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Integrating Clinical Services for HIV, Tuberculosis, and Cryptococcal Disease in the Developing World: A Step Forward with 2 Novel Diagnostic Tests

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Abstract

The success of antiretroviral therapy (ART) programs in the developing world is limited by the lack of adequate diagnostic tests to screen for life-threatening opportunistic infections such as tuberculosis (TB) and cryptococcal disease. Furthermore, there is an increasing need for implementation research in measuring the effectiveness of currently available rapid diagnostic tests. The recently developed lateral flow assays for both cryptococcal disease and TB have the potential to improve care and greatly reduce the time to initiation of ART among individuals who need it the most. However, we caution that the data on feasibility and effectiveness of these assays are limited and such research agendas must be prioritized.

Keywords

cryptococcosis, tuberculosis, point-of-care diagnostics, service integration

Despite global investments in HIV care over the past decade, the mortality rate within 12 months of starting antiretroviral therapy (ART) averages 15% to 20%.[1,2] Undiagnosed and untreated cryptococcosis and tuberculosis (TB), 2 leading causes of mortality among HIV-infected individuals worldwide,[2] significantly limit the success of ART programs. This is particularly true in sub-Saharan Africa, a region that receives over 50% of the total budget of the Global Fund to Fight AIDS, TB, and malaria[3] and where both cryptococcosis and TB are endemic.[4-6] Unmasking occult cryptococcosis and TB with the initiation of ART causes significant morbidity and mortality, particularly in central nervous system disease.[7-9] Additionally, the fear of unmasking those diseases results in an unnecessary delay in ART initiation among those who are not coinfected.

In endemic regions, effective screening and treatment for TB and cryptococcosis prior to initiation of ART could save thousands of lives a year. However, both cryptococcosis and TB lack diagnostic tools that are readily available in clinical settings with limited laboratory infrastructure.[10,11] This situation may be changing with the advent of 2 newly developed rapid diagnostic tests: the lateral-flow assay (LFA) to detect cryptococcal infection (cryptococcal antigen [CrAg] LFA; Immunomycologics, Norman, Oklahoma)[12] and the lipoarabinomannan (LAM) LFA (Determine TB-LAM Ag; Alere, Waltham, Massachusetts)[13] to detect TB. Using these 2 examples, we discuss how implementation research is critical in assessing the feasibility, scalability, and effectiveness in reducing HIV-associated mortality and promoting the integration of clinical services with those and similar rapid diagnostic tests.

Cryptococcosis

Cryptococcosis accounts for up to 18% of all deaths among HIV-infected individuals,[1,5] a figure that is likely underestimated. Despite the rapid scale-up of ART programs in the past decade, the decline in the incidence of and mortality associated with cryptococcosis has not been commensurate.[6] Reasons for this high mortality include the delayed diagnosis of HIV infection, suboptimal medical management of cryptococcal meningitis, and absent screening programs to identify curable cryptococcal infection before the onset of meningitis.

Asymptomatic cryptococcal infection is an independent risk factor for increased mortality during the first 12 weeks of ART initiation.[14] Screening asymptomatic individuals with CD4 count $\leq$100 cells/mm^3 and offering pre-emptive oral

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fluconazole therapy to positive cases prior to ART initiation are cost effective and decrease morbidity and mortality from cryptococcal meningitis.[15,16] However, the most commonly used serum-based screening tests for cryptococcosis, the latex agglutination test (LA) and the enzyme immunoassay (EIA), necessitate a laboratory infrastructure that is not available in most resource-poor settings. Such laboratory requirements include the ability to heat and cool specimens rapidly, the ability to pretreat with various enzymes such as pronase and, in the case of the EIA, the availability of spectrophotometry.[10,17] The recently developed LFA, which detects the same cryptococcal polysaccharide capsule glucuronoxylomannan as the LA and EIA using monoclonal antibodies, has been cleared by US Food and Drug Administration for use in serum and cerebrospinal fluid (CSF) specimens, given its close correlation (>99%) with the LA and EIA.[12,18] The serum LFA is 95.8% to 100% sensitive in the detection of cryptococcosis when compared with cryptococcal latex agglutination and culture.[12,18] Quantitative titers are ascertained by mixing the specimen (serum, CSF, or urine) with diluent solution.

The use of the LFA in urine or whole blood may offer a point-of-care strategy in resource-poor settings by obviating the use of centrifugation. Little is known of the test's characteristics in urine or whole blood in those with asymptomatic infection, but this remains an area of ongoing research.[10,19] The approximate cost of the LFA is US$2, and the test materials are stable at room temperature. The LFA is more cost effective in screening asymptomatic individuals with HIV/AIDS for cryptococcal disease than the LA.[20] In a region with an approximately 8% prevalence of cryptococcal antigenemia, the LFA would cost US$28.37 to detect one case of asymptomatic cryptococcal disease, or less than 15% of the cost of the LA, which would cost US$190.[20] Similarly, when taking into account fluconazole pre-emptive treatment, the LFA would cost US$39.73, while the LA would cost US$266 to prevent one death in a region of similar prevalence.[20]

How best to screen individuals for asymptomatic infection is a research priority, and outcome measurements should include surrogate end points (such as time to initiation of ART and frequency of immune reconstitution inflammatory syndrome) as well as mortality. Clinical algorithms have been proposed that focus on screening and treatment,[21] and these should be evaluated and tailored according to local resources (Figure 1).

