# Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults

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**Objective:** Despite the recognition of *Cryptococcus neoformans* as a major cause of meningitis in HIV-infected adults in sub-Saharan Africa, little is known about the relative importance of this potentially preventable infection as a cause of mortality and suffering in HIV-infected adults in this region.

**Design:** A cohort study of 1372 HIV-1-infected adults, enrolled and followed up between October 1995 and January 1999 at two community clinics in Entebbe, Uganda.

**Methods:** Systematic and standardized assessment of illness episodes to describe cryptococcal disease and death rates.

**Results:** Cryptococcal disease was diagnosed in 77 individuals (rate 40.4/1000 person-years) and was associated with 17% of all deaths (77 out of 444) in the cohort. Risk of infection was strongly associated with CD4 T cell counts $< 200 \times 10^6$ cells/l(75 patients) and World Health Organization (WHO) clinical stage 3 and 4 (68 patients). Meningism was present infrequently on presentation (18%). Clinical findings had limited discriminatory diagnostic value. Serum cryptococcal antigen testing was the most sensitive and robust diagnostic test. Cryptococcal antigenaemia preceded symptoms by a median of 22 days (> 100 days in 11% of patients). Survival following diagnosis was poor (median survival 26 days; range 0–138).

**Conclusions:** Cryptococcal infection is an important contributor to mortality and suffering in HIV-infected Ugandans. Improvements in access to effective therapy of established disease are necessary. In addition, prevention strategies, in particular chemoprophylaxis, should be evaluated while awaiting the outcome of initiatives to make antiretroviral therapy more widely available. 

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**Keywords: *Cryptococcus*, HIV, Africa, epidemiology, clinical presentation, cryptococcal antigen**

## Introduction

Infection with the saprophytic yeast *Cryptococcus neoformans* is a well-recognized complication of immunosuppression. During the 1990s, as a consequence of underlying HIV infection, cryptococcal meningitis has become the leading reported cause of adult meningitis in sub-Saharan Africa [1–7]. In addition, it is a leading cause of bloodstream infection in HIV-infected adults in most [8,9] but not all parts [10] of the developing world. Mortality from cryptococcal disease is inevitable in the absence of appropriate therapy.

Despite the frequent reports and seriousness of *C. neoformans* as a pathogen in regions of high HIV prevalence, there is no reporting of community-based incidence data. Consequently, the relative importance of this infection as a cause of morbidity and mortality is

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uncertain, and the role of prevention and treatment cannot be defined or prioritized. In the absence of antiretroviral therapy, care of HIV-infected adults in the developing world is focused on infection prophylaxis and treatment of concomitant infections. At present, specific recommendations exist for the use of isoniazid and co-trimoxazole. Primary chemoprophylaxis may be a suitable approach for cryptococcal disease prevention [11,12], particularly with the high cost of treatment and subsequent secondary prophylaxis. In addition, further evaluation of a previously reported protein conjugate cryptococcal polysaccharide vaccine may be warranted [13,14]. The potential benefit of these interventions for management may only be estimated with appropriate natural history data.

A cohort of over 1000 HIV-1-infected Ugandan adults was established in October 1995, initially to investigate the value of 23-valent pneumococcal vaccine for the prevention of invasive pneumococcal disease [15]. Participants' health was comprehensively surveyed throughout this study and we describe the natural history of cryptococcal disease in this population and discuss the implications for disease prophylaxis.

## Methods

### Study setting

The pneumococcal trial methodology has been described elsewhere [15]. In brief, the study was conducted at two community-based HIV care clinics (AIDS Support Organization of Uganda, TASO; and the Ministry of Health clinic, Uganda Virus Research Institute) in Entebbe, Uganda. HIV-1-infected adults (age 15 years or older) in World Health Organization (WHO) [16] clinical stages 1, 2 or 3 were invited to take part in a double-blind, randomized, placebo-controlled trial of 23-valent pneumococcal polysaccharide vaccine.

