International Journal of Infectious Diseases 81 (2019) 91–96

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Assessment of the Xpert MTB/RIF Ultra assay on rapid diagnosis of extrapulmonary tuberculosis

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ARTICLE INFO

Article history:
Received 20 December 2018
Received in revised form 27 January 2019
Accepted 31 January 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Mycobacterium tuberculosis
Xpert MTB/RIF Ultra
Xpert MTB/RIF
Extrapulmonary tuberculosis
Diagnosis

ABSTRACT

Objective: To evaluate the diagnostic performance of Xpert MTB/RIF Ultra for EPTB (Extrapulmonary Tuberculosis) patients on different types of extrapulmonary specimens from different anatomic sites.
Methods: Patients with suspected EPTB were prospectively included, extrapulmonary specimens were collected and subjected to culture, Xpert and Xpert Ultra assays in accordance with relevant guidelines.
Results: A total of 225 cases were included which contained 200 EPTB cases (43 culture-positive EPTB, 157 culture-negative EPTB which were diagnosed based on pathological results and a satisfied response to anti-TB treatment) and 25 non-EPTB cases. Sensitivities of Xpert Ultra and Xpert for culture-positive cases were 83.7% (95%CI, 68.7–92.7) and 67.4% (95% CI, 51.3–80.5) respectively. Specificities of Xpert Ultra and Xpert were 92.0% (95% CI, 72.5–98.6) and 96.0% (95% CI, 77.7–99.8) respectively. The sensitivities of Xpert Ultra, Xpert and culture for 200 EPTB cases were 52.5% (105/200, 95% CI, 45.4–59.6), 34.0% (68/200, 95% CI, 27.6–41.1) and 21.5% (43/200, 95% CI, 16.2–28.0) respectively. By comparison among different types of specimens, Xpert Ultra can detect 78.9% (56/71) of EPTB on fine-needle aspiration (FNA) tissues which was higher than that on pleural fluid (43.7% (45/103), $p < 0.05$.
Conclusions: Xpert Ultra assay had a higher sensitivity than those of Xpert and culture on extrapulmonary specimens, which could be a promising approach for rapid EPTB diagnosis.

Introduction

Tuberculosis (TB) is a global issue and a concerning important public health problem. In 2017, new TB cases were estimated to be 10.0 million worldwide and 1.3 million people died of TB (Organization, 2017a). *Mycobacterium tuberculosis* (MTB) mainly invades lungs and causes tuberculous lesions known as pulmonary TB (PTB), and occasionally also can invade other sites referred as extrapulmonary tuberculosis (EPTB) (Cukic and Ustamujic, 2018). Averagely, EPTB accounts for fourteen percent of TB reported in 2017, ranging from eight percent in the Western Pacific Region to twenty-four percent in the Eastern Mediterranean Region (Organization, 2017a). In Shanghai China, the proportion of EPTB to all TB was 9.7%–11.8% (Huang et al., 2000). EPTB diagnosis remains a great challenge due to subclinical or nonspecific clinical symptoms, low bacteriological positive rates and difficulties in obtaining qualified pathological specimens for MTB detection. The delayed diagnosis of EPTB may lead to untimely treatment and further severe consequences (Cukic and Ustamujic, 2018).

Xpert MTB/RIF (Xpert) is a rapid, semi-quantitative, in vitro nested real-time PCR assay on the GeneXpert platform for the simultaneous detection of MTB complex DNA and rifampin resistance-associated *rpoB* mutations, which was endorsed by World Health Organization (WHO) in 2010 (Organization, 2010). Previous studies have shown that Xpert has a good performance in the diagnosis of PTB with sputum specimens (Myo et al., 2018; Neto et al., 2018; Silva et al., 2018; Steingart et al., 2014). In contrast,

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https://doi.org/10.1016/j.ijid.2019.01.050

Xpert had a high specificity, but imperfect sensitivity for the diagnosis of EPTB (Denkinger et al., 2014; Moure et al., 2012; Penz et al., 2015; Theron et al., 2014; Zeka et al., 2011). The sensitivity of Xpert varies by specimen types, and is low in pleural fluid specimens but high in fine-needle aspiration (FNA) tissues, owing to the different bacillary loads of specimens (Penz et al., 2015).

