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Implementation of point-of-care testing and prevalence of cryptococcal antigenaemia among patients with advanced HIV disease in Mumbai, India

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ABSTRACT

Objectives To describe the implementation of screening for cryptococcal antigenaemia by point-of-care (POC) serum cryptococcal antigen (CrAg) lateral flow assay, measure the prevalence and factors associated with serum cryptococcal antigenaemia in the routine programmatic setting.

Design Cross-sectional study.

Setting Seventeen publicly funded antiretroviral therapy (ART) centres in Mumbai, India.

Participants Serum CrAg screening was offered to all adolescents (>10 years of age) and adults with advanced HIV disease (AHD) (CD4 <200 cells/mm^3 or with WHO clinical stage III/IV) regardless of symptoms of cryptococcal meningitis.

Primary and secondary outcome measures The primary outcome was to describe the implementation of serum CrAg screening and secondary outcome was to measure the prevalence of serum cryptococcal antigenaemia and its risk factors.

Results A total of 2715 patients with AHD were tested for serum CrAg by POC assay. Of these, 25 (0.9%) had a CrAg positive result. Among CrAg-positive patients, only one had symptoms. Serum CrAg positivity was 3.6% (6/169) and 1.6% (6/520) among those presenting with CD4 <100 cells/mm^3 in the treatment naïve and treatment experienced group, respectively. On multivariable analysis, CD4 count <100 cells/mm^3 (OR: 2.3, 95% CI 1.01 to 5.3; p=0.05) and people living with HIV who were treatment naïve (OR: 2.5, 95% CI 1.04 to 6.0; p=0.04) were significantly associated with a positive serum CrAg result. Lumbar puncture was obtained in 20/25 patients within 4 days (range: 1–4 days) of positive serum CrAg result and one person was confirmed to have meningitis. All serum CrAg-positive patients who had a negative cerebrospinal fluid CrAg were offered pre-emptive therapy.

Conclusions Implementation of a POC CrAg assay was possible with existing ART centre staff. Initiation of pre-emptive therapy and management of cryptococcal antigenaemia are operationally feasible at ART centres. The Indian National AIDS Control Programme may consider reflexive CrAg screening of all AHD patients with CD4 <100 cells/mm^3.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study was conducted in routine programme setting in contrast to prior studies from inpatient settings in India.
⇒ The study included both treatment experienced and treatment naïve patients with advanced HIV disease.
⇒ We included adults and adolescents over 10 years of age with CD4 count <200 cells/mm^3 and/or WHO clinal stage III or IV hitherto studies included people living with HIV with CD4 count <200 cells/mm^3.
⇒ The study had a small number of cases with cryptococcal disease.

INTRODUCTION

Cryptococcal disease is a serious opportunistic infection (OI) among people with advanced HIV disease (AHD).[1] Cryptococcal meningitis (CM) is the most common presentation representing 70%–90% of cryptococcal disease and globally accounts for 15% of all AIDS related deaths.[2] Within India, the reported mortality rates due to CM varies from 18% to 38%.[3] Factors associated with CM mortality in the low-income and middle-income countries include delay in HIV diagnosis, non-availability of rapid diagnostic assays, limited access to lumbar puncture (LP) and antifungal drugs, limited capacity to manage treatment toxicities, complications of raised intracranial pressure and immune reconstitution inflammatory syndrome associated with CM and antiretroviral therapy (ART).[4–8] Though improved access to ART has decreased the overall global mortality by half between 2005 and 2016, mortality remains an issue in people living with HIV (PLHIV) presenting late with advanced HIV.[1 9]

WHO recommends screening with serum cryptococcal antigen (CrAg) when initiating

or reinitiating ART for adults and adolescents >10 years of age living with HIV who have CD4 count <100 cells/mm^3 and conditionally recommends screening for PLHIV with CD4 <200 cells/mm^3. Context-specific recommendations are provided by WHO for diagnosis of CM depending on availability of LP.[10]

India's National AIDS Control Programme (NACP) implemented treat all policy in 2017,[11] however, nearly 35% of PLHIV still presented to care with AHD, increasing the likelihood of higher morbidity and mortality. Pooled estimated proportions of OIs among PLHIV in India ranks tuberculosis (TB) as the major OI (56.7%) with CM at 4.3%.[12] The prevalence of cryptococcal antigenaemia among adults with CD4 cells <100 cells/mm^3 varied between 0% and 12.6% in the studies from India.[13–18]

The Technical Resource Group of Indian NACP recommended CrAg screening by lateral flow assay for all PLHIV >10 years of age with CD4 <200 cells/mm^3 or WHO clinical stage III/IV as a modification to the WHO 2020 recommendation, with suggestions to further evaluate operational feasibility in the programmatic setting noting the paucity of data. We describe the implementation of screening for cryptococcal antigenaemia by point-of-care (POC) CrAg lateral flow assay, measure the prevalence and factors associated with serum cryptococcal antigenaemia in the routine programmatic setting as part of advanced disease package of services.

