

Prior Pulmonary Tuberculosis Is a Risk Factor for Asymptomatic Cryptococcal Antigenemia in a Cohort of Adults With Advanced Human Immunodeficiency Virus Disease

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The greater mortality risk among people with advanced human immunodeficiency virus disease and cryptococcal antigenemia, despite treatment, indicates an increased susceptibility to other infections. We found that prior tuberculosis was an independent risk factor for cryptococcal antigenemia (adjusted odds ratio, 2.72; 95% confidence interval, 1.13–6.52; $P = .03$) among patients with CD4 counts <100 cells/ μ L.

Keywords. advanced HIV disease; cryptococcosis; tuberculosis.

Tuberculosis (TB) and cryptococcal disease are leading causes of death among adults with advanced human immunodeficiency virus disease (HIV) [1]. Screening and treatment for both diseases are required before antiretroviral therapy (ART) is

initiated, re-commenced, or switched due to virological failure in individuals with low CD4 T-lymphocyte counts (CD4 counts) [2]. Cryptococcal antigenemia remains associated with an increased risk of mortality despite screening and preemptive treatment with fluconazole [3], possibly indicating that other opportunistic infections such as TB may be causing excess deaths among individuals with cryptococcal antigenemia. A shared immunological deficit (in addition to CD4 depletion) or pathophysiological mechanism might explain dual susceptibility to both pathogens in certain individuals with advanced HIV disease.

Previous studies have provided limited evidence of an association between previous TB and the occurrence of cryptococcal meningitis among patients with advanced HIV disease [4, 5]. However, the relationship between TB and cryptococcal antigenemia in patients without symptomatic cryptococcal meningitis is unclear, and data related to comorbid diagnoses and causes of death are sparse.

An understanding of the relationship between TB and cryptococcosis will help guide physicians in managing patients with advanced HIV disease. In this case-control study, we explored the association between a history of prior or prevalent TB and the presence of asymptomatic cryptococcal antigenemia in patients undergoing cryptococcal antigen (CrAg) screening.

METHODS

Retrospective data were collected for participants of a CrAg screening study described elsewhere [3]. Human immunodeficiency virus-positive adults with CD4 counts <100 cells/ μ L (both ART naive and experienced) were invited to participate during inpatient or outpatient attendance at 2 hospitals in Johannesburg, South Africa between June 2015 and October 2017. Patients with asymptomatic cryptococcal antigenaemia, and CrAg-negative patients with similar CD4 counts (± 10 cells/ μ L, at a 1:2 ratio), were consecutively enrolled if they provided consent.

Patients who had lumbar punctures performed revealing subclinical cryptococcal meningitis (cerebrospinal fluid positive for CrAg, India Ink microscopy, or culture) were included as long as they were asymptomatic at the time of enrollment.

Participants were asked about their medical history. If they reported a history of TB, the date, site of infection, and method of diagnosis (using clinical and laboratory records) was recorded, as well as treatment received. All participants were tested for prevalent TB (irrespective of symptoms) using pre- and/or postinduction sputum for auramine staining and microscopy, liquid culture (BACTEC MGIT; Becton Dickinson, Franklin Lakes, NJ), and molecular testing (Xpert MTB/RIF; Cepheid,

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Table 1. Results of Logistic Regression Analysis for Age, Sex, CD4 Cell Count, Prior Tuberculosis, and Cryptococcal Antigenemia

Variable parameters	n	CrAg Positive, % (n)	Unadjusted OR (95% CI)	P	Adjusted OR ^a (95% CI)	P
Age						
<39	99	35.3 (35)	Base		Base	
≥39	102	31.4 (32)	0.84 (.46–1.50)	.55	0.88 (.47–1.66)	.71
Sex						
Male	102	29.4 (30)	Base		Base	
Female	99	37.4 (37)	1.43 (.79–2.58)	.23	1.42 (.76–2.68)	.27
CD4 (<50)						
≥50	66	18.2 (12)	Base		Base	
<50	135	40.7 (55)	3.09 (1.52–6.31)	.002	3.37 (1.60–7.11)	.001
ART Status^b						
Naive/defaulted	172	29.7 (51)	Base		Base	
On ART	29	55.2 (16)	2.92 (1.31–6.51)	.009	2.58 (1.09–6.09)	.03
Prior TB						
No prior TB	172	20.2 (52)	Base		Base	
Prior TB	29	51.7 (15)	2.47 (1.11–5.49)	.03	2.72 (1.13–6.52)	.03

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; CrAg, cryptococcal antigen; OR, odds ratio; TB, tuberculosis.

^aAll variables adjusted for age, sex, CD4 count, ART status, and prior TB. There was no association between age and CrAg status if age was included as a categorical or continuous variable.

^bART status was categorized as naive or defaulted if they had not taken any ART in the past 3 months.

Sunnyvale, CA) and urine for lipoarabinomannan (LAM) (Determine TB LAM; Alere, Waltham, MA). Results were available immediately for urine LAM testing and within 24 hours for sputum Xpert MTB/Rif and microscopy. Culture results were reported when positive within 28 days incubation.