Enrolment followed signed informed consent sought in the local languages. Following a standardized clinical interview for clinical staging, a blood sample was drawn. Baseline CD4 T cell counts and haematological indices were measured. Follow-up clinical and haematological assessments were performed 1 month after enrolment and then 6 monthly. On each occasion, serum and plasma samples were archived. Participants had open access to the study clinics and were encouraged to attend whenever ill. Each episode of illness was investigated and managed according to set protocols recorded in the study procedure manual, and in accordance with standard clinical practice.

In the event of a patient failing to attend a routine clinic appointment, a study field-worker would visit the participant at home and encourage clinic attendance or arrange a visit from a trial physician. If he/she refused further participation in the cohort, had left the study area or could not be traced by virtue of an inaccurate address, he/she was deemed a defaulter. If the patient was dead, a verbal autopsy was performed. This included systematic questioning of a relative or carer about date of death and nature and duration of symptoms at the time of death.

If the terminal illness had not been managed by the study clinicians, or investigations had been incomplete, retrospective testing of a preterminal archived serum sample was undertaken to look for the presence of cryptococcal antigen (CRAG).

Therapy for cryptococcal disease was not routinely available in either community clinic. A charitable donation of itraconazole (50 mg capsules) was made during the course of the pneumococcal vaccine trial. A small number of individuals used this agent for therapy and secondary prophylaxis.

### Definitions

Cryptococcal infection was defined by the isolation of *C. neoformans* or a positive CRAG test at a titre of 1 in 8 or greater from blood or cerebrospinal fluid (CSF). A 'definite' diagnosis of cryptococcal disease was made if an individual was febrile with confirmed infection, in the absence of any other demonstrable explanatory pathogenic process. A 'probable' diagnosis of cryptococcal disease was made when a preterminal serum sample was positive for CRAG in the absence of confirmatory clinical information. Serum samples were screened if available within 3 months of the date of death.

### Laboratory methods

The HIV status of all participants was confirmed following enrolment using two standardized enzyme immunoassay [17] (Recombigen HIV-1/2, Cambridge Biotech Corp., Cambridge, Massachusetts, USA, and Welcozyme HIV-1/2, Welcome Diagnostics, Hertford, UK). CD4 T cell counts were performed using a FACScount system (Becton Dickinson, San Jose, California, USA). This system was unable to discriminate between technical failure and a true zero CD4 T cell count. Standard microbiological techniques were used for processing and investigating clinical specimens. Blood cultures used two bottles: one a brain heart infusion broth, the other a nutrient broth. CSF was cultured on sheep blood agar and chocolate blood agar and cultured at 35°C in 5% carbon dioxide. The laboratories participated in the UK external quality assessment scheme NEQAS.

The identity of all *C. neoformans* isolates were confirmed by a positive urease test, ability to grow at

37°C, brown colony formation on Bird Seed agar and the presence of CRAG. Serotyping was performed using the Cryptocheck agglutination test (Tatron Laboratories, Tokyo, Japan).

Cryptococcal antigen testing was performed on CSF or serum using a *Cryptococcus* latex test (Latex-Crypto test, Immuno-Mycologics, Norman, Oklahoma USA) in accordance with the manufacturer's guidelines.

### Statistical methods

Study data were collected by manual recording of information in a standardized format. Data entry was performed in duplicate in databases created in Foxpro(Microsoft, Redmond, Washington State, USA). Analyses were carried out using STATA statistical software version 5.0 (Stata Corp., College station, Texas, USA).

An individual's follow-up commenced on the day of enrolment and was censored at date of death, default or 1 January 1999 (study end date), whichever was the earliest. To allow for progressive changes in the clinical and CD4 T cell staging, person-years of observation (pyo) were calculated in 6-monthly blocks; the period under observation being ascribed to a particular CD4 T cell group or clinical stage on the basis of the measurement at the beginning of this period. The CD4 T cell count from the preceding steady-state visit was used when describing the association of clinical events and the status of HIV infection. CD4 T cell counts at the time of illness were not used as they may have been lowered by the intercurrent event [18].

A Cox proportional hazard approach [19] was used to investigate the strength of association, with multiple adjustments for potential confounders identified by a preliminary univariate analysis. All tests of statistical significance were two sided.