HIV-Associated TB

Worldwide, TB accounts for 5% to 44% of deaths among individuals with HIV infection,[1] and case finding remains a significant challenge. The most commonly used diagnostic and screening tests for TB in resource-limited settings include smear microscopy and a 4-point clinical symptom score (measuring the presence of cough, fever, night sweats, and/or weight loss). Both have remarkably poor performance in severely immunocompromised individuals, with 20% sensitivity for smear microscopy and 50% specificity for the 4-point symptom score.[11] More recently, the Xpert *Mycobacterium tuberculosis* (MTB)/rifampicin (RIF) assay, which can confirm the presence or absence of *M tuberculosis* and RIF susceptibility in specimens within 2 hours, has demonstrated high accuracy,[22] but it is costly (up to US$30 per specimen when taking all costs into account) and requires regular servicing, computer connectivity, and an electricity supply, all of which are often absent in low-resource settings.[13]

A new point-of-care assay detects LAM antigen (a cell wall component of *M tuberculosis*) in the urine. The TB-LAM antigen assay has a sensitivity of 66.7% at CD4 counts $\leq$50 cells/mm^3 and 51.7% at CD4 counts $\leq$100 cells/mm^3 (using liquid-based *M tuberculosis* culture of sputum as the gold standard) for the detection of *M tuberculosis*.[13] Similar to the cryptococcal LFA, the TB-LAM antigen assay functions as a dipstick (with colloidal antibodies on the strip binding to the LAM present in the urine[23]) and can yield a result in 25 minutes. The sensitivity of the test is increased when paired with sputum smear microscopy, comparable in accuracy with a single Xpert MTB/RIF sputum test.[24] The lower specificity of the TB-LAM antigen assay (87%-99%) when compared with *M tuberculosis* sputum culture may be a result of the ability of the assay to detect sputum culture-negative cases and/or extrapulmonary TB. Alternatively, nontuberculous mycobacteria and disseminated fungal infections might cross-react with the assay.[25]

In one cohort with a high prevalence of both TB and advanced AIDS, a high frequency of faint bands and low optical density were noted by enzyme-linked immunosorbent assay.[26] Furthermore, lower interobserver agreement (89.1% compared with the 99.6% noted in the original study) was also noted for these low-intensity bands. Although the original study only looked at binary results, whereby the faintest band was considered positive, there may be a need for stricter cutoffs to improve the specificity of the assay.[26] Such is an example of how better implementation research is imperative in assessing the robustness of this and other assays in different health care settings, and factors such as assay production batch and methods of urine collection and storage must be considered.[27]

Prior commercial serologic tests, including those that detect LAM, were not accurate in the detection of TB.[28] Given the limited sensitivity of the TB-LAM, the assay must not be used in lieu of smear microscopy[29]; and given the specificity, implementation of the urine LAM should only be considered in areas with a high prevalence of TB (and consequently where it will have a high-positive predictive value). However, if used in conjunction with existing screening protocols, the TB-LAM has the potential to increase case finding as well as to increase the number of patients who start ART sooner by rapidly excluding TB (Figure 1). In one recently reported study, adding the TB-LAM to microscopy in a high-prevalence setting resulted in the treatment of 80 additional individuals for every 1000 patients tested and averted 59 disability-adjusted life years.[30] The approximate cost of the TB-LAM antigen assay is US$3.50/test, making it cost effective in high-prevalence settings.[30] Furthermore, that assay would identify a subpopulation of patients with high *M tuberculosis* burdens and advanced