Xpert MTB/RIF Ultra (Xpert Ultra), a next-generation diagnostic on the GeneXpert platform, has a substantially lower limit of detection (LOD) than that of Xpert due to the use of multi-copy *IS1081* and *IS6110* insertion elements as MTB target sequences. For *M. tuberculosis* H37Rv, the LOD of Xpert Ultra in sputum has been down to 15.6 CFU/ml, compared to the 112.6 CFU/ml of Xpert (Chakravorty et al., 2017). Xpert Ultra incorporates melt analysis with nested real-time PCR and is equipped with a larger reaction chamber with a total capacity of 50 μl than that of (25 μl) Xpert. The novel cartridge can provide negative results automatically in 65 min and positive results in 77 min. The WHO has recommended the use of Xpert Ultra in all settings as a replacement of Xpert since March 2017 (Organization, 2017b).

However, so far, there is still limited data on the performance of Xpert Ultra on the diagnosis of EPTB with extrapulmonary specimens. We herein conducted a prospective study to evaluate the diagnostic performance of Xpert Ultra for EPTB patients with extrapulmonary specimens.

Material and methods

Studying population

This study was conducted in Shanghai Pulmonary Hospital (SPH), Tongji University School of Medicine. SPH is a tertiary-care hospital and also one of the national designated tuberculosis hospitals in China. Patients with suspected EPTB were enrolled between Nov 1, 2017 and June 30, 2018. The inclusion criteria were suspected EPTB cases aged ≥16 years old, HIV-negative and willing to receive aspiration or puncture procedures. Demographic information, including sex, age, medical history, and underlying diseases were recorded upon enrollment. Pathologic and microbiological testing results and follow-up were recorded as well.

The EPTB diagnostic criteria followed the WHO guidelines and was based on a combination of clinical symptoms, radiological evidence compatible with active TB, histological observations, lack of improvement in response to a course of broad-spectrum antibiotics (excluding anti-TB drugs such as fluoroquinolones and aminoglycosides), and a satisfactory response to all courses of anti-TB therapy observed by clinicians (Organization, 2010).

The included patients who were clinically diagnosed with EPTB or non-EPTB. The EPTB included both culture-positive and culture-negative EPTB. MTB cultures were performed with MGIT 960 from

