**Outcomes of reflex cryptococcal antigen (CrAg) screening in HIV-positive patients with CD4 counts of 100-200 cells/µL in Botswana**

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**ABSTRACT (50/50 words)**

Increasing the CD4-count threshold for cryptococcal antigen (CrAg) screening from ≤100 to ≤200 cells/µL resulted in a 3-fold increase in numbers screened. CrAg-prevalence was 3.5% at CD4 101-200 and 6.2% ≤100 cells/µL. Six-month mortality was 21.4% (9/42) in CrAg-positive CD4 ≤100 cells/µL and 3.2% (1/31) in CrAg-positive CD4 101-200 cells/µL.

**INTRODUCTION**

Advanced HIV disease (AHD) [CD4 ≤200 cells/µL] and associated opportunistic infections remain a common cause of mortality in persons living with HIV, with cryptococcal meningitis (CM) resulting in an estimated 15% of HIV-related deaths[1]. Cryptococcal antigen (CrAg) screening can detect early infection prior to the onset of clinical meningitis[2], and CrAg screening and targeted pre-emptive fluconazole is associated with a reduced risk of CM and all-cause mortality[3, 4]. Prior to 2018, international guidelines for CrAg screening focused on those with very advanced HIV (CD4 ≤100 cells/µL)[5] due to limited data on CrAg prevalence[6] and clinical outcomes[3] in patients with higher CD4 counts (101-200 cells/µL). Increasing the CD4 threshold for CrAg screening may prevent additional CM cases[7], as well as simplify AHD interventions by having a single CD4 cutoff of 200 cells/µL. In 2018, the World Health Organization conditionally recommended increasing the CD4 threshold for CrAg screening to ≤200 cells/µL, while emphasizing that it was a research priority to better understand outcomes in these CrAg-positive patients with higher CD4 counts[8].

In a cohort of patients receiving CD4 testing at the Botswana-Harvard HIV Reference Laboratory (BHHRL) in Gaborone, Botswana, we performed a study to determine clinical features and outcomes of patients undergoing CrAg screening at the increased CD4 threshold of 101-200 cells/µL and compared findings to those with CD4 ≤100 cells/µL.

**METHODS**

The screening cohort included sequential patients undergoing CrAg screening at BHHRL January 2018 through January 2019. BHHRL provides almost all CD4 testing for 27 ART clinics and a national referral hospital in greater Gaborone. From January-June 2018 residual EDTA blood samples from patients with a CD4 ≤100 cells/µL underwent real-time reflex CrAg screening at Botswana’s National Health Laboratory (NHL) using the IMMY lateral flow assay (Norman, OK); in June 2018 the CrAg screening threshold was increased to ≤200 cells/µL following the publication of updated WHO recommendations[8].

Non-pregnant CrAg-positive adults (≥18-years) attending local HIV clinics without prior CM were enrolled by the research team into a secondary “treatment” cohort and prospectively followed-up and managed by the study team. Patients ineligible for the treatment cohort were managed by the patients’ clinical care providers who were notified about CrAg results and referred to national treatment guidelines [9]. Recommended management was to offer lumbar puncture (LP) to all CrAg-positive individuals to assess for baseline CM (by cerebrospinal fluid CrAg, India ink, and culture), with pre-emptive fluconazole 1200mg/day for those without confirmed CM for two weeks, followed by standard consolidation and maintenance therapy. Those with CM were referred for hospitalization and amphotericin B-based treatment[9].

The national electronic medical record (EMR) was queried for history of prior HIV, CD4, and HIV viral load (VL) testing and to capture any diagnoses of CM by lumbar puncture within 6 months of CrAg screening for CrAg-screened patients. Six-month mortality was determined through active follow-up in patients enrolled in the treatment cohort, and using the EMR in the remainder; for those with unknown vital status on study completion the national death registry was queried using a unique national identification number.

We determined CrAg prevalence by CD4 strata (≤100 vs. 101-200 cells/µL) and compared characteristics of CrAg-positive patients with advanced (CD4 101-200 cells/µL) and very advanced (CD4 ≤100 cells/µL) HIV by chi-square or Wilcoxon rank-sum testing. We used Cox-proportional hazards models to evaluate mortality by CrAg and CD4 strata, with adjustment for covariates potentially associated with mortality risk (age, sex, current ART use, and CD4 count). Stata Version 13 (College Station, TX) was used for all analyses. Approval was obtained from institutional review boards at Botswana’s Ministry of Health and Wellness, the University of Botswana, and the University of Pennsylvania.

