Operating characteristics and effectiveness of a screening algorithm for the detection of active tuberculosis in adult outpatients with HIV infection in Mozambique

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A. Background and Study Rationale.

Tuberculosis kills nearly 2 million people each year and is the leading cause of mortality in individuals infected with Inv in Africa. Screening for active tuberculosis (TB) in patients with HIV may increase early detection that may, in turn, decrease morbidity and mortality. It is also important to rule out active tuberculosis prior to initiating treatment for latent tuberculosis infection in order to prevent the development of drug resistance. Screening and diagnostic methods are limited, however, in resource-poor regions with severe limitations in number of clinical staff, radiographic facilities, and laboratory resources. Thus, the implementation of low-cost, easy-to-use, and sensitive screening techniques is imperative until infrastructure can be established.

Mozambique carries a high tuberculosis burden, with an estimated disease related mortality of 129 per 100,000. With increasing prevalence of HIV, it accounts in part, for the declining healthy life expectancy, currently of only 37.1 years. Estimated prevalence of HIV is 9.4-15.7%.[i] Tuberculosis prevalence is 636 per 100,000; current incidence is estimated at 457 per 100,000.[2] Approximately 49% of patients with tuberculosis are co-infected with HIV. Notably, however, these prevalence and incidence estimates exclude the majority of the population with no access to tuberculosis diagnosis. Case detection rates are merely 33% of estimated incidence. Thus, efforts have intensified to increase detection through screening.

As part of a national effort by Mozambique's Ministry of Health to increase tuberculosis case finding, a screening questionnaire and diagnostic algorithm are scheduled to be implemented in some centers delivering HIV care in 2005 (see appendices 1 and 2). This screening instrument is designed to provide reliable means to screen for and diagnose TB in outpatient HIV facilities, including prevention of mother-to-child-transmission (pMTCT) sites, voluntary testing and counseling (VCT) centers (with 77,800 clients nationally in 2003), and antiretroviral treatment clinics referred to as day hospitals. Most of these sites have extremely limited laboratory, radiographic, and physician capacity. The degree to which these capacities are limited can vary from site to site. In general, few of the sites have access to mycobacterial cultures or diagnostic procedures such as bronchoscopy, thoracentesis, pleural biopsy or lymph node biopsy. Only some have access to chest radiography. There are, however, 206 laboratories in Mozambique capable of performing AFB smears.

Previous studies have validated the use of a simple symptom questionnaire, administered by nurses, to screen for active TB in a small sample of patients with advanced HIV in hospital HIV clinics in urban South Africa.[3] Patients with clinically advanced HIV disease (WHO stage 3 or 4) were screened for active tuberculosis using a symptom questionnaire, measured weight loss, chest radiography, sputum microscopy and culture prior to receiving tuberculosis preventive therapy. Tuberculosis was diagnosed in 11 of 129 (8.5%) screened. The screening questionnaire of two or more symptoms of measured weight loss, cough, night sweats or fever, had a sensitivity of 100% and specificity of 88.1%, and positive and negative predictive values of 44% and 100%, respectively. The South African study included culture as part of the diagnostic strategy, and was not concerned with comparing the combined yield of the questionnaire, chest radiography, and sputum microscopy with that of culture. Furthermore, the study did not measure lymph node aspirate smear or culture for detecting extra pulmonary TB. Finally, the study did not validate the symptom questionnaire for patients with early HIV disease.

