[Original Research]

CHEST

Accuracy of Xpert MTB/RIF Ultra for the Diagnosis of Pleural TB in a Multicenter Cohort Study

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BACKGROUND: The Xpert MTB/RIF (Xpert) assay has greatly improved the diagnosis of TB and identification of resistance to rifampicin (RIF). However, sensitivity of Xpert remains poor for pleural fluid detection. This study evaluated the performance of the novel next-generation Xpert MTB/RIF Ultra (Xpert Ultra) in comparison with Xpert for pleural TB diagnosis.

METHODS: Patients with suspected pleural TB were enrolled consecutively in four hospitals, and pleural fluids were subjected to smear, culture, and Xpert. Defrosted pleural fluid (−80°C) was examined using Xpert Ultra. Drug susceptibility testing (DST) was conducted for all of the recovered isolates.

RESULTS: In total, 317 individuals with suspected pleural TB were recruited; 208 of them were diagnosed with pleural TB according to the composite reference standard, which was composed of clinical, laboratory, histopathologic, and radiologic examination features and ≥ 12 months of follow-up data. The direct head-to-head comparison for *Mycobacterium tuberculosis* detection showed that Xpert Ultra (44.23%, 92 of 208) produced a higher sensitivity than culture (26.44%, 55 of 208, $P < .001$), Xpert (19.23%, 40 of 208, $P < .001$), and smear (1.44%, three of 208, $P < .001$). When Xpert Ultra outcomes were integrated, the percentage of definite pleural TB cases increased from 56.25% (117 of 208) to 64.90% (135 of 208). The specificities of smear, culture, Xpert, and Xpert Ultra were 100% (84 of 84), 100% (84 of 84), 98.67% (83 of 84), and 98.67% (83 of 84), respectively. Xpert Ultra was 100% concordant with phenotype DST for the detection of RIF resistance.

CONCLUSIONS: Xpert Ultra has great potential in diagnosis of pleural TB and its RIF resistance, which could speed up the initiation of appropriate treatment. CHEST 2019; ■(■):■-■

KEY WORDS: pleural fluid; TB; Xpert MTB/RIF Ultra

ABBREVIATIONS: AFB = acid-fast bacilli; CFU = colony forming unit; DST = drug susceptibility testing; RIF = rifampicin; Xpert = Xpert MTB/RIF; Xpert Ultra = Xpert MTB/RIF Ultra

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Drs Huang and Chen contributed equally to this manuscript.

FUNDING/SUPPORT: This work was supported by grants from National Natural Science Foundation of China [81703632, 81672065, 31600107], National Science and Technology Major Project [2017ZX09304009-004, 2017ZX10302301-003-004, 2017ZX10201301-004-002], Beijing Natural Science Foundation [7172050], Beijing Municipal Science & Technology commission [Z171100001017065], Beijing Tongzhou Municipal Science & Technology commission [KJ2019CX006], Beijing Municipal Administration of Hospitals' Ascent Plan [DFL20151501, DFL20181602] and Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support [ZYLX201809] and Key Project of the Department of Science and Technology Beijing, China [D141107005214003, D181100000418003].

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DOI: https://doi.org/10.1016/j.chest.2019.07.027

TB is one of the leading causes of death from a single infectious agent, with an estimated 10 million new cases and 1.6 million deaths in 2017.[1] Fourteen percent of the total 6.4 million reported TB cases were extrapulmonary,[1] among which pleural TB was the second most common type after lymph node TB.[2] Globally, pleural TB remains one of the most frequent causes of pleural exudates, particularly in high TB burden areas.[3] The definitive diagnosis of pleural TB depends on the detection of acid-fast bacilli (AFB) in sputum, pleural fluid, or pleural biopsy tissues.[4] Because of the paucibacillary nature of the disease, diagnosis sensitivities of all the available microbiological and molecular tests are poor.[5] As a consequence, the diagnosis of pleural TB was established based on clinical and radiologic features; laboratory examinations including cytologic, biochemical, and bacteriologic examination; and pleural biopsy occasionally.[4]

An early and accurate diagnosis of pleural TB is critical for its appropriate management.[6] The Xpert MTB/RIF (Xpert) (Cepheid) assay, a point-of-care technique, was a major step toward improved diagnosis of TB and resistance to rifampicin (RIF) globally. Besides for pulmonary TB, the World Health Organization endorsed this technique for extrapulmonary TB diagnosis in 2013.[7] However, various studies reported that Xpert had unsatisfactory performance for examinations of paucibacillary specimens, for example, Xpert demonstrated a sensitivity of 50.9% against culture and 21.4% against the composite reference standard in pleural TB diagnosis.[8-10] Furthermore, false-positive outcomes in the identification of RIF resistance also occur occasionally for paucibacillary specimens.[11]

