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ORIGINAL PAPER

GeneXpert of stool versus gastric lavage fluid for the diagnosis of pulmonary tuberculosis in severely ill adults

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Abstract

Purpose Stool is an alternative specimen matrix for tuberculosis (TB) tests, because *Mycobacterium tuberculosis* (MTB) can be swallowed and detected in the samples from digestive tract. We aimed to assess the performance of GeneXpert on stool and gastric lavage fluid (GALF) in diagnosing TB among patients with severe pulmonary TB.

Methods We enrolled adults with suspected pulmonary TB who were unable to produce sputum at visit between January 2016 and June 2018. Bacteriological samples consisted of one transtracheal aspirate sputum specimen, one stool specimen and/or one gastric lavage fluid specimen. Bacterial culture of transtracheal aspirate sputum provided the gold standard.

Results Of 65 individuals recruited for analysis, MGIT culture identified the presence of MTB in 32 samples. Overall, 29 of 32 stool samples from culture-positive cases were detected by the GeneXpert test, demonstrating a sensitivity of 90.6%. For GALF, 13 patients were detected as infected with MTB by GeneXpert, yielding a sensitivity of 56.5%. The statistical analysis revealed that GeneXpert showed significantly better sensitivity in detecting MTB from stool samples than GALF samples ($P=0.003$). Among individuals with GeneXpert-positive stool, the percentage of individuals with comorbid diabetes was significantly higher than among individuals with GeneXpert-negative stool (19.4% vs. 2.9%, $P=0.034$).

Conclusions In conclusion, our data reveal that GeneXpert provides a higher detection rate on stool compared to GALF, indicating stool should be considered as an alternative for adult TB patients unable to produce sputum. Individuals with diabetes are more likely to have positive GeneXpert stool than nondiabetic individuals.

Keywords *Mycobacterium tuberculosis* · Severe tuberculosis · Stool · GeneXpert

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB) complex, is a leading cause of death worldwide resulting from a single bacterial infection [1, 2]. In 2016, the World Health Organization (WHO) estimated the annual burden of tuberculosis to be 10.4 million cases and 1.67 million deaths in the world [1]. Early detection of TB is crucial to effective patient management and to TB control [3]. Despite the use of multiple available TB tests, only 57% of the pulmonary TB cases reported globally were bacteriologically confirmed [1]. In other words, approximate half of patients are treated empirically for TB, based on clinical symptoms, radiographic findings, and contact history with index patients. Therefore, there is an urgent need to develop a rapid and accurate diagnostic test for the detection of TB.

Sputum specimens remain the most important clinical samples used for current bacteriological test to confirm TB

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[4]. Their yield is expected to be suboptimal for patients with TB who are unable to expectorate sputum in the volume or quality required for sputum-based tests [5]. As an alternative, different specimen types have been studied to improve the sensitivity of bacteriological examination, such as induced sputum, bronchoalveolar lavage fluid, and gastric lavage fluid (GALF) [6–8]. Numerous previous reports have demonstrated that all three of these specimens have been shown to increase the diagnostic yield, especially for children with TB disease [6, 7]. Despite these advantages, the collection of induced sputum, bronchoalveolar lavage fluid and GALF are resource intensive and relatively unsafe for critically ill patients.

Stool is an alternative specimen matrix for TB tests, because MTB can be swallowed and detected in the samples from digestive tract [9, 10]. In particular, stool is easy to obtain for severe TB patients, the diagnosis of whom in more challenging [11]. WHO endorsed the automated nucleic acid amplification test (NAAT) GeneXpert MTB/RIF for the diagnosis of MTB from various specimen types [12]. In previous TB detection studies performed on stool, the GeneXpert assay yielded promising performance for the detection of TB bacilli from pediatric stool samples [11, 13]. To date, there are still limited data on the performance of GeneXpert MTB/RIF on stool for adult TB patients unable to produce sputum. The objectives of this study were to assess the performance of GeneXpert on stool and GALF in diagnosing TB among patients with severe pulmonary TB. We also aimed to determine the demographic and clinical factors associated with positive stool GeneXpert results.

