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Endemic fungal infections caused by *Cryptococcus neoformans* and *Penicillium marneffei* in patients infected with human immunodeficiency virus and treated with highly active anti-retroviral therapy

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ABSTRACT

This study compared the clinical presentations of 58 episodes of cryptococcosis in 50 patients and 26 episodes of penicillosis in 25 patients infected with human immunodeficiency virus (HIV) between June 1994 and June 2004, and assessed the safety of discontinuation of secondary prophylaxis for endemic fungal infections in those patients responding to highly active anti-retroviral therapy (HAART). Neurological symptoms were seen more commonly in patients with cryptococcosis, whereas respiratory symptoms, lymphadenopathy, hepatomegaly and/or splenomegaly, and non-thrush-related oral presentations were seen more commonly in patients with penicillosis. Patients with penicillosis were more likely to have abnormal chest radiography results and radiographic presentations of interstitial lesions, cavitations, fibrotic lesions and mass lesions. At the end of the study, maintenance antifungal therapy had been discontinued in 27 patients with cryptococcosis and in 18 patients with penicillosis in whom the median CD4 count had increased to 186 cells/μL (range, 9–523 cells/μL) and 95 cells/μL (range, 15–359 cells/μL), respectively, after HAART. Only one episode of penicillosis recurred (a relapse rate of 1.72/100 person-years; 95% CI, 1.44–2.10/100 person-years) after a median follow-up duration of 35.3 months (range, 2.6–91.6 months). No relapses occurred in patients with cryptococcosis after a median follow-up duration of 22.3 months (range, 1–83.4 months). These findings suggest that there are differences in the clinical presentations between endemic cryptococcosis and penicillosis in patients with HIV infection, and that it is safe to discontinue secondary antifungal prophylaxis for cryptococcosis and penicillosis in patients responding to HAART.

Keywords Cryptococcosis, *Cryptococcus neoformans*, highly active anti-retroviral therapy, human immunodeficiency virus, *Penicillium marneffei*, penicillosis

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INTRODUCTION

Clinical manifestations of invasive endemic fungal infections caused by *Cryptococcus neoformans* and *Penicillium marneffei* may be similar, resulting in difficulties in distinguishing between these two diseases in patients infected with human immunodeficiency virus (HIV) who travel to or reside in endemic areas, such as Southeast Asia. However, comparisons between these two diseases are reported rarely in the literature.

Following the introduction of highly active anti-retroviral therapy (HAART), the mortality and morbidity [1,2], as well as the incidence of AIDS-defining opportunistic infections (OIs) [3], have been reduced significantly [4]. Discontinuation of primary and secondary prophylaxis is safe for many OIs in HIV-infected patients whose immunity has been restored by HAART. Such OIs include *Pneumocystis carinii* pneumonia (*Pneumocystis jiroveci* pneumonia) [5–9], cytomegalovirus end-organ disease, disseminated *Mycobacterium avium* complex infection, and cerebral toxoplasmosis [10]. Discontinuation of secondary

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prophylaxis associated with invasive fungal infections has been reported for disseminated histoplasmosis [11], cryptococcal meningitis [12,13] and penicillosis [14]. Despite the dramatic decline in morbidity and mortality during the post-HAART era, OIs continue to occur in patients who are unaware of their HIV status, have limited access to HIV care, or show poor adherence to antimicrobial prophylaxis and HAART regimens. Therefore, it remains important for physicians to make timely diagnoses, based on clinical presentation and microbiological cultures, in order to start effective therapy.

The aims of the present study were to determine the clinical indicators required to differentiate between cryptococcosis and penicillosis in HIV-infected patients, and to assess the safety of discontinuation of secondary prophylaxis for cryptococcosis and penicillosis in HIV-infected patients responding to HAART.

MATERIALS AND METHODS

Between June 1994 and June 2004, 1047 HIV-infected patients sought medical care at the National Taiwan University Hospital, Taipei. A standard case record form was used to prospectively record patient demographics, AIDS-defining OIs, anti-retroviral therapy, antimicrobial therapy and laboratory test results, including plasma HIV RNA load (Amplicor v. 1.5; Roche, Branchburg, NJ, USA) and $CD4^+$ cell count [15]. Patients were enrolled in the study if a diagnosis of cryptococcosis and penicillosis was made. Clinical presentations, concurrent OIs, relevant laboratory results, treatment course and overall outcome (alive, relapse, death or loss to follow-up) of endemic fungal infections were recorded. Cryptococcosis was diagnosed if cultures of clinical specimens were positive for *C. neoformans*, or if India ink smears were positive, or if elevated cryptococcal antigen titres were present in the clinical specimens. Penicillosis was diagnosed if cultures of clinical specimens were positive for *P. marneffei*.

