Utilizing a Point of Care Test for Cryptococcal Screening Among Hospitalized HIV-Infected Adults in Addis Ababa, Ethiopia

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**Introduction/Background:** Cryptococcal meningitis (CM) is a major global public health problem that is responsible for significant morbidity and mortality among HIV-infected persons worldwide [1]. In Sub-Saharan Africa (SSA) there are an estimated 720,000 cases of HIV associated CM per year, with 530,000 resulting deaths suggesting mortality rates >50% [1]. This high rate compares to mortality rates of 10-20% in most developed countries [1]. A major contributor to the extremely high case fatality rate of CM in SSA is the lack of available diagnostics [2]. Early detection of cryptococcal antigenemia and prompt treatment can prevent the development of CM and improve the management of cryptococcal disease. The recent approval of a rapid and simple to use lateral flow assay (LFA) point of care cryptococcal antigen test offers much promise in improving the management of cryptococcal disease.

In light of the increasingly evident enormity of CM in resource limited settings (RLS), the World Health Organization (WHO) released rapid advice (interim) guidelines in 2011 to provide guidance in cryptococcal disease management. A major emphasis of the guidelines was the need to improve cryptococcal antigen (CRAG) screening. They recommended CRAG screening among antiretroviral therapy (ART)-naïve HIV infected adults with a CD4 <100 cells/mm³[3].

Prior CRAG screening studies have found high rates of cryptococcal antigenemia in several SSA countries including Uganda (5.8%) and South Africa (7%)[4]. Follow-up studies have demonstrated that cryptococcal antigenemia is significantly associated with mortality [4]. Most existing CRAG screening studies have used a latex agglutination-screening test, which requires a cold chain, high technical skill, and is too costly in many RLS. A recent study conducted by Emory Investigators, including my mentors, found a prevalence of cryptococcal antigenemia of 8.4% among 367 HIV-infected patients attending two ART clinics in Addis Ababa, Ethiopia [5]. The majority of CRAG screening studies to date evaluated HIV-infected patients in the outpatient setting and few have evaluated the prevalence of disease among hospitalized patients. One such study conducted in Thailand found that cryptococcus was the most common cause of acute respiratory infections among hospitalized HIV-infected patients [6]. Further work is needed to determine CRAG screening among hospitalized patients has a clinical benefit.

The recent FDA approval of a Lateral Flow Immunoassay (LFA) for the detection of cryptococcal antigenemia has the potential to revolutionize cryptococcal diagnosis. The LFA is a low cost, sensitive, and easy to perform test which requires minimal infrastructure and skill to perform and provides a result within 10-15 minutes[7]. The LFA test has all the attributes of a sustainable and effective point of care test (POCT). Initial studies have found the LFA to be more sensitive (can detect a lower concentration of cryptococcal antigen) than EIA and latex agglutination tests and accurate when compared to the gold standard of culture. A South African study found the LFA had a significant correlation (0.93 for serum, 0.94 for plasma and 0.94 for urine) with the EIA method for CRAG detection[8, 9]. When compared to the gold standard of culture the LFA demonstrated a sensitivity and specificity of 100% with a CI of 96-100%. While the LFA appears to be an excellent new tool in the fight against cryptococcal disease, operational research is needed to optimize the roll out and use of the LFA test.

Our proposed study will help provide important information on the utility of the LFA test in a hospital setting. Rates of cryptococcal disease among a high-risk group of hospitalized HIV-infected patients in Ethiopia and other sub-Saharan countries is not known. Our results will help inform forthcoming WHO guidelines on cryptococcal disease and on the local level will help determine the utility of hospital-based cryptococcal screening of HIV-infected persons. Ultimately, our study results will add to the growing body of literature on cryptococcal disease and hopefully help improve the prevention and management of HIV-infected patients inflicted with cryptococcal disease in Ethiopia and other RLS worldwide.

**Study Hypothesis:** A high prevalence of cryptococcal disease (>15%) exists among hospitalized HIV-infected patients in Addis Ababa, Ethiopia; the prevalence among HIV-infected
in-patients is much higher than reported in a previous study among HIV-infected patients at two outpatient HIV clinics (8.4% was recently reported by our group).

**Specific aims:**

1. To determine the prevalence of and risk factors for cryptococcal disease among HIV-infected adults admitted to Tikur Anbessa Hospital in Addis Ababa, Ethiopia utilizing an FDA-approved point of care cryptococcal antigen detection test (IMMY LFA).
2. To determine the utility of cryptococcal antigen screening among HIV-seropositive in-patients.
3. To determine the rate of cryptococcal meningitis among HIV-infected patients with a positive serum cryptococcal LFA test.
4. To determine the performance of the IMMY LFA cryptococcal antigen test on urine and finger stick whole blood as compared to serum LFA results.

**Study Design and Methods:** A cross-sectional study design will be utilized. Approval to carry out the study will be obtained from Addis Ababa University and Emory University IRBs prior to study initiation. All HIV-infected patients admitted to the medical wards of Tikur Anbessa (Black Lion) Hospital during May-August 2013 will be eligible to enroll. Tikur Anbessa is the major teaching hospital affiliated with Addis Ababa University and is the largest hospital in Ethiopia. It is estimated that 20-30% of all admitted medical patients are HIV-infected. All consenting HIV-infected adult (>18 years of age) patients will have serum (~1 ml), finger stick blood (~0.25 ml), and urine (~1 ml) samples collected. The LFA test will be performed on all samples and results will be provided to the treating physician. All management decisions including antifungal treatment and performing lumbar puncture will be at the discretion of the treating physician. Further data collection including information on clinical symptoms and HIV history will occur through a through an interview, and medical chart data abstraction.

**Data Analysis:** All data will be entered into an online REDCap database and analysis will be performed using SAS 9.3. Descriptive data analysis will be utilized to determine prevalence of cryptococcal antigenemia and distribution of study covariates. Multivariate logistic regression will be used to assess relationship between risk factors and a positive cryptococcal antigen test. The degree of agreement between test results (comparing results of LFA and CSF culture), and also between LFA results from different samples) will be assessed using the kappa (κ) statistic with a value of κ=1 denoting perfect concordance, and κ=0 denotes agreement by chance alone. A p-value of <0.05 will be considered statistically significant.

**Sample Size:** With an estimated prevalence of 15%; power of 80%, and 95% CI,

The following simple formula (Daniel, 1999) is used:

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n = \frac{Z^2 P(1-P)}{d^2}
\]

Where \( n \) = sample size; \( Z = Z \) statistic for a level of confidence =1.96;
\( P = \) expected prevalence = 0.15; \( d = \) precision (in proportion of one; if 5%, \( d = 0.05 \)).

According to the above formula and given variables the sample size will be about 196 patient samples. Screening this number of patients, we anticipate 30 positive specimens.

**Student Role:** I will travel to Addis Ababa, Ethiopia for a period of approximately 3 months to carry out the study. Dr. Russell Kempker, an Assistant Professor of Infectious Diseases at Emory University, will serve as my lead mentor and provide oversight for the project. While in Addis Ababa, I will be mentored on site by Dr. Admasu Tenna, Assistant Professor of Infectious Diseases at Addis Ababa University (AAU). I will assist in study design and logistics including
patient enrollment, perform the LFA test and instruct other investigators in test use. I will also conduct training sessions for Ethiopian research team members, hold research meetings every two weeks to assess study progress and identify challenges, and carry out data collection and entry. Furthermore, I will learn basic data analysis methods from my lead mentor and assist with data analysis.

References: