Europe PMC Funders Group
Author Manuscript
*Clin Infect Dis*. Author manuscript; available in PMC 2010 October 28.

Published in final edited form as:
*Clin Infect Dis*. 2009 September 1; 49(5): 702–709. doi:10.1086/604716.

# Rate of clearance of infection is independently associated with clinical outcome in HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients

**Tihana Bicanic**[1,2,3], **Conrad Muzoora**[4], **Annemarie E Brouwer**[5], **Graeme Meintjes**[3], **Nicky Longley**[1], **Kabanda Taseera**[4], **Kevin Rebe**[3], **Angela Loyse**[1], **Joseph Jarvis**[1,2,3], **Linda-Gail Bekker**[2], **Robin Wood**[2], **Direk Limmathurotsakul**[6], **Wirongrong Chierakul**[6], **Kasia Stepniewska**[6,7], **Nicholas J White**[6,7], **Shabbar Jaffar**[8], and **Thomas S Harrison**[1]

[1]Centre for Infection, St. George's University of London, London, UK [2]Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa [3]Infectious Diseases Unit, GF Jooste Hospital, Cape Town, South Africa and Department of Medicine, University of Cape Town, South Africa [4]Department of Medicine, Mbarara University Hospital, Mbarara, Uganda [5]Department of Internal Medicine and Infectious Diseases, University Medical Centre Nijmegen, The Netherlands [6]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand [7]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford [8]Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London

## Abstract

**Background—**Progress in therapy of cryptococcal meningitis has been slow because of the lack of a suitable marker of treatment response. Previously, we demonstrated the statistical power of a novel endpoint, the rate of clearance of infection, based on serial quantitative cultures of CSF, to differentiate the fungicidal activity of alternative antifungal drug regimens. We hypothesised that the rate of clearance of infection should also be a clinically meaningful endpoint.

**Methods—**We combined data from cohorts of patients with HIV-associated cryptococcal meningitis from Thailand, South Africa, and Uganda, for whom rate of clearance of infection was determined, and clinical and laboratory data prospectively collected, and explored the association between the rate of clearance of infection and mortality by Cox survival analyses.

**Results—**The combined cohort comprised 262 subjects. Altered mental status at presentation, a high baseline organism load, and a slow rate of clearance of infection were independently associated with increased mortality at 2 and 10 weeks. Rate of clearance of infection was associated with antifungal drug regimen and baseline CSF IFN-γ levels.

**Conclusions—**The results support use of rate of clearance, or early fungicidal activity, as a means to explore antifungal drug dosages and combinations in phase II studies. An increased understanding of how the factors determining outcome interrelate may help clarify opportunities for intervention.

### Keywords

Rate of clearance; early fungicidal activity; cryptococcal meningitis; Cryptococcus; outcome

Reprints or correspondence: Thomas S Harrison, Centre for Infection, St George's University of London, Cranmer Terrace, Tooting, London SW17 ORE, UK. Tel: 44 208 725 5828, fax 44 208 7253487 tharriso@sgul.ac.uk.

The authors have no conflicts of interest.

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

## Introduction

The acute mortality of HIV-associated cryptococcal meningitis remains unacceptably high [1-5], and, consequently, cryptococcal disease remains a leading cause of death in cohorts of HIV-infected individuals in Africa and Asia [6-8]. The urgent need to improve the acute management of cryptococcal meningitis is further reinforced by expanding access to antiretroviral therapy (ART). With access to ART, these patients have a good long-term prognosis, provided they survive the initial critical months [4].

After landmark studies in the 1990s [9,10], recent progress in therapy of cryptococcal disease has been slow because of the large numbers of patients needed for clinical endpoint trials and the lack of a suitable marker of treatment response. However, in a study carried out in Thailand, we demonstrated the feasibility and statistical power of a novel endpoint, the rate of clearance of cryptococcal colony-forming-units (CFU) from the cerebrospinal fluid (CSF), based on serial quantitative cultures of CSF from lumbar punctures over the initial 2 weeks of treatment [1]. The use of a summary statistic (the rate of fall in CFU), based on repeated quantitative measures in individual patients, means this endpoint is statistically more powerful than other markers of response previously used, for example, the proportion of patients with a positive CSF culture at 2 weeks [11]. In the study, the fungicidal activity of alternative combinations of antifungal drugs could be differentiated with only 16 patients enrolled per arm [1].