For patients with cryptococcosis, amphotericin B was prescribed at a daily dosage of 0.7–1.0 mg/kg as induction therapy for 2–3 weeks, followed by maintenance therapy with oral fluconazole 400 mg daily. For patients with penicillosis, amphotericin B was prescribed at a daily dose of 0.7–1.0 mg/kg for 2 weeks, followed by itraconazole 400 mg daily for 6–8 weeks. Survivors continued to receive HAART and itraconazole 200 mg daily as maintenance therapy. Relapse was diagnosed from clinical specimens by positive cultures or India ink smears for *C. neoformans*, or positive cultures for *P. marneffei*. Some of the cases included in this study have been reported previously [14,16].

HAART was defined as anti-retroviral therapy consisting of at least one protease inhibitor or non-nucleoside reverse transcriptase inhibitor, or abacavir plus two nucleoside reverse transcriptase inhibitors. All HIV-infected patients were given free access to HAART, which was introduced in Taiwan on 1 April 1997. The decision to discontinue maintenance therapy was at the discretion of the treating physicians. At discontinuation, data were collected concerning duration of antifungal and antiviral therapies, CD4 counts and plasma HIV RNA load. Clinical outcome was assessed on 31 December 2004, or at date of death or loss to follow-up.

All statistical analyses were performed with SAS software v. 8.1 (SAS Institute Inc., Cary, NC, USA) and Microsoft Office 2003. Categorical variables were compared using χ^2 or Fisher's exact test. Non-categorical variables were compared using Wilcoxon's rank sum test, and p values < 0.05 were considered significant. To estimate the mortality rate in the pre-HAART era (before 1 April 1997) and that in the post-HAART era (after 1 April 1997) in both groups, deaths of patients enrolled before 1 April 1997 were censored 6 months later, and for patients enrolled after 1 April 1997, deaths were censored 6 months after the end of study enrolment on 30 June 2004. The overall mortality rate for each group was calculated as the number of deaths/100 person-years (100 PY) from enrolment to the end of this study (31 December 2004), or to date of death or loss to follow-up. Exact 95% CIs for mortality rates were calculated on the basis of the Poisson distribution. The survival probabilities were estimated by the Kaplan–Meier method. The Cox proportional-hazards model was used to compare the mortality of the two endemic fungal infections, with adjustment for age, gender, year in which endemic fungal infection was diagnosed (i.e., pre-HAART or post-HAART), and baseline $CD4^+$ lymphocyte count. Hazard ratios and 95% CIs of risk for death between patients with cryptococcosis and those with penicillosis were calculated. The relapse rate for each group was calculated as the number of relapses/100 PY of observation. Exact 95% CIs for relapse rates were also calculated on the basis of the Poisson distribution.

RESULTS

During the 10-year study period, 58 episodes of cryptococcosis and 26 episodes of penicillosis were diagnosed in 50 and 25 HIV-infected patients, respectively. Thirteen (22%) episodes of cryptococcosis were diagnosed in 12 patients in the pre-HAART era, and 45 (78%) episodes were diagnosed in 38 patients in the post-HAART era. Six (23%) episodes of penicillosis were diagnosed in six patients in the pre-HAART era and 20 (77%) episodes were diagnosed in 20 patients in the post-HAART era. The baseline clinical characteristics are shown in Table 1. Patients with penicillosis had a lower median CD4 count at baseline compared with patients with cryptococcosis (9.5 cells/μL vs. 25 cells/μL; p 0.009), and were less likely to acquire HIV via homosexual activity (36% vs. 66%; p 0.01).

The clinical and microbiological characteristics at diagnosis of invasive endemic fungal infections are shown in Table 2. Most cases were diagnosed in patients with high plasma HIV RNA loads (>5 $\log_{10}$ copies/mL), reduced CD4 counts, and

Table 1. Baseline characteristics and outcomes of patients infected with human immunodeficiency virus (HIV) and with endemic fungal infections caused by *Penicillium marneffei* and *Cryptococcus neoformans*

