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Should we perform the serum cryptococcal antigen test in people living with HIV hospitalized due to a community-acquired pneumonia episode?

Adriana Paulino Silva[1], Carolina Toniolo Zenatti[1], Claudia Figueiredo-Mello[1,2], Marinella Della Negra[1], Anna S Levin[2], David R Boulware[3] and José Ernesto Vidal[2,4,5]

Abstract

Community-acquired pneumonia (CAP) is a common cause of hospitalization among people living with human immunodeficiency virus (PLWH), particularly those with severe immunosuppression. Pulmonary disease due to cryptococcosis is uncommonly reported and likely under-diagnosed. There is scarce information about cryptococcal antigen (CrAg) prevalence in PLWH with CAP. The objectives of this study were to identify among PLWH who were hospitalized with CAP: (i) the prevalence of serum CrAg positivity, (ii) the proportion with asymptomatic vs. symptomatic cryptococcosis; and (iii) the prevalence of serum CrAg positivity in CD4+ T-cell count <100 cells/mm^3. We performed a sub-analysis of a prospective cohort of hospitalized adults enrolled into a randomized clinical trial testing therapy for CAP. We included 202 participants who had serum CrAg testing performed. We found a 3.5% prevalence of serum CrAg-positivity overall, being higher (5.7%) in CD4+ T-cell count <100 cells/mm^3. Overall, asymptomatic and symptomatic cryptococcosis were present in 2.0% and 1.5%, respectively. This study identifies a target population for CrAg testing: PLWH hospitalized with diagnosis of CAP, particularly those with CD4+ T-cell count <100 cells/mm^3 where the number needed to test was 18 to detect 1 CrAg-positive person. This approach may facilitate the detection of asymptomatic cryptococcal infection and allow a timely diagnosis of symptomatic cryptococcal disease.

Keywords

Cryptococcosis, pneumonia, community-acquired pneumonia, cryptococcal antigen, diagnosis

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Introduction

Cryptococcal meningitis is the most common clinical manifestation of cryptococcosis among people living with human immunodeficiency virus (PLWH). Cryptococcosis continues to cause high rates of mortality, particularly in low- and middle-income countries.[1] Although the respiratory system is the portal of entry of *Cryptococcus neoformans* and *C. gattii*, isolated pneumonia without other organ involvement is infrequently reported and diagnosed in PLWH. In contrast, community-acquired pneumonia (CAP) is a common cause of hospitalization among PLWH, particularly those with severe immunosuppression.[2] In addition,

[1]Deparment of Infectious Diseases, Instituto de Infectologia Emílio Ribas, São Paulo, Brazil
[2]Department of Infectious Diseases, Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
[3]Department of Medicine, University of Minnesota, Minneapolis, MN, USA
[4]Department of Neurology, Instituto de Infectologia Emílio Ribas, São Paulo, Brazil
[5]Laboratório de Investigação Médica – 49, Instituto de Medicina Tropical, Universidade de São Paulo, São Paulo, Brazil

Corresponding author:
José Ernesto Vidal, Laboratório de Investigação Médica – 49, Instituto de Medicina Tropical, Universidade de São Paulo. Av. Dr. Enéas de CarvalhoAguiar, 470 – Jardim America, CEP 05403-000S, São Paulo, Brazil.
Email: josevibe@gmail.com

key strategies in the management of PLWH with severe immunosuppression include both the timely diagnosis of symptomatic cryptococcosis in order to initiate antifungal treatment and the identification of isolated cryptococcal antigenemia in order to initiate pre-emptive therapy.[3,4]

This study's three objectives were to identify among PLWH who were hospitalized with CAP: (i) the prevalence of serum cryptococcal antigen (CrAg) positivity, (ii) the proportion with asymptomatic vs. symptomatic cryptococcosis; and (iii) the prevalence of serum CrAg positivity in patients with CD4+ T-cell count <100 cells/mm^3.

