COMPARISON OF GeneXpert MTB/RIF ASSAY WITH CONVENTIONAL AFB SMEAR FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS IN NORTHEASTERN THAILAND

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Abstract. Among infectious agents, *Mycobacterium tuberculosis* remains one of the most significant causes of death worldwide. Rapid and accurate diagnosis of pulmonary tuberculosis (TB) remains a great challenge. GeneXpert MTB/RIF assay is a novel integrated diagnostic system for rapid diagnosis of TB and particularly of rifampicin-resistant strains. A study was conducted between January 2010 and December 2014 to compare the performance of the sputum GeneXpert MTB/RIF assay with the conventional sputum AFB smear for diagnosis of active pulmonary TB in Thailand, a country with a high burden of this disease. Of the 125 patients who had cough and/or prolonged fever together with abnormal chest radiograph, 63 were diagnosed as having pulmonary TB by mycobacterium culture assay, while the remaining subjects were considered of having TB-like conditions, *viz* non-tuberculous mycobacterium infection (NTM), bacterial pneumonia or bronchogenic carcinoma. Two-thirds of the patients had underlying diseases, *eg*, diabetes mellitus (19 patients), autoimmune diseases (14), and HIV (6). Among patients with positive diagnosis of *M. tuberculosis* infection, 30 were AFB smear positive and 53 by sputum GeneXpert MTB/RIF method; among patients negative for *M. tuberculosis* infection, 4 were AFB smear positive and 5 by GeneXpert MTB/RIF assay. Sensitivity and specificity of the sputum AFB smear and GeneXpertMTB/RIF assay test were 48% (95% CI: 35-61) and 84% (95% CI: 73-92), and 94% (95% CI: 84-98) and 92% (95% CI: 82-97), respectively. Diagnostic performance of the GeneXpert MTB/RIF assay among AFB smear positive patients was higher than among AFB smear negative patients (adjusted OR 6.7; 95% CI: 2.3-19.9). Earlier diagnosis of pulmonary TB using GeneXpert MTB/RIF assay will lead to earlier appropriate treatment and provide opportunities to interrupt TB transmission.

Keywords: *Mycobacterium tuberculosis*, GeneXpert MTB/RIF assay, AFB smear, pulmonary tuberculosis

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INTRODUCTION

Tuberculosis (TB) is one of the commonest infectious diseases worldwide with high morbidity and mortality (WHO, 2016). Pulmonary TB is the most common

presenting form and the form with the highest transmission rates. Rapid and accurate diagnosis of pulmonary TB followed by prompt treatment with effective anti-TB drugs is the cornerstone of TB management and elimination of transmission (Elkington and Zumla, 2015).

Thailand is among the 22 countries in the world with the highest TB burden (WHO, 2015a). With a population of nearly 67 million, Thailand has an overall estimated 160,000 cases of TB, 13% of which are HIV positive (WHO, 2015a). Therefore diagnosis of TB, especially pulmonary TB, is a challenge for national tuberculosis control. Delay in diagnosis and treatment may worsen the disease, increase mortality and enhance transmission in the community. A previous study found that patients with a delay of > 60 days are more likely to have unfavorable TB treatment outcome than patients with a delay of ≤ 60 days (adjusted OR 2.3; 95% CI: 1.0-5.3) (Gebreegziabher *et al*, 2016).

Some 5.2 million new or relapsed pulmonary TB cases were reported globally in 2014 (WHO, 2015a). Of these 3.0 million (58%) were confirmed by smear, culture or GeneXpert MTB/RIF assay. However, 42% were diagnosed using clinical, chest radiography and from the nature of their responses to anti-TB drug therapy. Among new cases of bacteriologically confirmed TB, 12% had access to drug susceptibility tests (DSTs), and among previously treated cases, 58% had access to DSTs (WHO, 2015a).