	Cryptococcosis	Penicillosis	p
No. patients (episodes)	50 (58)	25 (26)	
Female/male	1/49	5/20	0.007
Median baseline CD4 count, cells/μL (range)	25 (1–798)	9.5 (1–122)	0.009
< 100, *n* (%)	46 (93.9)	23 (95.8)	0.80
100–199	1 (2.0)	1 (4.2)	
≥ 200	2 (4.1)	0	
Median baseline PVL $\log_{10}$ copies/mL (range) (patients with available data)	5.39 (2.6-5.88) (29)	5.48 (2.6-5.99) (12)	0.68
Overseas Chinese, *n* (%)	7 (14)	7 (28)	0.14
Risk-factor for HIV, *n* (%)			
Heterosexual	14 (28)	15 (60)	0.007
MSM	33 (66)	9 (36)	0.01
IDU	1 (2)	1 (4)	
Transfusion	1 (2)	0 (0)	
Unknown	1 (2)	0 (0)	
Median age at diagnosis, years (range)	36 (24–82)	33 (27–82)	0.27
Median interval between initiation of effective treatment and admission, days (range)	2 (1–36)	8 (1–41)	0.006
Overall outcome, episodes (%)	58	26	
Pre-HAART period	13 (13/58, 22%)	6 (6/26, 23%)	0.95
Discontinuation	3 (3/13, 23%)	5 (5/6, 83%)	0.01
Alive without relapse	2	2	
Alive with relapse	0	1	
Death	1	2	
Alive with relapse	1 (1/13, 8%)	0 (0/6, 0%)	0.49
Death	9 (9/13, 69%)	1 (1/6, 17%)	0.03
Before 30 Sept. 1997	6	1	
Lost to follow-up	0	0	
Post-HAART period	45 (45/58, 78%)	20 (20/26, 77%)	0.95
Discontinuation	24 (24/45, 53%)	14 (14/20, 70%)	0.21
Alive without relapse	17	9	
Alive with relapse	0	0	
Death	1	2	
Lost to follow-up	6	3	
Alive with relapse episodes	7 (7/45, 16%)	0 (0/20, 0%)	0.06
Death	12 (12/45, 27%)	5 (5/20, 25%)	0.89
Before 31 Dec. 2004	12	5	
Lost to follow-up	1 (1/45, 2%)	0 (0/20, 0%)	0.50
Data unavailable	1 (1/45, 2%)	0 (0/20, 0%)	0.50
No treatment	0 (0/45, 0%)	1 (1/20, 5%)	0.13
Mortality rate within 2 weeks of hospitalisation, episodes (%)	6 (6/58, 10.3%)	3 (3/26, 11.5%)	0.87
Related to fungal infection	5 (8.6)	1 (3.8)	0.43
Mortality rate within 10 weeks of hospitalisation, episodes (%)	12 (20.7)	4 (15.4)	0.57
Related to fungal infection	5 (8.6)	1 (3.8)	0.43
Cause of death, episodes (%)	23	10	
Related to fungal infection	6 (6/58, 10.3%)	1 (1/26, 3.8%)	0.32
Enrolled before HAART	2	0	
Enrolled after HAART	4	1	
Related to other OI	4 (4/58, 6.9%)	3 (3/26, 11.5%)	0.48
Unrelated to OI	8 (8/58, 13.8%)	4 (4/26, 15.4%)	0.85
Unknown	5 (5/58, 8.6%)	2 (2/26, 7.7%)	0.89
Mortality rate, no. of episodes/ 100 person-years (95% CI)			
Overall	20.33 (19.53, 21.15)	12.46 (11.75, 13.21)	0.17
Pre-HAART	59.24 (55.01, 63.70)	9.46 (7.78, 11.40)	< 0.05
Post-HAART	[a]16.01 (15.19, 16.86)	[b]13.98 (12.98, 15.04)	0.77
Hazard ratio (95% CI) (cryptococcosis vs. penicillosis)			
Overall			
Adjusted[c]	1.377 (0.630, 3.009)		0.42
Pre-HAART			
Adjusted	3.503 (0.355, 34.596)		0.28
Post-HAART			
Adjusted	1.127 (0.406, 3.129)		0.82

HAART, highly active anti-retroviral therapy; IDU, intravenous drug user; PVL, plasma HIV RNA load; MSM, men who have sex with men; OI, opportunistic infection.
[a]Pre- vs. post-HAART, p 0.002.
[b]Pre- vs. post-HAART, p 0.71.
[c]Adjusted for age, gender, and baseline $CD4^+$ count.

