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Determinants of Mortality in a Combined Cohort of 501 Patients with HIV-associated Cryptococcal Meningitis: Implications for Improving Outcomes

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Abstract

Background. Cryptococcal meningitis (CM) is a leading cause of death in HIV-infected patients. Identifying factors associated with mortality informs strategies to improve outcomes.

Methods. 501 patients with HIV-associated CM were followed prospectively for 10 weeks during trials in Thailand, Uganda, Malawi and South Africa. South African patients (266) were followed for 1 year. Similar inclusion/exclusion criteria were applied at all sites. Logistic regression identified baseline variables independently associated with mortality

Results. Mortality was 17% at 2-weeks and 34% at 10-weeks. Altered mental status (OR 3.1, 95%CI 1.7-5.9), high cerebrospinal fluid fungal burden (OR 1.4 per logCFU/ml increase, 95%CI 1.0-1.8), older age (>50 years, OR 3.9, 95%CI 1.4-11.1), high peripheral white cell count (>10x10^9/L, OR 8.7, 95%CI 2.5-30.2), fluconazole-based induction treatment and slow clearance of CSF infection were independently associated with 2-week mortality. Low body weight, anemia (hemoglobin <7.5g/dL) and low CSF opening pressure were independently associated with mortality at 10 weeks in addition to altered mental status, high fungal burden, high peripheral white cell count and older age.
In those followed for 1-year, overall mortality was 41%. IRIS occurred in 13% of patients and was associated with 2-week CSF fungal burden (p=0.007) but not time to initiation of ART.

Conclusions. CSF fungal burden, altered mental status, and rate of clearance of infection predict acute mortality in HIV-associated CM. The results suggest earlier diagnosis, more rapidly fungicidal amphotericin-based regimens, and prompt immune reconstitution with ART are priorities for improving outcomes.

Introduction

HIV-associated cryptococcal meningitis (CM) is the commonest cause of adult meningitis in much of Africa[1-4]. Despite antifungal treatment, acute mortality in the developing world remains between 24-43%[5-7], and CM accounts for between 10 -20% of all HIV-related deaths in sub-Saharan Africa[8]. The median time to death following hospital admission with CM is 10-13 days[6]. To develop evidence-based interventions, it is essential to determine the key predictors of mortality. Using data from a cohort of 501 patients with CM from Thailand, South Africa, Malawi and Uganda, we describe the presenting clinical features and outcomes of patients with HIV-associated CM, and report the results of a predictive model used to identify the clinical and microbiological factors at baseline independently associated with mortality. We provide an analysis of factors associated with altered mental status, cerebrospinal fluid (CSF) fungal burden, CSF opening pressure at presentation, rate of clearance of infection and immune reconstitution inflammatory syndrome (IRIS).

Methods

The cohort comprised patients from nine trials conducted from 2002-2010 at five sites (Table 1a) in Thailand, South Africa, Malawi, and Uganda. The trials have been reported elsewhere, and represent all trials of HIV-associated CM published (at the time of analysis) using early fungicidal activity (EFA) as the primary outcome[5, 9-16]. A previous analysis of 262 patients explored the correlation between rate of clearance of infection and survival[17].Combining the data from the constituent trials into a combined cohort was done to obtain the power needed to reliably determine the predictors of mortality in patients with HIV-associated CM . All trials were sponsored by St. George's

University of London, and approved by the St. George's Research Ethics Committee and local ethics committees.

Participants and procedures

Following informed consent, HIV-positive adults with CM, diagnosed by CSF India-ink or cryptococal antigen testing (Meridian Cryptococcal Latex Agglutination System, Meridian Bioscience) were enrolled consecutively. All the trials had similar inclusion/exclusion criteria (Table 1a). Patients already receiving antiretroviral therapy (ART) and those with previous episodes of CM were excluded from this analysis. Induction therapy differed by study as described previously[5, 9-16]. Following 2-weeks' induction therapy, patients received 8 weeks of oral fluconazole consolidation (400-800mg/d) then maintenance therapy (fluconazole 200mg/d). Routine drug level monitoring was not performed. ART was started 2-6 weeks after starting antifungal therapy in accordance with local protocols (ART was not routinely available during the earliest trial[9]). Cohorts in Thailand, Uganda and Malawi were followed for 10 weeks, whilst those in South Africa for 12 months.

Evaluations and outcomes

On study enrolment, detailed history and clinical examination findings were recorded. Lumbar punctures (LPs) with opening pressure (OP) measurements and quantitative CSF cultures were performed on days 1, 3, 7 and 14. Patients with a markedly elevated OP (>30 cm) or symptoms of raised intracranial pressure had more frequent LPs[18]. CSF cell count, protein and glucose levels were determined. CSF interferon-γ (IFNγ), tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) concentrations were measured in patients from the Thai and South African sites using the Luminex multianalyte platform (Luminex) and Bio-Rad cytokine kits (Bio-Rad)[19]. Cryptococcal clearance was

calculated as the decrease in log colony forming units (CFU)/ml CSF/day derived from the slope of the linear regression of log CFU/ml against time for each patient[9]. Baseline blood tests included hematology, renal and liver function, CD4 cell counts, and, where available, HIV plasma viral load. The primary outcome in all studies was rate of decrease in CSF cryptococcal CFU, called early fungicidal activity (EFA). Secondary outcomes included mortality at 2 and 10 weeks. Cryptococcal meningitis IRIS (CM-IRIS) was diagnosed according to uniform criteria[20]. In patients who died presumed cause of death was ascertained by two study clinicians.