Materials and methods

Study design and participants

We conducted a prospective study in Beijing Chest Hospital, a National Clinical Center of TB in China. From January 2016 to June 2018, adults with a suspicion of severe pulmonary TB were screened for enrolment. The adults with suspected severe pulmonary TB had at least one of the following symptoms: (1) cough for more than 2 weeks; (2) TB contact history; (3) radiological features. In addition, these cases were unable to produce sputum due to the pre-existing unconsciousness. The patients receiving anti-TB treatment prior to current hospital admission were excluded from this study. The staff recorded all demographic and clinical details for recruited patients within 6 h of admission. Bacteriological samples were collected using standard collection methods that consisted of one transtracheal aspirate sputum specimen, one stool specimen and/or one GALF specimen. The transtracheal aspirate sputum was obtained with a suction tube connected to the aspirator system. For the patients without mechanical ventilation, the tongue depressor was used to pull the tongue forward and open the trachea. Then, the tube was introduced into the trachea through the mouth. The automated vacuum suction was connected to the tube to aspirate sputum. All procedures were performed without the use of sedatives and muscle relaxants, and no adverse effects were recorded among these patients. All the clinical samples were transported to laboratories for bacteriological examinations.

Laboratory examinations

The transtracheal aspirate sputum specimens were decontaminated with *N*-acetyl-L-cysteine and sodium hydroxide, and centrifuged [14]. Direct examination was performed using the Auramine O staining for acid-fast bacilli (AFB) [14]. In addition, the sample pellet suspensions were inoculated into BACTEC MGIT tubes (BD Microbiology Systems, USA). Positive culture from specimens was identified as *M. tuberculosis* complex by the sequence analysis of partial fragment of 16S rRNA gene.

In addition, the transtracheal aspirate sputum and gastric lavage fluid (GALF) samples were diluted 2:1 in sample reagent, and shaken vigorously. After incubation at room temperature for 15 min, 2 mL of the inactivated material was transferred to a cartridge for GeneXpert analysis.

Stool specimens were processed prior to GeneXpert MTB/RIF testing to remove the inhibitors of polymerase chain reaction. Briefly, 1 g of stool was homogenized with 5 mL of phosphate buffered saline (PBS) and vortexed. After steeling, 1 mL of supernatant was diluted in 2 mL of sample reagent, and loaded to the GeneXpert instrument.

Statistical analysis

Bacterial culture of transtracheal aspirate sputum provided the gold standard. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated to assess the performance of GeneXpert for stool and GALF samples. In addition, the chi-square test was performed to compare the performance of GeneXpert between stool and GALF specimens. Univariable analysis was performed to identify factors associated with GeneXpert-positive stool. All statistical analyses were done using SPSS version 14.0 (SPSS Inc., Chicago, IL), and differences were considered to be statistically significant at $P < 0.05$.

Results

Patients

In total, 83 individuals with suspected pulmonary TB who were unable to produce sputum at visit were recruited for

our analysis. Of 83 cases, 9 (10.8%) underwent mechanical ventilation. By reviewing the laboratory examination results, 18 patients (21.7%) were excluded, including 9 with no culture results from transtracheal aspirate sputum specimens, 9 with no GeneXpert results from stool and 2 with contaminated culture results (Fig. 1). As summarized in Table 1, 46 out of 65 patients included in final analysis were male, and the other 19 were female. Ages ranged from 18 to 85 years (median age 42 years, interquartile range 26–69). In addition, liver disease (8/65, 12.3%) was the most frequent comorbidity observed in the individuals enrolled with suspected pulmonary TB, followed by diabetes (7/65, 10.8%). IGRA testing yielded positive results in 73.8% (48/65) of subjects and negative results in 26.2% (17/65) of subjects.