Duration of cryptococcal antigenaemia was assessed by testing sequential archived serum samples. Antigen seroconversion was taken as the date of the first positive CRAG test.

To ensure cryptococcal antigenaemia was a specific marker of cryptococcal disease and not a consequence of advanced HIV-associated immunosuppression, enrolment serum samples from asymptomatic individuals with advanced HIV disease (CD4 T cell count $< 50 \times 10^6$ cells/l) were subjected to CRAG testing. To further ensure these individuals represented a suitable cryptococcal disease-free comparison group, they must have survived at least 6 months from enrolment.

### Ethics and trial conduct

The Liverpool School of Tropical Medicine Research Ethics Committee and the AIDS Research Subcommittee of the National Council of Science and Technology approved the pneumococcal vaccine trial. An independent data monitoring and ethics committee (DMEC) was established. In addition a Trial Steering Committee (TSC) supervised the trial progress and conduct, according to Medical Research Council good clinical practice guidelines [20].

## Results

Up to the beginning of January 1999, 1372 HIV-1-infected adults had been followed for a total of 1902 pyo, with a median follow-up of 1.2 years [interquartile range (IQR), 0.6–3.3]. Median age in the cohort was 30 (IQR, 26–36); 70% of participants were female. Nearly one-third of the cohort had died, 444 deaths (32%; rate 233/1000 pyo), and a further 130 participants (9.5%; rate 68/1000 pyo) defaulted. Deaths were strongly associated with low CD4 T cell count and advanced WHO clinical stage (Tables 1–3).

**Table 1.** Rates of death, default and cryptococcal disease by CD4 T cell grouping.

| | CD4 T cell groups ($\times 10^6$ cells/l) | | | |
|---|---|---|---|---|
| | $< 200$ | 200–499 | $\geqslant 500$ | 0[a] |
| Number of individuals at enrolment | 616 | 417 | 287 | 52 |
| Person-years of observation (pyo) | 716 | 633 | 464 | 88 |
| Deaths[b] | 362 | 51 | 10 | 21 |
| Death rate/1000 pyo | 505 | 80 | 21 | 238 |
| Defaults[b] | 54 | 35 | 34 | 7 |
| Default rate/1000 pyo | 75 | 55 | 73 | 80 |
| Cryptococcal disease cases[b] | 74 | 2 | 0 | 1 |
| Cryptococcal disease rate/1000 pyo | 103 | 3 | 0 | 11 |
| Definite/probable | 66/8 | 2/0 | 0/0 | 1/0 |
| Cryptococcal attributable deaths (%) | 20 | 6 | 0 | 5 |

[a]The methodology was unable to discriminate between a technical failure and an undetectable level of CD4 T cells.
[b]In relation to the staging criteria at the routine visit preceding the event.

Cryptococcal disease was diagnosed in 77 participants (69 definite and eight probable), an overall crude incidence rate of 40.4/1000 pyo (36.2/1000 definite cases). Rates of disease were strongly associated with low CD4 T cell count and advancing WHO clinical stage (Tables 1 and 2). The median CD4 T cell count at diagnosis of cryptococcal disease in 76 of 77 patients for whom counts were available was $16 \times 10^6$ cells/l (range, 1–365); in 66 (86%) the CD4 T cell count was $< 100 \times 10^6$ cells/l. The total lymphocyte count was $< 1000 \times 10^6$ cells/l in 18 (23%), and $< 2000 \times 10^6$ cells/l in 54 of the 77 patients. Cryptococcal infection was associated with 17% of all deaths in the cohort. Median survival from date of diagnosis was 26 days (range, 0–138) in the 69 definite cases. Median survival times were greater in 11 individuals who used itraconazole therapy: 38 days compared with 13 in those who received no therapy.

Demographic and clinical parameters were investigated for their association with cryptococcal disease. By univariate analysis, CD4 T cell count, WHO clinical stage, total lymphocyte count, male sex and herpes zoster infection were shown to be associated with cryptococcal disease. In a multivariate regression model, these variables, with the exception of total lymphocyte count, remained independent and strong predictors of cryptococcal disease (Table 3).