We hypothesised that the rate of clearance of infection should also be a clinically meaningful endpoint. The organism load at baseline is a powerful prognostic factor [1], and sterilization of the CSF at 2 weeks has previously been shown to be associated with clinical outcome at 10 weeks [12]. Herein, we investigate the association of rate of clearance of infection and acute mortality in HIV-associated cryptococcal meningitis, using data from a combined cohort of 262 patients from the Thai study and subsequent studies in Cape Town, South Africa, and Mbarara, Uganda.

## Patients and methods

Data for this study was combined from 4 trials of initial antifungal therapy for treatment of HIV-associated cryptococcal meningitis [1,4,13,14]. Studies were approved by the ethical and scientific review subcommittee of the Thai Ministry of Public Health; by the research ethics committee of The Faculty of Health Sciences, University of Cape Town, and The Medicines Control Council of South Africa; by the ethics committee of the University Hospital of Mbarara, Uganda; and by Wandsworth local research ethics committee, covering St George's Hospital, UK.

1. A randomised study of 63 ART-naïve HIV-seropositive patients treated with amphotericin B 0.7 mg/kg/d, alone or in combination with flucytosine (100 mg/kg/d), fluconazole (400 mg/d), or both, conducted in Ubon Ratchathani in Northeast Thailand [1].
2. An observational study of 54 ART-naïve or -experienced patients treated with amphotericin B alone, at 1 mg/kg/d, or fluconazole 400 mg/d, according to local protocol, in Cape Town, South Africa [4].
3. Randomised studies of ART-naïve patients receiving amphotericin B-based combination therapy in Cape Town, South Africa [13, ISRCTN68133435]. In step 1, amphotericin B 1 mg/kg/d was compared with 0.7 mg/kg/d, both with flucytosine 100 mg/kg/d (n=64, 13). In step 2, amphotericin B 1 mg/kg/d plus

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

flucytosine is being compared with amphotericin B 1 mg/kg/d plus fluconazole (at 800 mg/d or 1200 mg/d, n=21, recruitment ongoing).

4. A cohort, dose-escalation study of initial therapy with fluconazole at Mbarara University Hospital, Uganda, a setting where standard treatment was with fluconazole at 400 mg/d. 30 patients were treated with fluconazole at 800 mg/d, and 30 at 1200 mg/d [14].

For the randomised studies of amphotericin B combinations in Thailand and Cape Town, exclusion criteria were alanine aminotransferase (ALT) >5 times upper limit of normal (>200 IU), absolute neutrophil count<$500\times10^6$/L, platelets<$50{,}000\times10^6$/L, pregnancy, lactation, previous serious reaction to AmB, flucytosine, or fluconazole, and patients already on ART. In Mbarara, Uganda, exclusions were ALT >5 times upper limit of normal (>200 IU), pregnancy, and prior ART.

In trials 1, 3, and 4, above, after two weeks, therapy was fluconazole, 400 mg/d for 8 weeks and 200 mg/d thereafter. In study 2, amphotericin B was given for a median of 7 days prior to switching to fluconazole. After initial inpatient treatment, patients continued to be followed up in established HIV outpatient clinics at the study sites. At the time of the trial in Thailand, ART was not generally available in that country. In all more recent trials, patients have been counselled and started on ART at a median interval of 47 days (Cape Town) and 38 days (Mbarara) after starting antifungal therapy.

Lumbar punctures were done on days 1, 3, 7, and 14, for quantitative cultures to assess the rate of clearance of infection. Quantitative cultures of CSF were performed as previously described [1, 4]. The rate of decrease in log CFU/ml CSF per day was derived from the slope of the linear regression of log CFU against time for each patient [1, 4]. Population modelling confirmed that a linear model best fitted the CFU clearance data, and gave results consistent with the individual patient analysis. CSF cytokine levels (IFN-γ, TNF-α, IL-6) were determined using the Luminex multianalyte system (Luminex) and cytokine kits (Bio-Rad), and separate ELISA (Quantikine, R&D Systems), as previously described [4, 15]. Plasma viral load and CSF cytokine levels were not available for patients enrolled in Uganda.