Hudson, Wood, and Maartens[4] assessed the time to diagnosis and the yield and laboratory cost of diagnostic procedures in hospitalized patients already diagnosed with tuberculosis in a university-affiliated

hospital in urban South Africa. They found a low sputum smear yield (40% per sample), but higher yield of lymph node aspirate smears (50%) for detecting TB in patients with lymphadenopathy. Lymph node biopsy yielded 82%. The authors propose a streamlined diagnostic algorithm for HIV-infected patients with suspected tuberculosis. However, their algorithm includes the use of pleural aspirate/biopsy and lymph node excision biopsy if pleural effusion or lymphadenopathy is present, prior to mycobacterial culture. Though useful in referral hospital settings, these diagnostic techniques require significant procedural expertise, pose a moderate risk to the patient, and involve more intensive laboratory processing. For resource-poor areas where neither mycobacterial cultures nor procedures can be performed, studies are needed that assess the effectiveness of a symptom questionnaire used in combination with a simple diagnostic strategy to accurately diagnose both pulmonary and extra pulmonary tuberculosis in all HIV patients. The goals of such a strategy would be to increase active case detection, shorten delays in detection that should translate into earlier treatment, and appropriately rule out active tuberculosis prior to initiating treatment for latent TB, all without incurring a significant interest in cost.

With this validation study, we attempt to assess the operating characteristics and effectiveness of a screening tool composed of a symptom questionnaire and a simple clinical algorithm to rule in or rule out active TB in HIV-infected outpatients. The screening instrument will be compared to the current gold standard: mycobacterial culture in combination with nucleic acid amplification tests (NAAT) for *M. tuberculosis*. If the sensitivity and specificity of the instrument approaches that of culture/NAAT, the screening tool could be widely implemented in resource-poor areas of high tuberculosis prevalence, where positive and negative predictive values of such a tool would be high. The screening tool may, however, turn out not to be useful despite high sensitivity and specificity if it fails to increase case detection in HIV outpatients. Thus, we also plan to compare case detection rates for new outpatients during the year prior to implementation of the instrument, and during one year after initiating its use. If the screening tool has poor ability to rule in or rule out TB, we have strengthened the argument for the need for rapid establishment of laboratory infrastructure.

For our reference standard, we have chosen the BACTEC 960/MGTT broth method in combination with selective and non-selective Lowenstein-Jenson and NHIO solid media with supplemental GenProbe nucleic acid amplification test. The referred combination of

media currently constitutes one of two available reference standards for diagnosis of mycobacteria. The other standard is the radiometric BACTEC 460 system in combination with solid media, with 1% greater sensitivity and 0.3% greater specificity than the MGIT. However, the BACTEC 960/MGIT provides shorter times to detection of smear negative specimens and more convenient technology. In a meta-analysis comparing detection rates of the MGIT to BACTEC 460, sensitivity of MGIT plus solid media for detecting *M. tuberculosis* was 92% (87-95%).[5] Sensitivity of solid media alone, which is the method currently employed in the tuberculosis reference hospital of Mozambique is only 76% (73-79%).[6-7] Specificity of the MGIT is high, at 99.6%, but slightly less than the 460, at 99.9%. The supplemental Nucleic Acid Amplification Test (NAAT) adds both sensitivity and sensitivity to cultures. In a study reporting 96% sensitivity of cultures (BACTEC 460 and solid media), the addition of NAAT increased sensitivity to 99% and specificity to 100% with two specimens.[5] NAAT also has the advantage of rapid detection, as quick as within one day of sample receipt.

B. Study Design and Statistical Analysis

This validation study will be cross-sectional in design. Patients who are WV-infected and attending outpatient HIV care and treatment programs will have the screening tool administered to them. The screening instrument will consist of the questionnaire plus a clinical algorithm currently being implemented in Mozambique. The intervention, or application of reference standard, would consist of sputum cultures, NAAT, and, in the presence of lymphadenopathy, lymph node aspirate cultures. Primary outcome will be diagnosis of tuberculosis. Three sets of criteria will constitute a diagnosis of probable active TB: 1) at least one positive smear for acid-fast bacilli (AFB), 2) chest radiograph suggestive of tuberculosis combined with appropriate response to TB therapy, and 3) high clinical suspicion and appropriate response to TB

therapy. A positive culture for *M. tuberculosis* and/or a positive NAAT for *M. tuberculosis* will constitute a confirmed diagnosis of active TB, and will be used as the gold standard when determining operating characteristics of the screening tool.