Clinical presentation was often non-specific (Table 4). Headache was present in about half of all patients but classical signs of meningitis were present in only 13

**Table 2.** Rates of death, default and cryptococcal disease by World Health Organization (WHO) clinical stage.

| | WHO clinical stage | | | |
|---|---|---|---|---|
| | 4 | 3 | 2 | 1 |
| Number of individuals at enrolment (pyo) | 29 | 748 | 487 | 108 |
| Person years of observation | 39 | 757 | 848 | 258 |
| Deaths[b] | 36 | 287 | 108 | 13 |
| Death rate/1000 pyo | 923 | 379 | 127 | 50 |
| Defaults[b] | 6 | 70 | 43 | 11 |
| Default rate/1000 pyo | 153 | 92 | 50 | 42 |
| Cryptococcal disease cases[b] | 7 | 61 | 9 | 0 |
| Cryptococcal disease rate/1000 pyo | 179 | 81 | 11 | 0 |
| Definite/probable | 7/0 | 54/7 | 8/1 | 0/0 |
| Cryptococcal attributable deaths (%) | 19 | 21 | 8 | 0 |

[a]The methodology was unable to discriminate between a technical failure and an undetectable level of CD4 T-cells.
[b]In relation to the staging criteria at the routine visit preceding the event.

**Table 3.** Risk factors for the development of invasive cryptococcal disease. A Cox proportional hazards model was used to derive hazard ratios using a preliminary model without, and a subsequent model with, adjustment.

| Risk factor | Unadjusted analysis HR (95% CI) | Adjusted analysis[a] HR (95% CI) |
|---|---|---|
| CD4 T cell count $< 200$ against reference $\geq 200 \times 10^6$ cells/l | 3.70 (2.6–5.0) | 3.85 (2.5–5.9) |
| Clinical stage 3 or 4 against reference stage 1 or 2 | 3.67 (2.1–6.4) | 1.96 (1.1–3.6) |
| Total lymphocyte count $< 1000$ against reference $\geq 1000 \times 10^6$ cells/l | 3.23 (1.3–8.3) | 2.27 (0.9–5.9) |
| Male sex against reference female | 1.69 (1.1–2.6) | 1.96 (1.2–3.3) |
| Age $\geq 40$ against reference $< 40$ years | 0.83 (0.4–1.7) | – |
| Tribal group non-Bugandan against reference Bugandan | 0.83 (0.5–1.4) | – |
| Residence semi-urban[b] against reference rural | 0.89 (0.5–1.5) | – |
| Past history of herpes zoster | 1.78 (1.1–2.9) | 1.85 (1.1–3.2) |
| Past history of tuberculosis | 0.97 (0.5–1.8) | – |
| Past history of pneumonia | 0.96 (0.7–1.3) | |
| Alcohol consumption[c] | 1.28 (0.8–2.0) | – |
| Smoker[c] | 1.05 (0.6–2.0) | – |
| Fuel use electricity/charcoal against reference firewood/paraffin | 1.40 (0.9–2.2) | – |
| Domestic crowding against reference $< 1$ persons per room | | – |
| 1–3 | 1.05 (0.7–1.5) | |
| $> 3$ | 1.26 (0.7–2.2) | |

HR, hazard ratio; CI, confidence interval.
[a]Model incorporating factors associated with risk in the univariate analysis.
[b]Entebbe town and the settlements adjacent to the main Kampala road are categorised as semi-urban.
[c]Current users of tobacco and alcohol at the time of enrolment.