Statistical analyses

Data were analyzed using Stata (v.11, StataCorp). Variables were compared using t-tests, Kruskal-Wallis, χ^2, χ^2 for trend, or Fisher's exact tests. Relationships between continuous variables were examined using Pearson's correlation coefficient or Spearman's log-rank test. Multivariable logistic regression models were constructed using stepwise regression with the primary objective of determining the clinical and microbiological factors at baseline associated independently with all-cause mortality (as measured at 2 and 10 weeks). A predictive modeling strategy was used in which variables were selected for model inclusion based upon i) *a priori* knowledge from previous studies (CD4 cell count), and ii) association with outcome in univariable analysis. Variables associated with mortality in univariable analysis (p-value of <0.1) were included in the first fit of the multivariable model and retained, based on likelihood ratio testing, if they significantly improved model fit, to obtain the most parsimonious model identifying predictors of mortality. Clustering by individual study was accounted for using a hierarchical mixed effects model including a random-effects term for "study". An *a priori* decision was made to adjust the multivariable model for amphotericin (AmB) versus fluconazole-based

treatment as a potential confounder in the relationship between baseline factors and outcomes. Exploring the effect of treatment on outcome, after adjusting for other predictors, was a secondary objective. Patients with missing outcome data were censored from the main analysis, with sensitivity analyses performed assuming all patients lost were either a) dead or b) alive. Further models were constructed to examine the baseline factors associated with altered mental status, baseline fungal burdens, and CSF opening pressure; to examine the impact of ART timing and IRIS on longer-term outcomes; and describe the relationship between EFA and outcome. EFA was modeled both as a single linear term for each patient as previously described[17] and as a time-updated variable in a Cox regression. In the group with one-year follow-up data, Kaplan-Meier survival curves were compared using the Mantel-Haenszel log-rank test.

Results

Baseline characteristics and outcome

After screening 896 patients, 523 met eligibility criteria for inclusion in the clinical trials, consented to participation, and were included. Of these, we studied the 501 ART-naïve patients with a first episode of CM (table 1). The median age was 34 years, and 52% were male. All had confirmed HIV infection; 76% were known to be HIV-positive at time of presentation, diagnosed a median of 152 (IQR 44-745) days earlier; the remainder tested HIV-positive at study enrollment. Male patients presented with longer median reported duration of symptoms than female patients (14 versus 10 days, p=0.004). The median CD4 count was 23cells/μL. Amphotericin B deoxycholate (AmB 0.7-1mg/kg/day) induction treatment was used in 80% of patients, and 20% received fluconazole-based induction (median 1200mg/day) without AmB. All-cause mortality was 17% at 2-weeks

and 34% at 10-weeks (Table 2). Of patients in care at 2 weeks (n=410), 244 were started on ART a median of 30 days (IQR 26-42) after starting antifungal therapy. Nine patients were lost to follow-up at 2 weeks, and seventeen at 10 weeks.

Independent associations with outcome (table 2)

Four variables independently predicted mortality at 2 weeks in multivariable analysis: altered mental status, high baseline CSF fungal burden (measured by either quantitative cryptococcal culture (QCC) or cryptococcal antigen titre, which were closely correlated – figure s1), older age, and high peripheral white blood cell count. Patients with altered mental status at presentation had a three-fold increase in mortality at 2 weeks; there was an incremental 1.4 odds increase in mortality with each $\log_{10}$ CFU/mL increase in fungal burden; and patients 50 years or older were almost four-times more likely to die than those below 50 years.

Seven baseline parameters were associated independently with 10-week mortality: altered mental status, high baseline fungal burden, older age, low body weight, low CSF opening pressure (OP $\leq$ 25cmCSF), high peripheral blood white cell count and anemia. AmB treatment was associated independently with lower mortality at both 2 and 10 weeks, however it should be noted that fluconazole-treated patients were predominantly from the lowest resource settings (97% Uganda or Malawi).

Altered mental status

At presentation, 25% of patients had altered mental status. In a multivariable regression model including 409 patients with complete data, the strongest predictor of altered mental status was male sex (aOR 2.2, 95%CI 1.3-3.7, p=0.003). Additional variables associated independently with altered mental status were age >50 years (aOR 1.4, 95%CI 1.1-2.0, p=0.02) and very high CSF opening pressure (>30cmCSF, aOR 1.8, 95%CI 1.1-3.0,

p=0.02). Altered mental status was not associated with any other variables examined including baseline fungal burden, CD4 count or CSF white cell count in adjusted analyses.

Baseline CSF fungal burden

CSF QCCs were negatively correlated with CD4 count, CSF white cell count, CSF protein, and CSF pro-inflammatory cytokines (IL-6, IFNγ and TNFα). The strongest correlation was with CSF IFNγ (Pearson's r=-0.4, p<0.001, figure 1). There was no significant correlation between QCC and CSF OP (table 3).

CSF opening pressure

Raised baseline CSF OP (>25cm CSF) was present in 51% of the cohort (230). Raised pressure was associated with papilledema(OR 2.6, 95%CI 1.1-5.8, p=0.02), however, other than the association between very high CSF opening pressures (OP>30cm) and mental status described above, there were no other significant associations between high OP and clinical variables. Raised OP correlated with increasing CSF TNFα concentrations (Spearman's r=0.2, p=0.008), but not with IFNγ or IL-6. Although there was no significant correlation between QCC and baseline CSF OP, high baseline QCC was necessary but insufficient for development of a high day 1 and day 14 OP (figure s2).

Early fungicidal activity

EFA was associated with outcome, as shown previously among a subset of 262 patients[17]. A slope measurement was available in 450 of the 501 patients, and in 129 of the 163 patients who died. Mean(SD) EFA of those who died at 2 weeks was -0.24(0.25) $\log_{10}$CFU/ml/day versus -0.42(0.25) $\log_{10}$CFU/ml/day in survivors (p<0.001). In those who died at 10 weeks EFA was -0.34(0.27) $\log_{10}$CFU/ml/day versus -0.43(0.24) $\log_{10}$CFU/ml/day in survivors (p<0.001). EFA remained independently associated with

mortality after adjusting for altered mental status and fungal burden (mean difference in EFA between survivors and fatal cases at 2-weeks -0.15 log_{10}CFU/ml/day, 95%CI 0.07-0.22, p<0.001). When the serial counts were fitted as a time-dependent variable, the adjusted hazard ratio for death within the first 2 weeks was 1.8 (95%CI 1.2–2.5, p=0.002) for each unit increase in the log_{10}cfu count. The mean EFA was greater for amphotericin-based compared with fluconazole-based induction treatment (difference 0.32 log_{10}CFU/day; 95%CI 0.27–0.36, p<0.001). EFA was associated independently with amphotericin-based treatment, baseline organism count, and CSF-IFNγ level (Figure 1).