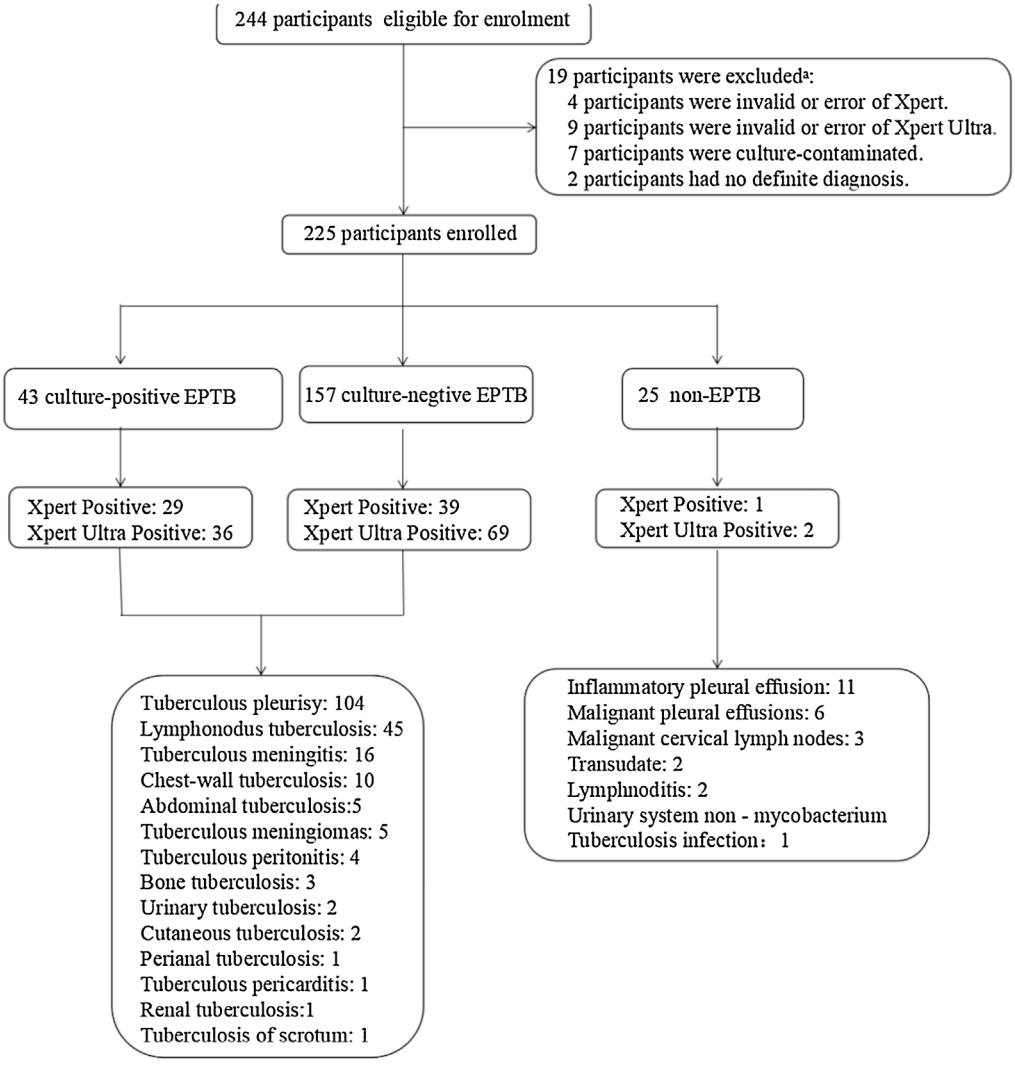

Figure 1. Enrollment of 200 EPTB patients and 25 non-EPTB patients.

clinical specimens. For culture-negative EPTB, diagnoses were achieved based on whether pathological results were consistent with TB, whether clinical findings were consistent with EPTB and whether patients showed response to anti-TB treatment within six months. Non-EPTB referred to the cases with other diagnosis.

Bacterial Culture, Xpert MTB/RIF Ultra, Xpert MTB/RIF

Laboratory examination was performed in the Clinical laboratory of SPH, an ISO 15189 accredited laboratory specialized in MTB detection and equipped with a full set of mycobacterium detection facilities.

The collected EPTB specimens included pleural fluid, FNA tissues from extrapulmoanry sites, cerebrospinal fluid, peritoneal fluid, urine and pericardial fluid. No less than 2 ml extrapulmonary specimens were collected and subject to culture, Xpert and Xpert Ultra assays in accordance with relevant guidelines. EPTB specimens with more than 5 ml were centrifuged at 3000 g for 15 min and the sediments were resuspended in 2 ml PBS buffer (pH=6.8) via vortexing for 30 s.

Sterile specimens were directly processed. Non-sterile specimens were pretreated with N-acetyl-L-cysteine-NaOH-Na citrate (1.5% final concentration) for 15 min at room temperature, and then neutralized with PBS buffer (40 ml final volume). After centrifugation at 3000 g for 15 min, the sediments were resuspended in 2 ml PBS buffer via vortexing for 30 s. 1 ml resuspension of non-sterile specimens and 1 ml sterile specimens were added to mycobacteria growth indicator tube (MGIT) with a Bactec 960 instrument (BACTEC MGIT, Becton Dickinson, Cockeysville, MD, USA) for liquid culture. Cultures were incubated for up to 42 days. The remaining 1 ml was used for Xpert and Xpert Ultra assays.

Xpert and Xpert Ultra assays were performed according to the manufacturer's instructions. 4 ml GeneXpert sample reagent was added to the remaining 1 ml of each specimen, then vortex for 30 s. After being incubated at room temperature for 15 min, 2 ml of digested sample was transferred to one Xpert and one Xpert Ultra cartridge and loaded onto four-module GeneXpert instruments. The results of the detection of MTB and rifampin resistance can be automatically generated by the instrument. The semi-quantitative results of the Xpert Ultra assay were read as trace, very low, low, medium, or high, while those of Xpert were read as very low, low, medium or high. The rifampin resistance results were read as detected, not detected, or indeterminate.

Patient follow-up

Follow-ups were conducted for all patients in the outpatient department for at least 6 months. Radiological and bacteriological examinations were performed every two months and the efficacy of the administered chemotherapy was evaluated by experienced physicians.