**Table 4.** Presenting clinical features of cryptococcal disease in 69 definite cases.[a]

| | No. (%) | Sensitivity (%) | Specificity (%) | Association[b] |
|---|---|---|---|---|
| Sex (female) | 42 (61) | | | |
| Age [median years (IQR)] | 27 [25–49] | | | |
| Seasonality (cases during wet season[c]) | 26 (37) | | | |
| Symptoms | | | | |
| Headache | 32 | 46 | 76 | $< 0.01$ |
| Confusion | 4 | 6 | 99 | NS |
| Feverish | 58 | 84 | 19 | NS |
| Cough | 23 | 33 | 55 | NS |
| Signs | | | | |
| Meningism | 13 | 18 | 99 | $< 0.01$ |
| Focal neurological deficit[d] | 6 | 9 | 98 | $< 0.01$ |
| Depressed conscious level[e] | 5 | 7 | 97 | NS |
| Respiratory abnormalities[f] | 12 | 16 | 76 | NS |
| Fits | 1 | 1 | 99 | NS |
| Temperature (axillary $> 37.5$°C) | 56 | 81 | 19 | NS |

IQR, interquartile range.
[a]Sensitivity and specificity of clinical symptoms and signs and their association with cryptococcal disease are derived from comparison to 1075 non-cryptococcal illness events.
[b]The association is expressed as a *P* value derived from a chi-square test.
[c]Wet seasons March to May, November and December.
[d]Cortical blindness was reported in a further three patients and was only found in association with cryptococcal infection.
[e]Glasgow Coma Score $< 13$.
[f]Chest radiographs were performed in 9 of the 12 patients with respiratory signs. Of these, four were abnormal (hilar or mediasteinal lymphadenopathy in two, segmental/lobar consolidation in one, interstitial shadowing in one). Radiographs were performed for 23 individuals without respiratory signs. Of these, 11 were abnormal (consolidation in two, lymphadenopathy in two, atelectasis in two, interstitial shadowing in three, pleural effusion in one, cardiomegaly in one).

(18%). *C. neoformans* was the principal cause of meningitis in the cohort. Meningitis, confirmed by CSF examination, was diagnosed in 21 individuals over the period of the study; the meningitis was cryptococcal in aetiology in 17 (81%). The four other patients had pneumococcal (one), tuberculous (one) and undiagnosed lymphocytic meningitis (two). When symptom frequency in the 69 patients with definite cryptococcal disease was compared with the 1075 investigated non-cryptococcal illness episodes, the presence of neurological symptoms and signs were specific markers of cryptococcal disease but were insensitive (Table 4). The positive and negative predictive values in this cohort in respect for cryptococcal disease was 76% and 95%, respectively, for meningism and 47% and 95%, respectively, for focal neurological deficit.

Confirmation of disease was based on blood culture in 19 patients, CSF examination in nine, blood and CSF examination in eight and CRAG testing in 33. The eight patients with probable cryptococcal disease were identified from screening stored preterminal serum samples available for 219 individuals out of 375 deaths that were not definitely known to be a result of cryptococcal disease. Serum CRAG testing was positive in all culture-proven patients. Blood cultures were performed on 51 of the 69 patients and were positive in 27 (53% sensitivity). White cell counts in CSF examinations were $< 4 \times 10^6$ cells/l in 14 specimens and 10, 47 and $415 \times 10^6$ cells/l in the remaining three. All 36 cryptococcal isolates were confirmed as *C. neoformans* var *grubii* (previously var *neoformans* serotype A).

Out of the total of 77 patients, 42 had stored serial serum samples and a seroconversion sample. Median minimum duration of CRAG positivity was 41 days (range, 1–297) in 34 individuals who had not received systemic antifungal therapy and 52 days (range, 22–241) in eight individuals who received itraconazole therapy. Median CRAG titre at death in these 42 individuals was 1 in 1024 (range 1 in 16 to 1 in 65 536). CRAG titre showed a significant inverse relationship with sampling time before death (Fig. 1). CRAG positivity preceded clinical symptoms by a median of 22 days (range, 5–234 for the 37 definite cases), four individuals (11%) being positive for more than 100 days. Seven of the individuals who developed cryptococcal disease were retrospectively identified as CRAG positive at the time of study enrolment. No CRAG antigenaemia was found in 58 individuals with advanced HIV disease without features of cryptococcal disease.