METHODS

Setting

This is a cross-sectional study among PLHIV enrolled for treatment and care in the NACP, Mumbai. The activity was implemented in 17 publicly funded ART centres in Mumbai, India, under the aegis of Mumbai Districts AIDS Control Society (MDACS) responsible for the implementation of NACP activities . During the period of November 2020–December 2021, nearly 38 000 PLHIV were on HIV treatment across ART centres in Mumbai. ART centres have a team of multi-disciplinary health staff including a trained physician, nurse, pharmacist, counsellor, data manager, lab technician and a community representative. Of the 17 ART centres selected for the study, 6 centres were in tertiary centres, 8 in secondary centres and 3 in primary healthcare facilities. In the programmatic setting, CD4 count is determined for all PLHIV on ART at baseline and every 6 months while on treatment.

Study population

Serum CrAg screening was offered to all adolescents (>10 years of age) and adults with AHD (CD4 <200 cells/mm^3 or with WHO clinical stage III/IV) regardless of symptoms of CM. We included treatment-experienced and treatment-naive patients presenting with AHD from inpatient and outpatient settings.

Patient and public involvement statement

Community members from the PLHIV network were one of the key stakeholders of the study conceptualisation, design, and implementation and they had proposed CrAg screening and further management of CM as a strategy to prevent death in patients presenting with AHD. The care coordinator and the peer counsellors at the ART centres are PHIV community members who were engaged in the navigation of the patients for CrAg testing and referral for Lumbar Puncture (LP). The status and results of the study were disseminated to the PLHIV network community members during the network and MDACS meetings.

Screening and diagnosis of CM

We developed job aids and standard operating procedures for use in the ART centre to ensure clinical and laboratory staff adhered to operational and technical aspects of cryptococcal screening and steps for subsequent referral for LP and management. Hands-on training was provided to nurses and laboratory technicians on the laboratory workflow with emphasis on the pre-analytical procedures for performing POC CrAg lateral flow assay. ART staff followed the operational flow chart (figure 1) for screening of symptoms for meningitis, which included headache. The nurse at ART centres counselled the AHD patients and referred patients for serum CrAg testing. Fresh blood specimens were collected, and serum CrAg assay was performed at the ART centres by a trained laboratory technician as per the manufacturer's instructions. MDACS provided on-site supportive supervision, mentoring and monitoring support to the ART centre staff.

We used the in vitro CrAg Lateral Flow Assay POC test (Immy, Norman, OK, USA), an immunochromatographic test system for the qualitative detection of capsular polysaccharide antigen of cryptococcus species complex in serum and cerebrospinal fluid (CSF). The results were reported by a trained laboratory technician and shared with the patient within 2 hours of sample collection for usage of the results by medical officer (figure 1).

Patients who were reactive for serum CrAg were referred for LP within the same institute or to a nearby institution. For patients eligible for an LP, CSF was drawn under aseptic conditions and the samples were processed in the laboratory. Patients who were positive for both serum and CSF CrAg were treated for CM. In addition to CSF CrAg, CSF analysis for meningitis indices, cell count, glucose, protein and other biochemical tests were performed for all patients. For patients with a negative serum or CSF CrAg result, but high clinical suspicion for CM, either CSF or serum sample was diluted, and then testing repeated considering the prozone and post-zone phenomenon.[19]

Management of CM

For patients with CSF CrAg negative or if an LP was not feasible and no suspicion or symptoms of meningitis, management started with pre-emptive treatment for asymptomatic cryptococcemia with fluconazole