Long-term outcomes, ART timing and immune reconstitution inflammatory syndrome

Among the 266 patients enrolled in South Africa, survival analysis was restricted to the 263 patients treated with AmB-based regimens. The median age was 33 years (IQR 29-39), 42%(110) were male, median CD4 count was 28 cells/μl (IQR 12-57), and 15% had altered mental status. Of 179 patients surviving to 10-weeks and in care, median follow-up was 352 days (IQR 209-409). Mortality was 13% at 2-weeks, 30% at 10-weeks and 41% at end of follow-up (figure 2). Of patients surviving to 2 weeks, 85%(171) started ART a median of 31 days (IQR 23-46) after antifungal therapy. IRIS developed in 13%(22) of patients a median of 29 days (IQR 23-45) after starting ART, of whom 18%(4) died. IRIS was associated with day 14 CSF fungal burden (p=0.007) but not with time to ART (p=0.4).

Presumed causes (as ascertained clinically by study physicians) were recorded for 91%(98/108) of deaths (figure 2). Deaths during the initial two weeks were primarily attributed to CM (85%, 29/34). Later deaths were mostly attributed to other HIV-related infections or complications (67%, 43/64). Survival in patients who started ART was not associated with IRIS (p=0.3) or time to ART (p=0.3) (fig 2b).

Discussion

This is the largest study examining factors determining outcome in HIV-associated CM. The results emphasize the high acute mortality in patients with CM, even among patients primarily treated with amphotericin-based therapy in research settings. Such patients are likely to have better outcomes than those managed in routine care; hence these results provide a *minimum* mortality estimate. One third of patients had died by 10 weeks after presentation. However, the median time to death of 13 days suggests that improved early interventions could prevent some of these fatalities. Acute deaths were attributed mainly to CM. High fungal burden and slow clearance of infection on treatment, together with altered mental status, were the most important drivers of acute cryptococcal-related mortality. After two weeks, other HIV-related causes of death predominated, and the risk factors for mortality in addition to high fungal burden, slow clearance of infection, and altered mental status, included older age, low CD4+ cell count, low weight, and anemia, which have been identified previously as predictors of mortality in HIV cohorts in general[21].

High baseline CSF fungal burden was one of the strongest risk factors for mortality. It was associated with a low peripheral CD4 count, reflecting the importance of cell-mediated immunity in controlling cryptococcal infection[22], and with a poor inflammatory response at the site of infection, as evidenced by low CSF white cell counts, and low levels of IFNγ, TNFα and IL6. The *rate of fungal clearance* was independently related to outcome, with slower clearance associated with higher mortality. This strongly supports the use of EFA as a clinically relevant pharmacodynamic endpoint

for phase II clinical studies[17]. Higher levels of pro-inflammatory CSF IFNγ were associated with more rapid rates of fungal clearance during treatment [17, 19], and lower fungal burdens at presentation, demonstrating the importance of IFNγ in the protective immune response, and reinforcing the rationale for augmentation of pro-inflammatory responses with IFNγ immunotherapy as a therapeutic approach[15].

Amphotericin-based treatments were more rapidly fungicidal than fluconazole-based treatments, and the mortality in fluconazole-treated patients was almost double that in amphotericin B-treated patients at 10 weeks. However the majority of fluconazole-treated patients were from the lowest-resourced settings, and it is possible that the association between fluconazole use and mortality is confounded by factors relating to study site; however the association remained statistically robust after adjustment for the other key variables associated with outcome, including abnormal mental status and baseline fungal burden. The clear association between slow rates of fungal clearance and poor outcomes provides a strong argument in favor of rapidly fungicidal initial treatments, and more widespread use of rapidly fungicidal amphotericin B combination therapy is likely to reduce early mortality.

The pathophysiological basis of altered mental status, the other main risk factor for mortality, remains unclear. The independent associations of altered mental status with male sex and older age have not been reported previously. High CSF opening pressure (>30cm) was associated independently with altered mental status, but was not contributory to altered mental status in the majority of cases; half of those with altered

mental status did not have markedly raised pressures. Of note, altered mental status was not associated with CD4 count, CSF white cell count or fungal burden.

High CSF opening pressure was not associated with increased mortality in this cohort, in contrast to earlier reports [23]. This may have been a result of management: all patients routinely had four LPs over the first two weeks of treatment, and raised pressures were managed according to established guidelines[18]. A novel finding of this analysis was that raised CSF opening pressures at baseline, in patients managed according to these guidelines, were associated with *improved* outcomes at 10 weeks. It is possible that pro-inflammatory CSF cytokine responses (TNFα was associated with raised pressure) may be protective in situations where raised OP is appropriately managed, or that large volume CSF drainage is beneficial over-and-above its role in reducing pressure[23]. These findings emphasize the importance of CSF pressure management in patients with CM, and highlight the need for widened access to manometers to manage pressure safely in centers in Africa with the highest burden of disease.

Long-term survival in the cohort of South African patients with access to amphotericin B and ART was good, provided patients survived the acute period. ART was usually started between 3 and 6 weeks after antifungal therapy. Within this time frame, there was no association between earlier ART initiation and the development of subsequent IRIS. Patients who developed IRIS did not have higher overall mortality. The majority of deaths after two weeks were attributed to other HIV-related illnesses that may have been preventable through earlier initiation of ART. In the context of amphotericin induction, ART initiation nearer to 3 rather than 6 weeks after starting antifungal

therapy may prevent some of the later HIV-related mortality, while not substantially increasing the risk of IRIS.

