Timing of antiretroviral therapy in cryptococcal meningitis: What we can (and cannot) learn from observational data

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HIV-associated cryptococcal meningitis continues to have acute mortality rates in excess of 15% even in well-resourced settings [1, 2]. Rapid restoration of immune function with early antiretroviral therapy (ART) in immunosuppressed patients could facilitate clearance of *Cryptococcus* and prevent other fatal opportunistic infections. However, this benefit must be balanced against the risk of immune reconstitution inflammatory syndrome (IRIS) if ART is initiated in the context of infection [3]. To address this uncertainty, a series of randomized controlled trials examining when to start ART in cryptococcal meningitis were conducted almost a decade ago [4-6], culminating in the definitive COAT trial, led by Boulware, that demonstrated early ART was associated with a 15% higher absolute mortality than delayed ART [7].

Should these randomized trials be the final word on ART timing in cryptococcal meningitis? The trials showing significant benefit of deferred ART were conducted in Africa, in patient populations who are not equivalent as in high-income settings. It is plausible that in the context of less severe disease and more fungicidal amphotericin and flucytosine based treatments, the risk/benefit balance may differ. In order to gain insights into the impact of ART timing on mortality in cryptococcal meningitis in high-income settings, Ingle et al. analyzed observational data drawn from three US/European cohorts {Ingle_CID_REFERENCE}. They conclude that their findings do not suggest increased mortality with early ART initiation. The key question is whether the observational data presented can support these conclusions? As we have seen during COVID, analyses of observational data have concluded that therapies such as hydroxychloroquine and ivermectin are effective, yet these have failed when tested in rigorous randomized trials [8, 9]. In the analyses presented by Ingle et al, several potentially important caveats exist.

Firstly, observational data are vulnerable to confounding by indication. How physicians practice medicine and make decisions cannot be fully accounted for with statistical adjustments, no matter how complex. In cryptococcal meningitis management, low-risk clinically stable patients deemed by a physician as ready to start ART are likely to have been given earlier ART, whereas clinically unstable (i.e. “sicker”) patients may have had their ART deferred, or never started -- pursuing hospice. Across the data reported in the current analysis, derived from 30 different observational cohorts over two decades from 1994-2012, individual physicians practice is impossible to standardize and quantify. Furthermore, the known markers of cryptococcal meningitis severity, e.g. Glasgow coma scale, CSF cryptococcal antigen titer, CSF quantitative culture, CSF white cell count, serum sodium, or hemoglobin [10-12] were not reported or included in statistical modeling. Mimicking trials using such observational data is not equivalent to randomized trials.

A second and perhaps even more consequential concern with this dataset is sampling bias. In this observational cohort of 630 Europeans and North Americans with cryptococcosis, 256 (41%) were lost to follow up with no outcome data and another 176 (28%) excluded for missing CD4 or HIV viral load. After further exclusions, Ingle et al report outcomes on just 190 individuals (30%), of whom 145 started ART. How many individuals started ART early during hospitalization then became “lost” is unknown. Prior experience strongly suggests that persons with a median CD4 count of 20 cells/μL with cryptococcal meningitis who are lost to follow up are often dead, creating a very probable ascertainment bias. It is unclear why these prospective cohort studies in high-income countries have such a high lost to follow up of consented research participants. Among three large, multinational African cryptococcal meningitis trials comprising 1712 participants, only 4 (0.2%) participants were lost [13-15]. As the excess mortality observed during the COAT trial was between 7-30 days after earlier ART initiation [7], persons given early ART and then “lost” could appreciably affect the study’s conclusion. Additionally, by excluding the 176 persons with missing CD4's or viral loads, the investigators removed a further 39 deaths from analysis; this excluded group’s mortality was 22.1% (39/176) which is significantly higher than those included in this study of 13.2% (25/190) (P=.028). Again, the authors do not report how many of those excluded initiated early ART.

Within the context of these major limitations, the authors reported no excess risk of death in a “mimicked trial” in which data from the 190 individuals not excluded from the initial cohort of 630 were cloned and included in each hypothetical trial arm; 56 initiated ART within 14 days and 68 delayed ART initiation after 14 days. In the mimicked trial, 13 deaths occurred with early ART compared to 20 with late ART, with no significant differences in survival between arms (95%CI for hazard ratio ranged from 0.64 to 2.56). A formal non-inferiority analysis with a pre-specified non-inferiority margin was not performed, which would be a standard expectation when declaring a strategy officially non-inferior.