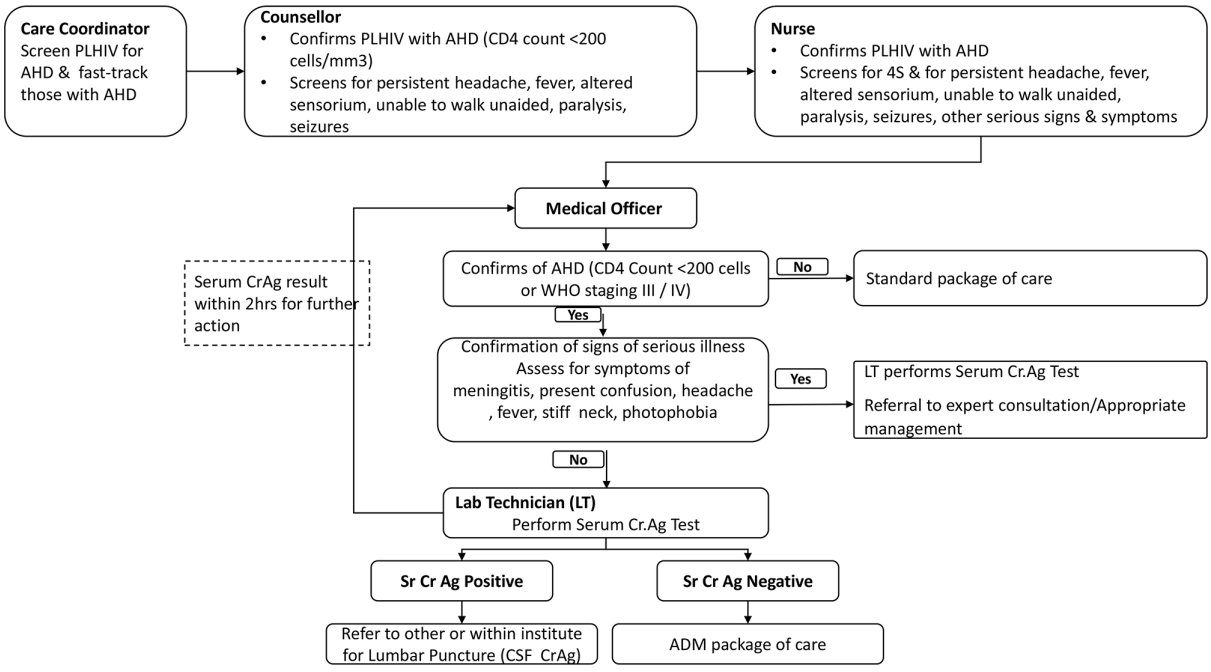

Figure 1 Operational flow for screening and management of cryptococcal meningitis. AHD, advanced HIV disease; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; LP, lumbar puncture; PLHIV, people living with HIV; ADM, Advanced Disease Management.

800 mg/day for 2 weeks, then 400 mg/day for 8 weeks and continued maintenance with fluconazole 200 mg/day. We followed the national guidelines for management of CSF CrAg positive among AHD patients. The induction phase included 2 weeks of amphotericin B deoxycholate (0.7–1 mg/kg)+fluconazole (1200 mg daily, 12 mg/kg/day for children and adolescents) and the consolidation phase included fluconazole (400–800 mg/day, 6–12 mg/kg/day for children and adolescents up to a maximum of 800 mg/ day) for 8 weeks. The maintenance phase/secondary prophylaxis included fluconazole (200 mg/day, 6 mg/kg/day for adolescents and children).[12] Criteria for discontinuing fluconazole maintenance therapy included all the following: PLHIV is adherent to ART and free of cryptococcal disease, maintenance given for at least 1 year, CD4 count >100 and having suppressed plasma viral load.

Data collection, management and analysis

Variables such as age, gender, patient setting (outpatient and inpatient), ART status (treatment-experienced and ART naïve) symptoms of headache (present or absent), duration of ART, CD4 count, serum and CSF CrAg results were collated from the patient records of routine programme at the ART centres. All data were entered in Microsoft Excel (Version MSO (16.0.10383.20027)) and analysed using IBM SPSS Statistics for Windows (V.23.0). Median and IQR as appropriate were calculated for continuous variables. Frequency and percentages were calculated for categorical variables. All proportions were reported with corresponding 95% CIs.

We calculated the prevalence of serum cryptococcal antigenaemia stratified by CD4 count at presentation, presenting symptoms and ART status. We used bivariate analysis to determine the association between serum CrAg status and age, gender, ART status, treatment duration, presence of headache, WHO clinical stage and CD4 count .

