# Short term mortality outcomes of HIV-associated Cryptococcal meningitis in antiretroviral therapy naïve and experienced patients in sub-Saharan Africa

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## Abstract

### Background

An increasing proportion of patients with HIV-associated cryptococcal meningitis have received antiretroviral therapy (ART) prior to presentation. There is some evidence suggesting an increased two-week mortality in those receiving ART for less than 14 days compared with those on ART for more than 14 days. However, presentation and outcomes for cryptococcal meningitis patients who have recently initiated ART, and those with virologic failure and/or non-adherence are not well described.

### Methods

678 adults with first episode of cryptococcal meningitis recruited into a randomized, non-inferiority, multicentre phase 3 trial in 4 sub-Saharan countries were analysed to compare clinical presentation and 2-and 10-week mortality outcomes between ART-naive and experienced patients, and between patients receiving ART for varying durations prior to presentation.

### Results

Over half (56% (381/678)) the study participants diagnosed with a first episode of cryptococcal meningitis were ART-experienced. All-cause mortality was similar at 2-weeks (17% vs 20%; HR 0.85, 95%CI 0.6-1.2, p=0.35), and 10 weeks (38% vs 36%; HR 1.03, 95%CI 0.8-1.32, p=0.82) for ART-experienced vs ART-naïve patients, respectively. Among ART-experienced patients, using different cut-off points for ART duration, there were no significant differences in 2- and 10-week mortality based on duration of ART.

### Conclusion

In this study, there were no significant differences in mortality at 2-and 10-weeks between ART-naive and experienced patients, and between ART-experienced patients according to duration on ART.



### Key words

HIV, Cryptococcal meningitis, Short-term mortality, Antiretroviral therapy, Sub-Saharan Africa, Treatment

## Background

Cryptococcal meningitis (CM) continues to cause significant morbidity and mortality in people living with HIV (PLHIV) despite the scale-up of antiretroviral therapy (ART) in sub-Saharan Africa (SSA) and an increasing proportion of patients with HIV-associated cryptococcal meningitis are now presenting on ART (1). Amongst ART experienced patients, a proportion will have unmasking immune reconstitution inflammatory response syndrome (usually those with ART initiation ~< 3 months prior to presentation), and a proportion will have virological and immunological failure due to ART failure (usually those on ART ~> 6 months) (2). Although it is well recognised that early initiation of ART during induction treatment of CM results in higher mortality (3, 4), data on outcomes for CM patients recently started on ART prior to presentation are few, and guidance on ART management (continuation, switch or interruption) in this group is based largely on expert opinion (5). Overall mortality appears to be similar comparing ART-experienced and ART-naïve cryptococcal meningitis patients (6-9). However, differences in presentation and outcomes for patients with 'unmasking' cryptococcal infection, where ART has been recently initiated, and those with virologic failure and/or non-adherence are not well described.

A recent study of 605 PLHIV diagnosed with CM in Uganda (10) found no difference in 2-week mortality between the ART-experienced and ART-naïve groups but there was increased short-term mortality in those receiving ART for less than 2 weeks compared with those on ART for more than 2 weeks. The study suggested the possibility that pre-existing subclinical meningitis in some patients at ART initiation may drive an early unmasking immune reconstitution inflammatory response syndrome (IRIS) and increased mortality. Here we have compared clinical presentation and short-term mortality outcomes for 678 ART-naïve and ART-experienced patients with a first episode of CM enrolled into the Advancing Cryptococcal meningitis Treatment for Africa (ACTA) trial in 4 countries in SSA (9). The impact of ART duration prior to presentation on mortality outcomes were also compared.

## Methods

### *Study setting and population*

From January 2013 to November 2016, 721 HIV-infected adults (≥18 years old) from centres in Malawi, Zambia, Tanzania and Cameroon, presenting with a first episode of CM were prospectively enrolled into a randomized, noninferiority, multicentre trial (Advancing Cryptococcal Meningitis Treatment for Africa (ACTA), as previously described (9). ART-

experienced patients were excluded at the beginning of the trial (4% of enrolment) but an amendment allowed inclusion of this population after noting that large number of patients exposed to ART were presenting with CM. A total of 678 participants were included in the final intention-to-treat analysis.