Statistical analysis

Statistical analysis was performed with the SPSS software version 17.0 (IBM SPSS, Armonk, NY). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated at the 95% confidence interval (CI). Comparison of Xpert and Xpert Ultra assays were performed with χ^2 test. A P-value <0.05 was considered statistically significant.

Results

Clinical characteristics

A total of 244 patients with suspected EPTB were enrolled in this study (Figure 1). Then 19 were excluded due to invalid Xpert or Xpert Ultra results, culture contamination, or obscure diagnosis. Finally 225 were included for analysis. Among these patients, 43 (19.11%) were culture-positive EPTB, while 157 (69.78%) were culture-negative EPTB and all had undergone six-month follow-up. The remaining 25 (11.11%) with no evidence of EPTB were diagnosed as non-EPTB.

The demographic characteristics of the included patients are shown in Table 1. Among these patients, 152 (67.56%) were males. Their ages ranged from 16 to 88 years old (mean: 42.5 years old). About half of patients (113, 50.2%) were between 20 and 50 years old. The types of extrapulmonary specimens included pleural fluid (122, 54.2%), FNA tissues (76, 33.8%), cerebrospinal fluid (16, 7.1%), pericardial fluid (5, 2.2%), urine (5, 2.2%) and pericardial fluid (1, 0.4%). The FNA tissues were obtained from different anatomic sites, including lymph node, thoracic wall, abdominal wall, skin, crissum and bone.

Diagnostic performance of the Xpert Ultra and Xpert

Among 225 included suspected EPTB, there were 43 culture-positive EPTB, 157 culture-negative EPTB, 25 non-EPTB. Among 43 culture-positive EPTB, the Xpert Ultra detected positive results in

Table 1
Demographic characteristics and extrapulmonary specimen types in this study (n = 225).

		Location	Total No Of samples	Culture Positive		Culture Negative	
				Xpert	Xpert Ultra	Xpert	Xpert Ultra
Characteristics							
Sex	Male		152	15	20	23	47
	Female		73	14	16	17	24
Age	≤20		26	6	7	4	9
	20–50		113	19	23	26	44
	>50		86	4	6	10	18
Specimen Types	Pleural fluid		122	13	17	9	30
	FNA tissues		76	15	18	30	38
		Lymph node	50	10	13	18	23
		Thoracic wall	15	2	2	9	10
		Abdominal wall	5	1	1	2	2
		Skin	2	0	0	0	2
		Crissum	1	1	1	0	0
		Bone	3	1	1	1	1
	Cerebrospinal fluid		16	0	0	0	2
	Peritoneal fluid		5	0	0	0	0
	Urine		5	0	0	1	1
	Pericardial fluid		1	1	1	0	0

36 cases (36/43, 83.7%) and the semi-quantitative readouts were 4 (4/36, 11.1%) trace, 8 (8/36, 22.2%) very low, 4 (4/36, 11.1%) low, 18 (18/36, 50.0%) medium, 2 (2/36, 5.6%) high. Meanwhile, the Xpert detected positive results in 29 cases (29/43, 67.4%) and the semi-quantitative readouts were 6 (6/29, 20.7%) very low, 14 (14/29, 48.3%) low, 6 (6/29, 20.7%) medium, 3 (3/29, 10.3%) high-positive.

Among the 157 culture-negative EPTB, Ultra detected positive results in 69 cases (43.9%, 69/157) and the semi-quantitative results were 25 (25/69, 36.3%) trace, 9 (9/69, 13.0%) very low, 10 (10/69, 14.5%) low, 22 (22/69, 31.9%) medium, 3 (3/69, 4.3%) high-positive. Meanwhile, the Xpert detected positive results in 39 cases (39/157, 24.84%) and the semi-quantitative results were 5 (5/39, 12.8%) very low, 15 (15/39, 38.5%) low, 19 (19/39, 48.7%) medium-positive.

The sensitivities and specificities of Xpert, Xpert Ultra and culture were as follows (Table 2). The sensitivities of Xpert Ultra and Xpert for culture positive specimens were 83.7% (95% confidence interval [CI], 68.7–92.7) and 67.4% (95% CI, 51.3–80.5) respectively. The specificities of Xpert Ultra and Xpert for the diagnosis of EPTB were 92.0% (95% CI, 72.5–98.6) and 96.0% (95% CI, 77.7–99.8) respectively. The sensitivities of Xpert Ultra, Xpert and culture for all EPTB participants including culture positive and probable cases with culture negative were 52.5% (95% CI, 45.4–59.6), 34% (95% CI, 27.6–41.1) and 21.5% (95% CI, 16.2–28.0) respectively. The corresponding receiver operating characteristic (ROC) curves for these three methods are shown in Figure 2. The areas under the curve (AUC) for culture, Xpert and Xpert Ultra were 0.608, 0.650 and 0.723, respectively.