## Statistics

Plasma viral load, baseline CSF CFU counts, and cytokine data, were log transformed. Continuous data were categorised into equal-sized groups and analysed using chi-squared tests. Rate of clearance of infection was analysed both as a continuous variable and categorized into quartiles. Survival analysis was conducted using Cox regression with time calculated from the date treatment was started to either 14 or 70 days, depending on the analysis, the date last seen or the date of death. Models were compared using the likelihood ratio test. Additional analyses were done with patients lost to follow-up classified as deaths. The findings from these analyses were similar, and so only the data with subjects who were lost-to-follow-up censored when last seen are presented here. Linear regression was used to explore associations of other variables with rate of clearance of infection [15] and Spearman's rank correlation coefficient to examine the association between cytokines and CD4 cell count. Analyses were conducted using STATA version 10 (Stata statistical software, Stata Corp., College Station, Texas, USA).

## Results

Table 1 shows the characteristics at baseline of 262 subjects recruited from the four cohorts. Five (2%) subjects were lost to follow-up within 2 weeks and a further 38 (15%) died. By 10

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

weeks, 9 (3%) subjects were lost to follow-up and 81 (32%) had died. Rate of clearance of infection could not be measured in 31 subjects who died or were lost to follow-up before a second CFU measurement; in the remainder 231 subjects, the rate of clearance of infection was approximately normally distributed with a mean (SD) clearance of −0.37 (0.27) log CFU per day (Figure 1).

### Associations with mortality at 2 and 10 weeks

Consistent with prior studies [1,9,12], altered mental status (defined as Glasgow Coma Score <15) at presentation and high baseline organism load were associated, very significantly, with mortality (Table 2, Figure 2). In addition, a slow rate of clearance of infection was associated with mortality in both univariate and multivariate analysis (Table 2, Figure 2). None of the other variables examined (CD4 cell count, sex, age, weight, CSF opening pressure, CSF white cell count, viral load) were associated with mortality.

The mean (SD) rate of clearance of infection of those who died and those who survived was −0.17 (0.27) and −0.40 (0.27) log CFU/d, respectively, at 2 weeks; and − 0.27 (0.27) and −0.41 (0.26) log CFU/d, respectively, at 10 weeks. These rates were significantly less rapid in those who died compared to those who survived at both 2 and 10 weeks (difference [95% CI] in mean rates of decline per day −0.23 [−0.35, − 0.11] and −0.15 [−0.22, −0.069] log CFU/d, respectively, p<0.001, both cases).

In multivariate models, including altered mental status, baseline organism count, and rate of clearance of infection, all 3 factors remained independently associated with mortality. When rate of clearance was fitted on a continuum scale, after adjusting for baseline count and altered mental status, the hazard ratio (95% CI) increase for each 0.1 log unit increase in rate of fall of CFU was 1.34 (1.06, 1.68; p=0.01) at 2 weeks and 1.18 (1.04, 1.33; p=0.008) at 10 weeks (Table 2).

We also fitted the serial CFU counts as a time-dependent variable. After adjusting for altered mental status at baseline, the hazard ratio for death was 1.91 (95% CI 1.28, 2.85; p<0.001) at 2 weeks, and 1.14 (95% CI 1.01, 1.30; p=0.04) at 10 weeks for each unit increase in the last log CFU count. When we adjusted also for baseline CFU count (in addition to altered mental status), these hazard ratios were 1.82 (95% CI 1.12, 2.96) (p=0.007) at 2 weeks and 1.13 (95% CI 0.65, 1.32) (p=0.14) at 10 weeks, for each unit increase in the last log CFU count.

### Associations of other variables with rate of clearance of infection

In previous work, based on the Thai study, only antifungal drug regimen and baseline CSF IFN-γ were independently associated with rate of clearance of infection [15]. Patients in this larger cohort were treated with a total of 9 different regimens containing amphotericin B, and 3 regimens of fluconazole. In univariate analysis, the strongest associations with rate of clearance of infection were with treatment that included amphotericin B, CSF IFN-γ, and baseline organism count (p <0.001, for all 3 associations). Consistent with the prior analysis, in a multivariate model including all 3 factors, only treatment with amphotericin B and CSF IFN-γ remained independently associated with rate of clearance. In a final model including these 2 factors, the mean rate of fall in CSF log CFU counts was more rapid for amphotericin B-containing regimens compared with fluconazole regimens (difference = 0·45 log CFU/d, 95%CI 0·26-0·63, $p < 0·001$). Log IFN-γ was significantly associated with a more rapid clearance (increase in rate of fall in CFU for each unit increment in log IFN-γ = 0.11 log CFU/ml CSF/day, 95% CI 0.06-0.15, $p < 0.001$).

