Incremental detection of pulmonary tuberculosis among presumptive patients by GeneXpert MTB/RIF® over fluorescent microscopy in Mwanza, Tanzania: an operational study

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Abstract

Laboratory confirmation among presumptive tuberculosis (PTB) patients is pivotal in ensuring prompt management. Limited information exists in Tanzania regarding the performance of GeneXpert MTB/RIF® in comparison with conventional methods. An operational study was conducted involving 806 PTB patients at Sekou Toure Hospital in Mwanza, Tanzania from June to November 2013. Patients' information was obtained and their respective sputum samples analyzed by light-emitting diode fluorescent microscopy (LED FM) and GeneXpert MTB/RIF®. The mean age of study participants was 39.6±16.0 years, with males accounting for 50.5%. The majority of patients (97.5%) were new cases. The proportions of PTB patients confirmed by LED FM and GeneXpert MTB/RIF® were 14.1% (114/806) and 23.7% (191/806) respectively, resulting into a 9.6% incremental detection rate by GeneXpert MTB/RIF® over LED FM. The detection rate among HIV positive individuals was also higher [23.6% (63/267) *vs* 14.2% (38/267), respectively], with an incremental detection of 9.4%. The incremental detection of PTB by GeneXpert MTB/RIF® over LED FM calls for expansion of its use to increase detection of smear negative PTB among people living with HIV.

Introduction

The escalating burden of tuberculosis (TB) in Tanzania in the midst of high prevalence of HIV/AIDS poses a negative social and economic impact in this developing country which is ranked 22nd among countries accounting for 80% of the global burden of TB.[1-3]

To avert continuous transmission, morbidity and mortality attributable to TB, laboratory confirmation among presumptive pulmonary tuberculosis (PTB) patients is pivotal in ensuring prompt management.[2,4] Ziehl-Neelsen (ZN)-based light microscopy which is the main stay and universally available diagnostic technique in Tanzania and other developing countries has long been shown to have low performance.[5,6] In the light of this, the World Health Organization (WHO) has recommended scaling up the use of light-emitting diode fluorescent microscopy (LED FM) which is on average 10% more sensitive in detection of TB compared to the conventional ZN-based light microscopy using culture as a gold standard.[5,7,8] This notwithstanding, LED FM coverage is still low in developing countries.[2] To address the low performance of microscopy-based detection methods for TB, a number of molecular based diagnostic methods have been validated by WHO to increase coverage and enhance timely detection of PTB patients,[8-10] but their utility is unevenly appreciated across countries mainly due to the installation and running costs as well as lack of expertize.[10,11]

Recently, WHO endorsed a new rapid molecular test called GeneXpert MTB/RIF® (Cepheid, Sunnyvale, CA, USA).[9] The dual function of the machine in simultaneously diagnosing TB and identifying resistance to one of the core first line anti-TB drug, rifampicin along with its high sensitivity and specificity, has revolutionized the diagnosis of TB globally.[9,12-15] The performance of GeneXpert MTB/RIF® has been shown to be better compared to LED FM in both smear positive and negative people living with HIV (PLWH), though variability exists depending on the population involved.[16-18] The rifampin resistance has been shown to vary in different countries from 0% in Mbeya (Tanzania), 10% in Harare (Zimbabwe) to as high as 35.1% in Moldova.[16,19] In response to WHO call to scale up the utilization of this new diagnostic, the Ministry of Health in the United Republic of Tanzania, through the National Tuberculosis and Leprosy Control Program (NTLP)[3,9] and other developmental partners, has cordially rolled out the GeneXpert MTB/RIF® machines to various regions. Apparently the target groups are smear negative PLWH, PTB patient who recently contacted multidrug resistant tuberculosis (MDR) patient and children.[3]

In Tanzania, Mwanza region is second to Dar Es Salaam in terms of TB case notification rates emphasizing the need to have reliable diagnostic methods in place.[3] Despite this, limited information exists in this region regarding the performance of the recently introduced GeneXpert MTB/RIF® in comparison with LED FM for the diagnosis of TB. Furthermore, the magnitude of rifampicin (RIF) resistance remains to be explored in this setting. Therefore, the present study aimed at determining the incremental detection of TB among PTB patients by GeneXpert MTB/RIF® and LED FM at Sekou Toure Regional Referral Hospital (SRRH) in Mwanza, Tanzania so as to offer baseline information crucial for future assessment of the diagnostic performance of the facility as well as the utility of the new technique in this local setting.

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Materials and Methods

Study design and area

This was an operational prospective laboratory based study carried out at SRRH in Mwanza, Tanzania from June 2013 to November 2013 involving 1946 PTB patients submitting their sputum for analysis at SRRH. Of these, 806 (41.4%) had dual results (*i.e.* LED FM and GeneXpert MTB/RIF® results) fulfilling the inclusion criteria, and 1140 (58.6%) patients were excluded for various reasons (Figure 1).