Methods

This is a sub-analysis of a randomized clinical trial that enrolled adults $\geq$18 years of age with clinically and radiologically suspected CAP who required antibiotic treatment (denoting a clinical diagnosis of bacterial pneumonia) and hospitalization. This trial included 224 PLWH (Brazilian Clinical Trials Registry: RBR-8wtq2b) at Instituto de Infectologia Emílio Ribas, a tertiary teaching infectious diseases hospital in São Paulo, Brazil.[5] An extensive work-up for etiology of pneumonia was available, including: blood samples for bacterial, fungal, and mycobacterial cultures; sputum for direct examination for *Pneumocystis jirovecii* and acid-alcohol-resistant bacilli, and cultures for bacteria, fungi, and mycobacteria; blood samples for serology for *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*; blood samples for *Streptococcus pneumoniae* and *Haemophilus influenzae* by PCR; urinary antigen test for *Legionella pneumophila* serogroup 1; nonquantitative PCR methods for *C. pneumoniae*, *L. pneumophila*, *M. pneumoniae*, *P. jirovecii*, and adenovirus in respiratory samples; and nasopharyngeal swabs were tested by real time-PCR for the following agents: parainfluenza viruses 1 to 3, respiratory syncytial virus, influenza viruses A and B, human coronaviruses CoV NL63, HKU1, OC43 and 229E, enterovirus, rhinovirus, adenovirus, bocavirus, human metapneumovirus, *C. pneumoniae, Bordetella pertussis*, and *M. pneumoniae*. In addition, a serum cryptococcal antigen latex agglutination test (CrAg LA) was performed.[6]

For this sub-study, we excluded participants missing serum CrAg test results (n = 12), those with a known history of cryptococcal disease (n = 5), or those with non-infectious cause for the pulmonary disease (n = 5). Lumbar punctures were routinely performed among CrAg-positive participants to determine the presence of central nervous system cryptococcosis. Clinical and laboratory data, including CD4+ T-cell counts, were prospectively collected during the parent clinical trial and from the electronic hospital database. We summarized categorical variables with n (%) and summarized quantitative variables with mean and standard deviation (SD), median and interquartile range (IQR), or median and minimum–maximum (min–max), as appropriate.

Results

We included 202 participants admitted with a diagnosis of CAP who had CrAg testing performed. Among participants, 68% (n = 137) were men and the mean ($\pm$ SD) age was 40 ($\pm$12) years. Overall, 80% (161/202) were antiretroviral therapy (ART) experienced, but only 17% (34/161) reported regular ART use. Among 184 participants with CD4+ T-cell count measured, the median (IQR) CD4+ T-cell count was 51 (16–234) cells/mm^3 with 57% (106/184) having a CD4+ T-cell count <100 cells/mm^3. Only 13% (n = 27) of hospitalized participants with pneumonia had human immunodeficiency virus (HIV) viral loads of <50 copies/mL.

The prevalence of serum CrAg positivity was 3.5% (7/202; 95% confidence interval [CI], 1.4% to 7.0%). Table 1 reports the main clinical and laboratory characteristics of these CrAg-positive participants. Four (2.0%) of 202 cases were classified as asymptomatic cryptococcal antigenemia, and three (1.5%) of 202 cases had symptomatic cryptococcosis. The median (min–max) CD4+ T-cell counts were 67 (19–920) cells/mm^3 and 12 (4–18) cells/mm^3 in asymptomatic cryptococcal antigenemia and symptomatic cryptococcosis, respectively (P = 0.034). Among symptomatic cryptococcosis, one had culture-proven symptomatic cryptococcal meningitis; one had culture-proven asymptomatic cryptococcal meningitis associated with clinical and radiological diagnosis of pneumonia but without a bacterial etiologic diagnosis (with pneumonia possibly caused by *Cryptococcus*); and one had pathology-proven symptomatic cryptococcal pneumonia. The prevalence of serum CrAg positivity in pneumonia patients with CD4+ T-cell count <100 cells/mm^3 was 5.7% (6/106; 95%CI, 2.1% to 11.9%). The other serum CrAg-positive participant had a CD4+ T-cell count of 920 cells/mm^3, HIV viral load of <50 copies/mL, and was adherent to ART. He was serum CrAg-positive and was classified as asymptomatic cryptococcosis; however, this may have been a false positive latex agglutination test.

None of the patients received preemptive cryptococcal treatment during hospitalization. Patient number 1 (Table 1) developed a culture-proven cryptococcal meningitis seven months later. Unfortunately, long-term follow-up was unavailable for the other participants.

Table 1. Characteristics of people living with HIV/AIDS hospitalized due to community-acquired pneumonia and positive serum cryptococcal antigen.