Despite the low sensitivity of acid-fast bacilli (AFB) staining for detecting *M. tuberculosis* (MTB), this remains the main diagnostic test in resource-limited countries, including Thailand, due to its rapid turnaround time of less than 24 hours (Singhal and Myneedu, 2015). However, 20% of positive AFB smears are actually due to non-tuberculous mycobacterium infection (NTM) (Singhal and Myneedu, 2015). Mycobacterial culture is the gold standard and the most sensitive method for TB diagnosis, but its use in clinical practice is limited due to a slow turnaround time of 6-8 weeks, biosafety requirements and high cost (WHO, 2015b).

GeneXpert MTB/RIF assay is an automated, rapid, PCR-based assay, providing a convenient platform over conventional smear and culture methods and simultaneously detects rifampicin-resistant bacilli (WHO, 2015b). As GeneXpert MTB/RIF method does not rely on trained personnel and provides results within 100 minutes (Boehme *et al*, 2010), it could potentially be used at the clinical point-of-care to accelerate diagnosis time and subsequent initiation of treatment (Lawn *et al*, 2013). However, GeneXpert MTB/RIF assay is a relatively new diagnostic test in Thailand, but one which is likely to enjoy widespread use.

Thus, this study compared the performance of sputum GeneXpert MTB/RIF assay with conventional sputum AFB staining in patients with clinically suspected pulmonary TB, using MTB culture as the reference standard.

MATERIALS AND METHODS

Study group

A cross sectional study was carried out at Srinagarind Hospital, Khon Kaen, Thailand, between January 2010 and December 2014 involving 125 subjects. Inclusion criteria were: 1) clinical signs and symptoms, including cough and/or prolonged fever of more than two weeks, 2) age ≥ 15 years, 3) abnormal chest radiograph, 4) available of sputum AFB smear, GeneXpert MTB/RIF assay and mycobacterium culture results, 5) received

treatment at Srinagarind Hospital, and 6) definite final diagnosis. Exclusion criteria were: 1) diagnosis of extrapulmonary TB and 2) contaminated mycobacterium culture. The study was approved by the Research Ethics Committee, Khon Kaen University (approval no. HE591027).

Study design

Demographic and clinical data of patients were recorded including age, sex, occupation, clinical signs and symptoms, underlying diseases, chest radiograph findings, sputum AFB smear, sputum GeneXpert MTB/RIF assay, sputum mycobacterium culture, MTB drug susceptibility, treatment and outcome. Diagnosis of pulmonary TB was based on MTB positive sputum culture. Other diagnosis depended on laboratory investigations.

Each sputum AFB smear and GeneXpert MTB/RIF assay were reported as positive or negative and whether rifampicin-resistance gene was detected. Culture was reported as positive for MTB, non-tuberculous mycobacterium, or no growth. Drug susceptibility test for first line anti-tuberculosis drugs, ofloxacin, and kanamycin, were performed by proportional method on LJ medium if culture was MTB positive.

Statistical analysis

Descriptive statistics were used to analyze demographic data. Mean and standard deviation (SD) was calculated for continuous data and number and percentage were used for the categorical data. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of sputum AFB smear and GeneXpert MTB/RIF assay were calculated, and 95% confidence interval (CI) determined. Univariate and multivariate analyses for factors associated with positive GeneXpert MTB/RIF assay were calculated and presented as crude and adjusted odds ratio (OR). Statistical analysis was performed using STATA version 10.1 software package (StataCorp, College Station, TX).

RESULTS

Sixty-three subjects (50%) were diagnosed with pulmonary TB based on sputum MTB culture results, among whom 30 and 33 were AFB smear-positive and -negative, respectively. The remaining 62 subjects were diagnosed with non-tuberculous mycobacterium infection (n = 17), bacterial pneumonia (n = 14), bronchogenic carcinoma (n = 6), bronchiectasis (n = 5), autoimmune disease (n = 3), pulmonary melioidosis (n = 2), lung abscess (n = 2), pulmonary nocardiosis (n = 2), pulmonary cryptococcosis (n = 2), *Pneumocystis jirovecii* infection (n = 2), metastatic lung cancer (n = 2), lymphoma (n = 2), pulmonary edema (n = 2), and germ cell tumor (n = 1). The most common organisms found in 17 subjects with non-tuberculous mycobacterium infection were *M. abscessus*, followed by *M. intracellulare* complex.