## Discussion

Cryptococcal disease is a common and important HIV-related problem across most of sub-Saharan Africa

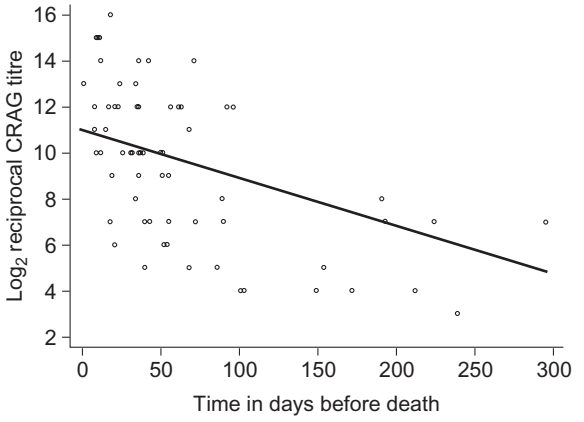


**Fig. 1.** The relationship of cryptococcal antigen titre (CRAG) with sampling time before death in 77 samples from 42 individuals. There is a strong linear association between $\log_2$ CRAG titre and time before death (regression coefficient −0.03/day; $P < 0.001$, or a doubling of the titre every 33 days).

[2,5–7,21] and is usually diagnosed in patients with meningitis. Our findings show that cryptococcal disease is one of the leading contributors to death in HIV-infected adults in Uganda and commonly presents without the specific features of meningitis. The scale of the problem would suggest further evaluation of preventive strategies to be appropriate.

Previous reports from Africa have focused on cryptococcal disease presenting as meningitis and emphasized the frequency of neurological abnormalities [3]. The findings from this community-based cohort suggest a broader and perhaps less-specific presentation of cryptococcal disease. Only half of the patients complained of headache and in only one-fifth was there clear physical signs of meningism. Whether individuals with non-neurological presentations also had central nervous system involvement is uncertain as systematic assessment of CSF was not undertaken when blood culture or CRAG testing was positive. Respiratory complaints were frequent and pulmonary cryptococcosis was almost certainly present in some of the patients as a component of a disseminated fungaemic infection. Chest radiographic abnormalities were present in 47% of films but these were performed on only 32 patients. Furthermore an abnormal chest radiograph bore no relationship to the presence of respiratory symptoms and signs and, as with the principal symptoms and signs, it would appear to have poor diagnostic discriminatory power. Although early presentation may explain in part the low frequency of clinical signs, a significant burden of cryptococcal disease may be going unrecognized if only neurological illness is investigated or if specific mycological investigations are not performed on febrile patients with advanced (stage 3/4) HIV/AIDS disease.

Cryptococcal antigen testing proved to be the most sensitive diagnostic test. We have previously shown it to be a specific discriminator of cryptococcal disease from other febrile illnesses [22]. By testing serum samples from individuals with CD4 T cell counts $< 50 \times 10^6$ cells/l and no apparent cryptococcal disease, we have also gone some way to confirming its specificity as a marker of cryptococcal infection and not a marker of advanced HIV disease.

CRAG positivity preceded the onset of clinically recognizable symptoms by a median of 22 days, although 11% of the patients had preceding cryptococcal antigenaemia for greater than 100 days. The serum CRAG titre was associated with survival time in this group; however, in practical clinical terms, a single titre probably carries little predictive prognostic value and routine titration of serum to record a level is unnecessary. Nevertheless, the overall strong temporal association between CRAG positivity and death supports the reliability of this test, not only as an indicator of systemic cryptococcal infection but also as a predictor of disease and subsequent death. This has been previously suggested by investigators in North America [23], although in their setting of unrestricted access to antifungal therapy, the treatment of asymptomatic antigenaemia obscured the relationship between antigenaemia and outcome. Our findings would support their view that the treatment of asymptomatic antigenaemia is correct. Whether routine screening for cryptococcal antigenaemia would be of value in an African population with highly restricted access to antifungal therapy is unclear: early diagnosis is only likely to be of benefit if it allows the use of simplified and less-expensive oral antifungal treatments. Further evaluation of this strategy is appropriate.

