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BRIEF REPORT

Outcomes of Reflex Cryptococcal Antigen (CrAg) Screening in Human Immunodeficiency Virus (HIV)-Positive Patients With CD4 Counts of 100–200 Cells/μL in Botswana

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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.

Keywords. advanced HIV; HIV/AIDS; cryptococcal antigen; antiretroviral therapy; HIV treat all.

Advanced human immunodeficiency virus (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 preemptive 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 (WHO) conditionally recommended increasing the CD4 threshold for CrAg screening to ≤200 cells/μL, while emphasizing that better understanding of outcomes among CrAg-positive patients with higher CD4 counts was a research priority [8].

In a cohort of patients receiving CD4 testing at the Botswana-Harvard HIV Reference Laboratory (BHHRL) in Gaborone, Botswana, we evaluated 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, which provides nearly all CD4 testing in the Gaborone region. From January to June 2018 residual EDTA blood samples from patients with a CD4 ≤100 cells/μL underwent real-time reflex CrAg screening using the IMMY lateral flow assay (Immuno-Mycologics, Inc., Norman, OK, USA); in June 2018 the CrAg screening threshold was increased to ≤200 cells/μL following updated WHO recommendations [8].

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 (inclusion criteria in Supplementary Table 1). The remainder were managed by the patients' local 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 preemptive fluconazole 1200mg/day for those without confirmed CM for 2 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 using unique patient identifiers recorded on CD4 requisitions for history of prior HIV, CD4, and HIV viral load (VL) testing, and to capture any diagnoses of confirmed CM (India ink, CrAg, or cryptococcal culture) by LP 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.

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We determined CrAg prevalence by CD4 strata (≤100 vs 101–200 cells/μL) and compared characteristics of CrAg-positive patients with CD4 101–200 cells/μL and CD4 ≤100 cells/μL by χ^2 or Wilcoxon rank-sum testing. We used Cox-proportional hazards models to evaluate mortality by CrAg and CD4 strata, adjusting for covariates potentially associated with mortality risk (age, sex, current antiretroviral therapy [ART] use, and CD4 count). Stata Version 13 (College Station, TX, USA) was used for all analyses. Ethics approvals were obtained from the Botswana Ministry of Health and Wellness, the University of Botswana, and the University of Pennsylvania.

RESULTS

CrAg Prevalence Within CD4 Strata

From January 2018 to January 2019, 2033 CrAg tests were performed in 1678 individuals with CD4 ≤200 cells/μL (355/2033 were repeat tests). During June 2018 to 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. Median age was 40 years (interquartile range [IQR], 33–46 years); 58% (969/1673) were male; 91% (1532/1678) outpatients; and 76% (1272/1678) ART-experienced (1180 reported currently taking ART and 92 had defaulted ART, Supplementary Table 2). 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 ($P = .01$); excluding those with prior CM, prevalence was 4.7% (34/720) with CD4 ≤100 cells/μL and 2.4% with CD4 101–200 cells/μL (22/936) ($P = .002$). 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 78 CrAg-positive individuals, 35% (27/78) were enrolled into the treatment cohort; 65% (51/78) were ineligible and managed by their routine providers. Reason for ineligibility included prior history of CM (n = 22), at nonparticipating site or hospitalized (n = 9), and aged <18 years (n = 3) [Supplementary Table 1].

Nineteen percent (15/78) of CrAg-positive individuals had confirmed baseline CM, although uptake of LPs was low. In the treatment cohort 33% (9/27) patients consented to LP, of whom 3 had CM; 1 symptomatic 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; details regarding LP refusal in the routine care cohort were not available. 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) postscreening. Preemptive fluconazole 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 6-month follow-up.

Characteristics and Outcomes of CrAg-positive Individuals With CD4 101–200 Cells/μL Versus ≤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], $P = .004$), to be on ART at baseline (94% [31/33] vs 62% [28/45], $P = .005$) and if on ART to have initiated >3 months previously (84% [25/31] vs 43% [12/28], $P = .002$), and had lower median CrAg titers (1:40; IQR, 1:10–1:160 vs 1:320; IQR, 1:80–1:2560, $P < .001$).

Six-month mortality data were available for 97% (1623/1678) (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) among those with 6-month outcomes. 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, .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 6-month mortality (interaction term $P = .75$).

DISCUSSION

Expanding the CD4 count thresholds for reflex CrAg screening in Botswana from ≤100 cells/μL to ≤200 cells/μL resulted in a 3-fold increase in the number of patients undergoing screening. As previously reported [6], CrAg prevalence was lower in the group with CD4 counts >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 2-fold increase in number of patients identified for preemptive fluconazole.