Sample collection, processing and data analysis

Sputum samples were collected from PTB patients following the NTLP Guidelines,[1] and analyzed based on the standard operating procedures by LED FM and GeneXpert MTB/RIF®.[8,9,15,20] For comparison purposes of the two diagnostic techniques, one sputum sample per patient was used. In case the sample was negative requiring the second sputum sample as per NTLP Guideline,[1] the latter was analyzed to guide patient's management but not used for the index study.

Patients' information was obtained from laboratory request forms and the TB registry book. Analysis was done using STATA software version 11 (College Station, TX, USA) according to the objectives of the study. Continuous variables were described as mean (±standard deviation). Categorical variables were described as proportions (percentages) and were analyzed to compare the distribution of PTB positive and negative patients with variables.

Study clearance and ethical considerations

The study was approved by the joint Bugando Medical Centre and Catholic University of Health and Allied Sciences Institutional Review Board. Permission to conduct the study was obtained from SRRH medical officer in charge, TB coordinator and laboratory manager. All patients' information was kept confidential and anonymous using study codes. Presumptive patients found to have PTB were treated in their respective treatment units basing on the NTLP guidelines[1] and those with RIF resistance were referred to Kibong'oto National Tuberculosis Hospital for confirmation and further expertize management.

Results

We involved 806 PTB patients in this study with the mean age (±standard deviation) of 39.6±16.0 years (age range 1-96 years); males accounted for 50.5% (407/806). Majority of patients (97.5%) were new cases and were residing within Mwanza City, 81.7% (658/806).

The proportion of PTB patients confirmed to have PTB disease by either FM or GeneXpert MTB/RIF® was 24.8% (200/806) (Table 1). Of these, 14.1% (114/806) and 23.7% (191/806) were detected by FM and GeneXpert MTB/RIF® respectively. This resulted into 9.6% incremental detection rate by GeneXpert MTB/RIF® over LED FM (Figure 1 and Table 1).

The detection rate of GeneXpert MTB/RIF® was higher compared to LED FM in both children (≤17 years) [8.3% (6/72) *vs* 4.2% (4/72)] and adults [25.2% (185/734) *vs* 15.1% (111/734)] respectively resulting into the incremental detections of 4.1% and 10.1% for children and adults respectively. The detection rate among HIV positive individuals was also higher using GeneXpert MTB/RIF® compared to FM [23.6% (63/267) *vs* 14.2% (38/267) respectively], with an incremental detection of 9.4%. Moreover, the GeneXpert MTB/RIF® detected 12.4% (86/692) and 12.2% (28/229) among all smear negative irrespective of HIV serostatus and smear negative HIV positive PTB patients were respectively.

The RIF resistance was found in 2 (1.1%) patients, 5 (2.6%) had indeterminate resistance, whereas in 184 (96.3%) there was no rifampin resistance detected. Of 200 PTB positive patients, majority were found to be in the age group of more than 18 years (96.5%), males (60%), residents of Mwanza city (69.4%), new cases (94.5%) and HIV positive (86.8%) (Table 2).

Discussion

The low performance of sputum smear microscopy in developing countries with high

Table 1. Diagnostic performance of GeneXpert MTB/RIF® *vs* light emitting diode fluorescent microscopy.

LED FM	GeneXpert MTB/RIF®		Total
	MTB detected	MTB not detected	
AFB detected	105	9	114
AFB not detected	86	606	692
Total	191	615	806

LED FM, light emitting diode fluorescent microscopy; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; AFB, acid fast bacilli.

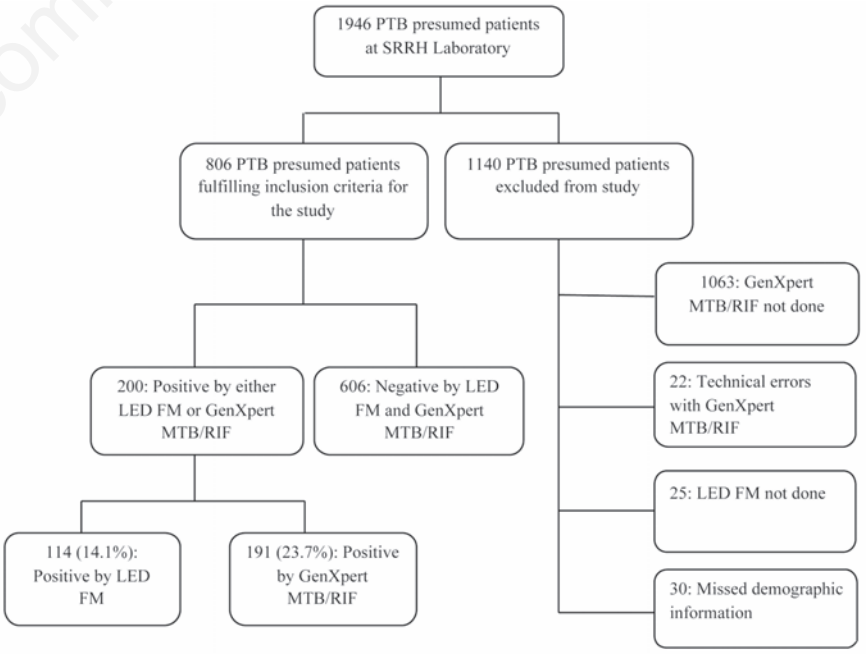

Figure 1. Flow chart showing series of events in the recruitment procedures and results. LED FM, Light emitting diode fluorescent microscopy; PTB, pulmonary tuberculosis; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; SRRH, Sekou Toure Regional Referral Hospital.

