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Asymptomatic cryptococcal antigen prevalence detected by lateral flow assay in hospitalised HIV-infected patients in São Paulo, Brazil

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Abstract

OBJECTIVE To determine the prevalence of asymptomatic cryptococcal antigen (CRAG) using lateral flow assay (LFA) in hospitalised HIV-infected patients with CD4 counts <200 cells/μl.

METHODS Hospitalised HIV-infected patients were prospectively recruited at Instituto de Infectologia Emilio Ribas, a tertiary referral hospital to HIV-infected patients serving the São Paulo State, Brazil. All patients were >18 years old without prior cryptococcal meningitis, without clinical suspicion of cryptococcal meningitis, regardless of antiretroviral (ART) status, and with CD4 counts <200 cells/μl. Serum CRAG was tested by LFA in all patients, and whole blood CRAG was tested by LFA in positive cases.

RESULTS We enrolled 163 participants of whom 61% were men. The duration of HIV diagnosis was a median of 8 (range, 1–29) years. 26% were antiretroviral (ART)-naïve, and 74% were ART-experienced. The median CD4 cell count was 25 (range, 1–192) cells/μl. Five patients (3.1%; 95%CI, 1.0–7.0%) were asymptomatic CRAG-positive. Positive results cases were cross-verified by performing LFA in whole blood.

CONCLUSIONS 3.1% of HIV-infected inpatients with CD4 <200 cells/μl without symptomatic meningitis had cryptococcal antigenemia in São Paulo, suggesting that routine CRAG screening may be beneficial in similar settings in South America. Our study reveals another targeted population for CRAG screening: hospitalised HIV-infected patients with CD4 <200 cells/μl, regardless of ART status. Whole blood CRAG LFA screening seems to be a simple strategy to prevention of symptomatic meningitis.

keywords Cryptococcus, cryptococcal meningitis, cryptococcal screening, HIV/AIDS, cryptococcal polysaccharide, prevalence

Introduction

Cryptococcus neoformans is a major cause of adult meningitis among HIV-infected persons around the world. Sub-Saharan Africa has the highest burden [1, 2], but Latin America has the third highest number of cases globally [3]. Currently, cryptococcal meningitis is the main cause of HIV-related opportunistic meningitis in Brazil [4] and in most low- and middle-income countries [5]. Mortality continues to be unacceptably high. In retrospective and prospective hospital-based studies performed in Brazil and Argentina, cryptococcal case fatality rates have ranged from 26% to 63% [4]. These results are similar to the 24–50% reported in prospective interventional trials in Africa and Asia [6]. These studies suggest that cryptococcosis is an important cause of

mortality in Latin America. Yet, it is potentially preventable.

Early diagnosis of cryptococcal infection is a keystone to improving outcomes. Detectable cryptococcal antigen (CRAG) in peripheral blood precedes symptomatic meningitis disease by weeks to months, offering an opportunity for early detection and pre-emptive intervention [7].

WHO recommends routine serum or plasma CRAG screening in antiretroviral therapy (ART)-naïve adults with CD4 counts <100 cells/μl, followed by pre-emptive antifungal therapy if CRAG positive, to prevent the development of cryptococcal disease. Similarly, persons failing ART due to non-adherence or virologic failure should also likely be CRAG-screened, thus some countries, such as South Africa, routinely screen all persons with a CD4 count <100 cells/μl. There is evidence suggesting that CRAG screening and pre-emptive therapy with fluconazole are a cost-saving intervention for HIV-infected persons with a prevalence of cryptococcal antigenemia of ≥3%, and this strategy is now recommended [7, 8].

To validate the cost-effectiveness of the implementation of WHO guidelines, it is necessary to know the local CRAG prevalence in subsets of patients. In most African studies, among ART-naïve persons with CD4 <100 cells/μl, CRAG prevalence is ≥3% [8]. Currently in South America, there are no published studies during the ART era of CRAG prevalence in HIV-infected persons living with AIDS, including in Brazil. In this study, we evaluated CRAG prevalence in HIV-infected patients with CD4 counts <200 cells/μl, either ART-naïve or experienced, who were admitted at a referral tertiary centre from São Paulo, Brazil.