We stratified serum CrAg positivity by ART status (treatment naïve (not on ART at the time of CrAg testing) and treatment experienced) and CD4 count with cut-off range to calculate the percentage difference.

Continuous variables (age, CD4 count and duration of ART) were compared across groups using non-parametric Mann-Whitney U test due to small numbers of samples (n=25) in outcome group and categorical variables were compared using χ^2 or Fisher's exact test. We applied Haldane-Anscombe correction by adding 0.5 to each cell when the observation was zero to calculate the OR. Bivariate analysis was used to individually assess the association of all covariates. ORs with 95% CIs were calculated. Since there were only 25 outcomes, following the 10 events per predictive variable rule we could only include two variables (CD4 count and ART status) with strongest association with outcome in the final logistic regression model to determine the independent risk factors associated with serum CrAg positivity. A p value of less than 0.05 was considered statistically significant. We described the diagnostic and treatment outcomes of CrAg-positive cases.

RESULTS

Demographic and clinical characteristics of the patients

Of the 2744 adolescents and adult PLHIV with AHD accessing care from Mumbai ART centres during November 2020–December 2021 2715 (98.9%)

underwent serum CrAg assay (clinician deferred CrAg assay (n=23), died prior to the assay (n=3), transferred out (n=3)).

The median age of the participants was 44 years of age (IQR: 36–50), 68.1% were male, and the majority (66.4%) were 40 years or older. A total of 360 (13.3%) were treatment naïve and among the 2355 (86.7%) who were treatment experienced, 57.6% were on ART for more than 24 months. The median duration on ART was 36 months (IQR: 5–87). Of the 2355 treatment-experienced PLHIV, 7 (0.3%) had treatment interruption (missed pills for >28 days from pill-pick up appointment). The cohort predominantly consisted of outpatients (98.4%; 2672/2715) and overall, 686 (25.3%) had CD4 count <100 cells/mm^3. Overall, 1984 (73.1 %) were in WHO clinical stage I and 35 (1.3%) of the cohort presented with headache (table 1). Among 2715 patients, 25 (0.9%) had a CrAg positive result and 24 patients (96%) with CrAg positive results were without symptoms of headache. Of the 35 patients out of 2715 with headache, 1 (2.9%) outpatient had a CSF CrAg positive result. Of the total 360 patients who were treatment naïve, 8 (2.2%) were positive for serum CrAg assay compared with 17/2,355 (0.7%) treatment experienced AHD patients.

Serum CrAg positivity was 3.6% (6/169) and 1.6% (6/520) among those presenting with CD4 <100 cells/mm^3 in the treatment naïve and treatment-experienced group, respectively (table 2). None of the seven patients with treatment interruption in the treatment experienced group had a serum CrAg positive result.

In the bivariate analysis, PLHIV with CD4 count <100 cells/mm^3 (OR: 2.7, 95% CI 1.2 to 6.2, p=0.02); WHO stage III and IV (OR: 2.6, 95% CI 1.1 to 6.1, p=0.05) and PLHIV who were treatment naïve (OR: 3.1, 95% CI 1.3 to 7.2, p=0.02) were more likely to have a positive serum CrAg result. Overall, the prevalence of cryptococcal antigenaemia was three times higher (2.2%, 8/357) in the treatment naïve patients compared with treatment-experienced group (0.7%, 17/235). This finding was also observed across the different strata of CD4 count (<50, 50–<100 and 100–<200 cells/mm^3) where the prevalence of CrAg was three times higher in the treatment naïve group compared with the treatment experienced group (table 2).

The CD4 count by strata and ART status was included in the multivariable logistic regression model; CD4 count <100 cells/mm^3 (OR: 2.3, 95% CI 1.01 to 5.3; p=0.05) and PLHIV who were treatment naïve (OR: 2.5, 95% CI 1.04 to 6.0; p=0.04) were significantly associated with a positive serum CrAg result (table 3).

Among the patients with symptoms of headache who did not have cryptococcal antigenaemia/CM, 29.4% (10/34) had TB and 24 did not have a definitive diagnosis. Of those without a definitive diagnosis, two died and one was lost to follow-up.