Several other caveats need to be considered in interpreting these results. In the early ART group in this observational cohort, the median time to start ART was zero days, which no guidelines have ever recommended. Same day ART initiation in people just diagnosed with HIV presenting with a life-threatening neurological infection has never been standard practice in our experience. An alternative explanation for such efficiency could be that differences in database coding across 30 cohorts have introduced an error as to how zero is coded in records going as far back to 1994. Second, the categories compared in the mimicked trial are not distinct groups. The data were dichotomized at precisely 14 days, which is not comparable to “delayed” ART of

4-6 weeks as studied in the randomized trials versus <4 weeks. Numerous deaths occurred between 2-4 weeks, all assigned to the late ART group. The fact that the actual raw data are not presented means that readers cannot see when actual mortality occurred in relation to ART timing in the underlying cohort, and the authors will not publicly share their deidentified dataset. Third, those who never started ART are included in the delayed ART group. Thus, much of the comparison is early ART versus no ART. Whether hospice intent was present in those never starting ART is unknown. Lastly, the analytic method utilized means that the vast majority of hypothetical participants were censored at day 14 in the early ART group meaning any deaths after this time point were only considered in the delayed ART group.

If the lack of association between early ART initiation and excess mortality in high income settings reported by Ingle et al. is genuine, is it biologically plausible? The 15% mortality difference observed in the COAT trial was driven by two key groups who did worse with earlier ART initiation. The first were those with altered mental status at time of starting earlier ART. Earlier ART did not rescue such patients but was associated with excess mortality. Second, those lacking CSF pleocytosis (<5 white cells/μL) had ~30% better survival with deferred ART. Lack of CSF pleocytosis is a known risk factor for paradoxical immune reconstitution inflammatory syndrome (IRIS), [16, 17], and earlier ART was associated with an influx of CSF white cells and macrophage/microglial activation in those without pleocytosis [18]. Could differences in outcomes be explained by the European and North American cohorts having more people with CSF pleocytosis and less altered mental status versus the COAT trial? While certainly possible, no meningitis-related clinical data are presented in these European and North American cohorts, including rates of altered mental status or CSF pleocytosis, meaning that this hypothesis cannot be examined. The potential benefits of early ART in terms of aiding clearance of cryptococcal infection and preventing other opportunistic infections would be expected to be more marked in African cohorts with more severe baseline disease and higher rates of other infections than seen in Europe and North America, making this an unlikely explanation for better outcomes with early ART outside of the African context.

Our Ugandan team's clinical practice since 2013 among the last 1625 cryptococcosis patients has been to defer ART for 4-6 weeks. This delay allows for focused diagnostics for opportunistic infections [19], initiating trimethoprim-sulfamethoxazole prophylaxis, giving TB preventive therapy (e.g. 1 month of isoniazid and rifapentine), allowing neurocognitive function recovery [20], and initiating consolidation fluconazole therapy at 800mg/day to assure CSF culture sterility before starting ART [17, 21]. Our paradoxical IRIS incidence has fallen to $\leq$5% with this strategy. The incidence of paradoxical IRIS in these US/European cohorts is unknown. With early ART in one randomized trial in Botswana, 7 of 13 who initiated ART at a median of 7 days developed paradoxical IRIS [5].

Clinical trials are difficult to conduct. Careful analysis of observational data plays an important role in informing clinical care. Mimicking clinical trials using "big data" is an interesting and increasingly used; however, complex statistical methods can never overcome the limitations inherent in observational datasets, especially when large amounts of missing data exist. Small observational studies cannot replace appropriately powered randomized clinical trials. To determine whether the impact of early ART initiation in cryptococcal meningitis differs in high-income countries and whether guidelines should change, the authors would need to conduct further randomized clinical trials.