Table 2. Clinical and microbiological manifestations of patients infected with human immunodeficiency virus and cryptococcosis or penicillosis

	Cryptococcosis	Penicillosis	p
Median CD4 at diagnosis of fungal infection, cells/μL (range)	24 (1–565)	5.5 (0–122)	<0.001
< 100, *n* (%)	48 (90.6)	25 (96)	0.79
100–199	2 (3.8)	1 (4)	
≥ 200	3 (5.7)	0	
Geometric mean of cryptococcal antigen level at diagnosis (range) (episodes with available data)	398.46 (0–131 072) (50)	NA	
Median PVL at diagnosis, $\log_{10}$ copies/mL (range) (episodes with data)	5.17 (1.7–5.88) (34)	5.54 (2.6–5.88) (14)	0.12
<5 $\log_{10}$, *n* (%)	15 (44.1)	3 (21)	0.20
Clinical manifestations, *n* (%)			
Fever	48 (82.8)	24 (92.3)	0.25
Constitutional symptoms[a]	29 (50.0)	14 (53.8)	0.74
Respiratory symptoms	23 (39.7)	21 (80.8)	<0.001
Neurological symptoms	43 (74.1)	4 (15.4)	<0.001
Skin lesions	12 (20.7)	8 (30.8)	0.32
Lymphadenopathy	9 (15.5)	18 (69.2)	<0.001
Hepatomegaly and/or splenomegaly	3 (5.2)	7 (26.9)	0.004
Non-thrush-related oral presentations[b]	2 (3.4)	6 (23.1)	0.005
Other concurrent OI	28 (49.1)	14 (53.8)	0.69
Chest X-ray findings			
Episodes with data	49	26	
Normal, *n* (%)	20 (40.8)	2 (7.7)	0.003
Interstitial	14 (28.6)	15 (57.7)	0.01
Patch	7 (14.3)	3 (11.5)	0.74
Nodular	7 (14.3)	3 (11.5)	0.74
Consolidation	3 (6.1)	1 (3.8)	0.68
Mass	0 (0)	2 (7.7)	0.05
Cavitations	7 (14.3)	9 (34.6)	0.04
Pleural effusion	4 (8.2)	3 (11.5)	0.63
Fibrotic lesions	0 (0)	4 (15.4)	0.005
Site of detection, episodes (%)			
Blood	52/55 (94.5)		
Culture	34/52 (65.4)	16/25 (64)	0.91
Antigen	47/48 (97.9)	NA	
Lung biopsy	6/7 (85.7)	7/8 (87.5)	
Culture	2/6 (33.3)	3/6 (50)	0.56
Pathology	4/5 (80)	4/4 (100)	0.28
Antigen	2/2 (100)	NA	
Sputum	1/26 (3.8)		
Culture	1/26 (3.8)	9/15 (60)	<0.001
Antigen	1/2 (50)	NA	
Bone marrow	4/8 (50)	11/14 (78.6)	
Culture	4/5 (80)	10/11 (90.9)	0.54
Pathology	1/7 (14.3)	8/13 (61.5)	0.04
Antigen	0/1 (0)	NA	
CSF	42/55 (76.4)	0/2 (0)	
Culture	40/55 (72.7)	0/2 (0)	0.03
Antigen	39/52 (75)	NA	
Skin	0/6 (0)	9/10 (90)	
Culture	0/5 (0)	6/8 (75)	0.008
Pathology	0/5 (0)	6/8 (75)	0.008
Lymph node	2/3 (66.7)	5/5 (100)	
Culture	1/3 (33.3)	5/5 (100)	0.04
Pathology	2/2 (100)	4/4 (100)	
Oral/pharyngeal lesions			
Culture	0/3 (0)	4/8 (50)	0.13
Urine	3/11 (27.3)	0/6 (0)	
Culture	1/11 (9.1)	0/6 (0)	0.45
Antigen	2/2 (100)	NA	

CSF, cerebrospinal fluid; OI, opportunistic infection; PVL, plasma HIV RNA load; NA, not applicable.
[a]Constitutional symptoms included malaise, weight loss, sweating and poor appetite.
[b]Non-thrush-related oral presentations included oral ulcers, sore throat, and dysphagia.

other concurrent AIDS-defining OIs. Patients with penicillosis had significantly lower CD4 counts than patients with cryptococcosis (5.5 vs. 24 cells/μL; $p < 0.001$). In both groups, most patients had fever, and half had constitutional symptoms. While neurological symptoms were seen more commonly in patients with cryptococcosis (74.1% vs. 15.4%; $p < 0.001$), patients with penicillosis were significantly more likely to present with respiratory symptoms (80.8% vs. 39.7%; $p < 0.001$), lymphadenopathies of the neck, mediastinum and peri-aortic areas (69.2% vs. 15.5%; $p < 0.001$), hepatomegaly and/or splenomegaly (26.9% vs. 5.2%; p 0.004), and non-thrush-related oral presentations (23.1% vs. 3.4%; p 0.005). Skin lesions were seen with similar frequency in each patient population, but only patients with penicillosis were diagnosed from skin investigations (90% vs. 0%; $p < 0.001$).