Mean age (SD) of the patients was 55 (SD 18) years and 83 (66%) were male. Major occupations were in agriculture, government service, and as employees (Table 1). A third of the subjects resided in Khon Kaen Province. The most common clinical symptom was chronic cough (75%), followed by fever (52%) and weight loss (38%). The median duration of symptoms prior to seeking medical advice was 28 days. Two-third of the patients had some underlying disease, the most common medical co-morbidity being diabetes mellitus (15% of cases). Serology for HIV was performed on 72% of cases, with 6 cases tested positive. Other medical co-

Table 1
Demographic data of patients with clinical signs and symptoms of suspected pulmonary TB, Srinagarind Hospital, Khon Kaen 2010-2014.

Characteristic	Pulmonary TB (n = 63)	Other diseases (n = 62)	Total (n = 125)
Age (years), mean (SD)	55 (19)	54 (16)	55 (18)
Male, *n* (%)	42 (67)	41 (66)	83 (66)
Occupations, *n* (%)			
Agriculture	17 (27)	24 (39)	41 (33)
Government service	12 (19)	10 (16)	22 (18)
Employee	6 (10)	7 (11)	13 (10)
Student	4 (6)	3 (5)	7 (6)
Business	2 (3)	3 (5)	5 (4)
Monk	2 (3)	2 (3)	4 (3)
No occupation	20 (32)	13 (21)	33 (26)
Duration of symptoms, days median (q1-q3)	28 (7, 56)	14 (14, 28)	28 (7, 56)
Symptom, *n* (%)			
Cough	43 (68)	51 (82)	94 (75)
Fever	32 (51)	33 (53)	65 (52)
Weight loss	29 (46)	18 (29)	47 (38)
Hemoptysis	10 (16)	14 (23)	24 (19)
Anorexia	11 (17)	8 (13)	19 (15)
Underlying disease, *n* (%)			
DM	13 (21)	6 (10)	19 (15)
Autoimmune diseases	4 (6)	10 (16)	14 (11)
HIV	2 (3)	4 (6)	6 (5)
Post-kidney transplantation	2 (3)	3 (5)	5 (4)
Solid-organ malignancy	1 (2)	3 (5)	4 (3)
Nephrotic syndrome	1 (2)	3 (5)	4 (3)
Cirrhosis	3 (5)	1 (1)	4 (3)
Hematologic malignancy	1 (2)	2 (3)	3 (2)

DM, diabetes mellitus; HIV, human immunodeficiency virus; SD, standard deviation.

morbidities included autoimmune diseases, post-kidney transplantation, solid-organ malignancy, nephritic syndrome, cirrhosis, and hematologic malignancy (Table 1). Age, sex, occupation, duration of symptoms, clinical presentation and underlying diseases did not differ between pulmonary TB patients and those with other diseases. The more common abnormal findings on chest X-ray were reticulonodular lesions (38%), patchy alveolar infiltration (34%) and cavitary lesions (14%) (Table 2).

The results of sputum samples for AFB smear, GeneXpert MTB/RIF assay, and MTB culture were shown in Fig 1. Of the 125 sputum samples, 63 (50%) were MTB culture positive, of which 30 (48%) were AFB smear positive and 53 (84%) GeneXpert MTB/RIF assay positive. Rifampicin resistance was detected by GeneXpert MTB/RIF assay in six patients, three of whom proven to be multidrug-resistant (MDR)-TB by culture and drug

Table 2
Chest radiography of suspected pulmonary TB patients, Srinagarind Hospital, Khon Kaen 2010-2014.

