Benefits of enhanced infection prophylaxis at antiretroviral therapy initiation by cryptococcal antigen status

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Objectives: To assess baseline prevalence of cryptococcal antigen (CrAg) positivity; and its contribution to reductions in all-cause mortality, deaths from cryptococcus and unknown causes, and new cryptococcal disease in the REALITY trial.

Design: Retrospective CrAg testing of baseline and week-4 plasma samples in all 1805 African adults/children with $CD4^+$ cell count less than 100 cells/μl starting antiretroviral therapy who were randomized to receive 12-week enhanced-prophylaxis (fluconazole 100 mg/day, azithromycin, isoniazid, cotrimoxazole) vs. standard-prophylaxis (cotrimoxazole).

Methods: Proportional hazards models were used to estimate the relative impact of enhanced-prophylaxis vs. standard-cotrimoxazole on all, cryptococcal and unknown deaths, and new cryptococcal disease, through 24 weeks, by baseline CrAg positivity.

Results: Excluding 24 (1.4%) participants with active/prior cryptococcal disease at enrolment (all treated for cryptococcal disease), 133/1781 (7.5%) participants were CrAg-positive. By 24 weeks, 105 standard-cotrimoxazole vs. 78 enhanced-prophylaxis participants died. Of nine standard-cotrimoxazole and three enhanced-prophylaxis cryptococcal deaths, seven and two, respectively, were CrAg-positive at baseline. Among deaths of unknown cause, only 1/46 standard-cotrimoxazole and 1/28 enhanced-prophylaxis were CrAg-positive at baseline. There was no evidence that relative reductions in new cryptococcal disease associated with enhanced-prophylaxis varied between baseline CrAg-positives [hazard-ratio = 0.36 (95% confidence interval 0.13–0.98), incidence 19.5 vs. 56.5/100 person-years] and CrAg-negatives [hazard-ratio = 0.33 (0.03–3.14), incidence 0.3 vs. 0.9/100 person-years; $P_{heterogeneity} = 0.95$]; nor for all deaths, cryptococcal deaths or unknown deaths ($P_{heterogeneity} > 0.3$).

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Conclusion: Relative reductions in cryptococcal disease/death did not depend on CrAg status. Deaths of unknown cause were unlikely to be cryptococcus-related; plausibly azithromycin contributed to their reduction. Findings support including 100 mg fluconazole in an enhanced-prophylaxis package at antiretroviral therapy initiation where CrAg screening is unavailable/impractical.

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Introduction

Cryptococcal disease continues to have high morbidity and mortality in advanced HIV disease in sub-Saharan Africa, despite improved antifungal regimens for treatment [1], and combination antiretroviral therapy (ART) [2]. The screening test of choice is for cryptococcal antigen (CrAg), for asymptomatic individuals in blood, and for symptomatic individuals in cerebrospinal fluid (CSF) to identify meningitis [3]. The global prevalence of cryptococcal antigenaemia in HIV-infected adults with $CD4^+$ cell count less than 100 cells/μl is ∼6%, although higher prevalences have been reported [4]. Using the CrAg latex agglutination assay, the average time between CrAg detection in blood and the onset of symptomatic cryptococcal disease is ∼3 weeks [5], and is likely even longer with more sensitive lateral flow assays (LFA) [6], allowing the opportunity to intervene with antifungal prophylaxis or treatment [5]. A recent cross-sectional study in South Africa [7] confirmed that 90% of CrAg-positive patients with headache as their only reported symptom had CrAg-positive CSF, as did 34% of asymptomatic CrAg-positive patients.

WHO guidelines [3] recommend a '(CrAg) screen and treat' approach to preventing cryptococcal disease, with CrAg-positive individuals receiving preemptive fluconazole treatment (800 mg/day for 2 weeks) then maintenance (400 mg/day for 8 weeks). This recommendation was based on the REMSTART trial [8] which showed significant mortality reductions in HIV-infected adults in Tanzania and Zambia initiating ART with $CD4^+$ cell count less than 200 cells/μl with this approach. One challenge with 'screen and treat' in high-risk populations is that CrAg testing kits are frequently unavailable in low and middle-income countries, especially at primary healthcare centres where ART is increasingly initiated. Furthermore, even when kits are available, waiting for CrAg results can considerably delay starting ART in patients at high risk of immediate morbidity/mortality, particularly if the CrAg test is not performed on the same residual specimen from $CD4^+$ testing, if the latter is requested [9].

An alternative approach is universal prophylaxis in high-risk populations. The REALITY trial (ISRCTN43622374) demonstrated that a package of enhanced-prophylaxis, comprising cotrimoxazole (as fixed dose combination with isoniazid/pyridoxine), fluconazole (100 mg/day for 12 weeks) azithromycin (500 mg/day for 5 days) and albendazole (single dose), significantly reduced all-cause mortality, deaths from cryptococcus and unknown causes, and incidence of new cryptococcal disease and tuberculosis (TB), compared with standard-cotrimoxazole prophylaxis alone. Patients were African adults and children more than 5 years initiating ART with $CD4^+$ cell count less than 100 cells/μl [10]. The total dose per week (700 mg) and duration of fluconazole used in REALITY was based on a previous trial in Uganda, showing benefit of fluconazole 200 mg three times weekly (total 600 mg/week) until $CD4^+$ count reached at least 200 cells/μl [11]. However, dosing was daily in REALITY to match ART dosing schedules.