Of the 25 PLHIV with positive serum CrAg result, 8 were treatment naïve and 1 had symptoms of meningitis. The CD4 count range was <100, 100–<200 and CD4 ≥200 cells/mm^3 for 12, 12 and 1 patient, respectively. Five patients could not be referred for LP (one died, one transferred out, two not willing and one had prior diagnosis of CM). Of the 20 patients who underwent LP, 11 patients were referred to the specialty department in the same tertiary institute and 9 patients had to be referred to the higher institute. All 20 patients were referred to LP within 4 days (range: 1–4 days) of positive serum CrAg result. The single patient who was positive on CSF CrAg completed consolidation phase and maintenance therapy. Eighteen PLHIV who were CSF negative and followed up were offered pre-emptive treatment within 3 days (range: 1–3 days) of CSF CrAg results (eight of them completed treatment, six are ongoing maintenance treatment, three died and one was lost to follow-up) (figure 2).

DISCUSSION

This is the first study to our knowledge conducted in a routine programme setting from India exploring the implementation of POC CrAg lateral flow assay among predominantly treatment- experienced AHD patients at public sector ART centres. We found an overall prevalence of cryptococcal antigenaemia of 0.92% and the prevalence was relatively higher among those with CD4 count <100 cells/mm^3 compared with those with CD4 count between 100 and <200 cells/mm^3, as found in prior studies.[20–22] Among those with a positive CrAg, we observed a difference in prevalence between those who were treatment naïve compared with treatment-experienced and this difference was also encountered in PLHIV with CD4 <100 cells/mm^3.

Prior studies from India report a higher prevalence of cryptococcal antigenaemia ranging between 0% and 12.6%.[13–17] The lower prevalence rate observed in our study could be attributed to the (a) screening of predominantly ambulatory patients in contrast to the tertiary hospital-based studies, which receive referral of sick patients and (b) inclusion of both treatment experienced and naïve patients presenting with CD4 <200 cells/mm^3 or WHO stage III and IV within the programme setting. In this regard, only a quarter of the patients in the programme setting had a CD4 count <100 cells/mm^3.

Higher prevalence of cryptococcal antigenaemia have been reported from India and in Southeast Asia in prior studies, with the prevalence range between 4.0% and 20.6% in both clinically suspected and asymptomatic PLHIV.[23 24] A pooled prevalence of 6.4% (CD4 count ≤100 cells/mm^3) and 2.0% (CD4 count of 100–200 cells/mm^3) cryptococcal antigenaemia was reported from a meta-analysis in 2018.[20] Similarly, another meta-analysis observed a pooled prevalence of 6% (CD4 count ≤100 cells/mm^3) and 5% (CD4 count of 100–200 cells/mm^3) cryptococcal antigenaemia in 2019 and 2020, respectively.[21 22] Similar from Sierra Leone indicated a prevalence of 4.7% among PLHIV with CD4 <100 cells/mm^3.[25] Prior study from South India in a tertiary care centre observed none of the sampled patients positive

Table 1 Baseline characteristics of people living with HIV with advanced HIV disease stratified by serum cryptococcal antigen results, Mumbai