Chest X-ray finding	Pulmonary TB (n = 63) n (%)	Other diseases (n = 62) n (%)	Total (n = 125) n (%)
Reticulonodular lesion	37 (59)	10 (16)	47 (38)
Patchy alveolar infiltration	19 (30)	23 (37)	42 (34)
Cavitary lesion	15 (24)	3 (5)	18 (14)
Lung mass	6 (10)	6 (10)	12 (10)
Bronchiectasis	2 (3)	7 (11)	9 (7)
Minimal fibropatchy infiltration	1 (2)	7 (11)	8 (6)
Honeycomb	3 (5)	3 (5)	6 (5)
Reticular infiltration	1 (2)	4 (6)	5 (4)
Pleural effusion	3 (5)	2 (3)	5 (4)
Miliary infiltrations	1 (2)	3 (5)	4 (3)
Atelectasis	3 (5)	1 (2)	4 (3)
Lymphadenopathy	2 (3)	0 (0)	2 (2)

susceptibility test. The remaining 62 MTB culture negative samples, 4 (6%) were AFB smear positive (but culture yielded non-tuberculous mycobacterium) and 5 (8%) GeneXpert MTB/RIF assay positive, which included 1 AFB smear positive sample. For the 63 active pulmonary TB cases, 47 (75%) were new cases and the remaining were previously treated cases.

Diagnostic performances of the sputum AFB smear test and the GeneXpert MTB/RIF assay using *M. tuberculosis* culture as a reference for active pulmonary TB showed that sensitivity of GeneXpert MTB/RIF assay was superior but specificity was comparable (Table 3). Positive predictive value of GeneXpert MTB/RIF assay was slightly better than the sputum AFB smear test and negative predictive value of the former test was clearly better.

Of the overall 125 patients, 34 were AFB smear positive. Of these 30 yielded culture results positive for MTB and 4 did not. Considering only these 34 individuals, the sensitivity, specificity, positive and negative predictive values of GeneXpert MTB/RIF assay were 90% (95% CI: 80-100), 75% (95% CI: 60-90), 96% (95% CI: 90-100), and 50% (95% CI: 33-67), respectively. For the 91 patients who were AFB smear negative; the sensitivity, specificity, positive and negative predictive values of GeneXpert MTB/RIF assay were 76% (95% CI: 70-85), 93% (95% CI: 88-98), 86% (95% CI: 79-93), and 87% (95% CI: 80-94), respectively.

Patients with positive AFB smear results were more likely to have a positive GeneXpert MTB/RIF assay (Table 4). On the other hand, diabetes mellitus, positive HIV serology, cavitary or bilateral lung lesions, and previously treated pulmonary TB were not associated with a positive GeneXpert MTB/RIF assay result.

DISCUSSION

People presenting with unexplained cough lasting two or more weeks or with findings suggestive of TB by chest radio-