Patients and methods

Patients were prospectively recruited and samples collected from 1 November 2014 to 30 October 2015 at the medical wards at Instituto de Infectologia Emilio Ribas, which is the main tertiary referral hospital for HIV-infected patients serving the São Paulo State, Brazil.

This study was conceived in the Brazilian Network of Cryptococcosis and approved by the Research Ethics Committee of the hospital. All study participants provided written informed consent. For unconscious patients in the hospital, primary relatives and/or those legally responsible provided written informed consent. We sequentially enrolled HIV-infected adults aged ≥18 years old who were hospitalised with CD4 counts <200 cells/μl without prior diagnosis of cryptococcal infection, without clinical suspicion of cryptococcal meningitis, and regardless of ART status.

Demographic, clinical and laboratory data were recorded at study enrolment and during hospitalisation through discharge or death to obtain the final diagnosis. Approximately 5 ml of whole blood was collected from all enrolled patients and sent to the Centro de Parasitologia e Micologia of the Instituto Adolfo Lutz where two technicians independently determined CRAG testing without access to participant clinical or laboratory information. Serum was tested for CRAG using the lateral flow assay (LFA) kits (Immuno-Mycologics Inc, Norman, OK, USA) following manufacturer's instructions. Positive results were cross-verified by performing CRAG LFA in whole blood (using the same initial sample or fingerstick) and urine. Positive serum CRAG results were reported to the responsible clinicians for further investigation and treatment at their discretion. If a lumbar puncture was performed by the responsible clinician, CRAG LFA was performed on cerebrospinal fluid (CSF).

Data were analysed using Stata, version 12.0 (StataCorp, College Station, Texas, USA).

Results

We screened 219 hospitalised participants and enrolled 163 without symptomatic meningitis with CD4 cell counts <200 cells/μl of whom 61% (n = 99) were men. A total of 56 of the 219 prospective participants had symptomatic meningitis, including 20 patients with culture-proven cryptococcal meningitis, and were excluded. The median age was 38.4 (±10) years. The duration of HIV diagnosis was a median of 8 (Range, 1–29) years, and 36% (59/163) had a prior history of opportunistic infection(s). The median CD4 cell count was 25 (Range, 1–192) cells/μl with 79% (n = 128) having a CD4 cells counts <100 cells/μl. Of the cohort, 26% (n = 42) were ART-naïve, and 74% (n = 121) were ART-experienced with irregular ART use and/or having defaulted from ART. Among the ART-naïve patients, 19 (45%) cases were newly diagnosed with HIV infection during hospitalisation. Overall among hospitalised patients, only 2.5% (4/160) had undetectable HIV-1 viral loads of <40 copies/ml.

Five of 163 participants without symptomatic meningitis (3.1%; 95%CI, 1.0–7.0%) were CRAG-positive with a median age of 41 (±5.6) years. The median duration of HIV diagnosis was 8 (Range, 0–14) years. Two of the five CRAG-positive patients had prior opportunistic infections. Among CRAG positive, the median CD4 cell count was 18 (Range, 6–192) cells/μl. One of

the five had a CD4 cell count of 192 cells/μl; the other 4 had CD4 cell counts <100 cells/μl. All CRAG-positive persons had detectable viral loads, and only one was ART-naïve. Details of these five patients are displayed in Table 1.

Among the five asymptomatic CRAG positive, three received fluconazole pre-emptive therapy of whom two survived and one was later re-hospitalised and died. Two person left against medical advice and did not receive pre-emptive treatment (unknown outcome, presumed dead). Although in an observational study these outcomes were not ideal, the cost of CRAG screening per life saved was $326 (95%CI, $91 to $2685) per hospitalised person without meningitis with CD4 <200 cells/μl, assuming a real-world cost of $4 per CRAG LFA (e.g. $2 test, $0.50 labour, $0.50 overhead, $0.20 shipping, $0.01 disposables, $0.29 profit) in most low- and middle-income settings. However, at present in Brazil, the CRAG LFA commercial cost is approximately US$8 per test (i.e. a 400% price mark up from the list price), which would increase cost estimates 2.5-fold.