Given these findings, current WHO cryptococcal guidelines [3] also recommend that, where there is no access to CrAg testing or delays in returning results, fluconazole can be offered as primary prophylaxis in advanced HIV at the time of ART initiation or switch, using the REALITY dose of 100 mg/day or alternatively 200 mg three times/week [3].

Baseline CrAg testing was not routinely performed in real-time in REALITY. Therefore, it was unknown whether reductions in cryptococcal disease and deaths were restricted to baseline CrAg-positives, and whether the significant reductions in deaths from unknown causes associated with enhanced-prophylaxis could have been due to missed cryptococcal disease (and hence plausibly reduced by fluconazole prophylaxis), or whether reductions might be driven by other components of the package. The aims of this substudy were therefore to estimate baseline CrAg prevalence, and to assess its contribution to the significant reductions in all-cause and cryptococcal-related/unknown mortality, and cryptococcal-related morbidity observed in the REALITY trial.

Methods

CrAg LFA qualitative and quantitative testing was performed retrospectively between May 2017 and February 2018, blinded to randomized group and patient characteristics, using 40 μl of frozen plasma samples

stored at baseline (day of enrolment) and 4 weeks after ART initiation, from all REALITY participants. If CrAg-positive, CrAg titre was estimated using the semiquantitative dilution technique as per package insert. Any sample that was positive on qualitative testing, but not using any of the dilution steps, was assigned a titre of 1 : 2.5 (half the lowest titre of 1 : 5). Results were verified, blinded to randomization, by central review of photographs of the testing strips. Testing was performed at one central laboratory within each country by staff trained in qualitative and semiquantitative CrAg testing using the IMMY LFA kits supplied by Alpha Laboratories Limited, Eastleigh, UK. CrAg testing was included in the main trial protocol and approved by Ethics Committees in Zimbabwe, Uganda, Malawi, Kenya and the United Kingdom.

All clinical events in the trial up to 48 weeks were ascertained at prespecified trial visits or additional visits for acute illness. Nurse visits at weeks 2, 4, 8, 12, 18, 24, 36 and 48 included a symptom checklist which included severe headache amongst solicited symptoms; history and examination by a clinician was performed at weeks 4, 12, 24, 36 and 48. All defaulting participants were traced through home visits and telephone calls. An Endpoint Review Committee (ERC) (majority independent members) adjudicated causes of death and nonfatal events (WHO 3/4 events/grade 3/4 adverse events/serious adverse events) using clinical narratives written by treating clinicians, incorporating imaging scans/reports and laboratory results, including CrAg-positive status (usually in blood) if this was measured locally in real-time. Definitive cryptococcal meningitis was defined as clinical meningitis (severe headache, meningism, photophobia) with a positive CSF CrAg test and/or CSF microscopy (positive India ink stain and/or CSF culture positive). A probable diagnosis was defined as a consistent clinical history and a positive plasma CrAg test (or fungaemia) in the absence of any CSF results. Events were adjudicated retrospectively by at least two ERC members, blinded to randomized groups, against protocol-defined criteria and grading tables [10,12]. Compatibility of fatal and nonfatal event(s) with immune reconstitution inflammatory syndrome (IRIS) was documented based on clinical and diagnostic information (often limited) and the time course after ART initiation. No distinction was made between paradoxical and unmasking IRIS given the limited information available. The ERC did not have access to viral load data as these were done retrospectively; the earliest post-randomization $CD4^+$ cell count results were at week 4. Therefore for early events, a clinical judgement was made using baseline data (including $CD4^+$ cell counts) and previously published definitions [13,14] in modified form, to determine whether event(s) represented an atypical/exaggerated presentation of an opportunistic infection or tumour soon after ART initiation (i.e. were IRIS-compatible).

Statistical analysis

As our first aim was to estimate the prevalence of latent cryptococcal infection prior to ART initiation with $CD4^+$ cell count less than 100 cells/μl, participants with a diagnosis of cryptococcal meningitis at or prior to baseline were excluded from all analyses; all were treated for cryptococcal disease. Logistic regression with backwards elimination ($P > 0.1$ to fit an exploratory model, including nonlinearity by fractional polynomials where $P < 0.05$) was used to identify independent predictors of baseline CrAg status in the remaining asymptomatic individuals, from age, sex, WHO stage, BMI, $CD4^+$, viral load (VL) and haemoglobin, adjusting for site of enrolment.

We then considered the time-to-event outcomes of mortality [all-cause, cryptococcal and from unknown causes as determined by the ERC (where enhanced-prophylaxis had significant benefits in the trial overall)], new cryptococcal disease (fatal and nonfatal), cryptococcal IRIS, and determined or undetermined central nervous system (CNS) events (fatal or nonfatal) (Supplementary Table 1, http://links.lww.com/QAD/B959). Analyses used competing risks methods for patients dying of other causes without having recorded the event of interest [15]. Absolute rates of each outcome by baseline CrAg status were calculated through 24 weeks on ART (time of the main trial primary endpoint) when most clinical events had occurred [16]. Proportional (sub)hazards models were used to estimate heterogeneity in the relative impact of enhanced-prophylaxis vs. standard-cotrimoxazole by CrAg status over the first 24 weeks using interaction tests.

Results

All 1805 REALITY participants (98% aged ≥13 years) had a baseline CrAg test using stored plasma. We excluded 23 (1.3%) participants being treated for local physician-identified active cryptococcal disease at baseline (on stored samples 22 were CrAg-positive with titres 1 : 1280–1 : 2560; one CrAg-negative) and one participant with previous cryptococcal disease (CrAg-positive on stored sample at enrolment, titre 1 : 2560, on 400 mg fluconazole), leaving 1781 participants without identified cryptococcal disease at baseline in the analyses (Table 1).

Prevalence of cryptococcal antigen positivity at baseline (antiretroviral therapy initiation)

133/1781 [7.5%, 95% confidence interval (CI) 6.3–8.8%] participants were CrAg-positive at ART initiation, 69/888 (7.8%) in the standard-cotrimoxazole vs. 64/893 (7.2%) in enhanced-prophylaxis group ($P = 0.65$, Table 1). In CrAg-positives, the median CrAg titre was 1 : 80 [interquartile range (IQR) 1 : 10–1 : 640] (range <1 : 5–

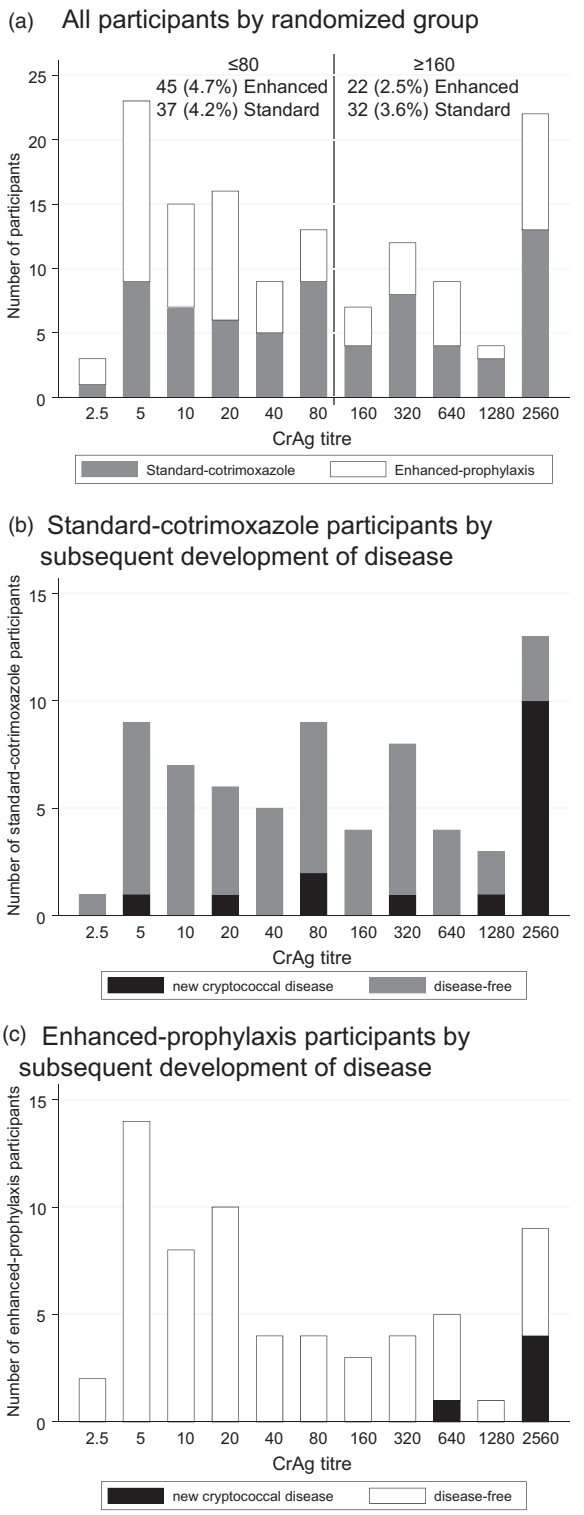
Table 1. Baseline characteristics of participants without active cryptococcal disease at antiretroviral therapy initiation in the REALITY trial.

