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Evaluation of the Xpert® MTB/Rif test, microscopic observation drug susceptibility test and nitrate reductase assay, for rapid and accurate diagnosis of smear-negative tuberculosis in HIV patients

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ABSTRACT

Diagnosis of smear-negative tuberculosis (TB), which is frequently seen in HIV-infected patients, is a challenge without conventional culture methods. Since 2007, the WHO (World Health Organization) has endorsed new or improved tests for increased and rapid diagnosis of TB. This study was undertaken in an effort to evaluate the accuracy of two rapid culture methods: the Microscopic Observation Drug Susceptibility assay (MODS) and Nitrate Reductase Assay (NRA), and the molecular based test Xpert® MTB/Rif (Xpert), for diagnosis of smear-negative TB in HIV patients using the mycobacteria growth indicator tube (MGIT) in the BACTEC™ MGIT™ 960 system as the reference test. 430 smear-negative patients with presumptive TB were enrolled in a cross-sectional study at a tertiary care facility in Uganda. Their sputum was tested on MODS, NRA, Xpert and MGIT. Of the 430 patients, 373 had complete results to compute test accuracy. *Mycobacterium tuberculosis* (MTB) was detected in 43 patients by MGIT. The sensitivity and specificity were 24.4% and 98.1% for MODS, 41.5% and 92% for NRA, 48.8% and 95.1% for Xpert, respectively. The low sensitivity of the tests implies that additional diagnostics such as chest X-ray and conventional liquid culture methods might still be needed to detect TB in smear-negative HIV patients. The high specificity of the tests is useful to confirm TB in HIV patients with symptoms suggestive of TB.

Introduction

The World Health Organization (WHO) estimates that 8.7 million people develop tuberculosis (TB) each year worldwide [1]. Of these, 13% are co-infected with HIV, while of the 1.4 million deaths that occur, 30% are HIV-related. The African region, especially sub-Sahara Africa, accounts for 80% of the HIV-associated TB cases worldwide. Unfortunately, diagnosis

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of TB in this region relies mainly on sputum microscopy, which due to its relatively low sensitivity, performs poorly in HIV-infected patients. This is owing to the paucibacillary nature of TB in HIV-infected patients; therefore, active TB disease is often not detected by smear examination [2,3].

Culture is a more sensitive way to detect paucibacillary forms of TB, which are common in HIV patients and children [4]. However, in several resource-limited settings, such as in sub-Sahara Africa, mycobacterial culture is inaccessible to the majority of the population. Even when available, the commonly used solid culture on Löwenstein–Jensen culture media (L–J) is time-consuming and growth of mycobacteria is typically detected after several weeks [5]. Commonly, therefore, diagnosis of TB in smear-negative HIV patients is made empirically. This is associated with delayed detection and initiation of TB treatment, and a higher mortality rate compared with patients who are smear-positive [6].

Since 2007, the pipeline for new TB diagnostics has expanded. In parallel, WHO has endorsed several new tests for increased and more rapid TB diagnoses. However, despite a major drive to incorporate these tests into the current diagnostic algorithms, data of their accuracy for diagnosis of smear-negative TB in HIV patients is still inadequate [7]. In this study, the accuracy of a molecular test and two rapid culture methods for diagnosis of smear-negative TB in HIV patients using the mycobacteria growth indicator tube (MGIT) in the BACTEC™ MGIT™ 960 system (Becton Dickinson Biosciences, Sparks, MD) as the reference test was evaluated [8].

Overview of tests

The molecular test Xpert® MTB/Rif (Cepheid GeneXpert System, Sunnyvale, CA) (Xpert) is based on quantitative real-time PCR for detection of TB. This is the first molecular method for TB detection to be fully automated and to integrate all the steps required for PCR-based DNA testing. Thus, results are reported within three hours and with high accuracy [9,10]. Both processed and unprocessed sputum can be used in this test.

The two rapid culture methods evaluated were: the Microscopic Observation Drug Susceptibility assay (MODS), and the Nitrate Reductase Assay (NRA). The tests are described as being low-cost and easily implemented, and WHO has recommended that both can be used as direct or indirect tests for rapid diagnosis of multi-drug resistant TB [11].

The MODS relies on two well-known properties of *Mycobacterium tuberculosis* (MTB): first, the rate of growth in liquid medium is considerably higher than that on solid medium. Secondly, the morphology in liquid culture is characteristic and recognizable, consisting of so-called cord-like structures. By using an inverted light microscope to examine culture plates inoculated with sputum, MTB growth can be detected within 7–10 days, for both smear-positive and -negative samples, compared with conventional solid culture that takes 3–8 weeks [12,13].

The NRA assay relies on the ability of MTB to reduce nitrate to nitrite. The presence of nitrite, indicating metabolically active mycobacteria, can easily be detected by the addition of what is known as the Griess reagent into the culture tube, producing a visually observed pink to purple color change [14]. The NRA test was modified by using Middlebrook 7H9 broth (Difco) as the culture media, since the rate of growth in liquid medium is considerably quicker than that on solid medium. As reported previously, results can be available within 10–14 days when liquid culture media is used. These studies were, however, based on examination of microscopy positive sputum samples with a higher content of bacteria [15].

Materials and methods

Ethical approval for the study was obtained from the research and ethics committee, of the College of Sciences of Makerere University (REC 2011-45).

Sample size

The sample size was calculated based on methods described earlier [16]. It was hypothesized that the culture and molecular methods would have similar sensitivities and specificities of 90% and 98%, respectively. The 95% confidence interval was adopted, and the corresponding Z value of 1.96 in the calculations, while the precision was set at ±10%. Based on data from the pilot study, it was expected that approximately 10% of HIV patients reported smear-negative would have a positive MTB culture when evaluated by fluorescent microscopy (FM). It was therefore required that at least 346 smear-negative patients be enrolled for the full study.

Patient recruitment

Using an observational and cross-sectional study design, patients with symptoms of TB between March and November 2012 were enrolled in the study. The study patients were in attendance at an adult out-patient HIV clinic or were admitted to the medical department at Mulago National Referral Hospital, Kampala, Uganda. At the hospital, patients are screened for TB using a standard WHO/Ministry of Health intensified TB case finding form. According to the form, patients are presumed to have TB if they had a cough for $\geqslant$2 weeks, with or without fever, night sweats, loss of weight, or blood-stained sputum. Following consent of the enrolled patients, they provided a spot and early morning sputum sample. Patients were excluded who were already on TB treatment or on quinolone medication, or were unable to produce sputum. Detailed HIV care information was obtained from the patients' clinical charts and recorded on a standardized case record form.

Laboratory methods

The collected sputum samples were maintained at 2–4 °C in an ice box at the clinic/ward, and were carried to a closely located bio-safety level 3, mycobacteriology research laboratory, twice daily. The laboratory is quality controlled by the National TB Reference Laboratory (NTRL).

Unprocessed sputum received in the laboratory was examined by fluorescent microscopy (FM) using standard auramine reagent at x40 objective (Olympus CX31 with LED attachment, Olympus Corporation, Tokyo, Japan). Samples found positive