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

### Correlations between CSF cytokines and CD4 T cell count

There was a positive correlation between CD4 count and log CSF IFN-γ levels (r = 0.4, p <0.0001, Figure 3), and between CD4 count and CSF TNF-α levels (r = 0.3, p = 0.001). CSF IL-6 levels were not correlated with CD4 cell count. In this dataset, CSF IFN-γ and TNF-α remained strongly positively correlated (r = 0.7, p = <0.0001), but, in contrast to earlier analysis [15], there was no statistically significant correlation between IFN-γ and IL-6 levels (r = 0.1, p = 0.08).

## Discussion

In this cohort of 262 patients, we have demonstrated an association of rate of clearance of infection with survival, independent of the other major prognostic factors, altered mental status at presentation and baseline organism load. The strength of the association in multivariate analysis was stronger with survival at 2 than 10 weeks. This may reflect the fact that deaths within 2 weeks are nearly all related to cryptococcal infection, whereas after this time point deaths are increasingly related to other complications of late-stage HIV infection. The results lend strong support to the use of rate of clearance as both a statistically powerful and clinically relevant marker of treatment response. The shape of this relationship, whether linear, or whether there is a cut-off above which more rapid clearance has little further benefit, remains to be defined by analysis of larger cohorts, although the data do suggest that there may be less impact on outcome at the most rapid rates of clearance. Larger, phase III cohorts, with larger numbers of patients on particular drug regimens, will also be needed to test whether rate of clearance fulfils the additional criteria of a surrogate marker of treatment response [16]. Larger cohorts will also be needed to explore with adequate power the possible effect of additional factors, such as fungemia, not examined in this study, on mortality.

Given the dependence of rate of clearance of infection on antifungal regimen, it is not possible to completely exclude the possibility that an association between rate of clearance and outcome could be observed in this cohort if fluconazole therapy were associated with higher mortality through a separate unknown mechanism, independent of its association with a slow clearance of infection. However, it seems more likely that prolonged exposure to the organism through a high organism load at baseline and slow clearance does directly impact outcome, as suggested by examination of prior trials [9, 10, 12, 17], in addition to this analysis.

The associations between variables in the cohort lead us to propose a model for how the factors determining rate of clearance of infection and mortality may interrelate (Figure 4). The proposed causal nature of the associations in the model remain speculative, although in one instance, the association of IFN-γ and rate of clearance of infection, causality could be tested by intervention studies, such as those published and ongoing to examine the effects of adjunctive therapy with IFN-γ [18, ISRCTN72024361].

Notable was the fact that in this cohort we could not demonstrate an association between baseline CSF opening pressure and survival, as has been found in some prior studies [19]. Efforts were made to ensure accurate measurement of opening pressure in all patients, and in all trials patients had a minimum of 4 lumbar punctures according to protocol, and further lumbar punctures if the opening pressure was raised. This aggressive approach to management of raised CSF pressure may have reduced its effect on outcome [20].

The size of this cohort enabled us to demonstrate an association of CSF IFN-γ but not IL-6 levels with CD4 count, consistent with the known reduction in IFN-γ but preservation of IL-6 release in late stage HIV-infection [21, 22].

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

An increased understanding of the factors determining rate of clearance and outcomes increases our ability to examine the impact of individual components. Thus, for example, variations in individual patient immunity (CSF IFN-γ) can be controlled for in trials examining the effect of novel drug regimens on clearance of infection. An increased understanding of how the factors determining outcome interrelate may also help clarify opportunities for intervention, for example, through more rapidly active drug combinations, adjunctive immunotherapy, or earlier diagnosis and treatment; and prioritise research questions, for example, understanding the pathophysiological basis of raised CSF pressure and altered mental status.