More patients with penicillosis had abnormal chest radiography results compared with patients with cryptococcosis (92.3% vs. 59.2%; p 0.003) (Table 2). Although there were no specific radiographic patterns, patients with penicillosis were more likely to have interstitial lesions (57.7% vs. 28.6%; p 0.01), cavitations with greater severity (34.6% vs. 14.3%; p 0.04), fibrotic lesions (15.4% vs. 0%; p 0.005) and mass lesions (7.7% vs. 0%; p 0.05) than patients with cryptococcosis.

C. neoformans and *P. marneffei* were isolated from various clinical specimens (Table 2). Diagnosis of cryptococcosis by culture from bone marrow provided the highest sensitivity (80%), followed by cerebrospinal fluid (72.7%), blood (65.4%), lung biopsy (33.3%) and lymph nodes (33.3%). Pathology also provided good diagnostic sensitivity with lymph node (100%) and lung biopsy (80%) specimens. Culture of bone marrow was more sensitive than pathology (80% vs. 14.3%; p 0.02), but the sensitivity of serum cryptococcal antigen detection was higher than that of culture for blood (97.9% vs. 65.4%; $p < 0.001$), sputum (50% vs. 3.8%; p 0.02) and urine (100% vs. 9.1%; p 0.005) specimens.

Diagnosis of penicillosis by culture provided the highest diagnostic yield with lymph node specimens (100%), followed by specimens from bone marrow (90.9%), skin (75%), blood (64%), sputum (60%), lung biopsy (50%) and oral and pharyngeal lesions (50%). Examination of the specimens by pathology also provided a high diagnostic yield for lymph node (100%), lung biopsy (100%), skin (75%) and bone marrow (61.5%) specimens.

Antifungal agents were initiated earlier following hospitalisation for patients with cryptococcosis than for patients with penicillosis (median interval, 2 vs. 8 days; p 0.006) (Table 1). All patients with cryptococcosis received antifungal agents following admission. The mortality rate within 2 weeks of hospitalisation with cryptococcosis was 10.3% (6/58 patients), with a median interval from admission to death of 5 days (range, 3–10 days). Among the six deaths, five were caused directly by cryptococcosis, while the other was caused by a pulmonary embolism. The mortality rate within 10 weeks of hospitalisation was 20.7% (12/58 patients), with a median interval from admission to death of 14 days (range, 3–52 days). No deaths that occurred between the third and the tenth weeks were caused by fungal infection. At the end of the observation period, the mortality rate in the pre-HAART era was significantly higher than that in the post-HAART era for patients with cryptococcosis, i.e., 59.24 vs. 16.01/100 PY (p 0.002). This finding might be related to the introduction of HAART, with fewer non-fungal-related deaths (20.0% vs. 61.5%; p 0.003) occurring in the post-HAART era.

The mortality rate within 2 weeks of hospitalisation in patients with penicillosis was 11.5% (3/26 patients), with the intervals from admission to death being 7, 10 and 11 days, respectively. A patient who died of pneumothorax on day 11 did not receive antifungal therapy before the diagnosis of penicillosis, while the other two patients died of pneumothorax and *Pneumocystis carinii* pneumonia, respectively. The mortality rate within 10 weeks of hospitalisation was 15.4% (4/26 patients), with the fourth death occurring from an unknown cause on day 50 following admission. At the end of the observation period, the mortality rate of penicillosis was similar before and after the introduction of HAART, i.e., 9.46 vs. 13.98/100 PY (p 0.71).

The overall mortality rate was similar for the cryptococcosis and penicillosis groups (20.33 vs. 12.46/100 PY; p 0.17). Although patients with cryptococcosis had a higher mortality rate than those with penicillosis in the pre-HAART era (59.24 vs. 9.46/100 PY; $p < 0.05$), the adjusted hazard ratios for death were similar between patients with cryptococcosis and penicillosis either before or after the introduction of HAART.