Factor	CrAg-negative, $N=1648$	CrAg-positive, $N=133$	P
Standard-cotrimoxazole	819 (92.2%)	69 (7.8%)	0.65
Enhanced-prophylaxis	829 (92.8%)	64 (7.2%)	
Country and site			0.048
Zimbabwe – Harare	517 (92.2%)	44 (7.8%)	
Uganda – Mbarara	210 (94.2%)	13 (5.8%)	
Uganda – Gulu	128 (92.1%)	11 (7.9%)	
Uganda – Fort Portal	132 (96.4%)	5 (3.6%)	
Uganda – Mbale	106 (89.8%)	12 (10.2%)	
Malawi – Blantyre	226 (89.0%)	28 (11.0%)	
Kenya – Eldoret	192 (92.3%)	16 (7.7%)	
Kenya – Kilifi	137 (97.2%)	4 (2.8%)	
Sex			0.21
Male	867 (91.7%)	78 (8.3%)	
Female	781 (93.4%)	55 (6.6%)	
Age at last birthday (years)	36 (29–42)	38 (31–44)	0.058
WHO stage			0.78
1	279 (93.0%)	21 (7.0%)	
2	510 (92.1%)	44 (7.9%)	
3	637 (92.2%)	54 (7.8%)	
4	222 (94.1%)	14 (5.9%)	
$CD4^+$ cell count[a] (cells/μl)	38 (17–64)	30 (12–52)	0.003
VL (log_{10} c/ml)	5.4 (5.0–5.8)	5.5 (5.0–5.8)	0.76
BMI (kg/m^2) ($N=1773$)	19.2 (17.3–21.4)	18.9 (17.1–21.2)	0.51
Haemoglobin (g/dl) ($N=1776$)	11.2 (9.6–12.7)	11.0 (9.6–13.2)	0.69
On fluconazole prior to randomization[b]			0.76
No	1489 (92.6%)	119 (7.4%)	
Yes	159 (91.9%)	14 (8.1%)	
Fluconazole prescribed at randomization[b]			1.00
No	1520 (92.5%)	123 (7.5%)	
Yes	128 (92.8%)	10 (7.2%)	
Reporting severe headache at enrolment			0.32
No	1579 (92.3%)	131 (7.7%)	
Yes	57 (96.6%)	2 (3.4%)	

Note: Showing *n* (row %) or median (IQR). Comparisons made using exact tests for categorical variables and rank sum tests for continuous variables. CrAg, cryptococcal antigen; IQR, interquartile range; VL, viral load.
[a]Mean of screening and enrolment values.
[b]Outside of the randomization for other reasons, generally treatment of oral candidiasis.

1 : 2560) (1 : 80 standard-cotrimoxazole vs. 1 : 20 enhanced-prophylaxis, $P=0.06$) (Fig. 1a).

As expected, the median $CD4^+$ was slightly lower in CrAg-positives (30 vs. 38 cells/μl in CrAg-negatives, $P=0.003$), but there was no evidence of differences in VL (median log_{10} VL 5.5 vs. 5.4, respectively, $P=0.76$). 173 (9.6%) participants enrolled in the trial had received fluconazole in the 14 days before randomization, [mostly (79%) 200 mg daily for oral candida infection]. However, baseline CrAg-positivity did not differ by receipt of prior fluconazole [14/173 (8.1%)] or not [119/1608 (7.4%), $P=0.76$, Table 1]. 59 (3.3%) participants reported severe headache at enrolment (as a nurse-solicited symptom),



Note: panels (B) and (C) indicate all new cryptococcal disease observed in the trial through week 48. Two standard-cotrimoxazole participants with titres 80 and 2560 developed new cryptococcal disease after week 24 and are therefore shown in panel (b) but not in Figures 2 or 3.

Fig. 1. Baseline cryptococcal antigen titres.

Table 2. Baseline cryptococcal antigen status in those experiencing different types of events before 24 weeks.

	Standard prophylaxis: baseline CrAg positive/Total (%)		Enhanced prophylaxis: baseline CrAg positive/Total (%)		All participants: baseline CrAg positive/Total (%)	
All deaths	11/105	(10%)	5/78	(6%)	16/183	(9%)
Deaths from cryptococcus	7/9	(78%)	2/3	(67%)	9/12	(75%)
Deaths from unknown causes	1/46	(2%)	1/28	(4%)	2/74	(3%)
Tuberculosis deaths	1/22	(5%)	1/17	(6%)	2/39	(5%)
Deaths from severe bacterial infections	0/9	(0%)	0/12	(0%)	0/21	(0%)
Deaths from other causes	2/19	(11%)	1/18	(6%)	3/37	(8%)
Cryptococcal disease	14/17	(82%)	5/6	(83%)	19/23	(83%)
Baseline titre 1 : 2560[a]	*9*		*4*		*13*	
Cryptococcal IRIS	13/16	(81%)	4/5	(80%)	17/21	(81%)
Determined CNS events	15/19	(79%)	6/11	(55%)	21/30	(70%)
Undetermined CNS events	0/18	(0%)	2/20	(10%)	2/38	(5%)

Note: Cause of death as determined by the Endpoint Review Committee. The same event could be counted in multiple categories, for example cryptococcal death could also be cryptococcal IRIS. See Supplementary Table 1, http://links.lww.com/QAD/B959 for definitions of CNS events.
CNS, central nervous system; CrAg, cryptococcal antigen; IRIS, inflammatory syndrome.
[a]See Fig. 1.

but baseline CrAg-positivity did not differ in those reporting [2/59 (3.4%)] and not reporting [131/1710 (7.7%)] severe headache ($P = 0.32$, Table 1). Considering factors in Table 1, the only independent predictors of CrAg-positivity were a lower $CD4^+$ cell count [odds ratio (OR) = 0.89 per 10 cells/μl higher (95% CI 0.83–0.95) $P = 0.001$] and being older [OR per 10 years older = 1.19 (1.00–1.42) $P = 0.046$] at ART initiation. Accounting for $CD4^+$ cell count and age, CrAg-positivity was significantly lower amongst individuals recruited from Kilifi, Kenya ($P = 0.03$). Even considering many other factors reflecting clinical status [17], only night sweats [OR = 1.67 (0.98–2.85) $P = 0.06$] added weak predictive power to the model.

