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# Cryptococcal meningitis in HIV-infected patients: a longitudinal study in Cambodia

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**Summary**

OBJECTIVE To describe the frequency of diagnosis of cryptococcosis among HIV-infected patients in Phnom Penh, Cambodia, at programme entry, to investigate associated risk factors, and to determine the incidence of cryptococcal meningitis.

METHODS We analysed individual monitoring data from 11 970 HIV-infected adults enrolled between 1999 and 2008. We used Kaplan–Meier naïve methods to estimate survival and retention in care and multiple logistic regression to investigate associations with individual-level factors.

RESULTS Cryptococcal meningitis was diagnosed in 12.0% of the patients: 1066 at inclusion and 374 during follow-up. Incidence was 20.3 per 1000 person-years and decreased over time. At diagnosis, median age was 33 years, median CD4 cell count was 8 cells/$\mu$l, and 2.4% of patients were receiving combined antiretroviral therapy; 38.7% died and 34.6% were lost to follow-up. Of 750 patients alive and in care after 3 months of diagnosis, 85.9% received secondary cryptococcal meningitis prophylaxis and 13.7% relapsed in median 5.7 months [interquartile range 4.1–8.8] after cryptococcal meningitis diagnosis (relapse incidence = 5.7 per 100 person-years; 95%CI 4.7–6.9). Cryptococcal meningitis was more common in men at programme entry (adjusted OR = 2.24, 95% CI 1.67–3.00) and fell with higher levels of CD4 cell counts ($P < 0.0001$).

CONCLUSIONS Cryptococcal meningitis remains an important cause of morbidity and mortality in Cambodian HIV-infected patients. Our findings highlight the importance of increasing early access to HIV care and cryptococcal meningitis prophylaxis and of improving its diagnosis in resource-limited settings.

**keywords** Asia, cryptococcosis, opportunistic infection, HIV, epidemiology

## Introduction

*Cryptococcus neoformans* is an encapsulated fungal pathogen that causes cryptococcosis, a life-threatening condition common in patients with defective cell-mediated immunity. It is the third most frequent opportunistic infection of the central nervous system in HIV-infected individuals (Powderly 1993). In the pre-antiretroviral therapy area, cryptococcosis was a leading cause of mortality in patients hospitalised with AIDS in Cambodia (Senya *et al.* 2003; Chhin *et al.* 2004; Kong *et al.* 2007; Madec *et al.* 2007). Reported prevalence among patients with AIDS varies from 2 to 10% in Europe (Dromer *et al.* 2004; Antinori *et al.* 2009) and North America (Sorvillo *et al.* 1997; Mirza *et al.* 2003), but it is more than 15% in Africa (Moosa & Coovadia 1997; Heyderman *et al.* 1998; French *et al.* 2002; Jarvis *et al.* 2009) and more than 20% in Asia (Thailand) (Chariyalertsak *et al.* 2001; Amornkul *et al.* 2003).

In this study, we describe the frequency of cryptococcal meningitis (CM) in a large cohort of HIV-infected individuals treated in the Médecins Sans Frontières (MSF)-supported HIV/AIDS programme in Phnom Penh, Cambodia at programme entry; and incidence of disease, CM-associated mortality, relapse rates and individual-level factors associated with cryptococcosis diagnosis at programme entry.

## Methods

All HIV-infected adults (≥15 years old) who received free medical care in the MSF-supported HIV/AIDS programme in Phnom Penh between February 1999 and November 2008 were eligible for inclusion. Hospital and outpatient care is offered at the Khmer-Soviet Friendship Hospital, the national referral centre for HIV in Cambodia. Free combined antiretroviral therapy (ART) provision was started in June 2001 and primary prophylaxis against CM in 2002.

Medical follow-up visits for patients on ART are scheduled every 3 months after clinical stabilisation. Primary prophylaxis with fluconazole (200 mg/day) is given to patients with CD4 cell counts <50 cells/$\mu$l, and <100 cells/$\mu$l since 2004. Diagnosis of CM is suspected in patients with severe headache, fever, neck stiffness, altered mental status, neurological deficit and/or coma. Confirmation is made with India ink direct examination of the cerebrospinal fluid. Since 2006, Pastorex Crypto Plus (Bio-Rad Laboratories, Hercules, CA, USA) has been used to detect serum cryptococcal antigen for patients with CD4 cell count below 100 cells/$\mu$l.