A potential limitation of this analysis, derived from multiple cohorts, is possible residual confounding due to unmeasured study specific effects, relating to temporal or geographic differences between studies. However a key strength of this cohort is the extensive prospectively collected baseline data, allowing adjustment to minimize confounding. There was little evidence of clustering by study within the hierarchical model, and the robustness of the key conclusions was further supported by consistency across univariable and multivariable analyses, and the sensitivity analyses performed. Levels of missing data among outcomes and the key predictor variables were low, reducing the risk of bias.

In summary, these data provide a rationale for several strategies to improve outcomes. Firstly earlier diagnosis of CM should be possible, resulting in lower fungal loads at presentation and a reduction in mortality. Clinicians should have a low threshold for lumbar puncture in HIV-positive patients presenting with headache. Novel point-of-care antigen tests[24, 25] should now facilitate earlier diagnosis. Given the high proportion of patients presenting with CM who have already been diagnosed with HIV (76%), screening for sub-clinical infection with point-of-care antigen tests and pre-emptive antifungal treatment, along with early ART initiation, could prevent a substantial proportion of clinical disease from developing[26-28]. Second, increasing access to the most fungicidal AmB-based regimens is a priority in settings with a high incidence of CM[29-31], in particular Sub-Saharan Africa. Lastly, prompt initiation of ART is

required to address the substantial proportion of deaths in these patients that are HIV but not CM-related.

NOTES

Author contributions

JNJ, TB and TSH wrote the manuscript with editorial input from all authors. JNJ and SJ performed the statistical analysis, with input from TSH, CvdH and DL. JNJ, TB, AL, DN, AJ, JCN, NL, CM, JP, KT, CK, DW, MCH, AEB, DL, NW, CvdH, RW, and GM performed the component clinical trials. The study was conceived and designed by TSH.

Role of the funding source

The funding source had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

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Conflicts of interest

None to declare.

Figure legend

Figure 1. Associations between CSF IFNγ concentrations and baseline fungal burden, rate of clearance of infection (EFA) and 2-week mortality in the 243 Thai and South African patients with CSF cytokine measurements. CSF IFNγ concentration was strongly associated with rate of clearance of infection, with a 0.10 $\log_{10}$CFU/ml/day (95%CI 0.06-0.15) increase in EFA for every $\log_{10}$picogram increment in IFNγ concentration.

Figure 2. Kaplan Meier survival curves showing a) survival in the 263 South African amphotericin B treated patients followed for 1-year, b) survival from enrollment in the subset of patients who started ART, stratified by ART timing (before and after the median of 31 days, p=0.15. Only showing patients surviving to ART initiation), and c) cause of death data (as determined by study clinicians) in the cohort of 263 South African amphotericin B treated patients followed up for 1-year, split by time from diagnosis. "Other" included tuberculosis (11), bacterial sepsis (8), bacterial pneumonia (7), non-specified infections (9), Kaposi's sarcoma (2) lymphoma (1), non-CM IRIS (3), ART toxicity (2), diarrhea/wasting syndrome (4) and decompensation of chronic liver disease (1).

Figures

Figure 1.