**RESULTS**

*CrAg prevalence within CD4 strata.*

From January 2018 - January 2019, 2033 CrAg tests were performed in 1678 individuals with CD4 ≤200 cells/µL (355/2033 were repeat tests from previously screened individuals). During the period June 2018-January 2019, when the CD4 threshold was raised to 200 cells/µL, 68% (1157/1711) of CrAg tests were performed on samples with CD4 counts 101-200 cells/µL. The median age of CrAg-screened individuals was 40 years (interquartile range [IQR]:33-46 years); 58% (969/1673) were male; 91% (1532/1678) were outpatients; and 76% (1272/1678) were ART-experienced (i.e. were taking or had previously taken ART). CrAg prevalence was 4.7% (78/1678) overall; 6.2% (45/731) among those with CD4 ≤100 cells/µL and 3.5% (33/947) in those with a CD4 101-200 cells/µL (p=0.01); excluding those with prior CM, CrAg prevalence was 4.7% (34/720) with CD4 ≤100 cells/µL and 2.4% with CD4 101-200 cells/µL (22/936) (p=0.002). During the period when the CD4 threshold was raised to 200 cells/µL, 57% (33/58) of CrAg-positive individuals had CD4 counts >100 cells/µL.

*Management of CrAg-positive individuals.*

Of the 78 CrAg-positive individuals, 35% (27/78) were enrolled into the treatment cohort and managed by the study team; 65% (51/78) were ineligible for the treatment cohort and managed by their routine care providers. Reason for ineligibility included prior history of CM (n=22), at non-participating site or hospitalized (n=9), aged <18 years (n=3), and died prior to contact (n=2) [details in Supplementary Table 1].

Nineteen percent (15/78) of CrAg-positive individuals had confirmed CNS disease (CM) at baseline, although uptake of LPs was low. In the treatment cohort 33% (9/27) patients consented to LP, of whom 3 had CM; one individual initially refused LP, but had clinical CM subsequently confirmed on LP in hospital. In the routine care cohort 29% (15/51) had a baseline LP, 11 of whom had CM. All patients with CM were treated as inpatients with amphotericin B-based therapy; 33% (5/15) died a median of 31 days (IQR 21-37) post CrAg-screening. Recommended treatment with fluconazole 1200mg/day in patients without CM was prescribed by the study team in the 23/27 patients without baseline CM in the treatment cohort. Fluconazole prescription data were not available in the routine care cohort. None of the 63 CrAg-positive individuals without CM at baseline were diagnosed with CM during six-month follow-up.

*Characteristics and outcomes of CrAg-positive individuals with CD4 101-200 cells/µL vs. ≤100 cells/µL.*

There were no significant differences in age, sex, hospitalization status, or baseline CM between CrAg-positive individuals with CD4 ≤100 cells/µL and those with CD4 101-200 cells/µL (Supplementary Table 2). Those with CD4 101-200 cells/µL were more likely to have been diagnosed with HIV infection >6 months previously (85% (28/33) vs. 53% (24/45), p=0.004) and to be on ART at baseline (94% (31/33) vs. 62% (28/45), p=0.005), and had lower median CrAg titers (1:40, IQR 1:10-1:160, vs. 1:320. IQR 1:80-1:2560, P<0.001).

Six-month mortality data were available for 97% (1623/1678) of individuals (Figure 1); overall 13.7% (10/73) CrAg-positive and 3.7% (57/1550) CrAg-negative patients died (adjusted hazard ratio [aHR] 3.08, 95% confidence interval [CI] 1.55-6.15) [Supplementary Table 3]. In the group with CD4 ≤100 cells/µL 21.4% (9/42) CrAg-positive and 6.7% (44/654) CrAg-negative patients died (aHR 3.15, 95%CI 1.51-6.59). In those with CD4 101-200 cells/µL 3.2% (1/31) CrAg-positive and 1.5% (13/896) CrAg-negative patients died (aHR 2.68, 95%CI 0.34-21.14). Assuming those lost to follow-up (LTFU) had died, 26.7% (12/45) CrAg-positive and 11.1% (76/686) CrAg-negative patients were dead/LTFU in the CD4 ≤100 cells/µL group (aHR 2.37, 95%CI 1.27-4.43), and 9.1% (3/33) CrAg-positive and 3.4% (31/914) CrAg-negative patients in the CD4 101-200 cells/µL group (aHR 4.49, 95%CI 1.31-15.39) [Supplementary Table 4]. There was no evidence for any interaction effect between CrAg status and CD4 category (≤100 vs. 101-200 cells/µL) on hazards of six-month mortality (interaction term p=0.75).

**DISCUSSION**

Expanding the CD4 count thresholds for reflex CrAg screening in Botswana from ≤100 cells/µL to ≤200 cells/µL resulted in a three-fold increase in the number of patients undergoing CrAg screening. As previously reported[6], CrAg prevalence was lower in the group with CD4 counts over 100 cells/µL (3.5% vs. 6.2% in those with CD4 ≤100 cells/µL). However, due to the increase in number screened, the higher CD4 threshold resulted in a two-fold increase in number of patients identified for pre-emptive fluconazole.