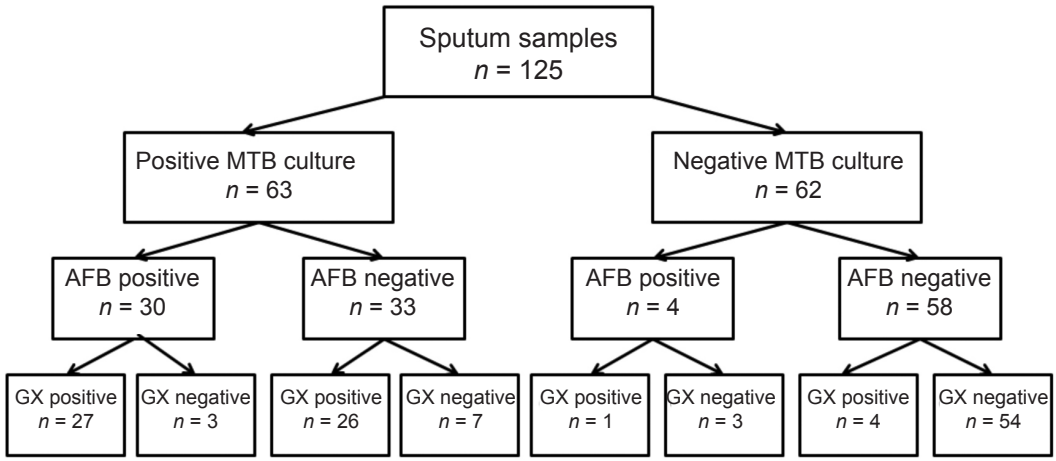

Fig 1–Laboratory results from 125 sputum samples. MTB, *M. tuberculosis*; GX, GeneXpert MTB/RIF.

graphy should be evaluated for the disease. However, many infectious and non-infectious diseases may mimic symptoms of TB, *eg*, cough, fever, and weight loss. The most common underlying diseases that predispose to TB infection and development of the disease are diabetes mellitus, autoimmune diseases, and HIV serology positive status (Erdozain *et al*, 2006; Jeon and Murray, 2008; Cain *et al*, 2010). However, one-third of TB patients have no medical co-morbidities. In this study the commonest abnormal chest radiograph finding for TB was reticulonodular lesions and the commonest mimicking TB was patchy alveolar infiltration. However, cavitary lesions can occur in both TB and other diseases. Among diseases mimicking TB, non-tuberculous mycobacterium and bacterial pneumonia were more common infectious diseases, and the commonest non-infectious disease was bronchogenic carcinoma. It is hard to diagnosis TB only by clinical presentation and chest radiograph, so laboratory investigation is needed for differentiation TB from other diseases (Davies and Pai, 2008).

Positive AFB smear test is sufficient to initiate anti-TB drug treatment to decrease transmission and disease progression (Singhal and Myneedu, 2015). The 50-60% sensitivity of AFB smear test for diagnosis of pulmonary TB is of concern (Lipsky *et al*, 1984) and moreover a positive AFB smear may be due to non-tuberculous mycobacterium (Jeon *et al*, 2005). Although the gold standard for diagnosis of active pulmonary TB is positive MTB culture, its application is hampered by the long turn-around time (WHO, 2015b). Among the 62 patients in this study with non-TB related diseases, 4 had positive sputum AFB smear results, but had positive non-tuberculous mycobacterium culture results. The combination of GeneXpert MTB/RIF assay with the sputum AFB smear test will greatly decrease the false positive rate, thus reducing the numbers of patients needlessly exposed to anti-TB drugs. Although many laboratory TB diagnostic methods have been developed to expedite diagnosis (Elkington and Zumla, 2015), delays in diagnosis remain a major problem in the clinical setting.

On account of its rapid and easy performance, the GeneXpert MTB/RIF assay has been recommended for initial testing or as an add-on to smear microscopy for

Table 3
Diagnostic performances of sputum AFB smear test and GeneXpert MTB/RIF assay for diagnosis of active pulmonary TB.

Performance	Sputum AFB smear % (95% CI)	GeneXpert MTB/RIF assay % (95% CI)
Sensitivity	48 (35-61)	84 (73-92)
Specificity	94 (84-98)	92 (82-97)
Positive predictive value	88 (72-97)	91 (81-97)
Negative predictive value	64 (53-74)	85 (74-93)
Positive likelihood ratio	7 (3-20)	10 (4-24)
Negative likelihood ratio	0.6 (0.4-0.7)	0.2 (0.1-0.3)

Table 4
Factors associated with positive results for GeneXpert MTB/RIF assay in diagnosis of active pulmonary TB.

Factor	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Positive AFB smear	9.5 (3.5-25.4)	6.7 (2.3-19.9)
DM	2.9 (1.0-8.2)	2.4 (0.7-8.0)
HIV	0.6 (0.1-3.2)	1.0 (0.2-6.8)
Cavitary or bilateral lung lesions	3.6 (1.2-10.8)	2.1 (0.6-7.6)
Previously treated pulmonary TB	10.3 (2.2-47.8)	3.2 (0.6-18.1)

AFB, acid-fast bacilli; CI, confidence interval; DM, diabetes mellitus; HIV, human immunodeficiency virus.