	Total (N=2715)	Positive (N=25)	Negative (N=2690)	OR (95% CI)	P value
Characteristics	**n (%)**	**n (%)**	**n (%)**		
Age group (in years)					
>10–19	59 (2.2)	0 (0)	59 (2.2)	0 (0.0 to 10.9)	1.00
20–29	272 (10.0)	5 (20.0)	267 (9.9)	2.2 (0.6 to 8.1)	0.25
30–39	580 (21.4)	5 (20.0)	575 (21.4)	Ref	
40–49	992 (36.5)	4 (16.0)	988 (36.7)	0.5 (0.1 to 2.2)	0.41
≥50	812 (29.9)	11 (44.0)	801 (29.8)	1.6 (0.5 to 5.1)	0.41
Median age in years (IQR)	44 (36–50)	45 (34–51)	44 (36–50)	1.01 (0.97 to 1.04)	0.92
Gender					
Male	1850 (68.1)	20 (80.0)	1830 (68.0)	1.8 (0.7 to 5.5)	0.22
Female	849 (31.3)	5 (20.0)	844 (31.4.0)	Ref	
TG	16 (0.6)	0 (0)	16 (0.6)	0 (0.0 to 61.8)	1.00
CD4 count (cells/mm^3) at enrolment into ADM					
<100	686 (25.3)	12 (48.0)	674 (25.1)	2.7 (1.2 to 6.2)	0.02
100–<200	1886 (69.5)	12 (48.0)	1874 (69.7)	Ref	
≥200	143 (5.3)	1 (4.0)	142 (5.3)	1.1 (0.0 to 7.5)	1.00
Median CD4 cells/mm^3 (IQR)*	147 (99–79)	114 (82–153)	147 (100–179)	0.01 (1.00 to 1.02)	0.01
WHO staging					
III or IV	385 (14.2)	8 (32.0)	377 (14.0)	2.6 (1.1 to 6.1)	0.05
II	346 (12.7)	1 (4.0)	345 (12.8)	0.4 (0.01 to 2.3)	0.51
I	1984 (73.1)	16 (64.0)	1968 (73.2)	Ref	
Duration on ART (in months)					
Treatment naïve	360 (13.3)	8 (32.0)	352 (13.1)	3.9 (1.5 to 10.5)	0.01
<6	343 (12.6)	5 (20.0)	338 (12.6)	2.5 (0.8 to 7.7)	0.12
6–<24	446 (16.4)	3 (12.0)	443 (16.5)	1.2 (0.2 to 4.7)	1.00
≥24	1566 (57.7)	9 (36.0)	1557 (57.9)	Ref	
Median duration on ART (IQR)**	36 (5–87)	0.6 (0–86)	36 (5–87)	1.0 (1.0 to 1.0)	0.02
ART status					
Treatment naïve	360 (13.3)	8 (32.0)	352 (13.1)	3.1 (1.3 to 7.2)	0.02
Treatment experienced	2355 (86.7)	17 (68.0)	2338 (86.9)	Ref	
Patient setting					
IPD	43 (1.6)	2 (8.0)	41 (1.5)	5.6 (0.6 to 24.0)	0.12
OPD	2672 (98.4)	23 (92.0)	2649 (98.5)	Ref	
Symptomatic					
Yes	35 (1.3)	1 (4.0)	34 (1.3)	3.3 (0.1 to 21.2)	0.56
No	2680 (98.7)	24 (96.0)	2656 (98.7)	Ref	

*ORs for continuous variables indicate the odds of an outcome event occurring change as the continuous variable change.
†P value is determined by the Mann-Whitney U test.
ADM, Advanced Disease Management; ART, antiretroviral therapy; IPD, Inpatient Department; OPD, Outpatient Department.

Table 2 Prevalence of serum cryptococcal antigenaemia among people living with HIV with advanced HIV disease by CD4 count, antiretroviral therapy status and serum CrAg results, Mumbai

	Treatment naïve (N=360)			Treatment experienced (N=2355)		
CD4 count range (cells/mm^3)	CrAg positive	CrAg negative	Total	CrAg positive	CrAg negative	Total
	n (%)	n (%)	n	n (%)	n (%)	n
<50	2 (3.3)	59 (96.7)	61	1 (0.7)	147 (99.3)	148
50–<100	4 (3.7)	104 (96.3)	108	5 (1.3)	367 (98.7)	372
100–<200	2 (1.1)	174 (98.9)	176	10 (0.6)	1711 (99.4)	1721
≥200	0 (0.0)	15 (100.0)	15	1 (0.9)	113 (99.1)	114
Total	8 (2.2)	352 (97.8)	360	17 (0.7)	2338 (99.3)	2355

CrAg, cryptococcal antigen.

for CrAg.[17] However, a study from a tertiary hospital in Mumbai, indicated a prevalence of 2.7% among PLHIV with CD4 <100 cells/mm^3.[18] Higher rates of cryptococcal infection among those with CD4 count <100 cells/mm^3 is due to impaired cell mediated immunity that contributes to dissemination of cryptococcal infection.[26]

Cryptococcal antigenaemia was identified in 0.9% (23/2557) of asymptomatic patients with CD4 count <200 cells/mm^3. Of the 25 patients who had a positive CrAg result, 24 were asymptomatic. In prior studies, which included only asymptomatic treatment naïve patients, the prevalence has been observed as high as 12.9%.[27 28] As an early indicator, CrAg is detectable in blood weeks to months prior to the development of overt clinical symptoms. A community cohort from Uganda demonstrated that the CrAg positivity preceded the onset of clinically recognisable symptoms by a median of 22 days, and 10% of the patients had preceding cryptococcal antigenaemia for greater than 100 days.[29] This prolonged subclinical period of asymptomatic infection presents an opportunity to identify persons with asymptomatic or early disease and a clinical window for early pre-emptive therapy.