Patients diagnosed with CM receive amphotericin B (0.7 mg/kg/day for 2 weeks), followed by fluconazole (400 mg/day for 8 weeks). Culture for *C. neoformans* is performed in patients with persistent symptoms after receiving 14 days of antifungal treatment to confirm clearance of the pathogen. Secondary prophylaxis with fluconazole (200 mg/day) is routinely provided after treatment completion and until CD4 cell counts exceed 100 cells/$\mu$l.

We analysed routine individual patient data collected using standardised forms and entered into the FUCHIA software (Epicentre, Paris, France). Data included birth date, sex, weight, height, new or recurrent opportunistic infections (e.g. CM, penicilliosis, tuberculosis or histoplasmosis), primary and secondary antifungal prophylaxis (e.g. fluconazole and itraconazole), ART regimen and starting date, CD4 lymphocyte counts with date of blood collection, and dates of visit, hospitalisation, death or transfer to another programme.

We categorised body mass index (BMI; <16, 16–18.4 and ≥18.5 kg/m$^2$), age (15–24, 25–39 and ≥40 years) and time period at programme inclusion (pre-ART, 2001–2002, 2003–2005, and 2006–2008). Measurements of CD4 cell count closest to and between 3 months before and up to 1 month after programme inclusion, and closest to and within 2 months of CM diagnosis, were grouped into <20, 20–49, 50–99, ≥100 cells/$\mu$l, and missing.

At cohort inclusion, we defined cases of CM as those diagnosed within 15 days of enrolment, incident cases as first recorded episodes diagnosed thereafter, and relapses as new episodes of CM diagnosed three or more months after the first recorded episode.

### Statistical analysis

Patient follow-up was right-censored at the earliest of the following dates: CM diagnosis (or relapse), death, transfer out or last visit. Overall incidence of first recorded episode of CM and of first relapse were then estimated with Poisson exact 95% confidence intervals. Incidence was also described by age group, sex, and calendar period at programme inclusion and was calculated in the subgroup of patients who had not been diagnosed with CM at programme entry. Relapse rates were estimated in the subgroup of patients with ≥3 months of follow-up after CM diagnosis. Kaplan–Meier methods were used to estimate probabilities of incident CM by CD4 count level at programme inclusion.

Probabilities of survival among patients who were not lost to follow-up and of retention in care were calculated using Kaplan–Meier naïve methods. These were then compared to patients with or without cryptococcal diagnosis using log-rank tests. Patients who missed their last scheduled appointment for ≥2 months were considered lost to follow-up. Finally, we identified factors associated with CM diagnosis at programme entry using multiple logistic regression and log-likelihood ratio tests for association and trend. *A priori* confounders were sex, age group and year of programme inclusion and models were further adjusted for factors associated with the outcome in univariable analyses ($P < 0.2$). Statistical analyses were performed using Stata 10.1 (Stata Corp., College Station, TX, USA). All significance tests were two-sided, and $P$ values of 0.05 or less were considered significant.

## Results

During the study period, 11 970 HIV-infected adults entered the programme and contributed 18 409 person-years to the analysis. Twenty-four patients (0.2%) with missing age, one diagnosed with CM, were excluded. At cohort inclusion, median age was 33 years [interquartile range (IQR) 29–38] and 42.8% of patients were women. Median duration of follow-up in the programme was 6.2 months [IQR 0.8–32.4] and 4339 patients initiated ART in median 6.9 months after programme inclusion [IQR 2.5–14.1]. At ART start, median CD4 cell count was 43 cells/$\mu$l [IQR 10–130; $n$ = 3631], and 77.5% of the 2471 patients who were eligible for primary prophylaxis against cryptococcosis received it. CM was diagnosed in 1440 patients (12.0% of the cohort).

**Table 1** Cryptococcal meningitis at programme inclusion, incidence of cryptococcal meningitis and cryptococcal relapse by calendar period of enrolment, sex and age group, Phnom Penh, Cambodia, 1999–2008