Mortality

Enhanced-prophylaxis significantly reduced all-cause mortality and deaths from cryptococcus and of unknown cause, with no evidence of effect on deaths from TB or other causes [10,16]. Of the 12 deaths before 24 weeks adjudicated by the ERC as due to cryptococcal disease, nine were CrAg positive at baseline on retrospective testing (7/9 deaths on standard-cotrimoxazole, 2/3 deaths on enhanced-prophylaxis; Table 2). In contrast, of the 74 deaths before 24 weeks adjudicated as due to unknown causes (many dying away from a healthcare facility) (Supplementary Fig. 2, http://links.lww.com/QAD/B958), only two were CrAg-positive at baseline (1/46 deaths on standard-cotrimoxazole, 1/28 deaths on enhanced-prophylaxis) (Table 2). Proportions who were baseline CrAg-positive were similarly low for deaths adjudicated to be from TB, severe bacterial infections or other causes (Table 2; Supplementary Fig. 2, http://links.lww.com/QAD/B958).

As expected, absolute rates of cryptococcal deaths were very high in those who were CrAg-positive at baseline (solid symbols in Fig. 2), and low in those who were CrAg-negative at baseline (hollow symbols in Fig. 2). However, there was no evidence that relative benefits from enhanced-prophylaxis differed by baseline CrAg status for cryptococcal deaths ($P_{\text{heterogeneity}} = 0.73$) (Fig. 3); nor was there any evidence of variation for all-cause mortality ($P_{\text{heterogeneity}} = 0.39$), or deaths from unknown causes ($P_{\text{heterogeneity}} = 0.67$).

Cryptococcal disease and cryptococcal immune reconstitution inflammatory syndrome-compatible events

Over the first 24 weeks on ART, new cryptococcal meningitis occurred in 17 standard-cotrimoxazole vs. six enhanced-prophylaxis participants ($P = 0.03$), diagnosed a median 20 days post-ART initiation (IQR 15–45) (16 vs. 5 adjudicated as cryptococcal-IRIS respectively). Two additional cases were diagnosed after 24 weeks, both in the standard-cotrimoxazole group (not included in time-to-event analyses through 24 weeks). Fourteen of 17 standard-cotrimoxazole vs. five of six enhanced-prophylaxis cryptococcal meningitis cases were CrAg-positive at baseline (13/16 vs. 4/5 cryptococcal IRIS-compatible cases, respectively) (Table 2). Most CrAg-positives who developed cryptococcal disease had baseline titres of 1 : 2560 (Table 2), with no clear gradient below 1 : 2560 (Fig. 1b/c). Of these 23 patients with new cryptococcal disease during the trial, 53% (9/17) and 50% (3/6) died in the standard-cotrimoxazole and enhanced-prophylaxis groups respectively (exact $P = 1.00$).

As expected, similarly to cryptococcal deaths, the absolute incidence of cryptococcal disease was significantly greater in those who were CrAg-positive vs. CrAg-negative at baseline ($P < 0.0001$), regardless of randomization (solid vs. hollow symbols respectively, Fig. 2). However, similarly to cryptococcal mortality, there was no evidence that the relative benefits of enhanced prophylaxis differed by baseline CrAg status ($P_{\text{heterogeneity}} = 0.95$ for cryptococcal disease, 0.97 for cryptococcal IRIS-compatible disease), with an overall