### *Study design*

Patients were randomised to 1 of 3 treatment strategies: oral fluconazole plus flucytosine for 2 weeks, 1-week Amphotericin B (AmB)-based therapy, and standard 2 weeks AmB-based therapy (9). Those in the AmB arms were further randomized to flucytosine or fluconazole in a 1:1 ratio, as the partner drug given with AmB. Patients received consolidation fluconazole therapy after the 2-week induction period: 800mg until ART initiation or switch at 4 weeks and 400mg to 10 weeks. The primary and secondary endpoints were all-cause mortality at 2-weeks and 10-weeks respectively. ART exposure was defined as ever having taken ART. Information on ART status and duration on ART was ascertained by self-report (or, where appropriate, from guardians) and confirmed by review of medical records. First line ART for adults were tenofovir based.

### *Study outcomes and analysis*

All-cause mortality at 2 and 10 weeks was compared based on ART status (experienced versus naïve) and ART duration at CM diagnosis. Baseline clinical and laboratory characteristics were compared across ART groups with Chi-square (when sample size is 30 or more) or Fisher exact tests (when sample size is less than 30) for categorical variables and Kruskal Wallis tests for continuous variables. Unadjusted and adjusted cox proportional hazards models, Kaplan Meier curves and log rank tests were used to examine the hazard of mortality between ART-experienced and ART-naïve patients and for those on ART for varying durations. Global test was conducted to test for non-proportional hazard assumption on the Kaplan Meier curves. Adjusted models included known prognostic markers for poor outcome: age, baseline cerebrospinal fluid (CSF) fungal count (quantitative cultures (QCCs) calculated as described elsewhere (11)), Glasgow Coma Score (GCS) scale, CSF white blood cell count, haemoglobin, antifungal treatment (flucytosine versus non-flucytosine regimens) and recruitment site.

A distinct cut-off for the duration a patient has been taking ART to help distinguish between patients experiencing 'unmasking' CM and those with virologic failure/non-adherence has not been clearly defined. Results from Rhein et al (10) suggested an increased mortality for patients receiving ART for ≤14 days compared to those on therapy for 15-182 days or >6 months. Therefore, we examined these time periods, and also conducted exploratory analyses to understand whether alternative time points with a cut-off of 1 month, 2 months, 3 months and 6 months could be used to distinguish sub-groups within the ART-experienced population and identify any differences in mortality. All analyses were performed in Stata version 15 ((StataCorp LP, College Station, TX)).

## Results

### *Outcomes by ART status*

A total of 678 patients were included in the study with 56% (381) ART-experienced at baseline (Figure 1). Overall, there was little difference in baseline demographics and clinical symptoms between ART-naïve and -experienced patients (Table 1), although both median plasma HIV viral load and median CSF fungal burden were higher for ART naive patients ($p < 0.001$). All-cause mortality was similar at 2-weeks (17% vs 20%, p=0.39; HR 0.85, 95%CI 0.6-1.2, p=0.35), and 10 weeks (38% vs 36%, p=0.64; HR 1.03, 95%CI 0.8-1.32, p=0.82) for ART-experienced vs ART-naïve patients, respectively, with similar results in adjusted analyses (data not shown).

### *Outcomes by ART duration*

Overall, 66% of patients (251/381) had data on ART duration (Figure 1). At recruitment study participants have been on ART for a median (IQR) time of 12 weeks (3 - 91 weeks). 19% (47/251) of patients had initiated ART within 14 days prior to presentation, and 61% (152/251) within 182 days (Table 1, Supplementary Table 1). There was similarity in the distribution of baseline demographics and clinical features between those taking ART for ≤14 days compared to those on ART for >14 days, though duration of headache was shorter for those taking ART for ≤14 days (median duration of 7 days vs 14 days; p=0.03) and CSF fungal burden was 1 log higher (Table 1). Comparing survival curves there was weak evidence of a trend towards increased mortality in those taking ART for ≤14 days, but there was no statistically significant difference in 2 week mortality (23% vs 15%; HR 1.70, 95% CI 0.85 to

3.39, p=0.13), and 10-week mortality was very similar (34% vs 38%; HR 0.92, 95% CI 0.54 to 1.57, p=0.76) (Fig 2), with similar results in adjusted analyses.

Comparable results were obtained when ART duration was divided into ≤2 weeks, 15-182 days and >182 days, and, with a 1-month cut-off (Supplementary. Table 1, Supplementary Figure 1). Higher viral loads and lower CD4 counts were identified for those reportedly on ART for >182 days compared to those on ART for shorter durations (Supplementary Table 1), consistent with the possibility that the former group had a higher proportion of non-adherent patients or patients who had developed ART resistance. There were no significant differences in 2- and 10-week mortality when exploring varying cut-off points for ART duration (Supplementary Table 2). Using a 1 month cut off, 2-week mortality was 23% for those taking ART for ≤1 month vs 14% for those taking ART for >1 month (p=0.08; HR: 1.74, 95%CI: 0.94 to 3.24, p=0.07) but this trend did not hold in the adjusted analysis (aHR: 1.44, 95%CI: 0.73 to 2.85, p=0.30).

## Discussion

Over half the patients with a first episode of CM included in this study were ART experienced with 14% having started ART in the 14 days prior to diagnosis. Whilst ART-naïve patients had higher median HIV plasma viral loads and fungal burdens, there was no evidence for an overall difference in mortality between ART-naïve and -experienced populations at both 2 weeks and 10 weeks. In exploratory analyses according to duration on ART, there was a trend in the unadjusted analysis of increased 2-week mortality for those taking ART for less than 1 month compared with those taking ART for >1 month, and the survival curves suggested the possibility of an increase in short-term mortality, between 1 and 2 weeks of antifungal treatment, in those taking ART for ≤2 weeks. Overall, however, we did not identify a cut off for ART duration that led to significant differences in short term mortality.

Our findings differ in some respects from the report of Rhein et al. The size of our study is similar to that of Rhein and colleagues in Uganda (10) and the findings are in many respects similar in terms of the patient populations and differences, for example, in CD4 cell counts and cryptococcal colony forming units (CFU) counts between groups. However, we did not find a significant difference in short-term mortality in those recently started on ART prior to presentation with cryptococcal meningitis, or as high a 2-week mortality in those who had

initiated ART within 2 weeks of presentation. This may simply be because both our studies were relatively small with only 51 and 47 patients respectively within the subgroup who had initiated ART within 2 weeks of presentation.

In addition, there were some differences in practice. In the study of Rhein, roughly a quarter of patients who had initiated ART within 2 weeks of presentation received corticosteroids and / or had ART withheld. In the ACTA trial, conducted prior to publication of the Uganda data, ART was not routinely withheld amongst participants who had commenced ART within 6 months of presentation with meningitis and who reported and were assessed as adherent to ART. Corticosteroids were also not used in this group, in the light of the results of the CryptoDex study (12), which found them not to be beneficial, including in the subgroup of patients who had initiated ART within 3 months of meningitis diagnosis.

It should be noted that results from this study were limited by missing data for a number of patients for duration of taking ART and the fact that the definition for ART exposure was broad. Therefore, the study was underpowered to detect differential mortality outcomes among the ART-experienced sub-groups. This is because assessing ART adherence retrospectively is very difficult. While several measures of checking adherence are operational in most ART programs in sub-Saharan Africa drug adherence continues to be an important problem with levels of non-compliance ranging between 2% and 70% (13).

Data from ongoing randomised studies may help clarify the most appropriate management for ART-experienced patients diagnosed with CM. The ongoing AMBITION-cm trial (14), and indeed data from all 3 studies combined (9, 10, 14) presents the opportunity to assess outcomes in patients on ART for various durations prior to meningitis. In the AMBITION-cm trial, based on a recent consensus among the investigators, including the team from Uganda, the current management strategy is to withhold ART for 4-6 weeks in those reportedly adherent and started or restarted on ART within the last 14 days, but to continue ART in those reportedly adherent and started or restarted on ART between 14 days and 6 months prior to presentation (5).

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## Patient Consent Statement

Written informed consent was obtained from all participants or their closest relative (for those with impaired mental status) prior to trial enrolment. The trial was approved by the London School of Hygiene and Tropical Medicine and country specific Research Ethics Committees.

## Potential Conflicts of Interest

Dr Hossenipour discloses receiving funding from Medical Research Council, UK. The rest of the authors have nothing to disclose.

### Author contributions

NK RH JE CKa CKu ET SM SL DC SL TN AC JVO MH OL SJ AL TH and SM conducted the study. TC and DW prepared the trial dataset. NK RH SM TH conceived this sub-study. NK and SM, with support from TC, analysed the data. NK SM RH TH drafted the manuscript. All authors critically reviewed and contributed to the final paper.

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Table 1: *Clinical presentation and outcomes by ART status and ART duration.*

| | ART status, no. (%) / median (IQR) | | | ART duration, no. (%) / median (IQR) | | |
|---|---|---|---|---|---|---|
| | **ART-naïve** (N=297) | **ART-experienced** (N=381) | **p-value** | **≤14 days** (N=47) | **>14 days** (N=204) | **p-value** |
| **Demographics** | | | | | | |
| Male | 173 (58%) | 217 (57%) | 0.74 | 30 (64%) | 118 (58%) | 0.45 |
| Headache duration (days) | 14 (7-21) | 14 (7-28) | 0.33 | 7 (7-20) | 14 (7-30) | **0.03** |
| Seizures (within 72 hours) | 54 (18%) | 65 (17%) | 0.70 | 9 (19%) | 32 (16%) | 0.56 |
| Confusion | 119 (40%) | 148 (39%) | 0.75 | 22 (47%) | 74 (36%) | 0.18 |
| Current TB | 36 (12%) | 62 (16%) | 0.13 | 8 (17%) | 37 (18%) | 0.86 |
| **Markers of HIV disease severity** | | | | | | |
| Anaemia (Hb<7g/dl) | 5 (2%) | 13 (4%) | 0.16 | 2 (4%) | 10 (5%) | 0.84 |
| CD4 count (cells/ml) | 25 (10-55) | 28 (10-68) | 0.51 | 41 (22-83) | 33 (12-78) | 0.10 |
| CD4 count <100 cells/ml | 250 (91%) | 312 (88%) | 0.10 | 33 (81%) | 164 (86%) | 0.38 |
| Viral load ($log_{10}$ copies/ml) | 5.4 (4.9-5.7) | 4.0 (2.5-5.0) | **<0.001** | 3.5 (2.9-3.9) | 3.8 (2.2-4.9) | 0.99 |
| **Markers of severe cryptococcal disease** | | | | | | |
| Age ≥50 (years) | 34 (12%) | 41 (11%) | 0.77 | 7 (15%) | 17 (8%) | 0.17 |
| GCS<15 | 76 (26%) | 87 (23%) | 0.41 | 15 (32%) | 42 (21%) | 0.10 |
| CSF opening pressure >25mmCSF | 125 (46%) | 159 (45%) | 0.73 | 21 (50%) | 94 (49%) | 0.88 |
| CSF WCC <5 cells/ml | 156 (56%) | 199 (54%) | 0.67 | 22 (51%) | 108 (55%) | 0.64 |
| CSF fungal count | 5.2 (4.2-5.7) | 4.7 (3.1-5.8) | **0.001** | 5.2 (3.9-5.9) | 4.2 (2.7-5.4) | **0.004** |