We defined prior TB as being diagnosed more than 2 months before study enrollment, and we defined prevalent TB as being diagnosed during the previous 2 months, or from samples taken on the day of enrollment using any of the diagnostics described. We assessed the risk of asymptomatic cryptococcal antigenemia in those with either prior or prevalent TB by calculating odds ratios (ORs) with 95% confidence intervals (CIs), using logistic regression modeling, adjusting for a priori confounders age, sex, CD4 count, and ART status.

Patient Consent Statement

Patient's written consent was obtained before participation in this study. The study protocol was approved by the Ethics Committees at University of the Witwatersrand, South Africa and the London School of Hygiene & Tropical Medicine, United Kingdom.

RESULTS

Sixty-seven CrAg-positive and 134 CrAg-negative patients were enrolled in the study. There were no differences in age, sex, serum C-reactive protein levels, body mass index, hemoglobin, or other opportunistic infections between CrAg-positive and CrAg-negative participants (Supplementary Table 1). However, CrAg-positive participants had lower CD4 counts (27 cells/μL [IQR 7–40] vs 41 cells/μL [IQR 16–64], $P = .002$), and they were more likely to have started ART (24% vs 10%, $P = .01$).

Prior TB was reported in 15 of 67 (22%) CrAg-positive patients and 14 of 134 (10%) CrAg-negative patients (OR, 2.47; 95% CI, 1.11–5.49; $P = .03$) (Supplementary Table 2). This association remained significant when adjusted for age, sex, ART status, and CD4 count (adjusted OR, 2.72; 95% CI, 1.13–6.52; $P = .03$) (Table 1). Laboratory records were only available for individuals who had been diagnosed with TB at the study sites. Among these patients, 5 (17%) were diagnosed on the basis of laboratory tests: staining and microscopy (1); culture (2); sputum Xpert MTB/RIF (2); urine LAM (1).

Prevalent TB was reported in 15 of 67 (22%) CrAg-positive and 32 of 134 (24%) CrAg-negative patients (adjusted OR, 0.90; 95% CI, .43–1.90; $P = 0.8$); 18 of 47 (38%) patients had been diagnosed with TB within the past 2 months, and 29 of 47 patients (62%) were diagnosed on the day of study enrollment (Table 1). Prevalent TB diagnoses were confirmed by laboratory tests in 43 of 47 (91%) patients: staining and microscopy (11); sputum culture (9), nonsputum culture (2), sputum Xpert PCR (16); nonsputum Xpert (1); histology (3); and urine LAM (38). Four (9%) patients were diagnosed with TB on the basis of radiological and clinical features only.

Prior TB was associated with a 2.7-fold higher odds of asymptomatic cryptococcal antigenemia in HIV-positive adults with CD4 counts of <100 cells/μL. Prevalent TB was common among both CrAg-positive and CrAg-negative patients; reported in over one fifth of patients. However, there was no evidence of an association between prevalent TB and cryptococcal antigenemia.

DISCUSSION

Our findings are consistent with previously reported evidence of an association between TB and cryptococcosis. In a

prospective cohort of 707 patients commencing ART in South Africa, 11 of 13 (85%) of those with cryptococcal meningitis had a history of TB. Prior (but not prevalent) TB within 2 years was a strong and independent predictor for the development of cryptococcal meningitis on multivariable analysis (OR, 6.6; 95% CI, 1.3–32.7, $P = .02$) [4]. A retrospective analysis of over 175 000 patients attending ART clinics in Zimbabwe, Zambia, and South Africa found that a history of TB was associated with cryptococcal disease when adjusted for age, sex, CD4 count, and treatment site (adjusted hazard ratio, 1.28; 95% CI, 1.05–1.55; $P = .015$) [5]. Furthermore, prospective cohort [6] and autopsy studies among HIV-positive adults have revealed a high incidence of coinfection with TB and cryptococcosis; post-mortems revealed cryptococcal pneumonia as the cause of death in 4 of 7 (57%) patients with known TB in South Africa [7].

Previous studies have also observed a relationship between TB and asymptomatic cryptococcal antigenemia. A prospective CrAg screening study in HIV-positive adults with CD4 <150 cells/ μ L in Tanzania found that a higher proportion of CrAg-positive patients had active (smear-positive) TB (4 of 28, 20%) than CrAg-negative patients (73 of 722, 12%) [8]. In Kenya, CrAg-positive patients were more likely to be started on antituberculous medication during the year after screening (22 of 59, 37% vs 109 of 455, 24%; $P = .04$) [9], although the method of TB diagnosis was not reported.

Individuals with advanced HIV disease become more susceptible to both TB and cryptococcal disease after CD4 depletion so dual infection is not unexpected. However, we found prior TB to be strongly predictive of cryptococcal antigenemia even after adjusting for CD4 count. This could be explained by immunological deficits in addition to HIV-associated CD4 depletion. Lower levels of proinflammatory cytokine production (interferon- γ and tumor necrosis factor- α), for example, have been associated with reduced elimination of both organisms [10, 11]. Both organisms are also known to “cause” innate and adaptive immunosuppressive effects [10, 11]. This may result in an interdependent susceptibility to *Mycobacterium tuberculosis* or *Cryptococcus*, when an individual becomes infected with either pathogen. An alternative explanation is that TB-related compromise of the respiratory mucosa results in an increased susceptibility to cryptococcal infection after exposure, facilitating the entry and dissemination of *Cryptococcus* inhaled from the environment or reactivation of latent cryptococcal infection. Furthermore, prolonged immune suppression may account for increased susceptibility to both organisms; the duration of advanced HIV infection was not known among this cohort.

Serological evidence suggests that exposure to *Cryptococcus* is likely to be almost universal, and infection can remain latent and then reactivate in the context of immune compromise [12]. However, only a small proportion of individuals with low CD4 counts develop disseminated cryptococcosis (global prevalence

of cryptococcal antigenemia is estimated to be approximately 6% in individuals with CD4 counts <100 cells/ μ L [13]). Because CrAg-positive patients have an increased risk of death despite fluconazole treatment, irrespective of whether they develop cryptococcal meningitis [3], an additional immune defect beyond CD4 depletion is plausible in the subpopulation of people with advanced HIV disease who develop cryptococcal antigenemia. This additional immune defect may increase susceptibility to other infections such as TB. We found that prior TB diagnosed at least 2 months before CrAg screening is a risk factor for cryptococcal antigenemia, indicating that host-related susceptibility is pre-existing, or perhaps a consequence of TB in some patients.

Our study is limited in numbers and by incomplete laboratory data for prior TB diagnoses. Although efforts were made to select comparable groups, CrAg-positive patients had lower CD4 counts and were more likely to be taking ART. Of note, the association between prior TB and cryptococcal antigenemia persisted despite adjustment for these confounders. Furthermore, more sensitive tests for TB are now available. However, because all patients were screened, these would not have affected the “difference” in positivity rates between cases and controls.

CONCLUSIONS

Both TB and cryptococcosis are associated with an increased risk of death among patients with advanced HIV disease [3, 14, 15]; patients with coinfection are at greater risk [6]. Our findings highlight the importance of comprehensive screening for TB among CrAg-positive patients, and of CrAg screening among HIV-positive adults with a prior history of TB, who may be at increased risk of cryptococcosis.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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References

1. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* **2008**; 22:1897–908.
2. World Health Organization. Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy. Geneva: World Health Organization, **2017**.
3. Wake RM, Govender NP, Omar T, et al. Cryptococcal-related mortality despite fluconazole preemptive treatment in a cryptococcal antigen screen-and-treat program. *Clin Infect Dis* **2020**; 70:1683–90.
4. Jarvis JN, Harrison TS, Corbett EL, Wood R, Lawn SD. Is HIV-associated tuberculosis a risk factor for the development of cryptococcal disease? *AIDS* **2010**; 24:612–4.
5. Fenner L, Reid SE, Fox MP, et al. Tuberculosis and the risk of opportunistic infections and cancers in HIV-infected patients starting ART in Southern Africa. *Trop Med Int Health* **2013**; 18:194–8.
6. Rutakingirwa MK, Cresswell FV, Kwizera R, et al. Tuberculosis in HIV-associated cryptococcal meningitis is associated with an increased risk of death. *J Clin Med* **2020**; 9:781.
7. Murray J, Sonnenberg P, Shearer SC, Godfrey-Faussett P. Human immunodeficiency virus and the outcome of treatment for new and recurrent pulmonary tuberculosis in African patients. *Am J Respir Crit Care Med* **1999**; 159:733–40.
8. Letang E, Müller MC, Ntamungiro AJ, et al. Cryptococcal antigenemia in immunocompromised human immunodeficiency virus patients in rural Tanzania: a preventable cause of early mortality. *Open Forum Infect Dis* **2015**; 2:ofv046.
9. Meyer A-CL, Kendi CK, Penner JA, et al. The impact of routine cryptococcal antigen screening on survival among HIV-infected individuals with advanced immunosuppression in Kenya. *Trop Med Int Health* **2013**; 18:495–503.
10. Vanham G, Toossi Z, Hirsch CS, et al. Examining a paradox in the pathogenesis of human pulmonary tuberculosis: immune activation and suppression/aneergy. *Tuber Lung Dis* **1997**; 78:145–58.
11. Tenforde MW, Scriven JE, Harrison TS, Jarvis JN. Immune correlates of HIV-associated cryptococcal meningitis. *PLoS Pathog* **2017**; 13:e1006207.
12. Goldman DL, Khine H, Abadi J, et al. Serologic evidence for *Cryptococcus neoformans* infection in early childhood. *Pediatrics* **2001**; 107:e66.
13. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* **2017**; 17:873–81.
14. Pac L, Horwitz MM, Namutebi AM, et al. Implementation and operational research: integrated pre-antiretroviral therapy screening and treatment for tuberculosis and cryptococcal antigenemia. *J Acquir Immune Defic Syndr* **2015**; 68:e69–76.
15. Manabe YC, Nonyane BAS, Nakiyingi L, et al. Point-of-care lateral flow assays for tuberculosis and cryptococcal antigenuria predict death in HIV infected adults in Uganda. *PLoS One* **2014**; 9:e101459.