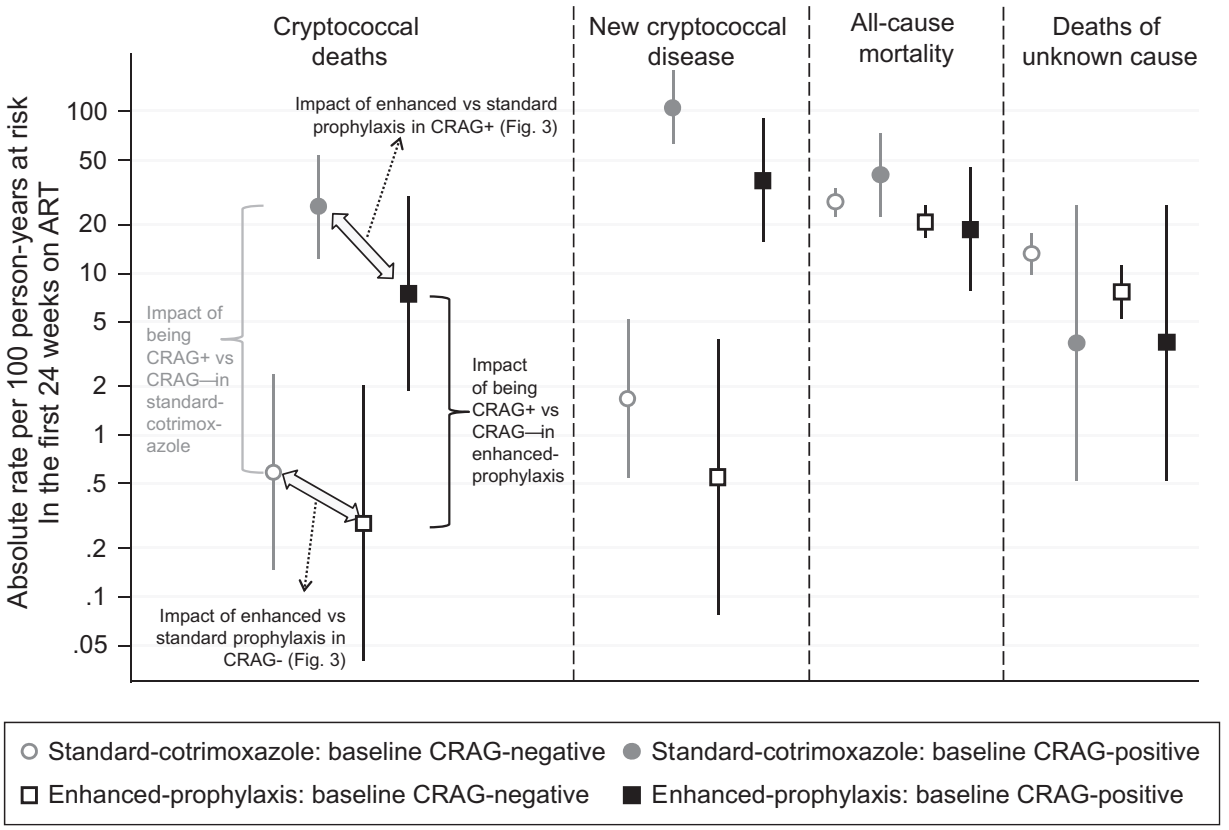


Fig. 2. Absolute rates of key events through week-24.

risk reduction of 0.36 (95% CI 0.13–0.98) in cryptococcal disease associated with enhanced-prophylaxis (including 100 mg fluconazole daily) in those CrAg-positive at baseline. Results were not influenced by the small proportion of participants prescribed fluconazole at enrolment outside of the randomization [138/1781 (7.7%), predominantly (83%) at a dose of 200 mg daily for oral/oesophageal candida (Supplementary Results, http://links.lww.com/QAD/B959)].

Determined CNS events (Supplementary Table 1, http://links.lww.com/QAD/B959) were predominantly cryptococcal meningitis, so results were similar to those for new cryptococcal disease. In contrast undetermined CNS events occurred similarly between the randomized groups (Table 2, Fig. 3).

Cryptococcal antigen titres

In baseline CrAg-positives with titres between 1 : 2.5 and 1 : 1280, cryptococcal disease occurred during the first 24 weeks on ART in five of 56 (9%) standard-cotrimoxazole vs. one of 55 (2%) enhanced-prophylaxis participants (Fig. 1b/c). At week 4, overall CrAg positivity was 7.9% (95% CI 6.7–9.3%) (130/1642 participants with data, excluding those developing cryptococcal disease between enrolment and week 4). Ninety-five (5.8%) were positive at both baseline and week 4, with median no change in doubling dilution (IQR 0 to +1; $P = 0.78$ comparing standard-cotrimoxazole vs. enhanced-prophylaxis) (Supplementary Fig. 1, http://links.lww.com/QAD/B958). Thirty-five (2.1%) became positive at week 4 having been negative at baseline (18 enhanced-prophylaxis, 17 standard-cotrimoxazole), whereas 15 (0.9%) became negative having been positive at baseline [nine enhanced-prophylaxis (one presumptively treated with 200 mg fluconazole for oral candida; others receiving 100 mg), six standard-cotrimoxazole (one presumptively treated with 1200 mg fluconazole daily for headache, others not receiving fluconazole)] (McNemar $P = 0.005$; Supplementary Results, http://links.lww.com/QAD/B959).

Discussion

In the four sub-Saharan African countries that enrolled participants with advanced HIV starting ART into the REALITY trial, we found a 7.5% prevalence of CrAg positivity with no clinically apparent cryptococcal disease. While CrAg positivity increased as baseline $CD4^+$ cell count decreased from 100 to 0 cells/μl, the impact of baseline $CD4^+$ cell count on CrAg positivity was relatively small. CrAg positivity rates were higher in