Table 1 Characteristics of patients with suspected severe pulmonary TB enrolled in this study

Characteristics	No. (%)
Sex	
Male	46 (70.8)
Female	19 (29.2)
Age (years)	
Median	42
Range	18–85
BMI(kg/m^2)	
< 18.5	45 (69.2)
> 18.5	20 (30.8)
Comorbidity	
No	43 (66.2)
Diabetes	7 (10.8)
Liver disease	8 (12.3)
Others	7 (10.8)
IGRA	
Positive	48 (73.8)
Negative	17 (26.2)

BMI body mass index, *IGRA* interferon gamma release assay

Performance of GeneXpert MTB/RIF in stool and GALF samples

Bacterial culture of transtracheal aspirate sputum provided the gold standard. As shown in Table 2, MGIT culture identified the presence of MTB in 32 (49.2%) samples,

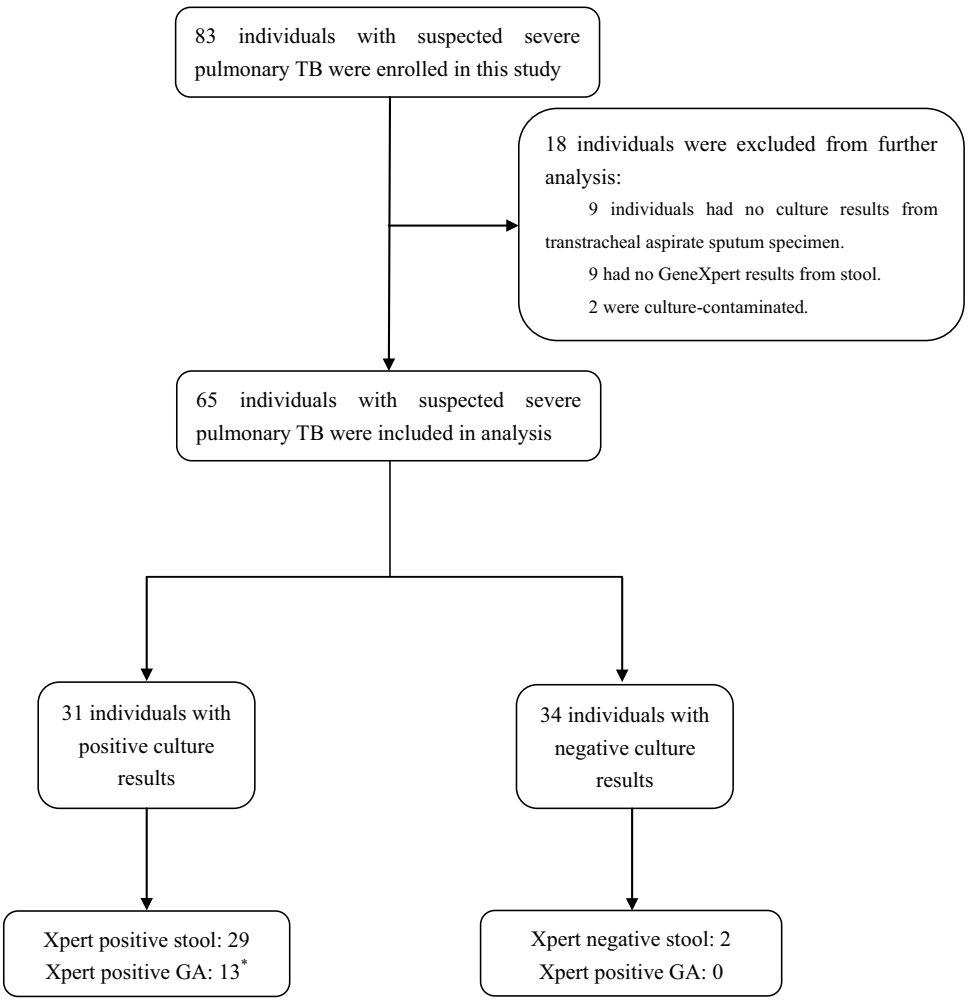

Fig. 1 Participant enrollment

while the remaining 33 (50.8%) were culture negative. Overall, 29 of 32 stool samples from culture-positive cases were detected by the GeneXpert test, demonstrating a sensitivity of 90.6% (95% CI 80.5–100.0%), and GeneXpert also identified 31 of 33 stool samples from culture-negative cases, demonstrating a specificity of 93.5% (95% CI 85.8–100.0%) (Table 2).