ID	Age (years)/ gender	CD4+ cells/mm^3	Clinical presentation	Radiological findings	Community-acquired pneumonia etiology and other diagnosis	Pneumonia treatment	Symptomatic cryptococcosis	CSF culture; CSF CrAg	Fungal blood cultures	Serum CrAg titer	Cryptococcal treatment	Hospital outcome
1	58/Male	85	Productive cough, pleuritic pain	Interstitial infiltrate and consolidation	*Rhodococcus* (culture-proven in sputum and blood); *Candida* esophagitis	Ceftriaxone + azithromycin + TMP-SMX	No	No lumbar puncture performed	Negative	1:16	None[c]	Discharge to home
2	51/Male	49	Dry cough, dyspnea, pleuritic pain	Interstitial infiltrate	*Pneumocystis* (PCR-proven in sputum); oral thrush	Ceftriaxone + azithromycin + TMP-SMX	No	No growth; negative CrAg	Negative	1:16	None	Discharge to home
3	43/Male	18	Productive cough, dyspnea, pleuritic pain	Interstitial infiltrate	No etiology identified	Ceftriaxone + azithromycin	Yes[a]	*Cryptococcus neoformans* and positive CrAg	Negative	1:512	Amphotericin + fluconazole	Death
4	43/Male	920	Productive cough, dyspnea, pleuritic pain, fever	Consolidation	Rhinovirus (swab-PCR FilmArray Respiratory Panel)[d]	Ceftriaxone	No	No growth; negative CrAg	Negative	Positive[b]	None	Discharge to home
5	47/Male	12	Dry cough, pleuritic pain, mental confusion	Interstitial infiltrate and consolidation	*Pneumocystis* (PCR-proven in sputum)	Ceftriaxone + azithromycin + TMP+SMX	Yes	*Cryptococcus neoformans*; positive CrAg	Positive	1:1024	Amphotericin + fluconazole	Discharge to home
6	50/Male	4	Productive cough, pleuritic pain, fever	Interstitial infiltrate and consolidation	Cryptococcosis (pulmonary pathology-proven)	Ceftriaxone + clarithromycin	Yes[a]	No growth; negative CrAg	Negative	Positive[b]	Amphotericin + fluconazole	Discharge to home
7	63/Male	19	Dry cough, dyspnea, pleuritic pain, fever	Interstitial infiltrate	*Pneumocystis* (PCR-proven in sputum); cytomegalovirus esophagitis	Ceftriaxone + azithromycin + TMP-SMX	No	No growth; negative CrAg	Negative	1:4	Amphotericin + fluconazole	Discharge to home

CSF: cerebrospinal fluid; CrAg: cryptococcal antigen latex agglutination test; PCR: polymerase chain reaction; TMP-SMX: trimethoprim-sulfamethoxazole; CAP: community-acquired pneumonia.
[a]Absence of neurological complaints.
[b]Only qualitative assay was performed.
[c]Developed culture-proven cryptococcal meningitis seven months later.
[d]BioFire Diagnostics, Salt Lake City, UT, USA.

Discussion

We found 3.5% of PLWH adults hospitalized with CAP were serum CrAg positive, and the prevalence of serum CrAg positivity was 5.7% in patients with CD4+ T-cell count <100 cells/mm^3.

Recently, the World Health Organization recommended a package of interventions for PLWH with advanced HIV disease including screening, treatment, and/or prophylaxis for major opportunistic infections, including cryptococcosis and tuberculosis.[4] Routine serum CrAg screening followed by pre-emptive antifungal therapy if CrAg-positive should be considered in patients with CD4+ T-cell count <100 cells/mm^3 to reduce the development of cryptococcal disease.[3,4]

Although this recommendation is derived from studies among ART-naïve persons, this strategy should be pursued among PLWH with advanced disease who are re-engaging into care after a period of ART interruption or who are failing ART and unwell.[4] The profiles of patients included in this study and the results of prior studies performed in Latin America and Africa reinforce this strategy.[7–10]