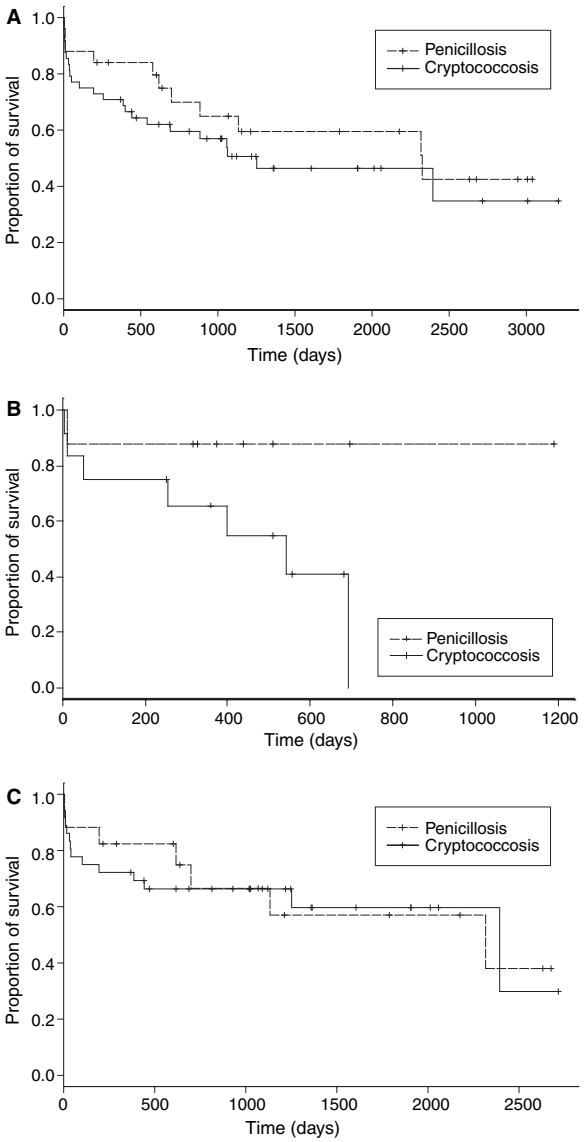

Fig. 1. (A) Overall Kaplan–Meier survival estimates for cryptococcosis and penicillosis patients. (B) Kaplan–Meier survival estimates for cryptococcosis and penicillosis patients before introduction of HAART. (C) Kaplan–Meier survival estimates for cryptococcosis and penicillosis patients after introduction of HAART.

The Kaplan–Meier survival estimates for cryptococcosis and penicillosis patients overall, and before and after the introduction of HAART, are shown in Fig. 1.

After HAART, maintenance therapy was discontinued for 27 episodes of cryptococcosis in 27 patients because of immune recovery (26 episodes) or loss to follow-up (one episode) (Table 3). At discontinuation, four (20%) of the 20 patients with available cryptococcal antigen data had achieved an undetectable level. The median cumulative treatment durations for the 27 patients with cryptococcosis were 13 days (range, 0–46 days) of amphotericin B, 16.1 months (range, 4.3–39.4 months) of fluconazole, and 18.7 months (range, 4.2–71.9 months) of HAART. The median CD4 count and plasma HIV RNA load at discontinuation were 186 cells/μL (range 9–523) and 1.7 $\log_{10}$ copies/mL (range 1.7–4.9), respectively; most (87.5%) patients had CD4 counts >100 cells/μL, and 83.3% had a CD4 increase of ≥100 cells/μL. At the end of the observation period, six patients were lost to follow-up and two patients died of pneumonia and an unknown cause, respectively. Most patients had good immune restoration, with a median CD4 count of 238 cells/μL (range 17–750), and 11 of 12 patients with data available still had detectable cryptococcal antigen. After a median observation period of 22.3 months (range, 1–83.4 months), there was no relapse (95% CI, 0–0.20/100 PY).