The next-generation Xpert MTB/RIF Ultra (Xpert Ultra) (Cepheid) was developed for improved performance. Xpert Ultra uses the same diagnostic platform as Xpert but incorporates several changes (eg, fully nested nucleic acid amplification, larger polymerase chain reaction chamber, incorporation of two multicopy polymerase chain reaction amplification targets [IS6110 and IS1081], use of melt curve analysis to detect RIF resistance).[11] These revisions have resulted in a lower limit of detection of the H37Rv strain in sputum of 15.6 colony forming units (CFUs)/mL for Xpert Ultra vs 112.6 CFUs/mL for Xpert.[11] Several clinical studies have also demonstrated that Xpert Ultra has evident promise for the diagnosis of paucibacillary TB, for example smear-negative pulmonary TB,[12] TB meningitis,[13] smear-negative extrapulmonary TB,[14,15] Xpert-negative TB,[16] and pediatric TB.[17] Data on the diagnostic performance of Xpert Ultra in pleural TB remain limited; therefore, additional data obtained under pragmatic conditions are required. The aim of this study was to analyze the diagnostic performance of Xpert Ultra for the detection of *Mycobacterium tuberculosis* in pleural fluid compared with culture or Xpert in a multicenter, head-to-head cohort study in a high TB burden and low HIV burden setting (far less than one per 10,000 population).

Materials and Methods

Ethical Approval

The ethical approval for this study was obtained from the Beijing Chest Hospital ethics committee (ethical approval No. BJXK-2015-08). Written informed consent was acquired from each participant.

Study Design and Participants

Pleural fluid specimens were prospectively collected in a cohort study to identify the appropriate algorithm for care of patients with pleural TB.[18] All of the patients were followed-up for a minimum of 12 months. Adults with suspected pleural TB were enrolled consecutively in four hospitals (Beijing Chest Hospital, Beijing Chao-Yang Hospital, Beijing Geriatric Hospital, and Beijing Hospital) in China from August 2016 to July 2017. Enrolled patients had not been treated with any anti-TB drugs in the past 6 months, had a detailed medical record, and presented a minimum of 50 mL pleural fluid volume. Sputum, collected from the patients who were able to produce samples spontaneously, was processed with smear, culture, and/or Xpert assay. Each pleural fluid specimen was subjected to smear microscopy, culture, Xpert, and routine biochemical examinations, simultaneously. Drug susceptibility testing (DST) was conducted for all of the isolates recovered. Meanwhile, 10-mL aliquot pleural fluid samples were stored in the Beijing Bio-Bank of Clinical Resources on Tuberculosis (Beijing Chest Hospital) at −80°C.

Patient Categories

Patients were divided into three groups according to the composite reference standard, which was composed of clinical, laboratory, histopathologic, and radiologic examinations and 12-month follow-up data. The first group included patients with definite or bacteriologically confirmed pleural TB, which was represented by positive outcome from at least one biological specimen (including pleural effusion, sputum, or pleural biopsy tissue) using any of the following: smear microscopy, culture, or Xpert. At the patient enrollment stage, Xpert Ultra was not performed; therefore, its result was not included. The second group included probable pleural TB indicated cases that did not fulfill the criteria for bacteriologic confirmation but had been diagnosed with active pleural TB by a physician according to clinical findings, thoracoscopic reports, radiologic imaging, and ≥ 12 months of follow-up outcome since the date of enrollment. The third group included non-TB indicated cases diagnosed as other diseases, or for which the laboratory testing was not suggestive of TB, and the patient improved without receiving antitubercular treatment.

Smear and Culture

Direct smear was prepared and stained with auramine and examined by light-emitting diode microscopy. The smear was read and interpreted in accordance with the International Union Against Tuberculosis and Lung Disease guidelines.[19] After processing with *N*-acetyl-L-cysteine and sodium hydroxide and centrifugation, the resuspended sputum pellet was subjected to cultivation on both solid Lowenstein-Jensen medium (Encode Medical Engineering Co, Ltd) and liquid medium using the MGIT 960 system (Becton, Dickinson and Co). The time to positivity was recorded. For all the isolates, MPT64 antigen testing was performed to confirm the presence of *M tuberculosis* complex.

Histopathologic Examination

Pleural biopsies were stored in saline solution for bacteriologic tests and in 4% formalin for pathologic examination. AFB or DNA were detected from the fixed specimens, either by Ziehl-Neelsen staining or by molecular testing. Pleural TB was diagnosed when AFB or its DNA was detected by different methods along with observation of typical granuloma. For patients with typical granuloma but no bacteriologic evidence, probable pleural TB was diagnosed.