A two by two table will be constructed to determine sensitivity, specificity, positive predictive value, and negative predictive value of the screening tool as a whole in reference to the gold standard. Tests for statistical significance of the difference of effect between the screening tool and gold standard will be performed using the chi-squared test. Separate two by two tables will be used to calculate the operating characteristics of the questionnaire and three sets of criteria employed for diagnosing TB compared to the reference standard.

For a set alpha of 0.05% and power of 80% to detect a difference between the screening instrument and gold standard if we assume sensitivity of the tool is 95% of culture/NAAT, we would need to find 95 cases of tuberculosis among WV-infected individuals. If we estimate a prevalence of tuberculosis of 7% among HIV positive patients (a conservative estimate compared to that detected at HIV clinics in South Africa) we would need to enroll 1357 patients.

As a correlate, subgroup analysis, we will calculate the difference in TB case detection before and after implementation of the screening tool. We will compare new cases of TB detected in new patients during the year prior to implementation, to new TB cases detected in new patients during the year following implementation of the tool. A chi-square test will be used to compare difference in proportions.

C. Study Procedure

Prior to submission for Columbia University Medical Center (CUMC) IRB review, approval will be obtained from the Mozambique Ministry of Health and the investigation site clinical supervisor. Following approval by the CUMC IRB, adult and adolescent patients receiving care at the HIV Day Clinic (Hospital de Dia) in Xai Xai, Mozambique will be approached for enrollment upon initial or follow up clinic visits. Informed consent will be obtained for each participant (see "Study Subjects" below). Recruited participants will provide a sputum sample for smear and culture on two consecutive days, and will receive compensation for their travel expenses. They then will be asked the six questions of the symptom questionnaire (appendix 1) and undergo a basic physical exam with attention to lymphadenopathy. If they answer one of six questions affirmatively, they will proceed with the diagnostic strategy which can be reviewed as appendix 2, also summarized below.

Sputum samples and lymph node aspirates in patients with adenopathy will be gathered from patients at the study site. They will immediately be labeled with a unique identifier, sample type, number 1 or 2, study name "Mozambique Screening Instrument Validation Study," and refrigerated between 2-8 degrees Celsius. A small amount of each sample will be separated for smear analysis by a local laboratory technician. The remaining (majority) quantity will be shipped on ice biweekly from Xai Xai Hospital de Dia laboratory to the mycobacterium laboratory at CUMC. Standard transport precautions and procedures will be observed. Only numerical identifiers and study name will accompany the samples.

On arrival to the CUMC laboratory, samples will be refrigerated and subsequently prepared for analysis. All samples will be cultured using the BACTEC 960/MGIT broth method in combination with selective and non-selective solid media Lowenstein-Jenson and NH10. Solid cultures (U and NH10) will be examined weekly for a total of 7 weeks. Liquid cultures will be monitored daily for 7 days and biweekly for 6 additional weeks by the laboratory staff under the supervision of the lab supervisor. The first collected specimen from each subject will be tested by NAAT on the day of arrival. If growth occurs or the NAAT is positive, the subject will receive the diagnoses of TB. Results of all positive cultures and NAAT will be faxed to Xai Xai Day Hospital tuberculosis program office. Patients with positive cultures will then be treated if they have not already received a diagnosis of TB per the screening instrument. If no growth occurs by 7 weeks, the patient will be considered to be culture negative, and not to carry the diagnosis of TB.