TB burden has been widely documented and if unchecked, it can result into uninterrupted transmission of this deadly infectious disease.[2,5,6] Despite a number of new technological advancement on the diagnosis of TB, the local evaluations of their performance remain a challenge in most developing countries.[10,11,13,21] The incremental detection of 9.6% among PTB patients at SRRH by GeneXpert MTB/RIF® over LED FM in the present study along with the 23% from a review involving 8880 participants in 21 studies,[12] 8.0% among children in Uganda,[17] and 9.7% in a recent multicenter, randomized controlled trial involving South Africa, Zimbabwe, Zambia and Tanzania,[16] emphasizes the utility of GeneXpert MTB/RIF® over microscopy in the diagnosis of TB patients. But the cost-related challenges for the universal introduction of GeneXpert MTB/RIF® in many health facilities in developing countries reiterate the need to continue strengthening the pre-existing microscopy-based TB diagnostic methods, so that the newer technique remains reserved to risky groups like smear negative PLWH, PTB patients who recently contacted MDR, and children.[3] The incremental detection of TB among smear negative PLWH in this and other studies[17,18,21,22] further justifies its utility in this risky group as recommended by the new NTLP guidelines.[3] The use of GeneXpert MTB/RIF® to detect RIF resistance as a surrogate marker of MDR has been suggested in many studies, with concordance ranging from 88 to 100%.[14,23,24] In the light of these, RIF resistance in the present study (2.2%) is higher than 0.86 (4/464) and 0.17% (2/1167) from a study in Mwanza and National survey in Tanzania respectively[25,26] but lower than 3.5 to 7.3% in different African countries.[19] Interestingly, no RIF resistance has been detected in three studies from Mbeya, Tanzania.[16,21,27] The finding of RIF resistance in Mwanza region which is second to Dar Es Salaam in terms of TB case notification calls for strengthening of surveillance system in this region to enable timely detection of patients with RIF resistant and MDR TB, thereby interrupting further transmission by provision of prompt management. Based on the nature of works and likelihood of exposure, the preponderance of males and city dwelling residents to be infected with PTB in this study is also similar to other reports.[3,28] The high proportion of PTB patients to be co-infected with HIV in the present study relates to another study.[17] These findings are also supported by other studies which have shown association of development of active TB with HIV/AIDS, smoking, co-morbidity such as diabetes mellitus, indoor air pollution and young age.[2,29]

Limitations

The culture method which is a gold standard for laboratory diagnosis of TB is not done at SRRH. Thus, this operational study did not compare the performance of GeneXpert MTB/RIF® and LED FM with culture. Also, the impact of other predictor variables on diagnostic performance such as CD4+ count was not evaluated.

Table 2. Distribution of pulmonary tuberculosis positive and negative patients with variables.

Variables	PTB patients (total=806)	
	Positive (total=200)*n (%)	Negative (total=606) n (%)
Mean age (years)	39.4±14.0°	39.7±16.7°
Age groups (years)		
≤8	4 (2.0)	17 (2.8)
9-17	3 (1.5)	48 (7.9)
≥18	193 (96.5)	541 (89.3)
Sex		
Female	80 (40.0)	319 (52.6)
Males	120 (60.0)	287 (47.4)
Residence		
Mwanza City	138 (69.4)	520 (85.8)
Outside Mwanza City	61 (30.6)	86 (14.2)
Treatment category		
New cases	189 (94.5)	597 (98.5)
Follow up	11 (5.5)	9 (1.5)
HIV serostatus#		
Positive	66 (86.8)	201 (87.0)
Negative	10 (13.2)	30 (13.0)

PTB, presumptive tuberculosis. *Diagnosed by either LED FM or GeneXpert MTB/RIF®; °continuous variable; #only 307 patients knew HIV serostatus.

Conclusions

There is an approximately 10% incremental detection of TB among PTB patients by GeneXpert MTB/RIF® compared to LED FM, with more detection also among smear negative PLWH who are apparently targeted by NTLP to be among beneficiaries of this new technology. Therefore, we recommend the expansion of its use to increase detection of PTB among smear negative PLWH at SRRH and other settings in the Lake Victoria zone. Evaluation of GeneXpert MTB/RIF® performance among people with extra pulmonary TB and the impact of various predictor variables on this diagnostic assay will be of interest to further delineate its utility in this setting.

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