We report a CrAg positivity of 3.6% (6/169) among those presenting with CD4 <100 cells/mm^3 in the treatment naïve group, underscoring the 2022 WHO guidance of screening PLHIV presenting with CD4 <100 cells/mm^3 for CrAg before initiating or reinitiating ART. We also observed a prevalence of 1.2% (6/520) among treatment experienced PLHIV with a CD4 <100 cells/mm^3. The higher positivity observed in both treatment naïve and treatment-experienced asymptomatic patients presenting with CD4 <100 cells/mm^3 raises the question of whether India should consider the evidence from South Africa and recommend laboratory-based reflexive screening, in which CrAg testing is routinely performed in the laboratory on any blood sample with a CD4 count <100 cells/mm^3 including treatment-experienced ADM patients.[10 11 30]

WHO conditionally recommends screening for PLHIV with CD4 <200 cells/mm^3. We found 2/177 (1.1%) and 10/1707 (0.6%) PLHIV with cryptococcal antigenaemia with CD4 counts between 100 and <200 cells who were treatment naïve, and treatment-experienced, respectively, underscoring the need for a tailored approach for CrAg screening in India with CD4 between 100 and <200 cells/mm^3. Serum CrAg may also serve as an important diagnostic test for screening of OIs among treatment experienced PLHIV presenting with immunological or virological failure at ART centres.[31]

Table 3 Risk factors for cryptococcal antigenaemia among people living with HIV with advanced HIV disease, multivariable analysis, Mumbai

	CrAg positive (N=25)	CrAg negative (N=2690)	P value	aOR (95% CI)
Characteristics	n (%)	n (%)		
CD4 count (cell/mm^3)				
<100	12 (48.0)	674 (25.1)	0.05	2.3 (1.0 to 5.3)
100–<200	12 (48.0)	1874 (69.7)	Ref	
≥200	1 (4.0)	142 (5.3)	0.93	1.1 (0.1 to 8.4)
ART status				
Treatment experienced	17 (68.0)	2338 (86.9)	Ref	
Treatment naïve	8 (32.0)	352 (13.1)	0.04	2.5 (1.0 to 6.0)

aOR, adjusted OR; ART, antiretroviral therapy; CrAg, cryptococcal antigen.