Whilst our findings highlight the potential resource implications of expanding CD4 count thresholds in reflex CrAg-screening programs, with no untreated control group we cannot definitively determine the clinical impact of CrAg-screening in the higher CD4 strata. CrAg-positive individuals with CD4 counts of ≥100 cells/µL were more likely to already be on ART, and had lower CrAg titers, both associated with lower risk of progression to meningitis and death[10]; thus, they may have had favorable outcomes in the absence of CrAg screening. Overall, cryptococcal antigenemia was associated with a three-fold increase in hazard of mortality, with no significant interaction between CD4 and cryptococcal antigenemia on hazard of mortality. The absolute risk of all-cause six-month mortality was markedly higher in CrAg-positive patients with a CD4 ≤100 cells/µL compared to 101-200 cells/µL (21% vs. 3%; or 27% vs. 9% in sensitivity analysis assuming those lost to follow-up had died).

In summary, increasing the CD4 count threshold for reflex CrAg screening to 200 cells/µL led to a large increase in the number of CrAg tests performed and more than doubled the number of CrAg-positive individuals identified. Further data are needed to determine the clinical benefits of screening individuals with CD4 counts >100 cells/µL.

**FUNDING AND CONFLICTS OF INTEREST**

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**Figure legend**

**Figure 1.** Six-month survival curves: A) comparing CrAg-positive and CrAg-negative patients with a CD4 count ≤100 cells/µL; B) comparing CrAg-positive and CrAg-negative patients with a CD4 count 101-200 cells/µL; C) comparing CrAg-positive and CrAg-negative patients with a CD4 count ≤100 cells/µL in a sensitivity analysis assuming those lost to follow-up died; D) comparing CrAg-positive and CrAg-negative patients with a CD4 count 101-200 cells/µL in a sensitivity analysis assuming those lost to follow-up died.

**Figure 1.**

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**Supplementary Table 1.** Reasons for ineligibility for CrAg treatment cohort

|  |  |
| --- | --- |
| **Reason for exclusion** | **Count (%)\***  **51 total patients** |
| Prior cryptococcal meningitis † | 22 (43%) |
| At non-participating site (excluding those hospitalized for cryptococcal meningitis) **§** | 9 (18%) |
| Screened during period when enrollment by study team was on hold | 8 (16%) |
| Being treated for cryptococcal meningitis at screening † | 6 (12%) |
| Unable to reach or refused enrollment | 6 (12%) |
| <18 years of age | 3 (6%) |
| Died before could be enrolled | 2 (4%) |

\* Total reasons for exclusion greater than total patient number as patients may have had more than one reason for treatment cohort exclusion

† One patient had a history of prior cryptococcal meningitis diagnosed in November, 2017 and was being treated for relapsed cryptococcal meningitis at the time of screening in August, 2018

**§** Among those at non-participating sites, 6 hospitalized and 3 received outpatient HIV care outside of the Gaborone city clinics; among hospitalized, 2 were also excluded for age <18 years 1 for history of prior cryptococcal meningitis, and 1 who was screened while treatment cohort study enrollment was on hold

**Supplementary Table 2.** Baseline characteristics of CrAg-positive study participants

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **CrAg-positive CD4 100-200 cells/µL**  **N=33** | **CrAg-positive CD4 ≤100 cells/µL**  **N=45** | **P-value** |
| CD4 count, cells/µL, median (IQR) | 165 (147 - 179) | 38 (23-63) | --- |
| Age, years, median (IQR) | 40 (36-44) | 39 (31-43) | 0.10 |
| Male, % (n) | 67% (22) | 67% (30) | 1.00 |
| Inpatient, % (n) | 15% (5) | 16% (7) | 0.96 |
| Time known HIV  >6 months, % (n)  >1 year, % (n)  >3 years, % (n) | 85% (28)  79% (26)  64% (21) | 53% (24)  44% (20)  33% (15) | <.0001 |
| ART at screening  On ART  Naïve  Defaulter | 94% (31)  6% (2)  0% (0) | 62% (28)  33% (15)  4% (2) | 0.005 |
| HIV viral load suppressed last 6 months, % (n) | 64% (21) | 24% (11) | 0.001 |
| Prior CM, % (n) | 36% (12) | 22% (10) | 0.17 |
| Baseline CM, % (n) | 15% (5) | 22% (10) | 0.43 |
| Death within 6 months, % (n) | 3% (1) | 21% (9) | 0.03 |
| Death within 6 months or lost to follow-up, % (n) | 9% (3) | 27% (12) | 0.05 |