| | Cryptococcal meningitis at programme entry | | Cryptococcal meningitis during follow-up* | | Cryptococcal meningitis relapse | |
|---|---|---|---|---|---|---|
| | Number of cases | Percentage of patients (95% CI) | Number of cases | Incidence per 1000 PY (95% CI) | Number of cases | Incidence per 100 PY (95% CI) |
| Total | 1066 | 8.9 (8.4–9.4) | 374 | 20.3 (18.4–22.5) | 103 | 5.7 (4.7–6.9) |
| Calendar period | | | | | | |
| 1999–2000 | 182 | 9.9 (6.6–9.0) | 58 | 36.8 (28.4–47.6) | 29 | 31.9 (22.2–45.9) |
| 2001–2002 | 316 | 8.9 (8.1–9.8) | 157 | 23.6 (20.2–27.6) | 40 | 5.4 (3.9–7.3) |
| 2003–2005 | 419 | 9.0 (7.9–9.8) | 137 | 16.5 (14.0–19.5) | 27 | 3.2 (2.2–4.7) |
| 2006–2008 | 149 | 7.8 (8.6–11.3) | 22 | 11.8 (7.8–17.9) | 7 | 5.1 (2.5–10.8) |
| Sex | | | | | | |
| Women | 264 | 5.1 (4.5–5.7) | 106 | 10.7 (8.9–13.0) | 17 | 4.4 (2.8–7.1) |
| Men | 802 | 11.7 (10.9–12.5) | 268 | 31.5 (27.9–35.5) | 86 | 6.0 (4.9–7.4) |
| Age group, years | | | | | | |
| 15–24 | 62 | 5.7 (4.3–7.1) | 26 | 13.9 (9.5–20.4) | 5 | 6.0 (2.5–14.4) |
| 25–39 | 799 | 9.7 (9.0–10.3) | 272 | 21.4 (19.0–24.1) | 86 | 6.0 (4.9–7.5) |
| ≥40 | 205 | 7.8 (6.8–8.8) | 76 | 19.7 (15.8–24.7) | 12 | 3.9 (2.2–6.8) |

95%CI, 95% binomial exact confidence interval; PY, person-years of follow-up.
*Only first diagnosed incident episode of cryptococcal meningitis considered among patients with no cryptococcal meningitis at programme entry.

### Cryptococcal meningitis diagnosis at programme entry and incidence

At cohort inclusion, 1066 patients (8.9% of patients) were diagnosed with CM (Table 1). Frequency of cryptococcal diagnosis remained stable over time and, in all age groups, was higher in men than in women. The highest frequency was observed in the 25–39 year group.

A total of 374 individuals were diagnosed with CM during follow-up. This represents an incidence rate of 20.3 per 1000 person-years (95% CI 18.4–22.5). The highest incidence was observed in the pre-ART era (1999–2000) with 36.8 per 1000 person-years, which decreased gradually to 11.8 per 1000 person-years in the 2006–2008 period. As with prevalence, incidence was higher in men than in women for all age groups, and higher rates were observed in patients aged 25 years or older. At the time of diagnosis, 45 (12.0%) individuals were receiving primary antifungal prophylaxis for a median of 66 days [IQR 29–136]. Cumulative probability of incident CM increased with lower CD4 count levels at programme entry (Figure 1).

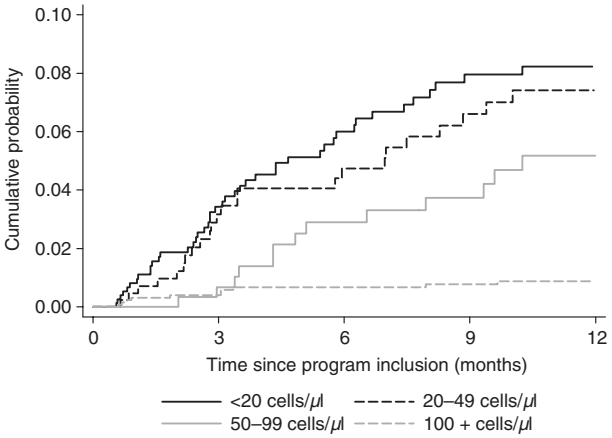



**Figure 1** Cumulative probability of incident cryptococcal meningitis per CD4 cell count level at programme inclusion, Phnom Penh, Cambodia, 1999–2008.

### Characteristics of patients at cryptococcal meningitis diagnosis

Of the 1440 patients ever diagnosed with CM, 370 (25.7%) were women. At the time of cryptococcosis diagnosis, median age was 33 years [IQR 29–38], and 34 (2.4%) patients were receiving ART for a median of 35 days [IQR 20–92] (Table 2).

Median CD4 cell count at cryptococcosis diagnosis was 17 cells/$\mu$l [IQR 7–34; $n$ = 189], and 80.4% of patients had counts less than 50 cells/$\mu$l. Men and patients with lower CD4 count levels at programme inclusion were more likely to be diagnosed with CM than women (adjusted odds ratio 2.2, 95%CI 1.7–3.0; $P < 0.0001$), and patients with higher CD4 cell counts ($P$ for trend <0.001; Table 3).