Rates of cryptococcal disease in this cohort were high, double those seen in North American reports (17–20/1000 pyo) [24,25] that predated the introduction of potent antiretroviral therapy. This excess risk persisted, with the exclusion of the seven patients who were asymptomatic but CRAG positive at the time of their enrolment. There is no reason to suspect geographic differences in HIV-associated immunosuppression in sub-Saharan Africa. Therefore, the rates in this cohort are likely to reflect the situation in much of the rest of Africa. However, cryptococcal disease is absent or infrequently reported in some African settings [10], suggesting either failure of diagnosis or an important exposure/environmental component of risk.

Advanced HIV-related immunosuppression proved to be the strongest independent risk factor for disease. Male sex was also shown to be a risk, similar to reports from the United States [24]. This finding may represent incomplete adjustment for stage of disease in the multivariate analysis, as in general men enrolled in the

cohort with more advanced HIV disease. However, male sex has been associated with increased risk of *C. neoformans* var *gatii* infection in non-HIV-related populations in Australasia [26], supporting a possible sex-linked (behavioural, biological or environmental) risk factor. The association with past herpes zoster infection was also unexpected. However, work in a separate cohort of HIV-infected adults in Uganda showed the cumulative incidence of herpes zoster infection had a linear relationship with duration of HIV infection and was not associated with more rapid progression or death [27]. Therefore, herpes zoster is a marker of duration of HIV infection and immunosupression; once again, the association may represent incomplete adjustment for stage of disease in the multivariate model.

*C. neoformans* var *grubii* was responsible for all events and this is a consistent feature of HIV-associated cryptococcal disease [28]. The reasons for the preponderance of this serotype are unclear. Some authors have suggested the possibility of a single global clone, with specific pathogenicity for individuals with HIV-related immunosuppression [26,29]. It is believed to be widespread in the environment, unlike other serotypes that have a limited environmental habitat, and its frequency of isolation may reflect this. A better understanding of the environmental residence of cryptococci and its relationship to disease is needed to help to plan appropriate avoidance and prevention strategies.

Survival following the development of clinical disease was poor. Effective management of established cryptococcal disease was not available to us and this continues to be the situation for most HIV-infected individuals in sub-Saharan Africa. Amongst the 69 definite cases, cryptococcal infection was believed to be the primary pathology leading to death. We did not identify alternative explanations antemortem and the lack of postmortem examinations and the advanced state of HIV-associated immunosupression does not allow the cause of death to be definitively ascribed. Irrespective of the contribution to death, cryptococcal meningitis is a distressing and painful illness that is compounded by rudimentary palliative care and difficulties in prescribing opiate analgesia in much of Africa. Reappraisal and further evaluation of prevention strategies are appropriate, given the frequency of disease, difficulties of providing therapy and poor outcome.

Intermittent fluconazole therapy was effective at preventing cryptococcal infections in studies in the United States [12]. However, it shows little impact on survival [11] in a setting where cryptococcal disease is a small contributor to overall HIV mortality. The principal concerns over Fluconazole use in an African population are cost and generation of widespread azole resistance in other fungi. Appropriate targeting of at-risk groups would help to limit both. Use of a CD4 T cell count $< 200 \times 10^6$ cells/l would be an appropriate marker of risk, but is unworkable in practice because of the lack of both funds and appropriate laboratory facilities. Clinical stage 3 and 4, while a less-sensitive indicator of risk than CD4 T cell counts, is simple and inexpensive for use in resource-limited health-care settings. Total lymphocyte counts were of limited value used in isolation and added little discriminatory power when combined with clinical stage (data not shown). A vaccine-based prevention strategy would, in principle, be the most relevant approach. In the absence of access to potent antiretroviral therapy in the foreseeable future for the majority of African populations, possible vaccine strategies and a previously described vaccine construct should be evaluated further [13,14].

Coinfection with *C. neoformans* is a frequent and serious problem in HIV-infected Africans. Under-recognition of the infection will occur if diagnostic tests are confined to patients with meningitis. Therapy of established disease is likely to remain difficult even with greater access to oral azole antifungal drugs. Strategies to use these agents as prophylaxis need assessment in the developing world; in the longer term, vaccine development should be deemed a priority in the control and prevention of cryptococcal disease.

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