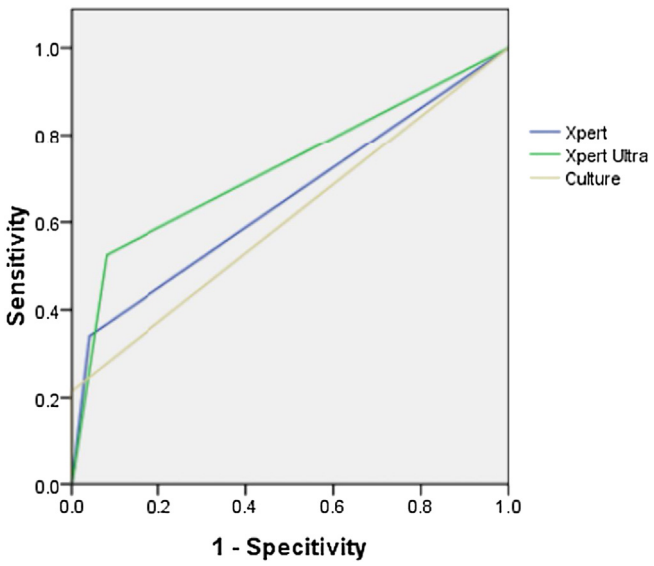

Figure 2. Receiver operating characteristic (ROC) curves of culture, Xpert and Xpert Ultra.

Different detection rate of Xpert Ultra, Xpert and culture

All data are shown in the supplementary Table. Of the 200 EPTB patients, 30 culture-negative specimens only showed positive results with Xpert Ultra, 24 of which were read as trace and indeterminate rifampin resistance. Of 25 non-EPTB patients, 23 showed negative results with all three assays, one was only Xpert Ultra-positive and one was both Xpert- and Xpert Ultra-positive.

The detection rate of Xpert Ultra for EPTB participants with culture-positive and culture-negative specimens was 52.5% (95% CI, 45.4–59.6) which was significantly higher than that (34.0%, 95% CI, 27.6–41.1) of Xpert, $\chi^2 = 138.161$, $P < 0.001$, and also was significantly higher than that (21.5%, 95% CI, 16.2–28.0) of culture, $\chi^2 = 27.878$, $P < 0.001$ seen in Table 2.

Comparison of detection rates among different types of EPTB specimen by Xpert Ultra

There was a significant difference in diagnostic performance of Xpert Ultra tested on different types of specimen types (seen in Table 3 and Figure 3). Xpert Ultra had a detection rate of 43.7% (45/103) on pleural fluid and 78.9% (56/71) on FNA tissues, respectively. The detection rate of Xpert Ultra on FNA tissues was significantly higher than that on pleural fluid. But they were both higher than those of Xpert (Table 3 and Figure 3) using the corresponding specimens.

Table 3
The detection rate of Xpert, Xpert Ultra and culture on pleural fluid and fine-needle aspiration.

Method	Pleural fluid[a] Sensitivity(%, 95% CI)	Fine-needle aspiration[b] Sensitivity(%, 95% CI)
Xpert	21/103 (20.4, 13.3–29.7)	45/71 (63.4, 51.0–74.2)
Xpert Ultra	45/103[a] (43.7, 34.0–53.8)	56/71[b] (78.9, 67.3–87.3)
Culture	23/103 (22.3, 15.0–31.8)	19/71 (26.8, 17.3–38.8)

[a] $\chi^2 = 50.177$, $P < 0.001$ (Xpert Ultra vs. Xpert).
[b] $\chi^2 = 42.279$, $P < 0.001$ (Xpert Ultra vs. Xpert).

Table 2
The performance of Xpert, Xpert Ultra and culture on extrapulmonary specimens.