**Table 2** Characteristics of HIV-infected patients with cryptococcal meningitis at diagnosis of disease, Phnom Penh, Cambodia, 1999–2008

| | Cryptococcal meningitis | | |
|---|---|---|---|
| | Total (*N* = 1440) | Cases diagnosed at programme entry (*N* = 1066) | Incident cases (*N* = 374) |
| Median age, years [IQR] | 33 [29–38] | 33 [29–38] | 33 [29–38] |
| Median BMI, kg/m$^2$ [IQR] | *n* = 489<br>17.9 [15.8–19.8] | *n* = 318<br>18.0 [16.0–20.1] | *n* = 171<br>17.7 [15.4–19.5] |
| CD4 cell count, cells/$\mu$l | *n* = 458 | *n* = 306 | *n* = 152 |
| Median [IQR] | 8 [3–23] | 8.5 [3–22] | 7 [3–25] |
| <20 (%) | 327 (71.4) | 218 (71.2) | 109 (71.7) |
| 20–49 (%) | 76 (16.6) | 49 (16.0) | 27 (17.8) |
| 50–99 (%) | 32 (7.0) | 27 (8.8) | 5 (3.3) |
| ≥100 (%) | 23 (5.0) | 12 (3.9) | 11 (7.2) |
| Receiving ART at diagnosis (%) | 34 (2.4) | – | 34 (9.1) |
| Hospitalised during episode (%) | 1389 (96.5) | 1,038 (97.4) | 351 (93.8) |
| Median time of follow-up, months* [IQR] | 3.4 [0.6–21.6] | 3.0 [0.5–15.6] | 5.2 [1.0–46.7] |
| Number of deaths (%) | 557 (38.7) | 418 (39.2) | 139 (37.2) |
| Median time to death*, days [IQR] | 26 [6–154] | 19 [4–147] | 45 [14–188] |

ART, combined antiretroviral therapy; BMI, body mass index; IQR, interquartile range.
*Time since cryptococcosis diagnosis.

Calendar year, age, or BMI at inclusion were not associated with diagnosis of CM at programme inclusion.

Almost all patients diagnosed with CM (96.5%) required hospitalisation for a median duration of 21 days [IQR 14–27]. Concomitant opportunistic infections diagnosed were pulmonary (*n* = 188; 13.1%) and extrapulmonary tuberculosis (*n* = 218; 15.1%).

### Treatment outcomes, relapse rates, and secondary prophylaxis

By November 2008, 338 (23.5%) of patients diagnosed with CM were alive and followed in the cohort; 47 had been transferred to another HIV programme in median 37 months after diagnosis; 498 (34.6%) had been lost to follow-up after a median time of 52 days [IQR 21–151]; and 557 (38.7%) had died after 26 days [IQR 6–154]. Programme retention was lower and mortality higher in patients diagnosed with CM than in other patients (log-rank test $P < 0.0001$; Figures 2a,b). For patients diagnosed with CM, probabilities of retention in care at 6, 12, 24, and 36 months of enrolment were 0.50, 0.37, 0.29, and 0.27, respectively.

Among the 750 patients alive and in care after 3 months of CM diagnosis, 644 (85.9%) received secondary prophylaxis for cryptococcosis and 151 (20.1%) received ART. A total of 103 patients (13.7%) relapsed in median 5.7 months [IQR 4.1–8.8] after cryptococcosis diagnosis. This represented a relapse rate of 5.7 per 100 person-years (95% CI 4.7–6.9). Patients who experienced relapse had median CD4 cell counts of 6 cells/$\mu$l at ART initiation [IQR 2–14; *n* = 53] and 14.5 cells/$\mu$l [IQR 6–34.5; *n* = 28] at first recorded cryptococcosis diagnosis. Seventy-one (69%) patients were receiving secondary cryptococcosis prophylaxis at the time of relapse, and 36 (35%) had started ART in median 3.8 months before the time of relapse [IQR 1.7–7.4]. Forty-three (41.7%) patients died in median 3.2 months [IQR 0.6–7.1] after relapse diagnosis, and 25 (24.3%) were lost to follow-up after 3.0 months [IQR 1.0–4.9].