The study demonstrates that rate of clearance of infection is not only a statistically powerful endpoint but also a clinically meaningful one. The results support the use of rate of clearance, or early fungicidal activity, as a means to explore antifungal drug dosages and combinations in phase II studies that can prioritize novel regimens for testing in phase III clinical endpoint trials.

**summary**

262 cryptococcal meningitis patients had rate of clearance of infection determined using serial quantitative CSF cultures. Altered mental status, a high baseline organism load, and a slow rate of clearance were independently associated with mortality at 2 and 10 weeks.

## Acknowledgments

This work was supported by the MRC (UK) grant G0501476, a British Infection Society Fellowship to TB, a Wellcome Trust Training Fellowship in Tropical Medicine to AEB (069991), and was part of the Wellcome Trust-Mahidol University-Oxford Tropical Medicine Research Programme.

We thank Supraphada Pinpraphaporn, Bina Maharjan, Anna Checkley, Vanaporn Wuthiekanun, Premjit Amornchai, Nongluk Getchalarat, Pissamai Manupan, Jintana Suwanpruek, Nick Day, Sharon Peacock, for help with the study at Sappasithiprasong Hospital. We thank Nomqondiso Sidibana, Tom Crede, Vanessa Burch, Anthony Williams, Noxolo Mahlaza, Elma de Vries for help with the studies at GF Jooste hospital; and James Mwesigye, Joselyne Rwebembera, Ali Chakera, Emma Wall, and Irene Andia for help with the study at Mbarara hospital.

## References

1. Brouwer AE, Rajanuwong A, Chierakul W, Griffin GE, Larsen RA, White NJ, Harrison TS. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet. 2004; 363:1764–67. [PubMed: 15172774]
2. Lortholary O, Poizat G, Zeller V, et al. Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. AIDS. 2006; 20:2183–91. [PubMed: 17086058]
3. Schaars CF, Meintjes GA, Morroni C, Post FA, Maartens G. Outcome of AIDS-associated cryptococcal meningitis initially treated with 200 mg/day or 400 mg/day of fluconazole. BMC Infect Dis. 2006; 6:118. [PubMed: 16846523]
4. Bicanic T, Meintjes G, Wood R, Hayes M, Rebe K, Bekker L-G, Harrison TS. Fungal burden, clearance of infection, and outcome in cryptococcal meningitis in antiretroviral-naïve and -experienced patients treated with amphotericin B or fluconazole. Clin Infect Dis. 2007; 45:76–80. [PubMed: 17554704]
5. Kambugu AD, Meya DB, Rhein J, et al. Outcomes of cryptocccal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clin Infect Dis. 2008; 46:1694–1701. [PubMed: 18433339]
6. French N, Gray K, Watrea C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS. 2002; 16:1031–38. [PubMed: 11953469]