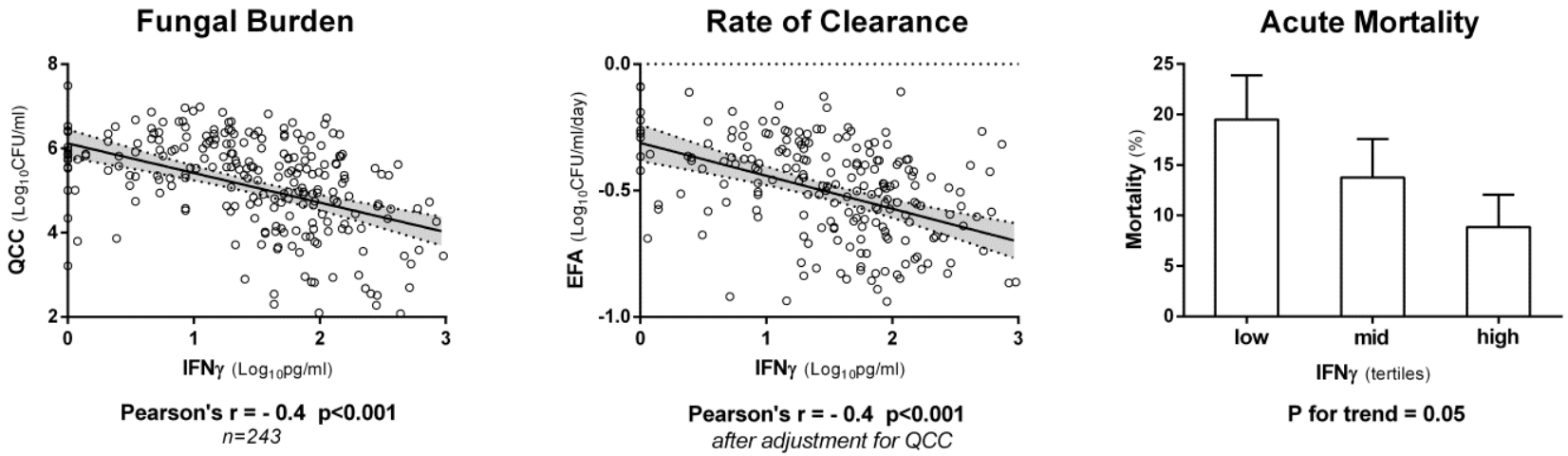
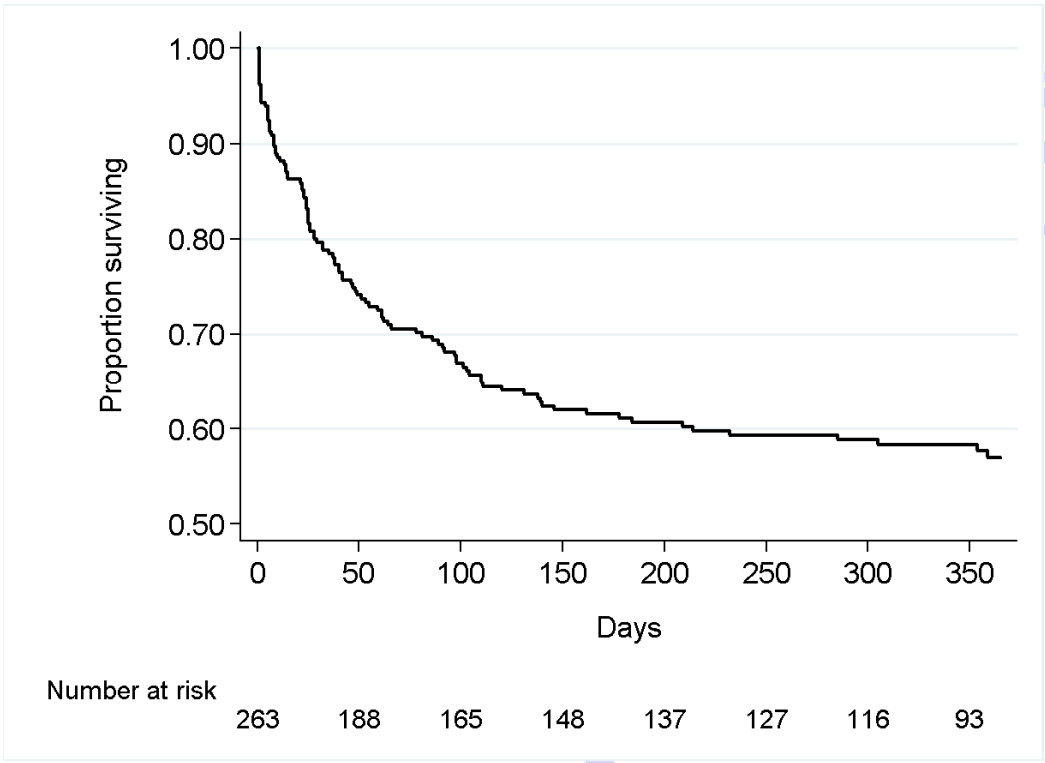
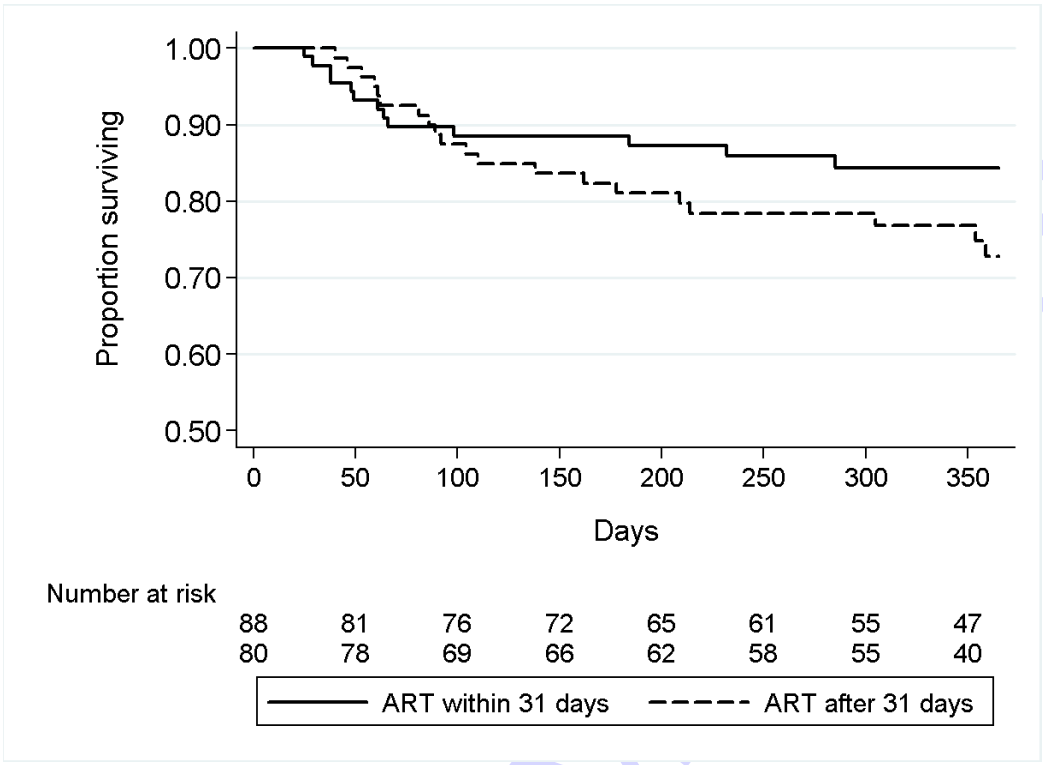


Figure 2

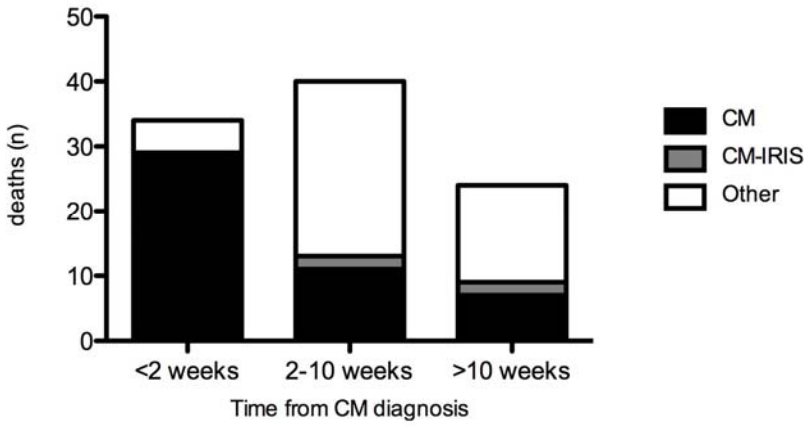
a)



b)



c)



Tables

Table 1a. Component studies contributing to the combined cohort.