Although our findings highlight the potential resource implications of expanding CD4 count thresholds in reflex CrAg-screening programs, with no untreated control group

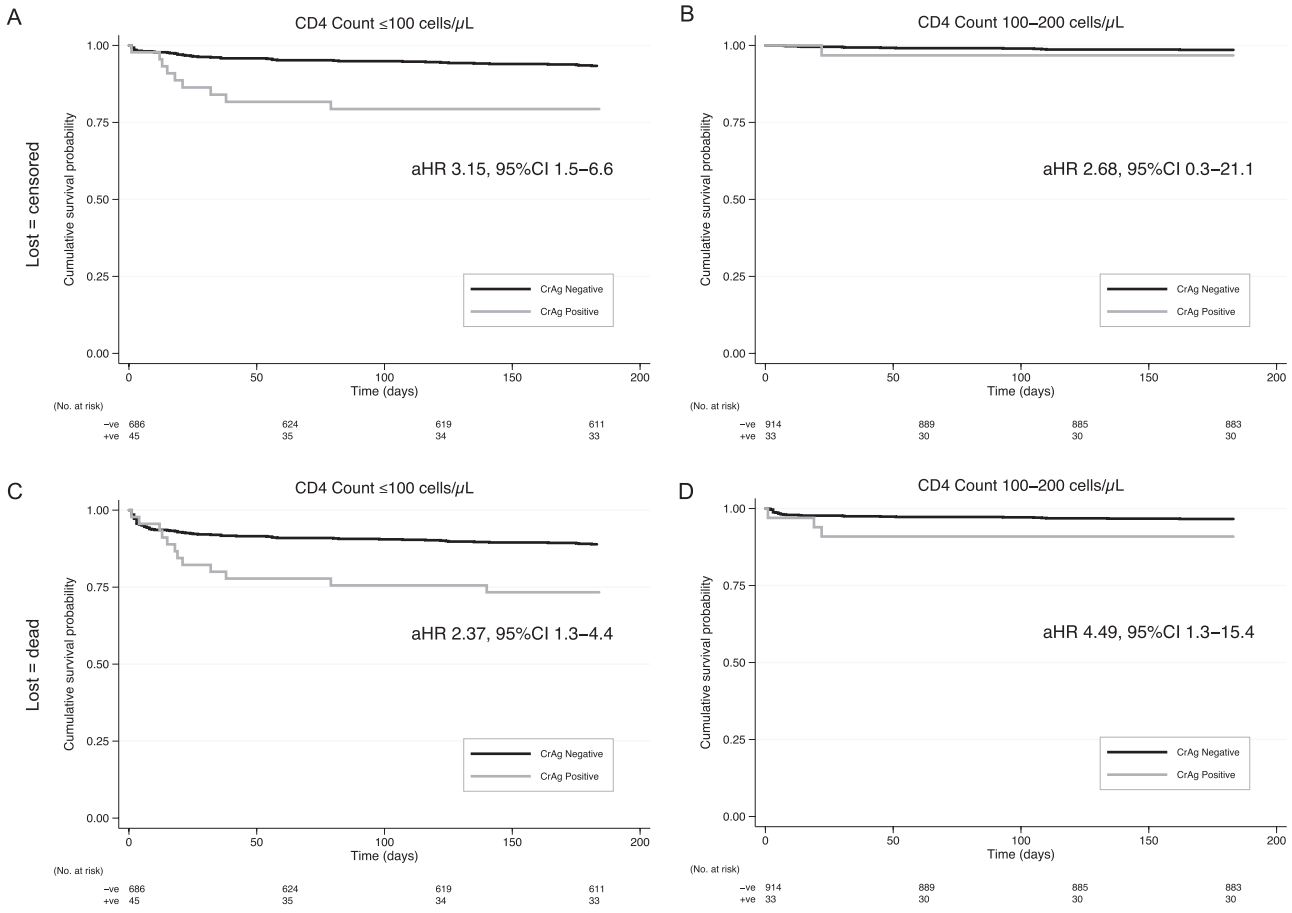

Figure 1. Six-month survival curves: (*A*) Comparing CrAg-positive and CrAg-negative patients with a CD4 count ≤100 cells/μL; (*B*) comparing CrAg-positive and CrAg-negative patients with a CD4 count 101–200 cells/μL; (*C*) comparing CrAg-positive and CrAg-negative patients with a CD4 count ≤100 cells/μL in a sensitivity analysis assuming those lost to follow-up had 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 had died. Patients were considered lost to follow-up if 6-month outcome data were missing from both active follow-up and electronic medical records, and they did not have a valid national identity number to enable death registry linkage. Data were censored at the date patients were last known to be alive. Abbreviations: aHR, adjusted hazards ratio; +ve, positive; -ve, negative; CI, confidence interval; CrAg, cryptococcal antigen.

we cannot definitively determine the clinical impact of CrAg-screening in the higher CD4 strata, and further cost-effectiveness analyses are needed to definitively guide programmatic implementation of expanded CrAg screening. 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. Use of a comprehensive EMR and national death registry means we are unlikely to have missed confirmed CM cases or deaths; however, as in prior cohorts, lumbar puncture refusal was high despite counseling [11], and early CM in asymptomatic CrAg-positive patients may not have been fully ascertained [12]. Overall, cryptococcal antigenemia was associated with a 3-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 6-month mortality was markedly higher in CrAg-positive patients with a CD4 ≤100 cells/μL compared to 101–200 cells/μL (21% vs 3%; 27% vs 9% in sensitivity analysis assuming those LTFU 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.

Notes

Disclaimer. The views expressed are those of the authors and not necessarily those of the United States (US) Centers for Disease Control and Prevention (CDC), National Institutes of Health (NIH), United Kingdom (UK) National Health Service (NHS), National Institute for Health Research (NIHR), the Department of Health and Social Care, or other funding entities.

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