Europe PMC Funders Author Manuscripts

7. Okongo M, Morgan D, Mayanja B, et al. Causes of death in a rural, population-based human immunodeficiency virus type 1 natural history cohort in Uganda. Int J Epidem. 1998; 27:698–702.
8. Corbett EL, Churchyard G, Charalambos S, et al. Morbidity and mortality in South African gold miners: impact of untreated disease due to human immunodeficiency virus. Clin Infect Dis. 2002; 34:1251–8. [PubMed: 11941552]
9. Saag MS, Powderly WG, Cloud GA, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. N Eng J Med. 1992; 326:83–9.
10. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. N Engl J Med. 1997; 337:15–21. [PubMed: 9203426]
11. Matthews JNS, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. BMJ. 1990; 300:230–235. [PubMed: 2106931]
12. Robinson PA, Bauer M, Leal ME, et al. Early mycological treatment failure in AIDS-associated cryptococcal meningitis. Clin Infect Dis. 1999; 28:82–92. [PubMed: 10028076]
13. Bicanic T, Wood R, Meintjes G, et al. High dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV: a randomised study. Clin Infect Dis. 2008; 47:123–130. [PubMed: 18505387]
14. Longley N, Muzoora C, Taseera K, et al. Dose-Response Effect of high dose fluconazole for HIV-associated cryptococcal meningitis in Southwest Uganda. Clin Infect Dis. 2008; 47:1556–61. [PubMed: 18990067]
15. Siddiqui A, Brouwer AE, Wuthiekanun V, et al. Interferon-γ at the site of infection determines rate of clearance of infection in cryptococcal meningitis. J Immunol. 2005; 174:1746–50. [PubMed: 15661940]
16. Weir CJ, Walley R. Statistical evaluation of biomarkers as surrogate endpoints: a literature review. Statist Med. 2006; 25:183–203.
17. Larsen RA, Leal ME, Chan LS. Fluconazole compared with amphotericin B plus flucytosine for cryptococcal meningitis in AIDS. Ann Intern Med. 1990; 113:183–187. [PubMed: 2197908]
18. Pappas PG, Bustamante B, Ticano E, et al. Recombinant interferon-γ1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. J Infect Dis. 2004; 189:2185. [PubMed: 15181565]
19. Graybill JR, Sobel J, Saag M, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. Clin Infect Dis. 2000; 30:47–54. [PubMed: 10619732]
20. Bicanic T, Brouwer A, Meintjes G, et al. Relationship of raised CSF opening pressure, fungal burden and outcome in HIV-associated cryptococcal meningitis patients managed with serial lumbar punctures. AIDS. 2009 in press.
21. Zhang M, Gong J, Iyer DV, Jones BE, Modlin RL, Barnes PF. T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. J Clin Invest. 1994; 94:2435. [PubMed: 7989601]
22. Breen EC, Rezai AR, Nakajima K, et al. Infection with HIV is associated with elevated IL-6 levels and production. J Immunol. 1990; 144:480–484. [PubMed: 2295799]

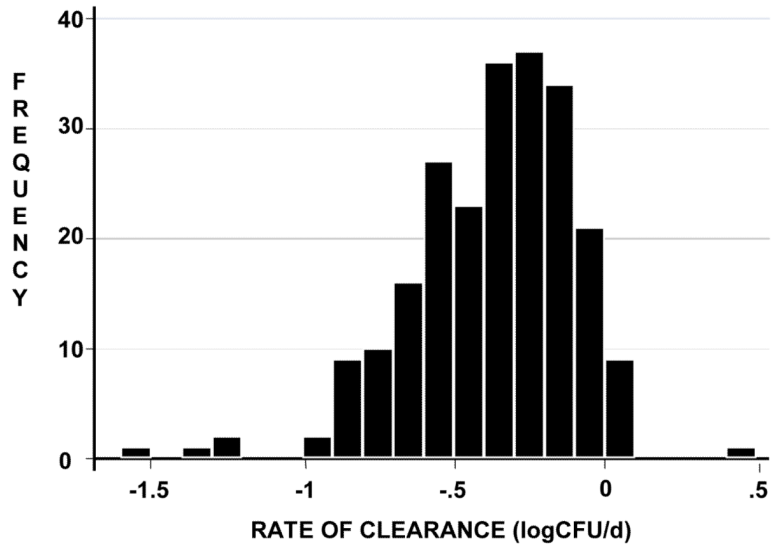



**Figure 1.**
Frequency distribution for rate of clearance of infection (logCFU/ml CSF/day) in the combined cohort

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

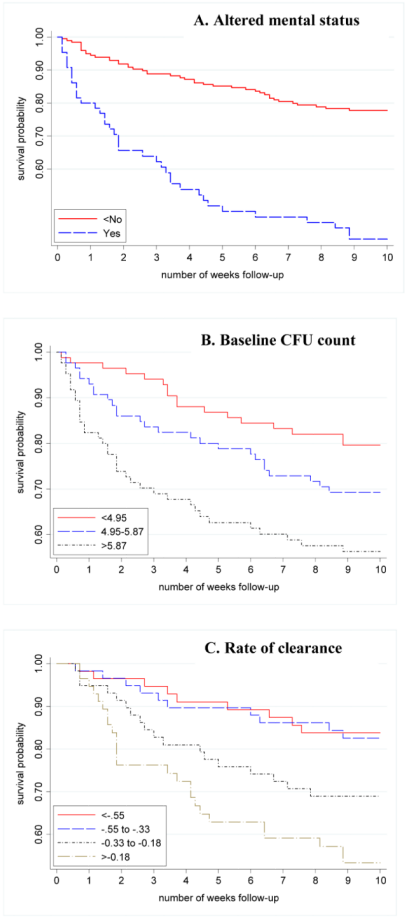



**Figure 2.**

Kaplan-Meier survival curves by A. altered mental status at presentation (yes or no), B. baseline CSF organism load (categorized into tertiles: <4.95, 4.95-5.87, and >5.87 log CFU/ml CSF), and C. rate of clearance of infection (categorized into quartiles: >−0.18, −0.18-0.33, −0.33-0.55, <−0.55 log CFU/ml CSF/day)



Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

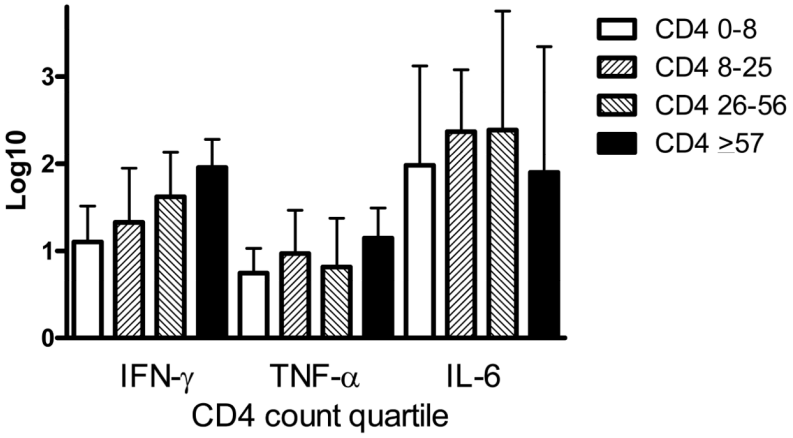



**Figure 3.**
Association of baseline CSF cytokine levels (median, IQR) and CD4 cell counts. CD4 cell counts were categorized into quartiles: 1st quartile 0-8, 2nd quartile 9-25, 3rd quartile 26-56, 4th quartile ≥57, × $10^6$cells/L

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

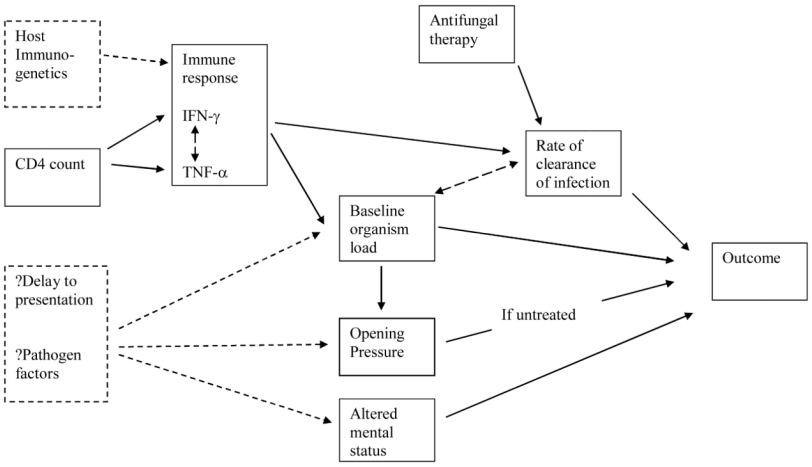



**Figure 4.**

A model illustrating possible relationships between factors associated with rate of clearance of infection and survival. Proposed causal links are shown with solid arrows, non-causal associations with long-dashed arrows, and speculative associations with short-dashed arrows.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

## Table 1

Baseline clinical and laboratory characteristics and clinical outcomes of the combined cohort. Results are median (IQR) unless indicated

| | **Patient characteristics** |
|---|---|
| N | 262 |
| Male (%) | 119 (46%) |
| Age (yrs) | 33 (29-38) |
| mean (SD) weight (kg) | 52 (11) |
| Abnormal mental status (%) | 65 (25%) |
| CD4 count ($\times 10^6$/L) | 26 (9-56) |
| VL (copies/ ml)[a] | 150997 (48000-372475) |
| *CSF data at baseline* | |
| Opening Pressure (cm $H_20$)[a] | 27 (17-36) |
| White cell count | 14 (1-54) |
| Fungal burden (CFU/ml CSF) | 271371 (38963-1112423) |
| IFN-γ (pg/ml) [b] | 30 (7-96) |
| TNF-α (pg/ml) [b] | 9 (3-22) |
| IL-6 (pg/ml) [b] | 192 (43-1417) |
| *Rate of clearance of infection* mean (SD) log CFU/d | −0.37 (0.27) |
| *Deaths* [c] | |
| 2 weeks (%) | 38 (15%) |
| 10 weeks (%) | 81 (32%) |