| | | | | | | |
|---|---|---|---|---|---|---|
| ($log_{10}$CFU/ml) | | | | | | |
| **Clinical management** | | | | | | |
| 5FC-based antifungals | 187 (63%) | 226 (70%) | 0.06 | 35 (75%) | 137 (67%) | 0.33 |
| Number LPs received | 3 (2-3) | 3 (2-3) | 0.9 | 3 (2-5) | 3 (3-4) | 0.68 |
| CSF clearance rate | 0.34 (0.23-0.50) | 0.33 (0.22-0.50) | 0.82 | 0.36 (0.27-0.63) | 0.32 (0.22-0.47) | 0.06 |
| **All-cause mortality** | | | | | | |
| 2 weeks | 59 (20%) | 66 (17%) | 0.39 | 11 (23%) | 30 (15%) | 0.15 |
| 10 weeks | 107 (36%) | 144 (38%) | 0.64 | 16 (34%) | 78 (38%) | 0.59 |

Abbreviations: LP: Lumbar puncture 5FC: Flucytosine WCC: White cell count Hb: Haemoglobin CFU: Colony forming units CSF: Cerebrospinal fluid

**Figure 1: Study Cohort**

**Figure 2: Kaplan Meier survival plot by ART status**

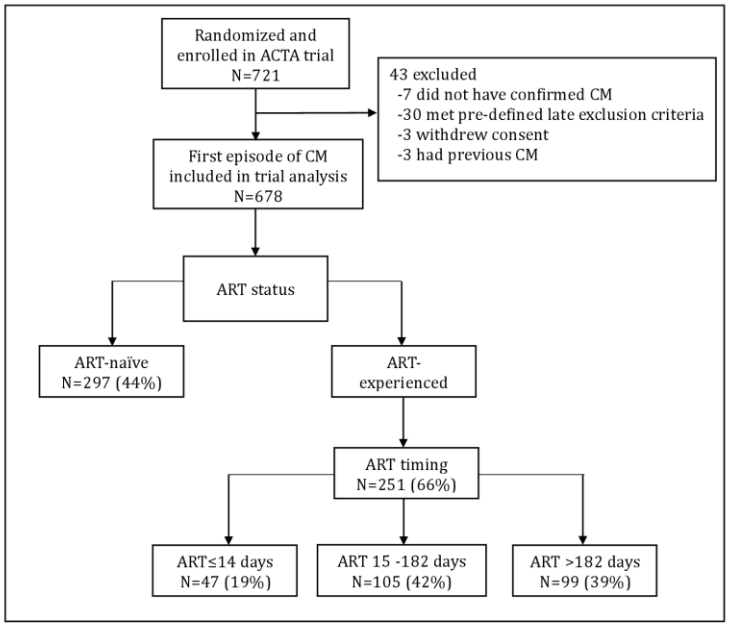


*Figure 1: Study cohort*

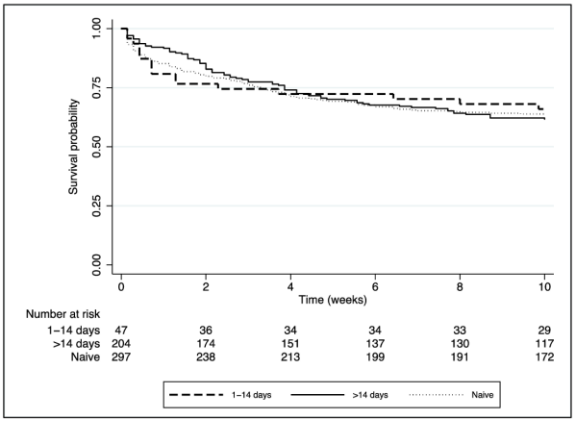


Figure 2: *Kaplan Meier surivival plot by ART status.*