In addition, 40 patients provided GALF samples for GeneXpert and MGIT culture testing, including 23 culture-positive and 17 culture-negative patients determined by transtracheal aspirate sputum cultures. Out of 23 culture-positive patients, 13 patients were detected as infected with MTB by GeneXpert, yielding a sensitivity of 56.5% (95% CI 36.3–76.8%). The statistical analysis revealed that GeneXpert showed significantly better sensitivity in detecting MTB from stool samples than GALF samples ($P=0.003$) (Table 2).

Factors associated with having GeneXpert-positive stool

Univariable analysis of demographic and clinical characteristics and their association with GeneXpert-positive stool is summarized in Table 3. Among individuals with GeneXpert-positive stool, the percentage of individuals with comorbid diabetes (19.4%, 6/31) was significantly higher than among individuals with GeneXpert-negative stool (2.9%, 1/34), indicating that diabetes was associated with having positive stool GeneXpert results (OR [95% CI] 10.125 [1.116–91.879], $P=0.034$). In contrast, sex, age, BMI and

Table 2 Performance of GeneXpert MTB/RIF assay for detecting MTB from stool and gastric lavage fluid specimens

Specimen	GeneXpert MTB/RIF	Reference standard		Total	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)
		Pos	Neg					
Stool	Pos	29	2	31	90.6 (80.5–100.0)	93.9 (85.8–100.0)	93.5 (84.9–100.0)	91.2 (81.6–100.0)
	Neg	3	31	34				
	Total	32	33	65				
Gastric lavage fluid[a]	Pos	13	0	13	56.5 (36.3–76.8)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	63.0 (44.7–81.2)
	Neg	10	17	27				
	Total	23	17	40				

PPV positive predictive value, *NPV* negative predictive value

[a]Fourty individuals with results from gastric lavage fluid were enrolled in analysis

Table 3 Factors associated with positive stool GeneXpert among suspected severe pulmonary tuberculosis patients

Characteristics	Positive GeneXpert ($n=31$)		Negative GeneXpert ($n=34$)		OR (95% CI)	*P* value
	No.	Col %	No.	Col %		
Sex						
Male	23	74.2	23	67.6	1.375 (0.468–4.043)	0.562
Female	8	25.8	11	32.4	1.000	Ref.
Age group (years)						
< 25	9	29.0	6	17.6	1.000	Ref.
25–64	14	45.2	15	44.1	0.622 (0.176–2.202)	0.460
> 64	8	25.8	13	38.2	0.410 (0.106–1.594)	0.194
BMI (kg/m^2)				0.0		
< 18.5	23	74.2	22	64.7	1.568 (0.539–4.566)	0.408
> 18.5	8	25.8	12	35.3	1.000	Ref.
Comorbidity						
No	16	51.6	27	79.4	1.000	Ref.
Diabetes	6	19.4	1	2.9	10.125 (1.116–91.879)	0.034
Liver disease	5	16.1	3	8.8	2.813 (0.591–13.374)	0.249
Others	4	12.9	3	8.8	2.250 (0.445–11.365)	0.416

OR odds ratio, *CI* confidence interval, *BMI* body mass index

other comorbidities were not significantly associated with stool GeneXpert results ($P > 0.05$).