Of the 219 persons presenting to hospital without symptomatic meningitis (n = 163) and symptomatic meningitis (n = 56), 11.4% (25/219) were CRAG-positive. Thus, among the hospitalised persons with a CD4 count <200 cells/μl, the number needed to test (NNT) to detect one CRAG-positive case was 8.8 (95%CI, 7.5–16.4). If all hospitalised patients with CD4 <200 cells/μl (n = 219) were CRAG-screened, the cost per case detected would be $35 (95%CI, $24 to $53).

The CRAG prevalence among asymptomatic persons with CD4 cell counts <50 cells/μl was 8% (2/25), <100 cells/μl was 3.1% (4/128), and among people with CD4 counts of 100–200 cells/μl, it was 2.9% (1/35). The CRAG prevalence in ART-naïve patients was 2.4% (1/42); in ART-experienced patients, it was 3.3% (4/121) (P = 0.62).

Among all 163 hospitalised participants without symptomatic meningitis, none of the 158 CRAG-negative patients developed cryptococcal meningitis during hospitalisation. Among CRAG-negative outcomes, 87% (n = 138) were discharged home, 9.5% (n = 15) died during hospitalisation, and 3% were lost to follow-up (n = 5).

A CD4 test before admission was available in 128 (79%) of participants. Of these, 106 (83%) had CD4 cell counts <200 cells/μl. Among the 22 (17%) cases with antecedent CD4 cell counts >200 cells/μl, 14 (64%) participants had CD4 tests performed at least 6 months before the admission. All these 22 cases presented with detectable viral loads. Interestingly, the asymptomatic CRAG-positive person with a CD4 count of 192 cells/μl had 444 cells/μl at the preceding clinic visit 4 years earlier.

Discussion

Cryptococcal antigenemia was found in 3.1% of asymptomatic hospitalised patients with CD4 cell counts <200 cells/μl, irrespective of ART status, admitted to a referral centre in São Paulo, Brazil. Among all hospitalised persons with a CD4 <200 cells/μl, 11.4% were CRAG-positive. Our study reveals another targeted population for CRAG screening: hospitalised HIV-infected patients with CD4 cells counts <200 cells/μl, regardless of ART status.

We evaluated hospitalised patients, 74% of them ART-experienced and 21% of patients with CD4 counts between 100 and 200 cells/μl, a profile not contemplated in prior WHO recommendations. Interestingly, in our setting, most of the patients admitted with opportunistic infection(s) have a prior HIV diagnosis and approximately half have prior ART use with either virologic failure or defaulting from care [9]. In this hospitalised population, the CRAG prevalence of 2.4% in ART-naïve patients was similar to the 3.3% prevalence in ART-experienced patients. This CRAG prevalence excluded those presenting with symptomatic meningitis. Including all hospitalised patients with CD4<200 gave an overall CRAG prevalence of 11.4%.

This study builds upon three other studies (two unpublished) regarding CRAG prevalence in South America. From the pre-ART era, Negroni reported a 6.2% asymptomatic CRAG prevalence by latex agglutination among 193 HIV-infected persons with CD4 <300 cells/μl in Argentina [10]. The first retrospective study was conducted in Lima, Peru among 368 ART-naïve adults with CD4 of ≤100 cell/μl, without a history of cryptococcosis. In Lima, 3.6% (n = 13; 95%CI, 1.7–5.5%) were CRAG-positive. Three of the 13 were culture-positive for *Cryptococcus*. Thus, 2.7% (10/368) presented with an isolated CRAG-positive result [11]. The second was a prospective study conducted in Buenos Aires, Argentina using HIV-infected persons with CD4 ≤100 cells/μl, without regular ART, without prior cryptococcosis and without antifungal therapy in the prior 14 days. Among 114 patients evaluated, 10 (8.8%; 95%CI, 4.3–15.5%) were CRAG-positive. Six of these 10 patients presented with symptomatic cryptococcal meningitis. Thus, 3.5% (4/114) presented as asymptomatic CRAG-positive [12]. Taken together, these results suggest that CRAG screening with pre-emptive treatment of asymptomatic, early disseminated cryptococcal infection, would be advantageous in

Table 1 Details of HIV-infected patients with positive serum cryptococcal antigen (CRAG LFA)