## Discussion

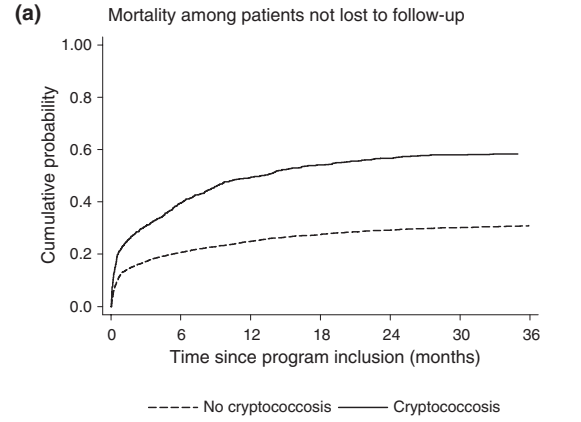
In this observational open cohort followed between 1999 and 2008 in Cambodia, 9% of HIV-infected patients were diagnosed with CM at programme inclusion; incidence was 20.3 per 1000 person-years and decreased over time from 36.8 per 1000 person-years in the pre-ART period to 11.8 per 1000 person-years in 2006–2008, and relapse rate was 5.7 per 100 person-years.

In our setting, CM remained a common opportunistic infection among HIV-infected patients at programme inclusion. Even if the proportion of patients diagnosed with CM at programme entry remained stable over time, the

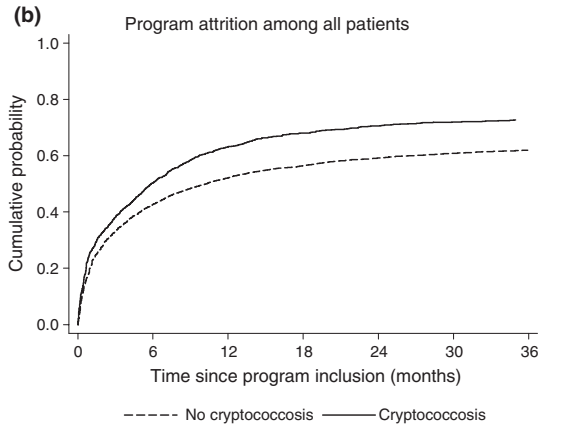
**Table 3** Associations between characteristics at programme inclusion and cryptococcal meningitis diagnosed at programme entry, Phnom Penh, Cambodia, 1999–2008

| | Adjusted OR | 95% CI | *P*-value |
|---|---|---|---|
| Sex | | | |
| Women | Ref | – | |
| Men | 2.24 | 1.67–3.00 | <0.0001 |
| CD4 cell count, cells/μl | | | |
| <20 | Ref | – | <0.0001 |
| 20–49 | 0.54 | 0.38–0.75 | |
| 50–99 | 0.28 | 0.18–0.43 | |
| ≥100 | 0.07 | 0.03–0.12 | |
| Calendar year | | | |
| 1999–2000 | Ref | – | 0.54 |
| 2001–2002 | 1.02 | 0.51–2.09 | |
| 2003–2005 | 1.10 | 0.55–2.20 | |
| 2006–2008 | 0.87 | 0.42–1.78 | |
| Age, years | | | |
| 15–24 | Ref | – | 0.10 |
| 25–39 | 1.80 | 0.92–3.50 | |
| ≥40 | 1.48 | 0.72–3.00 | |
| BMI, kg/m$^2$ | | | |
| ≥18.5 | Ref | – | 0.17 |
| 16–18.4 | 0.93 | 0.69–1.24 | |
| <16 | 1.29 | 0.92–1.81 | |

95% CI, 95% confidence interval; BMI, body mass index; OR, odds ratio from multiple logistic regression adjusted for age, BMI, CD4 cell count, year of inclusion and sex; *P*-value from likelihood ratio test for association.




| Number at risk | | | | | | |
|---|---|---|---|---|---|---|
| No CM | 4021 | 3628 | 3242 | 2859 | 2607 | 2401 |
| CM | 540 | 441 | 384 | 342 | 314 | 297 |




| Number at risk | | | | | | |
|---|---|---|---|---|---|---|
| No CM | 5365 | 4301 | 3662 | 3129 | 2789 | 2531 |
| CM | 685 | 497 | 416 | 363 | 328 | 305 |

**Figure 2** Cumulative mortality and attrition after programme inclusion in patients diagnosed or not with cryptococcal meningitis, Phnom Penh, Cambodia, 1999–2008. (a) Mortality among patients not lost to follow-up. (b)Programme attrition among all patients.