Initial cryptococcal infection is most often asymptomatic, but cryptococcosis can present as subacute pneumonia. CrAg may precede by several weeks to months the manifestation of meningitis. However, in clinical practice, routine CrAg testing is uncommon when a patient presents with pulmonary disease without neurologic symptoms. Cryptococcal lung involvement can be observed among 38–78% of autopsies of PLWH with disseminated cryptococcosis.[11] However, isolated cryptococcal pneumonia is less frequent even in autopsy studies (for example, 7% in autopsies of PLWH with cryptococcosis).[11] In the present study, only one patient had confirmed pulmonary cryptococcosis and all but one of the other six CrAg-positive patients had other confirmed etiologies to explain the respiratory findings. Interestingly, the single patient without an etiologic diagnosis of pneumonia had cryptococcal meningitis; therefore, pulmonary cryptococcosis cannot be excluded. This patient had no neurologic complaints, and cryptococcal meningitis was confirmed only after lumbar puncture was performed in response to the serum CrAg. Accordingly, a recent study reported that up to one-third of asymptomatic CrAg-positive patients have concurrent central nervous system cryptococcal involvement.[12] This is particularly true as the CrAg titer rises above 1:160.[13] These cases of 'subclinical cryptococcal meningitis' are likely underrecognized. World Health Organization guidelines do not specifically recommend lumbar punctures among asymptomatic CrAg-positive patients in 2011,[14] but this recommendation was updated in 2018.[3] Thus, lumbar puncture should be used routinely to investigate cryptococcal meningitis in all CrAg-positive patients, even those without symptoms.[3,4,12] In the same study, blood CrAg titer using lateral flow assay (LFA) (≥1:160) was significantly associated with cryptococcal meningitis in both asymptomatic and symptomatic patients.[12] In accordance with this, our patient with cryptococcal meningitis but without neurological manifestations showed CrAg latex agglutination titres of 1:512 equating to an approximate CrAg LFA titer of ~1:1280.[15]

In the present study, two patients with confirmed cryptococcal meningitis had *P. jirovecii* pneumonia, which pulmonary cryptococcosis can mimick.[16–18] There is scarce information about the frequency of cryptococcal antigenemia in hospitalized PLWHA with PAC, but cryptococcal pneumonia is rarely reported; also, rarely is CrAg reported as part of diagnostic pulmonary testing.[5] Contrary to this general assumption, Harris et al. studied PLWH in rural Thailand demonstrating among patients hospitalized for an acute respiratory illness, 13.1% were serum CrAg-positive and 40% may have had cryptococcal pneumonia.[19] Similarly, in Uganda, among 563 hospitalized PLWH with cough for ≥2 weeks and suspected tuberculosis, 5.7% were CrAg positive.

In this study, the patients had no clinical suspicion of cryptococcal disease. Interestingly, none of the CrAg-positive patients who underwent bronchoscopy were diagnosed with cryptococcal pneumonia.[20]

Our results from Brazil as well as the studies from Thailand and Uganda suggest that when tested for, CrAg-positivity is relatively common among PLWH with pulmonary disease, particularly in patients with severe immunosuppression. In our study, the population was patients with probable bacterial CAP who were enrolled into a clinical trial of bacterial pneumonia therapy. Thus, we did not include patients with other radiographic patterns (e.g. clustered nodular pattern, solitary pulmonary nodule, mass with/without cavitation, or scattered nodules) where cryptococcal disease is a concern.[17,18,21,22] For future research studies of bacterial pneumonia or tuberculosis in PLWH, CrAg testing should always be included as part of the initial evaluation.

The limitations of this study correspond to its retrospective design. However, we have been able to retrieve the information needed to achieve the desired goals.

In addition, CrAg-positive serum only demonstrates the presence of cryptococcal antigen, but does not necessarily indicate cryptococcal pneumonia. Ideally, all CrAg-positive patients included in this study should have undergone bronchoscopy with bronchoalveolar

lavage in order to systematically assess the diagnosis of cryptococcal pneumonia. Finally, considering that LFA performance is more sensitive than any other diagnostic test for cryptococcosis, it is possible that some cases may have been underdiagnosed in this study.

In conclusion, we found a high prevalence (3.5%) of CrAg-positivity in PLWH admitted with a diagnosis of CAP; 2.0% of the cases were classified as asymptomatic cryptococcal antigenemia and 1.5% had symptomatic cryptococcosis. The prevalence of CrAg-positivity was higher (5.7%) in persons with CD4+ T-cell count <100 cells/mm^3 where 1 in 18 patients had cryptococcal antigenemia. This study demonstrates another targeted population for CrAg testing: PLWH hospitalized with a diagnosis of CAP, particularly those with CD4+ T-cell counts <100 cells/mm^3, regardless of ART status. In the absence of timely CD4+ T-cell count results, we recommend all PLWH with pulmonary disease be CrAg screened to detect and preemptively treat cryptococcal infection while asymptomatic or to enable a timely diagnosis of symptomatic cryptococcal disease.

Declaration of conflicting interests

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