Study*	**Site and Year**	***n***	**Induction Treatment**[†]	**ART available**	**EFA** (mean and SD log_{10}CFU/ml/day)
Brouwer et al.[9]	Thailand	*64*	Amphotericin B 0.7mg/kg/d (n=16)	No	-0.31 (0.18)
RCT	2002		AmB 0.7mg/kg/d plus 5FC 100mg/kg/d (n=16)		-0.54 (0.19)
			AmB 0.7mg/kg/d plus Fluconazole 400mg/d (n=16)		-0.39 (0.15)
			AmB 0.7mg/kg + 5FC 100mg/kg + Fluc 400mg/d (n=16)		-0.38 (0.13)
			(All for 14 days)		
Bicanic et al.[10]	South Africa	*54*	AmB 1mg/kg/d for 7 days then Fluc 400mg/d (n=49)	Yes	-0.48 (0.28)
Cohort	2005		Fluconazole 400mg/d for 14 days (n=5)		-0.02 (0.05)
Bicanic et al.[5]	South Africa	*64*	AmB 0.7mg/kg/d plus 5FC 100mg/kg/d (n=30)	Yes	-0.45 (0.16)
RCT	2005-6		AmB 1mg/kg/d plus 5FC 100mg/kg/d (n=34)		-0.56 (0.24)
			(Both for 14 days)		
Longley et al.[11]	Uganda	*60*	Fluconazole 800mg/d (n=30)	Yes	-0.07 (0.17)
Cohort	2005-7		Fluconazole 1200mg/d (n=30)		-0.18 (0.11)
			(Both for 14 days)		
Nussbaum et al.[12]	Malawi	*41*	Fluconazole 1200mg/d (n=20)	Yes	-0.11 (0.10)

RCT	2008		Fluconazole 1200mg/d plus 5FC 100mg/kg/d (n=21)		-0.28 (0.17)
			(Both for 14 days)		
Loyse et al.[13]	South Africa	*80*	AmB 1mg/kg/d plus 5FC 100mg/kg/d (n=21)	Yes	-0.41 (0.22)
RCT	2006-8		AmB 1mg/kg/d plus Fluconazole 800mg/d (n=22)		-0.38 (0.18)
			AmB 1mg/kg/d plus Fluconazole 1200mg/d (n=24)		-0.41 (0.35)
			AmB 1mg/kg/d plus Voriconazole 600mg/d (n=13)		-0.44 (0.20)
			(All for 14 days)		
Muzoora et al.[14]	Uganda	*30*	AmB 1mg/kg/d for 5 days plus plus Fluconazole 1200mg/d	Yes	-0.3 (0.11)
Cohort	2008-9		for 14 days (n=30)		
Jackson et al.[16]	Malawi	*40*	AmB 1mg/kg/d for 7 days plus Fluconazole 1200mg/d for	Yes	-0.39 (0.20)
RCT	2009-10		14 days (n=20)		
			AmB 1mg/kg/d for 7 days plus Fluconazole 1200mg/d and		-0.49 (0.15)
			5FC 100mg/kg/d for 14 days (n=20)		
Jarvis et al.[15]	South Africa	*90*	AmB 1mg/kg/d plus 5FC 100mg/kg/d (n=31)	Yes	-0.49 (0.15)
RCT	2007-2010		AmB 1mg/kg/d plus 5FC 100mg/kg/d plus IFNγ 100μg day		-0.64 (0.27)
			1 & 3 (n=29)		
			AmB 1mg/kg/d plus 5FC 100mg/kg/d plus IFNγ 100μg days		-0.64 (0.22)
			1, 3, 5, 8, 10 & 12 (n=30)		

(AmB plus 5FC for 14 days in all arms)

* The 9 studies were conducted in 5 sites: Sappasitprasong Hospital, Ubon Ratchathani, Thailand; GF Jooste Hospital, Cape Town, and Edendale Hospital, Pietermaritzburg, South Africa; Kamuzu Central Hospital/ University of North Carolina Project, Lilongwe, Malawi; and Mbarara University Hospital, Uganda. Exclusion criteria at all clinical trials were an alanine aminotransferase >5 times the upper limit of normal (> 200 IU/ml), neutrophil counts < 500 x 10^6 cells/L, platelet counts < 50,000 x 10^6 cells/L, pregnancy, lactation, previous serious reaction to study drugs, or concomitant medication contraindicated with study drugs (cisapride and the class of antihistamines including terfenadine and astemizole). Eight hundred and ninety six patients were screened for inclusion in the clinical trials; 523 met eligibility criteria. Reasons for exclusion were ART use in 162, inability to obtain consent in 65 (usually due to reduced GCS), prior CM in 36, patient refusal in 18, death prior to consent in 12, and other reasons including prior antifungal use, and study exclusion criteria in 80.

† Amphotericin B was administered by intravenous infusion in all studies, and fluconazole by the oral or nasogastric route. Following 2 weeks of induction therapy, patients received 8 weeks of oral fluconazole consolidation therapy (400-800mg/d) then maintenance therapy (fluconazole 200mg/d).

Table 1b. Baseline characteristics of the cohort.