absolute number of cases diagnosed doubled between 1999–2000 and 2003–2005. Incidence was highest in the earlier calendar period and decreased over time to 11.8 per 1000 person-years in 2006–2008. This decrease might partly be explained by the observed gradual increase in patient CD4 count levels at programme entry, which in turn would lead to reduce the length of time when patients are at increased risk of developing cryptococcosis (<50 cells/μl). High proportions of patients diagnosed with CM at presentation have also been reported in previous studies conducted in Cambodia (11.8% to 18%) (Pichith *et al.* 2001; Senya *et al.* 2003; Chhin *et al.* 2004; Kong *et al.* 2007; Micol *et al.* 2007); Thailand and Vietnam (18% to 36% of patients with AIDS) (Chariyalertsak *et al.* 2001; Amornkul *et al.* 2003; Louie *et al.* 2004; Jongwutiwes *et al.* 2007; Manosuthi *et al.* 2007). The observed high frequency of diagnosis at programme inclusion is the result of the late presentation of HIV patients for treatment. Median CD4 cell count at programme inclusion was below 50 cells/μl and, as in other countries (Darras-Joly *et al.* 1996; Sungkanuparph *et al.* 2005; Antinori *et al.* 2009), most patients with CM arrived with severe neurological symptoms (e.g. coma, confusion) and were diagnosed with both HIV and cryptococcosis at hospitalisation. As previously reported, we found that men were twice as likely as women to be diagnosed with CM at programme inclusion (Sorvillo *et al.* 1997; Amornkul *et al.* 2003; Dromer *et al.* 2004; Micol *et al.* 2007), which might relate to differences in either level of exposure or susceptibility to the pathogen.

Cryptococcosis-related deaths represented 25% of all recorded deaths in our cohort, and case fatality rate among patients diagnosed with CM was 39%, confirming that CM is a leading cause of early mortality among patients with HIV/AIDS in Phnom Penh. Besides promoting earlier HIV testing, diagnosis, and treatment for HIV-infected patients to prevent late presentation of CM disease, patient education on signs and symptoms of severe illness should routinely be provided. Systematic testing of serum cryptococcal polysaccharide has been shown to be clinically valuable for detection of subclinical cryptococcosis in patients with CD4 counts <100 cells/$\mu$l (Micol *et al.* 2007), but this test is technically complicated to perform and costly for routine use in resource-limited countries. It is therefore also important that adequate diagnostic tools for CM are made available in resource-limited settings.

Other contributing factors to high mortality in our setting are likely to be related to the difficulties in managing severe cases at hospital level, including implementation of intravenous amphotericin B treatment in settings where renal function cannot easily be monitored. Moreover, in our cohort, 66% of the patients who were diagnosed with CM at programme inclusion and survived 2 months after diagnosis had not been started on ART. Starting ART as soon as the condition of the patient allows should be encouraged to increase patient survival.

Primary chemoprophylaxis with fluconazole is recommended for patients with CD4 cell counts <100 cells/$\mu$l (Singh *et al.* 1996; Havlir *et al.* 1998; Micol *et al.* 2007). In our cohort, 78% of eligible patients actually received it and only 6% of these developed CM. This finding stresses the importance of a prompt start of primary prophylaxis against cryptococcosis.

The relapse rate observed in our setting was similar to that reported in a multicentre cohort of patients in France (4.6 per 100 person-years) (Lortholary *et al.* 2006), but episodes were diagnosed earlier during follow-up (median of 6 compared with 12 months in the French cohort). Earlier relapse episodes could be explained by late start of ART, unavailability of flucytosine for cryptococcosis treatment, and/or inadequate adherence to antifungal therapy or secondary prophylaxis.

In this analysis of a large cohort of HIV-infected patients, we analysed routinely collected individual patient data. We lacked detailed information on case severity (e.g. presence of coma or impossibility to eat), results of cerebrospinal fluid examinations (detection and quantification of *C. neoformans* antigen), or information related to prescribed antifungal therapy. However, the availability of good-quality data collected over an 8-year period and the large sample size achieved allowed describing trends in both CM diagnosis at programme entry and incidence over time and obtaining precise estimations in the risk factor analysis.

## Conclusions

Our findings indicate that efforts still need to be made to increase early diagnosis and treatment of HIV infection and disease, provide primary and secondary prophylaxis against cryptococcosis, and early initiate ART for patients with CM. In parallel, early diagnosis of CM and improvement in acute management of the disease are needed. New strategies are therefore called for to reinforce and maintain treatment adherence and prophylaxis to decrease cryptococcal-related case fatality and relapse in resource-limited countries.

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