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References:

1. Rajasingham R, Govender NP, Jordan A, et al. The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. *Lancet Infect Dis* **2022**; 22: 1748-55.
2. Hevey MA, Presti RM, O'Halloran JA, et al. Mortality After Cryptococcal Infection in the Modern Antiretroviral Therapy Era. *J Acquir Immune Defic Syndr* **2019**; 82: 81-7.
3. Boulware DR, Meya DB, Bergemann TL, et al. Clinical features and serum biomarkers in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis: a prospective cohort study. *PLoS Med* **2010**; 7: e1000384.
4. Zolopa A, Andersen J, Powderly W, et al. Early antiretroviral therapy reduces AIDS progression/death in individuals with acute opportunistic infections: a multicenter randomized strategy trial. *PLoS One* **2009**; 4: e5575.
5. Bisson GP, Molefi M, Bellamy S, et al. Early versus delayed antiretroviral therapy and cerebrospinal fluid fungal clearance in adults with HIV and cryptococcal meningitis. *Clin Infect Dis* **2013**; 56: 1165-73.
6. Makadzange AT, Ndhlovu CE, Takarinda K, et al. Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-saharan Africa. *Clin Infect Dis* **2010**; 50: 1532-8.
7. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med* **2014**; 370: 2487-98.
8. Skipper CP, Pastick KA, Engen NW, et al. Hydroxychloroquine in Nonhospitalized Adults with Early COVID-19 : A Randomized Trial. *Ann Intern Med* **2020**; 173: 623-31.
9. Bramante CT, Huling JD, Tignanelli CJ, et al. Randomized Trial of Metformin, Ivermectin, and Fluvoxamine for Covid-19. *N Engl J Med* **2022**; 387: 599-610.
10. Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis* **2014**; 58: 736-45.
11. Tugume L, Fieberg A, Ssebambulidde K, et al. Association of Hyponatremia on Mortality in Cryptococcal Meningitis: A Prospective Cohort. *Open Forum Infect Dis* **2022**; 9: ofac301.
12. Tugume L, Morawski BM, Abassi M, et al. Prognostic implications of baseline anaemia and changes in haemoglobin concentrations with amphotericin B therapy for cryptococcal meningitis. *HIV Med* **2017**; 18: 13-20.
13. Sloan D, Dlamini S, Paul N, Dedicoat M. Treatment of acute cryptococcal meningitis in HIV infected adults, with an emphasis on resource-limited settings. *Cochrane Database Syst Rev* **2008**; *In Press*. doi:10.1002/14651858.CD005647.pub2CD005647.
14. Jarvis JN, Lawrence DS, Meya DB, et al. Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis. *N Engl J Med* **2022**; 386: 1109-20.
15. Molloy SF, Kanyama C, Heyderman RS, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. *N Engl J Med* **2018**; 378: 1004-17.
16. Boulware DR, Bonham SC, Meya DB, et al. Paucity of initial cerebrospinal fluid inflammation in cryptococcal meningitis is associated with subsequent immune reconstitution inflammatory syndrome. *J Infect Dis* **2010**; 202: 962-70.

17. Chang CC, Dorasamy AA, Gosnell BI, et al. Clinical and mycological predictors of cryptococcosis-associated immune reconstitution inflammatory syndrome. *AIDS* **2013**; 27: 2089-99.
18. Scriven JE, Rhein J, Hullsiek KH, et al. Early ART After Cryptococcal Meningitis Is Associated With Cerebrospinal Fluid Pleocytosis and Macrophage Activation in a Multisite Randomized Trial. *J Infect Dis* **2015**; 212: 769-78.
19. Cresswell FV, Ellis J, Kagimu E, et al. Standardized Urine-Based Tuberculosis (TB) Screening With TB-Lipoarabinomannan and Xpert MTB/RIF Ultra in Ugandan Adults With Advanced Human Immunodeficiency Virus Disease and Suspected Meningitis. *Open Forum Infect Dis* **2020**; 7: ofaa100.
20. Carlson RD, Rolfes MA, Birkenkamp KE, et al. Predictors of neurocognitive outcomes on antiretroviral therapy after cryptococcal meningitis: a prospective cohort study. *Metab Brain Dis* **2014**; 29: 269-79.
21. Rolfes MA, Rhein J, Schutz C, et al. Cerebrospinal Fluid Culture Positivity and Clinical Outcomes After Amphotericin-Based Induction Therapy for Cryptococcal Meningitis. *Open Forum Infect Dis* **2015**; 2: ofv157.