	Variable	*n*	Median (IQR) or % (n)
Demographics	Age (years)	*499*	34 (29-39)
	Sex (% male)	*501*	52% (260)
History	Concurrent tuberculosis (%)	*419*	25% (123)
	Duration of symptoms (days)	*458*	14 (7-21)
Symptoms	Headache (%)	*496*	99% (489)
	Febrile symptoms (%)	*497*	57% (280)
	Visual symptoms (%)	*493*	51% (250)
	Hearing loss (%)	*415*	14% (60)
	Seizures (%)	*496*	19% (94)
	Nausea & vomiting (%)	*494*	54% (266)
	Cough (%)	*494*	35% (173)
Signs	Fever (>37.5°C)	*479*	23% (112)
	Tachycardia (>100bpm)	*491*	19% (91)
	Hypotension (<90/50mmHg)	*485*	3% (15)
	Tachypnoea (>20bpm)	*463*	19% (89)
	Altered mental status	*499*	25% (123)
	Meningism (%)	*492*	75% (369)
	Papilloedema (%)	*311*	12% (36)
	Decreased visual acuity (<6/6)	*361*	39% (141)
	Cranial nerve lesion (%)	*434*	13% (57)
Investigations	*Raised OP >25cmCSF*	*450*	51% (230)
	Raised OP >30 cmCSF	*450*	38% (173)
	CSF white cell count (x10^6/L)	*461*	15 (1-57)
	CSF protein (g/dL)	*392*	0.7 (0.4-1.3)
	CSF glucose (mmol/L)	*374*	2.2 (1.4-2.8)
	CSF CRAG (titre)*	*247*	1:1024 (1:512-4096)
	Log_{10}QCC (CFU/mL)*	*496*	5.30 (4.5-5.9)
	CD4 (cells/μL)	*456*	24 (10-50)
	Log_{10}VL (copies/mL)	*368*	5.15 (4.7-5.5)
Outcomes	2-week mortality (%)	*492*	17% (82)
	10-week mortality (%)	*484*	34% (163)
	Time admission to death (days)	*161*	13 (5-310)

OP=opening pressure, CSF=cerebrospinal fluid, CRAG=cryptococcal antigen, QCC=quantitative cryptococcal culture, VL=HIV viral load.

*see supplementary figure s1 describing the relationship between CSF CRAG and QCC.

Table 2a. Associations between baseline variables and 2-week mortality.

Variable	Category	*n*	2 week mortality	OR (95% CI) Univariable	*p*	aOR (95% CI) Multivariable* *(adjusted for treatment, CD4 count, age, mental status and fungal burden†. Number included in final model = 445)*	*p*
Age	<50 years	*462*	16% (73)	1	*0.009*	1	*0.02*
	≥50 years	*24*	38% (9)	3.2 (1.3-7.8)		3.9 (1.4-11.1)	
Sex	Female	*237*	15% (36)	1	0.5		
	Male	*255*	18% (46)	1.2 (0.7-2.0)			
Seizures	No	*397*	14% (56)	1	*0.007*		
	Yes	*91*	27% (25)	2.2 (1.2-3.9)			
Mental status	Normal	*372*	11% (39)	1	*<0.001*	1	*<0.001*
	Abnormal	*119*	36% (43)	4.8 (2.9-8.0)		3.1 (1.7-5.9)	
Weight	<50 kg	*175*	17% (30)	1	*0.13*		
	≥50 kg	*282*	10% (30)	0.7 (0.4-1.1)			
Pulse	≤100 bpm	*395*	15% (58)	1	*0.033*		
	>100 bpm	*88*	24% (21)	1.9 (1.1-3.3)			
Respiratory rate	≤20 bpm	*368*	13% (49)	1	*0.002*		
	>20 bpm	*87*	26% (23)	2.6 (1.4-4.7)			
CD4 cell count	<25 cells/μL	*229*	17% (39)	1	*0.05*	1	*0.07*
	25-49 cells/μL	*106*	8% (8)	0.4 (0.2-0.9)		0.4 (0.2-0.9)	
	50-99 cells/μL	*74*	7% (5)	0.4 (0.1-0.9)		0.5 (0.2-1.4)	
	≥100 cells/μL	*39*	13% (5)	0.7 (0.3-1.9)		1.1 (0.4-3.2)	

Hemoglobin	≥7.5 g/dL	*429*	15% (65)	1	*0.02*		
	<7.5 g/dL	*28*	32% (9)	2.8 (1.2-6.7)			
White cell count	≤10 x10^9/L	*382*	14% (53)	1	*<0.001*	1	*0.002*
	>10 x10^9/L	*21*	48% (10)	6.7 (2.6-17.7)		8.7 (2.5-30.2)	
CSF opening pressure	≤25 cmCSF	*216*	18% (38)	1	0.488		
	>25 cmCSF	*226*	16% (37)	0.8 (0.5-1.4)			
CSF white cell count	≤20 x10^6/L	*272*	20% (54)	1	*0.017*		
	>20 x10^6/L	*183*	11% (20)	0.5 (0.3-0.9)			
Quantitative cryptococcal culture (QCC)	1st tertile	*163*	9% (15)	1	*<0.001*	1.4 (1.0-1.8) (per Log_{10} CFU/ml increase)‡	*0.02*
	2nd tertile	*162*	14% (22)	1.5 (0.8-3.1)			
	3rd tertile	*163*	27% (44)	3.6 (1.9-6.8)			
Treatment	Fluconazole	*99*	26% (26)	1	*0.005*	1	*0.05*
	Amphotericin	*393*	14% (56)	0.5 (0.3-0.8)		0.5 (0.3-1.0)	

Table 2b. Associations between baseline variables and 10-week mortality.