If a smear is positive for acid fast bacilli by Ziehl-Neelson or auramine stain, the patient will be considered to have a diagnosis of tuberculosis, and will be treated with the current DOT drug regimen

prescribed in Mozambique (see "Study Drugs" below). If both smears are negative and the patient has lymphadenopathy on exam, a lymph node aspirate will be performed and sent for smear and culture. If the aspirate smear is positive, the patient will be diagnosed with tuberculosis and will be treated. If there is no lymphadenopathy on exam or if the smear is negative, the patient will receive a chest radiograph. If the chest radiograph shows cavitary disease, a miliary pattern, or pleural effusion, the patient will be presumed to have tuberculosis and will receive an empiric course of therapy. If patients improve with therapy within four weeks, the diagnosis of tuberculosis will be considered as confirmed. If they do not respond to therapy, however, reassessment will be performed. If the chest radiograph does not have the features described, but there remains a high clinical suspicion for either pulmonary or extra pulmonary TB, the patient will receive an empiric course of treatment. If the patient improves, he or she will be presumed to carry a diagnosis of tuberculosis. If there is no improvement within four weeks, therapy will be stopped, and reassessment with re-culture with sensitivities and search for other diagnoses will be carried out. If there is not a high suspicion for TB, the patient will be considered not to carry a diagnosis of tuberculosis per the clinical algorithm.

D. Study Drugs

The study will not involve the use of investigational drugs. In the case of detected tuberculosis, the patient would be treated with the current standard of care regimen in Mozambique (2 months Rifampin, INH, PZA, and ethambutol, followed by 6 months ethambutol and INH), directly observed therapy.

E. Medical Devices

This study will not require the use of investigational devices. All device use including Ziehl-Neelson and auramine stains of sputum and lymph node aspirate, BACTEC 960, Lowenstein-Jenson, 7H10 agar culture media, *M. tuberculosis* nucleic acid amplification test, chest radiograph, and lymph node aspiration procedure will follow standard clinical and laboratory procedures described elsewhere.

F. Study Questionnaire

Title: Questionario e esquema pela localizaciio de casos de tuberculose (TB) ativa em individuos infectados pelo HIV. See appendix 1.

G. Study Subjects

The study will involve adolescents and adults age 14 and above enrolled at the Hospital de Dia, an outpatient HIV/AIDS care site in Xai Xai, Mozambique. The vast majority of patients are very poor, a large proportion is illiterate, and all could be classified as vulnerable. The predominant spoken languages are Portuguese, Ronga, or Chopi. All patients have previously had an HIV diagnosis confirmed. All eligible patients will undergo an informed consent process with Portuguese, Ronga, or Chopi consent forms read to them in a private setting. Consent forms will be translated by a neutral translation service not affiliated with the investigators. For specific issues regarding subject minors, refer to "Minors in Research Study" section below. No treatment will be withheld for any patient in the study.

Patients currently receiving treatment for tuberculosis or other mycobacterial infection, and patients who have been treated for tuberculosis within the past 1 year will be excluded. Any HIV negative individual will be excluded. Pregnant subjects will be included, but will not have chest radiographs.

H. Recruitment of Subjects

Recruitment of subjects will be performed by a study nurse at Hospital de Dia in Xai Xai. Recruitment will not be performed by the investigators or the patient's health care provider. A list of all

patients approached, enrolled, and followed to response to treatment (either positive of negative) will be compiled and stored at Hospital de Dia. A copy of this list will be held by the principal investigator.

I. Confidentiality of Study Data

Names of subjects will be on consent forms only. Each subject will be assigned a unique identifier number (DIN). Name/UIN pairs will be stored as a separate ledger under lock and key with the primary investigator. All data and samples gathered will be paired with the identifier number, and communication between investigators and clinicians at the

study sites will be through use of the identifier. Questionnaire, smear, and radiographic data will be kept in confidential storage at the participating clinic sites. Culture data will be maintained at confidential storage sites within the CPMC mycobacterial laboratory. Positive culture data will be relayed via fax using the identifier number.

J. Potential Conflict of Interest

The investigator declares no conflict of interest. I have no financial investment or affiliation with the following: Becton Dickinson and Company, producer of the BACTEC960/MGIT and BACTEC 460, any producer of Lowenstein Jenson media or NHIO media, or GenProbe, Inc, producer of the NAAT.