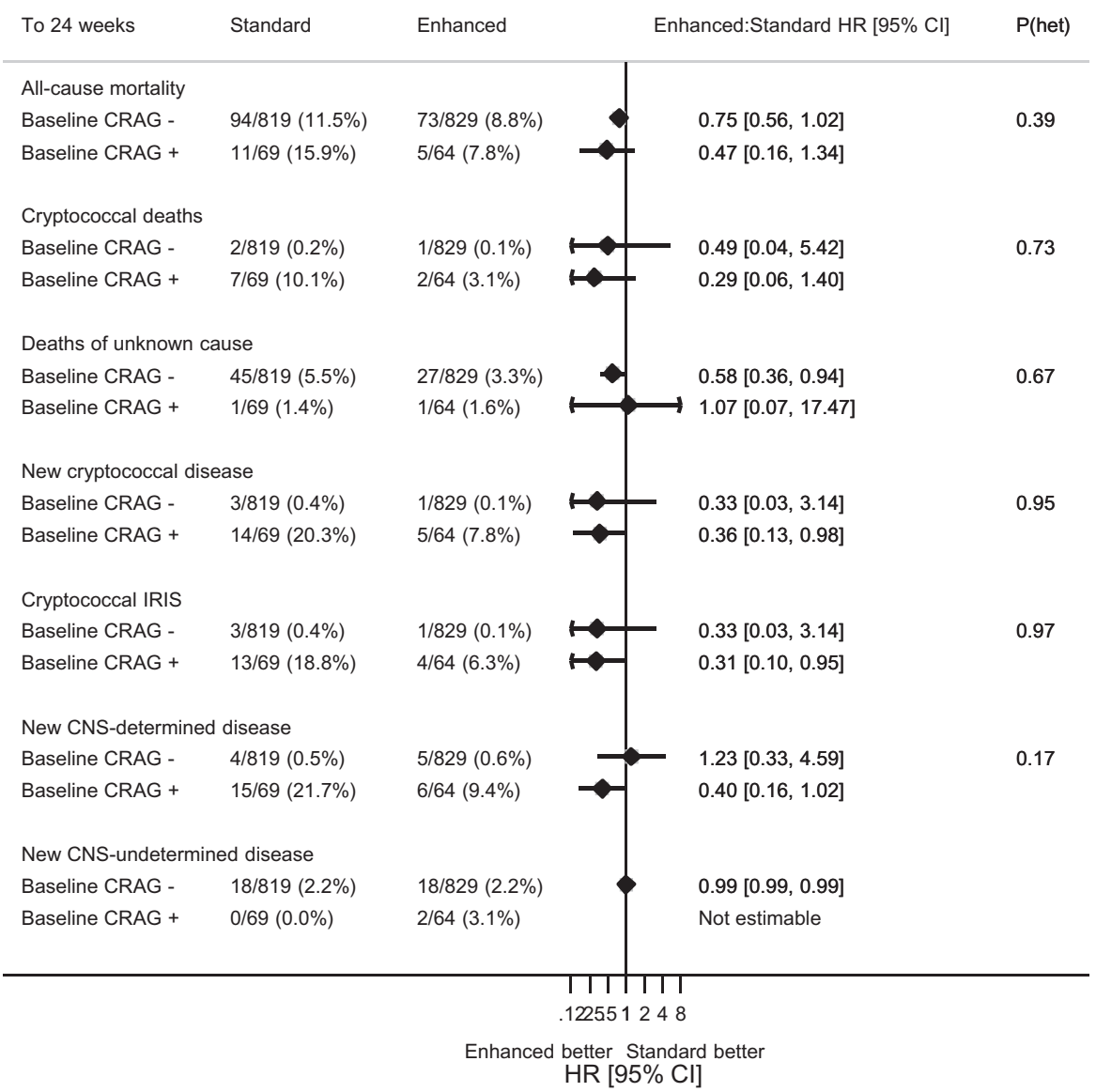


Fig. 3. Relative impact of enhanced-prophylaxis vs. standard-cotrimoxazole through 24 weeks by baseline cryptococcal antigen status.

older individuals, consistent with the known epidemiology [18]. We found no other predictors of CrAg positivity that could be used to target fluconazole prophylaxis or preemptive treatment where CrAg screening is not available.

As previously reported [10,16], the REALITY enhanced-prophylaxis package was associated with significantly lower mortality from ERC-adjudicated cryptococcus and unknown causes of death. Here we demonstrate that undiagnosed cryptococcus at baseline was not a driver of reductions in early deaths from unknown causes, since very few of these participants were CrAg-positive. As the CrAg test we used is both highly sensitive and specific, precedes clinical disease by several weeks and, in turn, remains positive for several weeks, this finding strongly suggests that deaths from unknown causes were not predominantly due to cryptococcus. Instead, the reduction in early deaths from unknown causes in the enhanced-prophylaxis group is plausibly due to another component of the enhanced-prophylaxis package. Possible candidates are isoniazid or azithromycin. TB was a relatively common diagnosis in the trial [16], with most sites using GeneXpert. Enhanced-prophylaxis was associated with reductions in TB disease, but not TB-related deaths, making this a less likely explanation, although TB can be difficult to diagnose in this population with advanced HIV. Azithromycin is a broad-spectrum macrolide with a long intracellular half-life in macrophages [19] and potential efficacy against severe respiratory and gastrointestinal bacterial infections common in Africa, especially in advanced HIV. In this setting,

azithromycin could also have had activity against toxoplasmosis [20], atypical mycobacteria [21], malaria [22] and/or as an anti-inflammatory agent [19,23]. However, it is also theoretically possible that fluconazole could have contributed to reduction in unknown deaths through noncryptococcal pathways, for example by affecting other fungi (e.g. candida oesophagitis leading to bacterial translocation) or through gut microbiome changes [24].

While there was no evidence that the relative clinical benefits of enhanced-prophylaxis differed among CrAg-positive and CrAg-negative participants, as expected the absolute benefits were much greater amongst CrAg-positives, who are at much higher absolute risk of developing overt cryptococcal disease. Reasons for observing clinical benefits from fluconazole prophylaxis in baseline CrAg-negative participants include false-negatives at baseline, unmasking of cryptococcal disease post-ART initiation (particularly given the low $CD4^+$ cell counts at ART initiation), or new acquisition of cryptococcus after ART initiation. False-negative CrAg are relatively rare, but even a 0.5% false-negative rate would have led to eight false-negatives in our population. The latter two scenarios (i.e. unmasking of cryptococcal disease post-ART initiation or new acquisition of cryptococcus after randomization) are supported by the fact that 2% of participants converted from being CrAg-negative at enrolment to CrAg-positive at week 4. A disease incidence of 10–20% over 24 weeks (Fig. 2) in the 35 participants who CrAg-converted could account for the new cases we observed postenrolment in those CrAg-negative at baseline.

There were fewer cryptococcal deaths in baseline CrAg-positives in the enhanced-prophylaxis group (two deaths) vs. the standard-cotrimoxazole group (seven deaths). We cannot directly assess the contribution of the emergence of fluconazole resistance to these deaths because no samples were stored; nor are resistance data available from those with nonfatal cryptococcal disease. However, although numbers are small, 50% (3/6) enhanced-prophylaxis participants with incident cryptococcus survived vs. 47% (8/17) standard-cotrimoxazole participants, suggesting that receipt of low-dose fluconazole does not increase the risk of treatment failure with current standard-of-care for treatment in Africa, that is high-dose fluconazole monotherapy or high-dose fluconazole and amphotericin. While a recent study suggested that 100 mg/day fluconazole could lead to subtherapeutic levels for treating cryptococcal disease in 40% of patients [25], in REALITY this fluconazole dose was given synchronously with ART, which was associated with substantial early immune reconstitution [10]. In those patients who were CrAg-positive at baseline, 24 week all-cause mortality was 7.8% with enhanced-prophylaxis (and immediate ART) and 15.9% with standard-prophylaxis (and immediate ART) (Fig. 2), only slightly lower than the ~20% in a pooled analysis of four CrAg-positive cohorts with titre 1 : 80 or less [26]. This is consistent with generally better outcomes observed in trials, either due to more consistent management (e.g. no stockouts, little delay in ART initiation) or less sick patients being enrolled (although death rates were very high shortly after enrolment in REALITY [16] suggesting the trial was not doing this to a large degree). Moreover, time from screening to trial enrolment was very short [median only 5 days (IQR 2–8)], meaning there was little opportunity for sites to recruit only 'nonprogressors', and CrAg testing was done on the sample taken at enrolment (day of ART initiation), not screening. However, it is possible that cryptococcal disease was more likely to be identified at trial screening than in a general programmatic setting.

An important study limitation includes the limited diagnostic information available in some cases, reflecting real-world settings, but making it difficult to distinguish between newly acquired, latent or undiagnosed cryptococcal infection in those without clinically apparent disease at baseline, or between paradoxical or unmasking IRIS. However, practically the distinction between these is probably small. Although delaying ART initiation for 5 weeks after starting treatment with amphotericin B and 800 mg daily fluconazole for cryptococcal meningitis was associated with improved survival [27], all participants in our study initiated 100 mg daily fluconazole at the same time as ART, so we cannot assess whether reductions in cryptococcal disease/death in CrAg-positives would have been even greater had ART been delayed.

The REALITY trial was designed to be pragmatic and relevant to real-life settings. As such, the trial did not mandate CrAg screening in the inclusion criteria. Ideally a cheap point-of-care CrAg test may become available. However, even then, our findings show that an enhanced-prophylaxis package containing fluconazole at 100 mg/day for 12 weeks is effective in this population of HIV-infected adults, adolescents and older children without overt cryptococcal disease, when started concurrently with first-line combination ART. Moreover, the finding of significant benefit in reducing early deaths from unknown causes in those CrAg-negative at baseline suggests that another component of the enhanced-prophylaxis package, possibly azithromycin, is providing this benefit, and supports the use of the enhanced-prophylaxis package in its entirety, in these populations with advanced HIV.

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Authors contributions: S.L.P., L.J.H. and L.A.B. designed the cryptococcal substudy. R.N., G.N., S.B., I.D., J.K., G.S., S.K., K.M.C., C.K. carried out the assays and also conducted laboratory testing for the main trial. M.S. organized sample retrieval and quality control of assays. D.M.G., J.H., A.S.W., J.A.B., R.S.H. contributed to design of the overall trial. J.H., G.M., J.A.B., A.H. collected data for the trial. A.S.W. analysed the data; A.S.W. vouches for data and analysis and is the guarantor; S.L.P. and A.S.W. wrote the first draft; all authors approved the final version and decided to publish.

Data sharing: The REALITY trial data are held at MRC CTU at UCL, which encourages optimal use of data by employing a controlled access approach to data sharing, incorporating a transparent and robust system to review requests and provide secure data access consistent with the relevant ethics committee approvals. All requests for data are considered and can be initiated by contacting mrcctu.ctuenquiries@ucl.ac.uk.

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Conflicts of interest

There are no conflicts of interest.

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