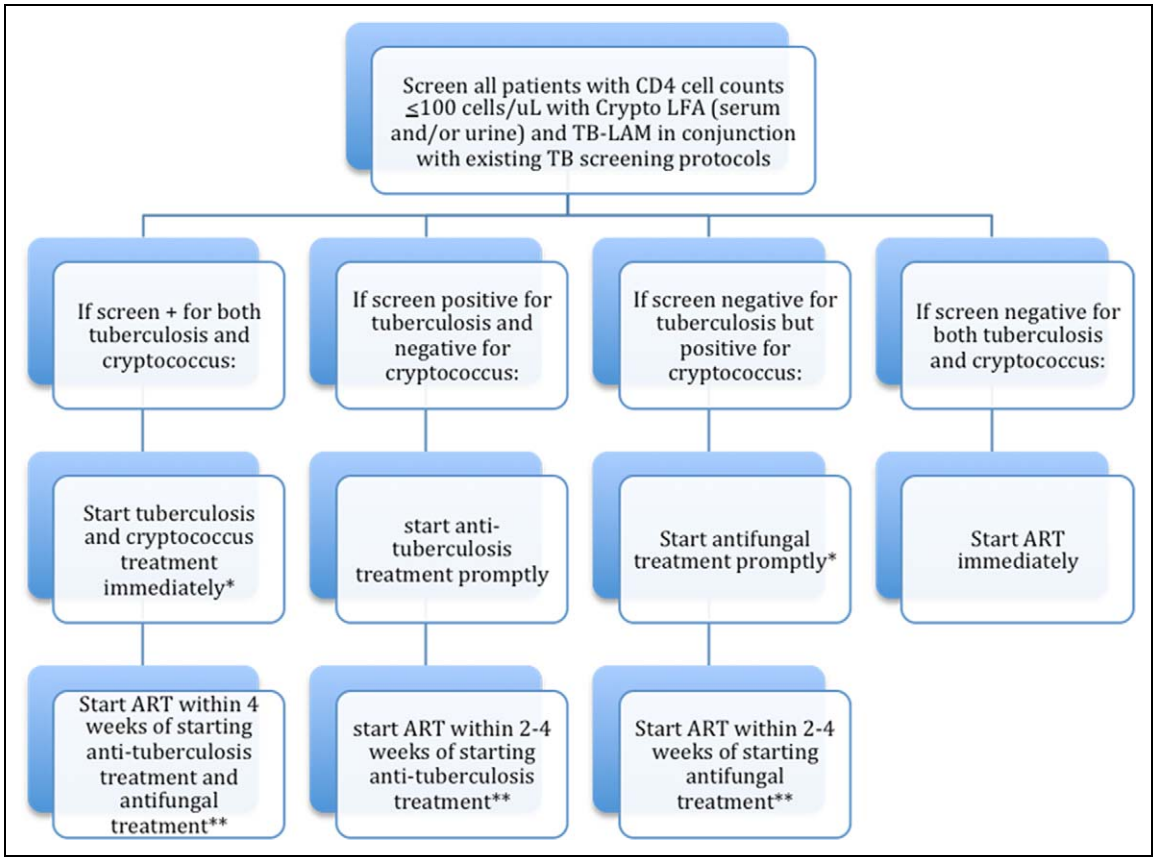

Figure 1. Pre-initiation of antiretroviral therapy screening algorithm. LFA, lateral-flow assay; TB, tuberculosis; LAM, lipoarabinomannan; ART, antiretroviral therapy.
*May need to perform lumbar puncture to rule out cryptococcal meningitis if symptomatic or treat empirically for cryptococcal meningitis.
**Optimum timing of ART has been better defined in TB than in cryptococcal meningitis.

immunosuppression[31] who are at greatest risk of developing and dying from severe immune reconstitution inflammatory disease. Implementation of the assay could decrease mortality among those individuals who are at greatest risk by enabling the early use of both anti-TB and ARTs.

Conclusion

Tuberculosis and cryptococcosis affect similar populations of individuals with advanced AIDS who have a high risk of premature death even with the initiation of ART. As others have noted, the CrAg LFA and the TB-LAM have the potential as screening tests to significantly improve clinical outcomes through early diagnosis and treatment.[24] Both the cryptococcal LFA and the TB-LAM meet many of the World Health Organization Affordable, Sensitive/Specific, User-friendly, Rapid/Robust, Equipment-free, Delivered (WHO ASSURED) criteria for being affordable, sensitive, specific, user-friendly, robust and rapid, equipment-free, and deliverable to those who need it the most. Although the accuracy of the TB-LAM is not as high as it is desired, and the need for better diagnostic tests for TB continues to be a major gap in our care for HIV-infected individuals, the TB-LAM can be useful in the appropriate clinical setting and the low cost makes it a tool that is easily scalable.

Several challenges remain in the systematic implementation of such diagnostic tests. While current policies have been fragmented with respect to the management of cryptococcosis, TB, and HIV, program integration at the research and service level must be a priority. A recent systematic review of the costs and efficiency of integrating HIV/AIDS services with other health services suggested that integration has the potential to achieve favorable health outcomes at a low cost.[32] The majority of the studies reviewed found that integration was cost effective, including the cost savings from preventive therapy.[32] To this end, research efforts should focus on program effectiveness using clinical end points such as time to initiation of ART (and/or fluconazole pre-emptive and isoniazid preventive therapies), retention in care, and mortality. Furthermore, it is not sufficient to look at the effectiveness of the TB-LAM and cryptococcal LFA independently as implementing both assays together have the potential to significantly decrease HIV-associated morbidity and mortality.[24]

Similarly, clinical efforts should prioritize screening for both cryptococcosis and TB, especially in those individuals with CD4 counts $\leq$100 cells/μL (Figure 1). It is not difficult

to envision the scale-up of HIV programs that are able to provide point-of-care CD4 and viral load testing with reflex point-of-care screening for both cryptococcosis and TB. Such a program has the potential to improve retention in care as well as reduce delays in ART initiation.[33]

We are at a crossroads in the management of HIV disease. Focusing efforts on the integration of clinical and research priorities for HIV, cryptococcosis, and TB will enhance existing international efforts to scale-up HIV care in the developing world. Point-of-care diagnostic tests might facilitate the integration of clinical services and improve retention in care. The early diagnosis and treatment of cryptococcosis and TB among individuals with HIV/AIDS can save thousands of lives and enable the earlier initiation of ART for those who need it the most.

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