K. Location of the Study

The study will take place at the Hospital de Dia in Xai Xai, Gaza Province, Mozambique. This Day Hospital is an outpatient facility designed for caring for individuals with HIV/AIDS. It also maintains home care services. From January 2003 to December 2004, the clinic had carried out 11,971 consultations and 3,264 patients 14 years and older were enrolled for follow up and treatment. The 24 Day Hospitals throughout Mozambique have been selected to provide antiretrovirals.

L. Potential Risks

Risks to subjects include discomfort in producing sputum samples, bleeding, infection, and minor scarring from lymph node aspiration (though this will be performed as part of routine operations, not specifically for the study), and accident on the way to the study site to provide samples. Subjects may also incur risk from exposure to tuberculosis treatment drugs if diagnosis is made by culture/NAAT, but not by routine algorithm. In this case, the diagnosis would have been made through participation in the study, and thus, exposure to anti-TB drugs would come as a direct result of the study. Furthermore, if TB diagnosis results from culture/NAAT, the patient is exposed to external stigma and cultural attitudes toward TB as a direct result from the study, which may have negative implications in their relationships or employment.

M. Potential Benefits

Despite the risks listed above, patients may benefit from being diagnosed with tuberculosis by culture/NAAT, where the diagnosis may have been missed by the screening instrument. Diagnosis will allow patients to be treated with anti-TB therapy, and may reduce significant morbidity and mortality. Significantly, all first line TB drugs are classified as safe in pregnancy.

N. Alternative Therapies

The study does not involve investigative drugs and there are no alternative therapies.

O. Compensation of Subjects

The cost of transport, storage, and processing of mycobacterial cultures will be covered by the study. Patients will be compensated for travel to return to the Hospital de Dia to provide the second sputum sample, and lymph node aspirate if required. They will also be compensated for any cost they incur as a direct result of the study. For example, if cultures are lost or damaged at the site or in transport, and patients need to return to clinic another time to provide an additional sample, their travel costs will be compensated.

P. Minors in Research Study

Adolescents 14-17 attend the Hospital de Dia. They will be approached to enroll in the study. They will be informed that their parents or guardian will need to be contacted to provide consent. The adolescents will be asked for assent. To protect confidentiality, adolescents have the right to refuse that the study nurse approaches their parents. In this case, they refuse all participation in the study.

Q. Radiation or Radioactive Substances

Patients will not be exposed to radiation or radioactive substances in addition to what is the current clinical practice of chest radiography for suspected cases of tuberculosis with negative AFB smears.

R. References

1. World Health Organization. 3 by 5, Mozambique Country Report, July 2004. http://www.who.int/3by5/en/cp moz.pdf.
2. World Health Organization, Global Tuberculosis Control. WHO Report, Mozambique Country Profile, 2005. http://www.whoint/GlobalAtlas/PDFFactory/TB/PDF Files/MZ 2003 Detailed.pdf
3. Mohammed A, Ehrlich R, Wood R et al. Screening for tuberculosis in adults with advanced HIV infection prior to preventive therapy. Int J Tuberc Lung Disease 2004; 8(6):792-795.
4. Hudson CP, Wood R, Maartens G. Diagnosing HIV-associated tuberculosis: reducing costs and diagnostic delay. Int J Tuberc Lung Dis, 2000; 4:240-245.
5. Cruciani M, Scarparo C, Malena M et al. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. Journal of Clinical Microbiology, 2004; 42: 2321-2325.
6. See Cruciani M as above.
7. Nunes EA, De Capitani EM, Coelho E, et al. Patterns of anti-tuberculosis drug resistance among HIV-infected patients in Maputo, Mozambique, 2002-2003. Int J Tuberc Lung Dis 2005; 9(5):384-500.
8. Moore DF, Guzman JA, Mikhail LT. Reduction in turnaround time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. Diagnostic Microbiology and Infectious Disease, 2005; in press.