Variable	Category	*n*	10 week mortality	OR (95% CI) Univariable	*p*	aOR (95% CI) Multivariable* *(adjusted for treatment, CD4 count, age, mental status, weight and fungal burden[§]. Number included in final model = 413)*	*p*
Age	<50 years	*454*	33% (148)	1	*0.014*	1	*0.009*
	≥50 years	*24*	58% (14)	2.9 (1.2-6.8)		4.0 (1.4-11.4)	
Sex	Female	*231*	34% (79)	1	0.815		
	Male	*250*	34% (84)	1.0 (0.6-1.4)			
Seizures	No	*389*	33% (127)	1	0.912		
	Yes	*91*	37% (34)	1.0 (0.6-1.6)			
Mental status	Normal	*366*	25% (90)	1	*<0.001*	1	*<0.001*
	Abnormal	*117*	62% (73)	5.2 (3.3-8.3)		2.8 (1.6-4.7)	
Weight	<50 kg	*173*	39% (68)	1	*0.003*	1	*0.004*
	≥50 kg	*277*	25% (68)	0.5 (0.3-0.8)		0.6 (0.4-1.0)	
Pulse	≤100 bpm	*389*	31% (121)	1	*0.010*		
	>100 bpm	*86*	45% (39)	1.9 (1.2-3.1)			
Respiratory rate	≤20 bpm	*363*	30% (110)	1	*0.006*		
	>20 bpm	*84*	45% (38)	2.0 (1.2-3.4)			
CD4 cell count	<25 cells/μL	*226*	35% (80)	1	*0.03*[‖]	1	*0.781*
	25-49 cells/μL	*102*	30% (30)	0.7 (0.4-1.2)		0.8 (0.5-1.4)	
	50-99 cells/μL	*73*	23% (17)	0.6 (0.3-1.0)		0.8 (0.4-1.5)	

	≥100 cells/μL	*39*	23% (9)	0.5 (0.2-1.2)		0.7 (0.3-1.9)	
Hemoglobin	≥7.5 g/dL	*423*	31% (133)	1	*0.008*	1	*0.02*
	<7.5 g/dL	*27*	56% (15)	3.0 (1.3-6.4)		3.0 (1.2-7.4)	
White cell count	≤10 x10^9/L	*377*	30% (114)	1	*0.001*	1	*0.02*
	>10 x10^9/L	*21*	63% (13)	4.7 (1.8-12.2)		4.0 (1.3-12.6)	
CSF opening pressure	≤25 cmCSF	*213*	39% (83)	1	*0.009*	1	*0.002*
	>25 cmCSF	*223*	30% (66)	0.6 (0.4-0.9)		0.4 (0.3-0.7)	
CSF white cell count	≤20 x10^6/L	*268*	35% (93)	1	0.461		
	>20 x10^6/L	*179*	31% (55)	0.9 (0.6-1.3)			
Quantitative cryptococcal culture (QCC)	1st tertile	*161*	24% (38)	1	*<0.001*	1.3 (1.1-1.7) (per Log_{10} CFU/ml increase)‡	*0.007*
	2nd tertile	*161*	32% (52)	1.5 (0.9-2.4)			
	3rd tertile	*158*	46% (72)	2.8 (1.7-4.5)			
Treatment	Fluconazole	*99*	53% (52)	1	*<0.001*	1	*0.02*
	Amphotericin	*385*	29% (111)	0.4 (0.2-0.6)		0.5 (0.3-0.9)	

Odds ratios and 95% confidence intervals for both univariable and multivariable associations are adjusted for clustering by study using a random effects term for "study" in a hierarchical mixed effects logistic regression model. There was very little evidence for significant clustering by study in either the 2-week or 10-week model (LR test of rho=0, p=0.498 at both 2 and 10 weeks).

Numbers of patients included in each analysis are indicated in the table. A complete records analysis was performed rather than multiple imputation as there were relatively few missing data points in the key exposure and outcome variables, and missing variables in important exposure variables such as CD4 cell count were thought to be missing not at random, meaning imputation would not

provide less biased results. It was suspected that lower values were associated with more advanced disease, and that blood tests were deferred in the sickest patients until they could consent to CD4 testing meaning patients with the lowest values may have been less likely to have a baseline test.

A sensitivity analysis in which all patients lost to follow-up were assumed either to be alive or dead did not alter the findings of either the 2 or 10-week model.

* Only variables included in the multivariable model are shown.

† Peripheral white cell count was significantly associated with 2-week mortality after adjustment, but was not included in the final model as observations were missing for 90 patients. Its inclusion in a model considering only the patients with complete data (n=370) did not alter the magnitude or significance of the associations seen in the full model.

‡ QCC is shown in the univariable analysis as categorical variables for ease of interpretation, but was included in the multivariable model as continuous variables to give a better fit.

§ Peripheral white cell count, anemia and raised CSF opening pressure were significantly associated with 10-week mortality after adjustment but not included in the final model to prevent missing observations markedly limiting the size of the model. Inclusion in a model considering only the patients with complete data (n=391 for hemoglobin, n=343 for peripheral white count, and n=374 for raised CSF opening pressure) did not alter the magnitude or significance of the associations seen in the full model.

‖ Test for trend.

Table 3. Correlations between baseline CSF fungal burden, derived from quantitative cryptococcal cultures (QCC, log_{10} colony forming units/ml CSF).

Correlation with QCC*	***n***	**Correlation coefficient (*r*)**	**95% CI**	***p***
CD4 cell count (cells/μL)	*452*	-0.24	-0.33 - -0.15	*<0.001*
CSF opening pressure (cm CSF)	*446*	0.05	-0.05 - 0.14	0.330
CSF white cell count ($x10^6$/L)	*458*	-0.30	-0.39 - -0.21	*<0.001*
CSF protein (g/dL)	*389*	-0.29	-0.38 - -0.19	*<0.001*
CSF TNFα (log_{10}picog/ml)	*242*	-0.20	-0.32 - -0.07	*0.002*
CSF IL-6 (log_{10}picog/ml)	*241*	-0.15	-0.27 - -0.02	*0.024*
CSF IFNγ (log_{10}picog/ml)	*243*	-0.40	-0.50 - -0.29	*<0.001*

*Correlations were assessed using Spearman's Log Rank test for CD4 count, CSF opening pressure, CSF white cell count and CSF protein; and Pearson's correlation coefficient for TNFα, IL-6 and IFNγ which were approximately normally distributed when log-transformed.

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