In the penicillosis group, antifungal maintenance therapy was discontinued in 19 episodes in 18 patients because of immune recovery (14 episodes), abnormal liver function tests (two episodes) or loss to follow-up (three episodes) (Table 3). The median cumulative treatment durations were 13 days (range, 0–24 days) of amphotericin B, 10.8 months (range, 2.9–18.9 months) of itraconazole, and 9.9 months (range, 3–61.6 months) of HAART. At discontinuation, the median CD4 count was 95 cells/μL. Most (94.7%) patients with penicillosis had CD4 counts <200 cells/μL at discontinuation of secondary antifungal prophylaxis, and ten (52.6%) patients had CD4 counts <100 cells/μL. In comparison with patients with cryptococcosis, patients with penicillosis had a less robust increase in CD4 count (89 vs. 159.5 cells/μL; p 0.004), with significantly fewer patients having an increase of ≥100 cells/μL (42.1% vs. 83.3%; p 0.005). At the end of the observation period, the median CD4 count was 245.5 cells/μL (range, 1–558 cells/μL) and the plasma HIV RNA load was 1.7 $\log_{10}$ copies/mL (range, 1.7–5.86 $\log_{10}$ copies/mL). At this time, 12 patients remained alive without relapse; four (21.1%) had died because of disseminated *M. avium* complex infection, upper gastrointestinal tract bleeding, pulmonary tuberculosis, and acute renal failure, respectively, and two were lost to follow-up. After a median duration of observation of 35.3 months (range, 2.6–91.6 months),

Table 3. Clinical outcome for patients infected with human immunodeficiency virus and cryptococcosis or penicillosis who discontinued secondary prophylaxis

	Cryptococcosis	Penicillosis	p
No. of patients who discontinued antifungal agents (episodes)	27 (27)	18 (19)	
Reasons for discontinuation, episodes (%)			
Immune recovery	26 (96.3)	14 (73.7)	
Lost to follow-up	1 (3.7)	3 (15.8)	
Abnormal LFT	0 (0)	2 (10.5)	
Median CD4 at discontinuation, cells/μL (range) (episodes with data)	186 (9–523) (24)	95 (15–359) (19)	0.001
< 50, *n* (%)	1 (4.2)	5 (26.3)	0.01
50–99	2 (8.3)	5 (26.3)	0.01
100–199	11 (45.8)	8 (42.1)	
200–349	7 (29.2)	0 (0)	
≥ 350	3 (12.5)	1 (5.3)	
Median increase of CD4 count at discontinuation, cells/μL (range)	159.5 (8–381)	89 (− 4 to 349)	0.004
Increase of CD4 ≥100, *n* (%)	20 (83.3)	8 (42.1)	0.005
Increase of CD4 ≥200	7 (29.2)	1 (5.3)	0.06
Median PVL at discontinuation, $\log_{10}$ copies/mL (range) (episodes with data)	1.70 (1.7–4.9) (23)	2.34 (1.7–5.23) (14)	0.18
PVL ≥400 copies/mL, *n* (%)	2 (8.7)	3 (21.4)	0.35
PVL <5 $\log_{10}$	22 (95.65)	11 (91.67)	1.00
Geometric mean of cryptococcal Ag level at discontinuation (range) (episodes with data)	38.5 (0–512) (20)	NA	
Patients with undetectable cryptococcal antigen (episodes) (%)	4 (20)		
Median duration of treatment at discontinuation			
Amphotericin B, days (range)	13 (0–46)	13 (0–24)	0.72
Azole, months (range)	16.1 (4.3–39.4)	10.8 (2.9–18.9)	0.001
Total, months (range)	16.5 (4.3–40.9)	10.8 (3.3–19.4)	
Median duration of HAART at discontinuation, months (range)	18.7 (4.2–71.9)	9.9 (3–61.6)	0.06
Median duration of observation, range (months)	22.3 (1–83.4)	35.3 (2.6–91.6)	0.08
Latest data, median (range)			
CD4 cells/μL	238 (17–750)	245.5 (1–558)	0.72
CD8 cells/μL	839 (330–1979)	768 (115–1473)	0.23
PVL $\log_{10}$ copies/mL	1.7 (1.7–4.99)	1.7 (1.7–5.86)	0.38
Geometric mean of cryptococcal Ag level	21.93 (0–256)	NA	
Outcome, episodes (%)			
Alive without relapse	19 (70.4)	12 (63.2)	0.61
Alive with relapse	0 (0)	1 (5.3)	0.23
Dead	2 (7.4)	4 (21.1)	0.18
Lost to follow-up	6 (22.2)	2 (10.5)	0.30
Relapse rate, events/100 patient-years (95% CI)	0 (0, 0.20)	1.72 (1.44, 2.10)	0.35

Ag, antigen; HAART, highly active anti-retroviral therapy; NA: not applicable; LFT, liver function test; PVL, plasma HIV RNA load.

there was one relapse after discontinuation of maintenance therapy for 329 days, probably resulting from poor compliance with HAART; the relapse rate for penicillosis was 1.72/100 PY (95% CI, 1.44–2.10/100 PY).