	Method	Total			
		Sensitivity(%, 95% CI)	Specificity(%, 95% CI)	PPV[a](%, 95% CI)	NPV[b](%, 95% CI)
For patients with culture positive[c]	Xpert	29/43 (67.4, 51.3–80.5)	24/25 (96.0, 77.7–99.8)	29/30 (96.7, 82.8–99.9)	24/38 (63.2, 46.0–78.2)
	Xpert Ultra	36/43 (83.7, 68.7–92.7)	23/25 (92.0, 72.5–98.6)	36/38 (94.7, 82.2–99.4)	23/30 (76.7, 57.7–90.1)
For patients with culture positive and negative[d]	Xpert	68/200 (34.0, 27.6–41.1)	24/25 (96.0, 77.7–99.8)	68/69 (98.6, 91.1–99.9)	24/156 (15.4, 10.3–22.2)
	Xpert Ultra	105/200 (52.5, 45.4–59.6)	23/25 (92.0, 72.5–98.6)	105/107 (98.1, 92.8–99.7)	23/118 (19.5, 13.0–28.0)
	Culture	43/200 (21.5, 16.2–28.0)	25/25 (100.0, 83.4–100.0)	43/43 (100.0, 89.8–100.0)	25/182 (13.7, 9.3–19.8)

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.
[c] $\chi^2 = 54.211$, $P < 0.001$ (Xpert Ultra vs. Xpert).
[d] $\chi^2 = 138.161$, $P < 0.001$ (Xpert Ultra vs. Xpert).

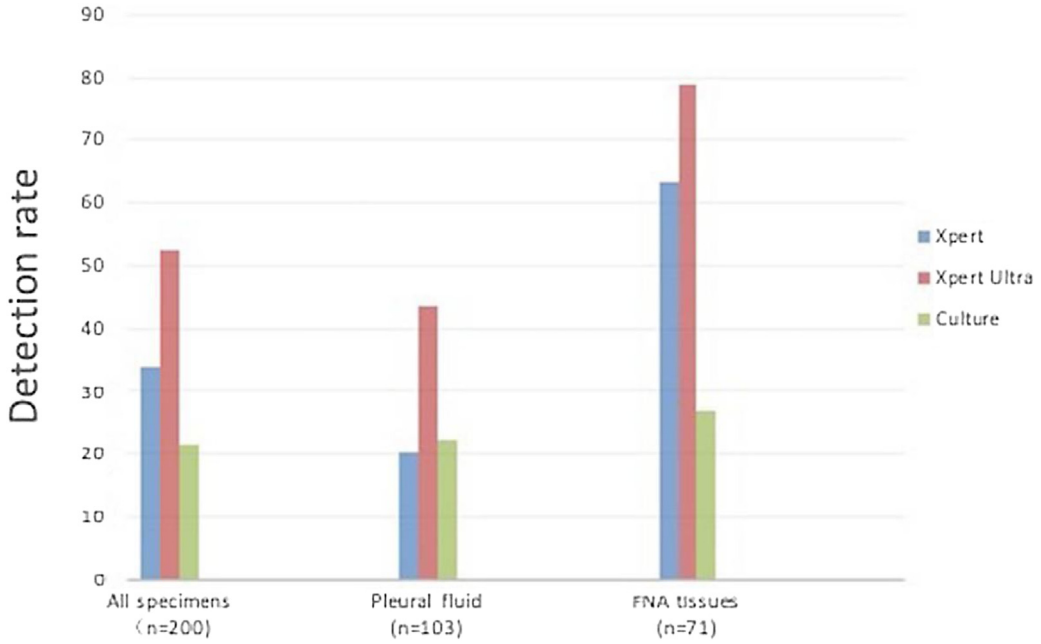

Figure 3. The detection rates of culture, Xpert and Xpert Ultra in different types of specimens.
The histograms on the left are overall detection rates of culture (21.5%, 43/200), Xpert (34.0%, 68/200) and Xpert Ultra (52.5%, 105/200) on all EPTB specimens (n = 200). The histograms in the middle are detection rates on pleural fluid (n = 103), which are 22.3% (23/103) for culture, 20.4% (21/103) for Xpert and 43.7% (45/103) for Xpert Ultra. The histograms on the right are detection rates on FNA tissues (n = 71), which are 26.8% (19/71) for culture, 63.4% (45/71) for Xpert and 78.9% (56/71) for Xpert Ultra.
The detection rates of other types of specimens (including cerebrospinal fluid, pericardial fluid, urine and pericardial fluid) were not displayed as the numbers of cases were limited for calculation.