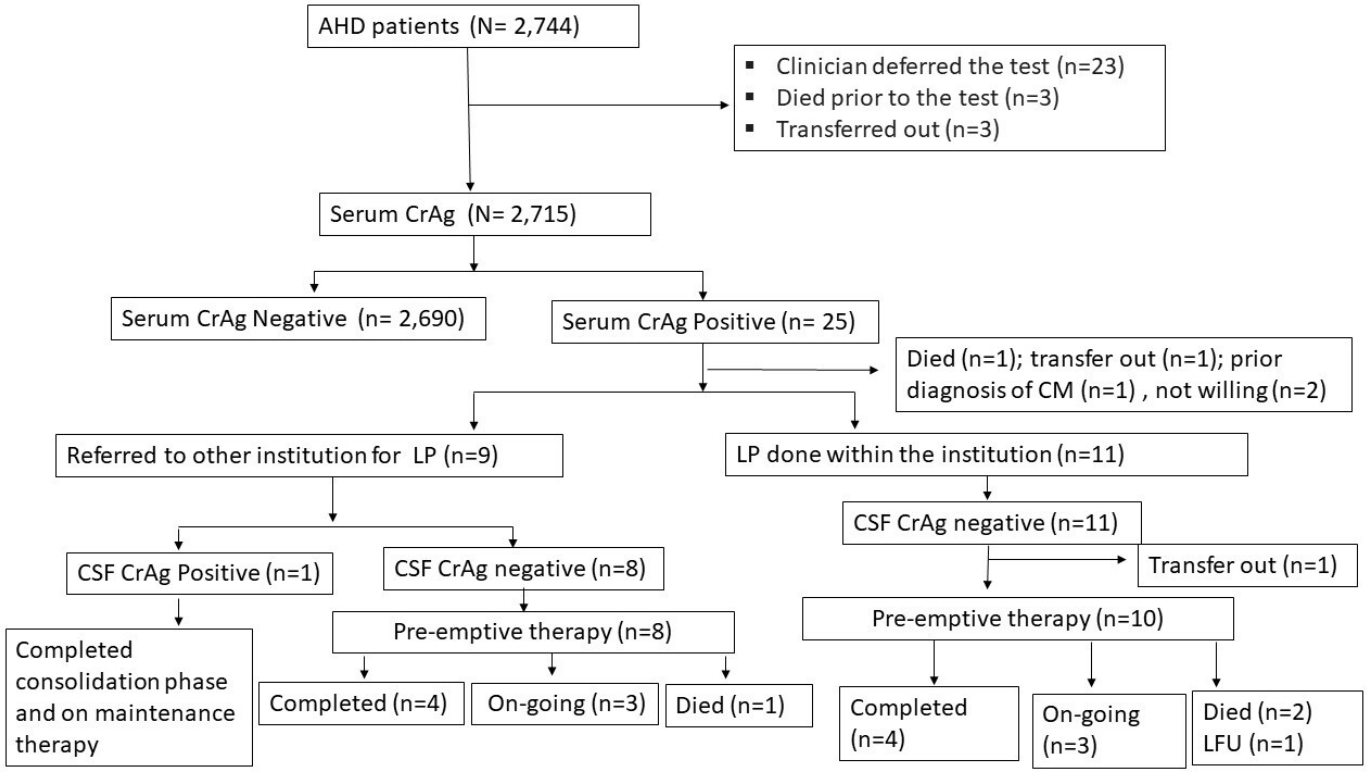

Figure 2 Cascade of cryptococcal meningitis (CM) management and outcomes of patients screened positive for serum cryptococcal antigen. AHD, advanced HIV disease; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; LFU, lost to follow-up; LP, lumbar puncture.

In this study, all serum CrAg-positive patients who had a negative LP were provided with pre-emptive treatment and have completed the therapy. WHO recommends screening for CrAg followed by pre-emptive antifungal therapy among serum CrAg-positive people to prevent the development of invasive cryptococcal disease for adults and adolescents living with HIV who have a CD4 cell count <100 cells/mm^3. Pre-emptive treatment was provided to CrAg-positive patients (6/169) only among newly diagnosed AHD patients with CD4 <100, optimising the usage of fluconazole prophylaxis. However, we faced a challenge in updating the status of PLHIV on pre-emptive treatment due to inadequate recording in the existing programmatic setting hence, a monthly review mechanism was needed to understand the status of PLHIV on pre-emptive treatment.

In the programmatic setting, we observed that it is possible to ensure serum CrAg assay results were available to the clinician within 2 hours of specimen collection and the results were used for patient management. Further with counselling and follow-up by the ART centre staff, all patients eligible for pre-emptive treatment completed the course.[23 32]

Referrals for LP were possible in the programmatic setting and 20 had an LP done within 4 days (range: 1–4 days) of positive serum CrAg result. However, in the routine programme setting, it may not be always possible to refer for LP due facility proximity. In our study, where patients did not have a facility in proximity, there were challenges in the referral for LP. To remedy this situation, we incorporated a peer navigation for referral and the medical team collected the follow-up details by phone in a follow-up visit.

Where LP is not readily available/feasible, a positive serum CrAg will have increased utility for management of asymptomatic patients with AHD in the prevention of CM deaths through early preemptive antifungal treatment.[32] The implementation of the POC CrAg assay process in the public setting may be of greater utility as the ART centres serve patients from primary or tertiary care hospitals.

Limitations

The study had a small number of cryptococcal cases making it hard to demonstrate robust associations. The ART staff were trained to ask for symptoms of CM, but we could only capture symptom of headache from the programme records. The cohort consisted of predominantly treatment-experienced patients. The prevalence from this study in Mumbai may not be representative of the rest of India in part due to the ubiquitous nature cryptococcus in certain geographies and the fact that Mumbai has a relatively better access for evaluation and for treatment.

CONCLUSION

We conclude that implementation of POC CrAg lateral flow assay as part of ADM package of services was a feasible

strategy to routinely screen AHD patients at public ART centres with existing staff. Initiation of pre-emptive therapy and management of cryptococcal antigenaemia as per context specific recommendations are operationally feasible at public ART centres. Though the overall prevalence was low, higher prevalence was observed among patients with CD4 <100 cell/mm^3 and/or ART naïve. The national programme in India may consider reflexive CrAg screening of all AHD patients regardless of duration on ART presenting with CD4 <100 cells/mm^3.

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