ART = antiretroviral therapy; CM = cryptococcal meningitis; CrAg = cryptococcal antigen

**Supplementary Table 3.** Predictors of mortality for patients by CD4 strata

|  |  |  |
| --- | --- | --- |
| **Variable** | **Crude HR (95% CI)** | **Adjusted HR (95%CI)\*** |
| **CD4 0-200 cells/µL** | | |
| CrAg-positive | 3.88 (1.98 - 7.60) | 3.08 (1.55 - 6.15) |
| 10 cells/µL increase in CD4 | 0.84 (0.80 - 0.88) | 0.85 (0.81 - 0.90) |
| Male sex | 1.52 (0.94 - 2.46) | 1.54 (0.94 - 2.51) |
| Age ≥40 years | 0.90 (0.56 - 1.45) | 1.07 (0.66 - 1.74) |
| On ART at screening | 0.36 (0.22 - 0.58) | 0.59 (0.35 - 0.97) |
| **CD4 0-100 cells/µL** | | |
| CrAg-positive | 3.40 (1.66 - 6.96) | 3.15 (1.51 - 6.59) |
| 10 cells/µL increase in CD4 | 0.80 (0.73 - 0.89) | 0.83 (0.75 - 0.92) |
| Male sex | 1.42 (0.83 - 2.44) | 1.58 (0.91 - 2.74) |
| Age ≥40 years | 0.97 (0.57 - 1.67) | 1.08 (0.63 - 1.87) |
| On ART at screening | 0.50 (0.29 - 0.86) | 0.56 (0.32 - 0.99) |
| **CD4 101-200 cells/µL** | | |
| CrAg-positive | 2.24 (0.29 - 17.10) | 2.68 (0.34 - 21.14) |
| 10 cells/µL increase in CD4 | 0.78 (0.64 - 0.94) | 0.78 (0.64 - 0.95) |
| Male sex | 1.49 (0.52 - 4.25) | 1.47 (0.51 - 4.23) |
| Age ≥40 years | 0.91 (0.32 - 2.59) | 1.01 (0.35 - 2.94) |
| On ART at date of screening | 0.58 (0.18 - 1.85) | 0.71 (0.21 - 2.35) |

ART = antiretroviral therapy, CrAg = cryptococcal antigen

\* Adjusting for other variables included in table, e.g. CrAg status adjusted for CD4 count, sex, age, and ART status

**Supplementary Table 4.** Predictors of mortality for patients by CD4 strata in sensitivity analysis assuming patients lost to follow-up died

|  |  |  |
| --- | --- | --- |
| **Variable** | **Crude HR (95% CI)** | **Adjusted HR (95%CI)\*** |
| **CD4 0-200 cells/µL** | | |
| CrAg-positive | 3.02 (1.76 - 5.20) | 2.56 (1.47 - 4.45) |
| 10 cells/µL increase in CD4 | 0.87 (0.85 - 0.90) | 0.89 (0.86 - 0.93) |
| Male sex | 1.30 (0.91 - 1.86) | 1.24 (0.86 - 1.79) |
| Age ≥40 years | 0.68 (0.47 - 0.98) | 0.77 (0.53 - 1.12) |
| On ART at screening | 0.38 (0.26 - 0.54) | 0.57 (0.39 - 0.83) |
| **CD4 0-100 cells/µL** | | |
| CrAg-positive | 2.55 (1.39 - 4.69) | 2.37 (1.27 - 4.43) |
| 10 cells/µL increase in CD4 | 0.85 (0.79 - 0.91) | 0.88 (0.81 - 0.95) |
| Male sex | 1.36 (0.89 - 2.07) | 1.40 (0.91 - 2.14) |
| Age ≥40 years | 0.81 (0.53 - 1.24) | 0.88 (0.57 - 1.35) |
| On ART at screening | 0.61 (0.40 - 0.93) | 0.69 (0.45 - 1.07) |
| **CD4 101-200 cells/µL** | | |
| CrAg-positive | 2.77 (0.85 - 9.06) | 4.49 (1.31 - 15.39) |
| 10 cells/µL increase in CD4 | 0.83 (0.74 - 0.94) | 0.85 (0.75 - 0.97) |
| Male sex | 0.97 (0.48 - 1.95) | 0.83 (0.41 - 1.68) |
| Age ≥40 years | 0.52 (0.26 - 1.06) | 0.59 (0.29 - 1.21) |
| On ART at screening | 0.27 (0.14 - 0.52) | 0.31 (0.15 - 0.65) |

ART = antiretroviral therapy, CrAg = cryptococcal antigen

\* Adjusting for other variables included in table, e.g. CrAg status adjusted for CD4 count, sex, age, and ART status