Discussion

In this study, we demonstrated the diagnostic value of GeneXpert MTB/RIF assay on stool samples for the diagnosis of TB in adult individuals. Our data have demonstrated that GeneXpert produces excellent performance in detecting MTB from stool samples in severe TB patients, with an overall sensitivity of 90.6%. Similar to our observation, a recent report by Rahman and colleagues revealed that the sensitivity of the stool GeneXpert assay is 94.8% in adults [15]. Numerous studies show that GeneXpert testing of stool yields promising performance in cohorts of children with high probability of pulmonary TB, with sensitivities of 41–89% compared with culture of respiratory samples [11, 13, 16], which were lower than our observation. The lower sensitivity reported compared to our results in adults may be explained in several possible ways. On one hand, a laboratory study by Blakemore and colleagues indicated that the positive GeneXpert results require higher bacillary load in stool samples than in respiratory samples [17]. Therefore, given that the presence of tubercle bacillus in stool comes from swallowed sputum, the paucibacillary nature of childhood TB may be associated with the limited sensitivity of GeneXpert from stool samples in children. On the other hand, this is possibly because of the severe spectrum of TB in individuals included in this study, who had higher MTB bacillary load.

Interestingly, we observed that stool samples have been shown to yield higher sensitivity in detecting MTB than GALF samples when tested with GeneXpert assay. We hypothesize that the longer stay duration of sputum in intestinal tract compared to stomach may result in the increased frequency of collection of specimens with MTB from stool samples. In addition to the promising performance, another advantage of stool test is that stool samples can be obtained from severe pulmonary TB patients without their active collaboration. Therefore, they are more conventional compared to GALF samples, especially for the patient population at high risk of rapid disease progression. Taken together, stool is a more suitable specimen type than GALF for accurately detecting MTB in clinical settings.

Evidence in human studies suggests that diabetes is associated with an increased risk of TB [18]. More importantly, diabetic guinea pigs infected with MTB presented severe and rapid progressive TB, such as shorten survival interval and a higher bacterial burden compared with nondiabetic controls [19]. In line with previous findings [20], we observe that individuals with diabetes are more likely to have positive GeneXpert stool than nondiabetic individuals, reflecting that this population with both diseases present with a higher bacillary load. Although the exact reason remains unclear, the increased bacillary load in the diabetes could be attributed to their impaired immunity against TB, thereby resulting in the uncontrolled bacterial growth and delayed bacterial clearance [20]. In view of the increasing overlap of populations at risk for TB and diabetes in recent years, TB patients with diabetes should be considered as important targets for early diagnosis and treatment interventions, which will bring a beneficial impact on TB control.

There are several limitations to our study. First, despite the great effort in the enrollment of patients, the small number of patients associated with a low prevalence of severe pulmonary TB weakens the overall significance of our study conclusion. Therefore, there is an urgent need to elucidate the diagnostic accuracy of GeneXpert on stool samples with a larger sample size. Second, the reference mycobacterial culture was only carried out on transtracheal aspirate sputum rather than stool tested by the GeneXpert assay, because mycobacteria culture was not routinely performed on stool samples in this study. Third, there is strong evidence that testing multiple stool samples could increase the sensitivity of GeneXpert [21], whereas only a single sample was analyzed in this study due to high cost of this cartridge. Fourth, given that lung tissues are the habitat of tubercle bacilli, it is more convenient to obtain a respiratory sample by tracheal suction for severe TB patients with mechanical ventilation. Nevertheless, our evaluation results demonstrate that GeneXpert is equally effective with transtracheal aspirate sputum, which could be used as standard sample for severe pulmonary TB patients.

In conclusion, our data reveal that the GeneXpert assay provides a higher detection rate on stool compared to GALF, indicating stool should be considered as an alternative for adult TB patients unable to produce sputum. Individuals with diabetes are more likely to have positive GeneXpert stool than nondiabetic individuals, reflecting that this population with both diseases presents with a higher bacillary load. Further studies are urgently needed to verify our results through the enrollment of more severe pulmonary TB patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This study was approved by the Ethic Committee of Beijing Chest Hospital affiliated to Capital Medical University. Written informed consent was obtained from the immediate relative on behalf of each patient.

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