Discussion

Xpert MTB/RIF Ultra has been applied in some areas or countries since it was recommended by WHO. According to the published study results, Xpert Ultra was better for TB detection capabilities and it has been demonstrated to have high sensitivities for the rapid diagnosis of pulmonary tuberculosis in children, smear-negative EPTB, tuberculous meningitis in HIV-infected adults, patients with paucibacillary disease and HIV (Bahr et al., 2018; Bisognin et al., 2018; Dorman et al., 2018; Nicol et al., 2018; Perez-Risco et al., 2018). Also, Xpert Ultra was performed on urine to identify disseminated TB in an HIV-infected woman in Uganda (Atherton et al., 2018). However, so far only a few reports have shown the diagnostic performance of Xpert Ultra for EPTB especially in China.

In this study, we enrolled 225 cases with suspected EPTB into final analysis and found that the sensitivity of Xpert Ultra (83.7%) was higher than that of Xpert (67.4%). If sensitivity was calculated for all EPTB patients, both culture-positive and culture-negative, the sensitivity of Ultra (52.5%) was significantly higher than that of Xpert (34%) and culture (21.5%), these results demonstrated that Xpert Ultra has an advantage of higher sensitivity over the first generation of the Gene Xpert platform in detection MTB from extrapulmonary specimens in previous studies (Dorman et al., 2018; Perez-Risco et al., 2018). The improved sensitivity of Xpert Ultra is due to the use of multi-copy *IS1081* and *IS6110* insertion elements specific to MTB as target sequences, allowing an additional semi-quantitative category "trace" that corresponds to the lowest bacillary load for MTB detection (Chakravorty et al., 2017; Sankar et al., 2011; Van Soolingen et al., 1992). The "trace" means that only the *IS1081* and *IS6110* targets were detected, but not the TB-specific regions in the *rpoB* gene. Consistently, 30 extrapulmonary specimens with negative culture and negative Xpert results showed positive results in the Xpert Ultra assay, demonstrating an improved EPTB detection.

The second point we found in this study was that Xpert Ultra had varied detection rates of MTB from different types of specimens. The detection rate was relatively low (43.7%) on pleural fluid but high (78.9%) on FNA tissues, which should be explained by the fact that tissues of aspiration from the lump or abscess had more bacillary load than exudative pleural fluid.

For specificity, we found that one false-positive Ultra result was seen on a culture-negative and Xpert-negative non-EPTB specimen. The existence of a few false-positives from Ultra had been reported in an adult with a history of prior tuberculosis treatment (Dorman et al., 2018; Kendall et al., 2017; Arend and van Soolingen, 2018), Ultra had two cases of false-positive results with 92% specificity while Xpert had one case of false-positive result with 96% specificity, therefore, we should be alert, although the specificity of Ultra deceased a little bit.

In addition to the above results, we found that Xpert Ultra had another advantage over Xpert and culture in shorter turnaround time (less than 90 min) for detection MTB on extrapulmonary specimens.

The limitation of the present study was that the types of specimens were not distributed on average; most of them focused on FNA tissues and pleural fluid. Other types were in a limited number of cases such as peritoneal fluid, urine and pericardial effusion. Larger studies will be required to further evaluate the performance of Xpert Ultra on those types of specimens.

In conclusion, Xpert Ultra had a significantly higher sensitivity in the diagnosis of EPTB than that of Xpert, however, specificity was slightly decreased. It has a higher sensitivity on FNA tissues than on pleural fluid. Based on the results of the present study we think the extrapulmonary specimens from suspected EPTB patients should be firstly tested using Xpert Ultra rather than Xpert.

Acknowledgments

We are very grateful to Cepheid for providing us with Xpert MTB/RIF Ultra reagents. Cepheid had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Statements

This study was approved by the Ethical Committee of Shanghai Pulmonary Hospital (approval number: K17-164) and informed consent was obtained from each patient.

Contributions

L.F and F.Y designed the study. X.W wrote the manuscript. L.F, F. Y and D.B modified the manuscript. G.T and X.W did the statistics. R.G and Y.G did laboratory examination. L.F and L.Y collected the samples. L.F and F.Y supervised the project.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2019.01.050.

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