSF, cerebrospinal fluid; CXR, chest radiography; D/C, discharged; FC, following commands; G, glucose (CSF/serum); GCS, Glasgow Coma Scale; Hb, hemoglobin in g/dL; L, lymphocytes; LAD, lymphadenopathy; LOS, length of stay; MRI, magnetic resonance imaging; N, neutrophils; P, protein in g/dL (normal, <0.45 g/dL); Plt, platelets in value $\times 10^3$ cells/ μ L; R, red blood cells/ μ L; T, temperature; U/S, ultrasonography; W, nucleated cell.

^a Patient 3 had *Salmonella* recovered from blood culture.

However, data on the efficacy and tolerability of high-dose fluconazole during longer consolidation therapy for these mycoses is limited [17].

This case demonstrates that in resource-poor settings, fluconazole following amphotericin B induction may be an acceptable choice for consolidation treatment of *C. neoformans* and *P. marneffei* coinfection. ART likely plays an important role in the successful management of this case, although the optimal timing of ART in fungal opportunistic infections is not yet defined [18, 19].

Acknowledgments

Financial support. Fogarty International Clinical Research Fellowship (to T.L.) and Wellcome Trust Major Overseas Programmes (to T.L., P.S.L., and J.N.D.).

Potential conflicts of interest. All authors: no conflicts.

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