TB diagnosis (Lawn *et al*, 2013; Van Rie *et al*, 2013; Opota *et al*, 2016). Moreover, this technique can detect resistant strains (Lin and Desmond, 2014). A Cochrane Database Systematic Review evaluated the role of GeneXpert MTB/RIF assay used as an initial test replacing smear microscopy (15 studies with 7,517 samples) or as an add-on test following negative smear microscopy (14 studies with 5,719 samples) (Steingart *et al*, 2014). The majority of studies (56%) were performed in low- and middle-income countries. When GeneXpert MTB/RIF assay was used as an initial test replacing smear microscopy, the pooled sensitivity was 88% and pooled specificity was 98% (Steingart *et al*, 2014). As an add-on test following a negative smear microscopy result, GeneXpert had a pooled sensitivity of 76% and pooled specificity of 98% (Steingart *et al*, 2014). In this study, sensitivity of the GeneXpert MTB/RIF assay, which as rapid as the AFB smear for initial testing, was higher than that of AFB smear; 84% (95% CI: 73-92) for GeneXpert MTB/RIF assay *vs* 48% (95% CI: 35-61) for AFB sputum smear. In addition, the specificity of both tests was high (> 90% and comparable). In subgroup analysis, sensitivity of GeneXpert MTB/RIF assay was higher for adult patients with smear positive than that for smear negative. In this study, we identified 26 of 63 (41%) smear-negative but GeneXpert MTB/RIF-positive pulmonary TB patients, such patients also constitute a

transmission risk (Opota *et al*, 2016).

WHO guidelines recommended two AFB sputum-smear tests, one on the day of attendance and another in the early following morning, for diagnosis of pulmonary TB (WHO, 2010). Being rapid and easy to perform, repeated AFB smear tests are recommended in cases of suspected TB. An incremental yield (8.4%) in positive results with the second specimen and 3.5% for a third specimen were reported (Castro *et al*, 2015). In addition, sensitivity of AFB smear test is higher among patients with pulmonary cavitation (Castro *et al*, 2015). However, molecular diagnosis techniques, such as GeneXpert, are currently widely available and will have an important role for diagnosis of pulmonary TB, especially in high-prevalence and high-burden TB countries.

The only factor associated, in this study, with positive GeneXpert MTB/RIF result was positive AFB smear. Other factors, *eg*, diabetes mellitus, HIV serology positive, cavitary or bilateral lung lesions, and previously treated pulmonary TB were not associated with a positive GeneXpert MTB/RIF result. However, CDC recommends screening of diabetes mellitus and HIV patients for TB, these being risk groups (CDC, 1995). In addition, WHO endorses the use of GeneXpert MTB/RIF for the rapid diagnosis of TB as well as detection of rifampicin resistance among HIV-infected individuals suspected of TB infection (WHO and STOP TB Department, 2010). We suggest the use of GeneXpert MTB/RIF assay to augment positive AFB test in patients suspected of having MDR-TB because of the former increased rate of detection of MTB and rifampicin resistant strains. However the benefit of using the GeneXpert MTB/RIF assay for screening in diabetes mellitus and HIV patients in Thailand requires additional study.

In conclusion, the GeneXpert MTB/RIF assay is sensitive and specific for use as an initial diagnostic test for TB. The assay may also be valuable as an add-on test following microscopy for patients previously been found to be smear-negative. Use of the GeneXpert MTB/RIF assay in routine and peripheral health care settings, and at the point-of-care in high TB burden countries should prove beneficial in providing evidence on the actual prevalence of TB and rifampicin resistance. Furthermore, early diagnosis of pulmonary TB will lead to earlier appropriate treatment and provide opportunities to interrupt TB transmission, especially in high burden TB countries.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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