DISCUSSION

Although patients with cryptococcosis and penicillosis often presented with indistinguishable symptoms, such as fever and constitutional symptoms, neurological symptoms were seen more frequently in patients with cryptococcosis, while respiratory symptoms, lymphadenopathy, hepatomegaly and/or splenomegaly, and non-thrush-related oral presentations were more common in patients with penicillosis. Such findings are consistent with previous reports of patients with cryptococcosis [17] and penicillosis [18]. Patients with cryptococcosis often have neurological signs and symptoms, such as headache (77%) and nuchal rigidity (29%) [17], but such symptoms were not observed by Supparatpinyo *et al*. [18] among patients with penicillosis.

Previous reports described skin lesions (71%), hepatomegaly (51%) and splenomegaly (16%) among penicillosis patients, but not among cryptococcosis patients [17,18]. Skin specimens were found to be more sensitive (90%) for isolation of *P. marneffei* in a series of cases in Thailand [18]. Although the patients belonging to both groups in the present study had skin lesions at similar frequencies (cryptococcosis 20.7% vs. penicillosis 30.8%; p 0.32), only patients with penicillosis had microbiological or histopathological evidence of skin invasion (75% vs. 0%; p 0.008). Therefore, skin biopsy and culture are likely to provide a higher diagnostic yield for patients with penicillosis than for patients with cryptococcosis.

Numerous HIV-associated pulmonary complications, including bacterial, fungal, protozoal and

mycobacterial infections, as well as neoplasms and idiopathic conditions [19], can result in cavitations, and the aetiology of cavitary lesions may vary with geographical area and immune status. On comparison of the two case series of patients with cryptococcosis and penicillosis, more cavitary lesions (14.6% vs. 0%) and intrathoracic adenopathies (10.4% vs. 0%) were observed in patients with cryptococcosis [17] than in patients with penicillosis [18]. However, consistent with the respiratory symptoms, more patients with penicillosis had abnormal chest radiography findings, especially cavitation and interstitial patterns, than those with cryptococcosis.

Although patients with penicillosis had a greater degree of immunosuppression than patients with cryptococcosis, both at baseline and at diagnosis of fungal infection in terms of CD4 counts, the 2-week, 10-week and overall mortality rates of the two diseases were similar (Table 1). In this study, a substantial (5/6) proportion of patients died directly from cryptococcosis within 2 weeks of diagnosis, although initiation of antifungal therapy was earlier for cryptococcosis than for penicillosis. These deaths may be attributable to the propensity of cryptococcosis to involve the central nervous system, causing increased intracranial pressure and related morbidity and mortality [20,21]. During the subsequent follow-up, most deaths in the two study populations were caused by other OIs because of insufficient immune restoration after a short course of HAART.

The present findings, along with those of previous studies [12,13], support the recommendation to discontinue secondary prophylaxis for cryptococcosis when the CD4 cell count increases to ≥ 100 cells/mL after HAART [22]. Despite detectable cryptococcal antigenaemia in 11 patients with cryptococcosis at the end of the observation period, no relapse was detected after antifungal therapy and HAART.

Compared with cryptococcal meningitis, the optimal CD4 count to guide discontinuation of antifungal prophylaxis for penicillosis remains unclear, since it is a rare OI of HIV-infected patients in developed countries. In the present study, compared with patients with cryptococcosis, patients with penicillosis discontinued maintenance therapy at lower CD4 counts (95 vs. 186 cells/μL; p 0.001) and had a shorter duration of maintenance therapy (10.8 vs. 16.1 months; p 0.001) (Table 3). Furthermore, 94.7% of patients had a CD4 count of ≤ 200 cells/μL, and ten (52.6%) patients even had a CD4 count of < 100 cells/μL, which is lower than current criteria for discontinuation of therapy for any opportunistic disease [22]. Despite a lower CD4 count at discontinuation of antifungal prophylaxis, the relapse rate (1.72/100 PY; 95% CI 1.44–2.10) remained low among the patients who continued to receive HAART. The only relapse episode, with a CD4 count of 10 cells/μL, was attributed to poor compliance with HAART. Therefore, it is suggested that cessation of secondary prophylaxis may be considered for patients with penicillosis who achieve a good immunological response to HAART.

In conclusion, there are differences in the clinical presentations of cryptococcosis and penicillosis in patients with HIV infection and AIDS. After appropriate management of acute infection, it appears to be safe to discontinue secondary prophylaxis for penicillosis and cryptococcosis in patients with HIV infection and AIDS who respond to HAART with immune reconstitution.

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