[a] Normal range 7-18 cm $H_20$

[b] VL and cytokine data were not available from the Uganda cohort

[c] 5 and 9 patients were lost to follow-up before 2 and 10 weeks, respectively

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts





*Clin Infect Dis*. Author manuscript; available in PMC 2010 October 28.

**Table 2**

Variables associated with mortality at 2 and 10 weeks

| | | 2-week mortality[a] | | | | | 10-week mortality[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Univariate | | Multivariate | | | Univariate | | Multivariate | |
| | | No (%) | HR (95% CI) | p-value | HR (95% CI) | p-value | No (%) | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Baseline CFU | | | | | | | | | | | |
| | < 4.95 | 3/85 (4) | 1.0 | | 1.0 | | 17/85 (20) | 1.0 | | 1.0 | |
| | 4.95-5.87 | 12/86 (14) | 4.1 (1.2, 14.5) | | 8.2 (1.0, 68.5) | | 26/86 (30) | 1.6 (0.9, 3.0) | | 1.8 (0.9, 3.6) | |
| | >5.87 | 22/85 (26) | 8.3 (2.5, 27.6) | <0.0001 | 11.3 (1.4, 90.6) | 0.006 | 36/85 (42) | 2.7 (1.5, 4.8) | 0.0004 | 2.3 (1.2, 4.5) | 0.02 |
| Altered mental status | | | | | | | | | | | |
| | No | 16/197 (8) | 1.0 | | 1.0 | | 43/197 (22) | 1.0 | | 1.0 | |
| | Yes | 22/65 (34) | 4.9 (2.6, 9.4) | <0.0001 | 3.4 (1.3, 8.5) | 0.01 | 38/65 (58) | 3.9 (2.5, 6.0) | <0.0001 | 3.4 (2.0, 5.8) | <0.0001 |
| Slope | | | | | | | | | | | |
| | <−0.55 | 2/57 (4) | 1.0 | | 1.0 | | 9/56 (16) | 1.0 | | 1.0 | |
| | −0.55 to −0.33 | 2/59 (3) | 1.0 (0.1, 6.8) | | 0.6 (0.08, 4.3) | | 10/59 (17) | 1.1 (0.4, 2.7) | | 0.9 (0.4, 2.3) | |
| | −0.33 to −0.18 | 5/58 (9) | 2.5 (0.5, 12.8) | | 1.3 (0.2, 7.0) | | 18/55 (33) | 2.1 (1.0, 4.7) | | 1.7 (0.8, 3.8) | |
| | >−0.18 | 13/57 (23) | 7.0 (1.6, 31.2) | <0.0001 | 2.6 (0.5, 12.4) | 0.04 | 25/56 (45) | 3.6 (1.7, 7.7) | <0.0001 | 2.1 (0.9, 4.7) | 0.02 |
| CD4 | | | | | | | | | | | |
| | ≤ 25 | 14/113 (12) | 1.0 | | - | | 34/112 (30) | 1.0 | | - | |
| | >25 | 6/113 (5) | 0.41 (0.16, 1.07) | 0.06 | | | 27/109 (25) | 0.73 (0.44, 1.20) | 0.2 | | |

Note: The p-values refer to tests for trend.

[a] 2 weeks: Slope on continuum: univariate hazard ratio increases by 1.52 (95% 1.24, 1.85; p<0.0001). After adjusting for baseline count, altered mental status, it was 1.34 (1.06, 1.68; p=0.01). CD4 count not significant in multivariate analysis (p=0.8).

[b] 10 weeks: Slope on continuum: univariate hazard ratio increases by 1.27 (95% 1.13, 1.43; p<0.0001). After adjusting for baseline count, altered mental status, it was 1.18 (1.04, 1.33; p=0.008). CD4 count was not significant in multivariate analysis (p=0.5).