Patient	Age, Sex	Diagnosis during hospitalisation	CD4 cells/μl	HIV-1 Viral Load copies/ml	Antiretroviral Therapy Duration	Blood Culture	CrAg LFA Whole blood/Urine	CSF Analysis India Ink/LFA/ Culture	Antifungal treatment	Outcome
1	45, male	Presumptive cerebral toxoplasmosis	6	13 815	Experienced	Negative	+/–	Not performed	None	Left Against Medical Advice
2	46, female	Confirmed disseminated tuberculosis	95	33 706	Experienced	Negative	+/–	–/–/–	None	Left Against Medical Advice
3	34, male	Diarrhoea, Bacterial pneumonia*	18	251 339	Naïve	Positive *Cryptococcus*	+/+	–/–/–	Fluconazole	Re-hospitalised, Died†
4	36, male	Confirmed pulmonary tuberculosis	192	866 912	Experienced	Negative	+/+	–/–/–	Fluconazole	Discharged home
5	44, female	Intestinal cryptosporidiosis, isosporiasis, oesophageal candidiasis	10	69 416	Experienced	Negative	+/+	–/–/–	Fluconazole	Discharged home

*This patient had clinical and radiological features (lobar pneumonia) compatible with bacterial pneumonia, received antibiotics and was discharged to home after unequivocal improvement. Bronchoalveolar lavage was not performed in this admission.

†This patient was readmitted one week after to be discharged to home due to respiratory complaints. He received intensive care support and a broad spectrum coverage for pneumonia (piperacillin–tazobactam, trimethoprim–sulfamethoxazole and amphotericin plus fluconazole). Despite this, he eventually died two weeks after admission, in consequence of respiratory failure. The aetiology of pneumonia was not found. None microorganism, including fungus, was identified in direct examination and cultures from bronchoalveolar lavage.

South America, in multiple subsets of HIV-infected patients.

The majority of data regarding cryptococcal antigenemia concerns outpatients in sub-Saharan Africa. In Africa, persons with CD4 cell counts ≤100 cells/μl have a reported CRAG prevalence between 2.2% and 21% with an average of 6.8% (95%CI, 6.5–7.2%) in studies including only asymptomatic, ART-naïve outpatients [13]. In Southeast Asia, CRAG prevalence in asymptomatic ART-naïve persons with CD4 cell counts ≤100 cells/μl is reported in 4–12.9% [14]. The WHO recommends CRAG screening in high prevalence regions, defined as ≥3% based CRAG-latex agglutination and the cost of amphotericin therapy alone [15], but other analyses have reported that screening may be cost-effective even at a prevalence as low as 1% based on the lower cost of CRAG LFA compared to the traditional latex agglutination CRAG [8, 15]. Considering the cost of hospitalisation in middle or high-income countries, CRAG screening likely is cost-saving [15]. The excessively inflated commercial cost of CRAG LFA tests in Brazil in 2016 (400% over U.S. list price) reflect an ongoing market failure, which likely requires further intervention, perhaps by organisations such as UNITAID or the Clinton Health Access Initiative.

A prior study performed in Ethiopia suggests the importance of considering CRAG screening in different profiles of patients [16]. In the Ethiopian study, the overall prevalence of cryptococcal antigenemia was 10% (26/254). As expected, CRAG positivity was associated with greater immunosuppression with CD4 cells counts ≤50 cells/μl at 25% (8/32); ≤100 cells/μl at 22% (13/59); 101–200 cells/μl at 14.7% (10/68); and 201–350 cells/μl at 5.8% (3/52). Different than our study, the prevalence of CRAG in the Ethiopian predominantly outpatient population was higher in ART-naïve patients at 14.2% (18/127) than in ART-experienced ones at 4.1% (5/121) [16]. These results reinforce the importance of CRAG screening in several epidemiologic and immunologic scenarios, including hospitalised HIV-infected patients.

In conclusion, 3.1% of HIV-infected inpatients with CD4 cells counts <200 cell/μl without symptomatic meningitis had cryptococcal antigenemia in São Paulo, suggesting that routine CRAG screening may be beneficial in similar settings in South America. Among hospitalised HIV-infected persons with CD4 cell counts <200 cell/μl and without symptomatic meningitis, approximately, 1 in 33 may likely have their cryptococcosis diagnosis missed. More outcome studies are needed to better determine how best to implement CRAG screening in a variety of settings. Whole blood CRAG LFA screening seems to be a simple strategy to prevention of symptomatic meningitis.

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