Xpert and Xpert Ultra

Pleural fluid samples were defrosted (−80°C) for Xpert Ultra assay. The Xpert and Xpert Ultra assays were performed as per the manufacturer's instructions. Briefly, 1-mL uncentrifuged pleural fluid specimen was mixed with 2-mL sample reagent, vortexed for at least 10 s, and incubated at room temperature for 10 min. The mixture was vortexed for another 10 s and incubated at room temperature for 5 min. A total of 2 mL of the mixture was transferred into the cartridge and loaded into the GeneXpert instrument (Cepheid). The automatic detection procedure was then run. For an invalid result, a repeat Xpert and/or Xpert Ultra test was performed on the same sample. Semiquantitative estimation of the *M tuberculosis* load was also determined by Xpert Ultra as high, medium, low, very low, or trace, depending on the cycle threshold value.

DST

Culture positive samples were also subjected for DST with proportion method using Lowenstein-Jensen medium. The critical concentration of 40 μg/mL was used for RIF.

Statistical Analyses

The sensitivity, specificity, positive predictive value, and negative predictive value of different assays were calculated against the reference standard. We used SPSS version 19.0 (IBM) to compare baseline clinical characteristics and demographic data by diagnosis via Mann-Whitney *U* test for continuous variables and $\chi 2$ test for categorical variables. Logistic regression was performed to examine the potential influence of other factors on the sensitivity of Xpert Ultra. Differences were considered statistically significant at $P < .05$.

Results

Patient Characteristics

In total, 317 patients with suspected pleural TB were enrolled at the four sites. Twenty-five participants were excluded from the analysis because of contaminated cultures (n = 13), indeterminate Xpert results (n = 3), indeterminate Xpert Ultra results (n = 2), or default during the follow-up term (n = 7). Therefore, the final sample size for analysis was 292 patients (Fig 1), which included 208 patients with pleural TB (71.23%) (including 117 definite cases and 91 probable cases) and 84 patients without TB (28.77%). The 84 patients without TB included 42 malignant pleural effusion cases,

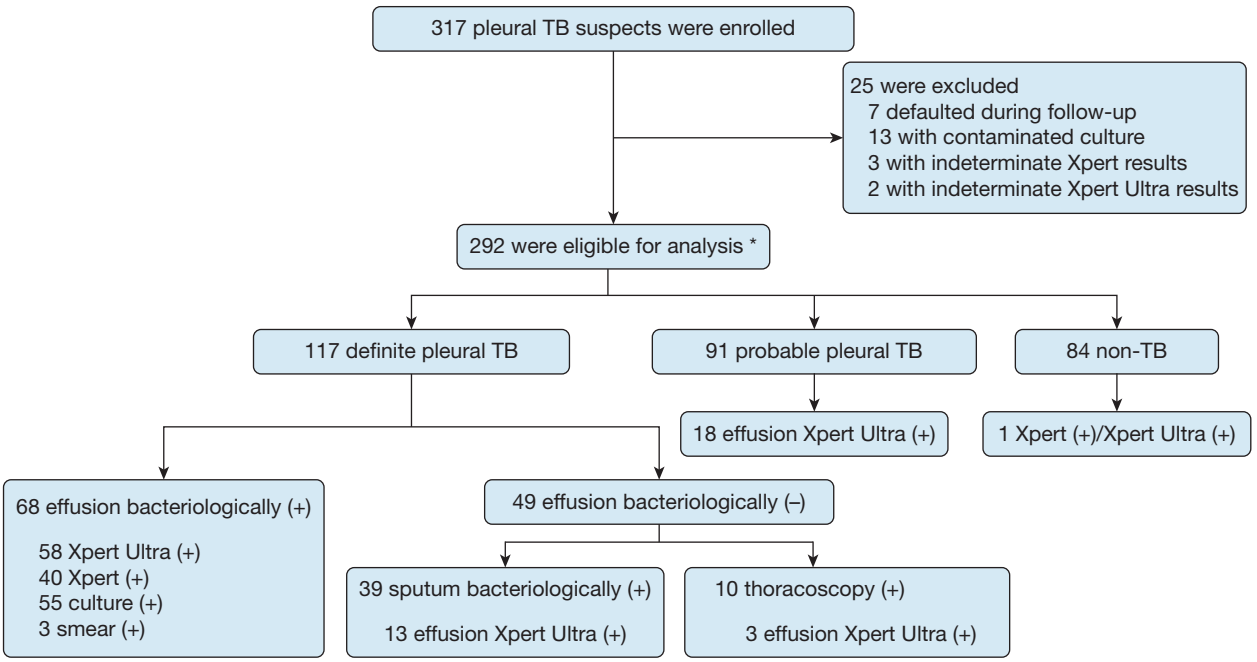

Figure 1 – *Recruitment and diagnostic classification of the participants. The asterisk represents patients who were classified according to composite reference standard criteria that does not include Xpert Ultra results. Xpert = Xpert MTB/RIF; Xpert Ultra = Xpert MTB/RIF Ultra.*

33 exudative pleural effusion cases, five parapneumonic effusion cases, three bacterial pneumonia cases, and one *Mycobacterium intracellulare* infection case. Patients with pleural TB were younger than patients without TB ($P < .001$). A high proportion of patients with pleural TB (14.90%) also had diabetes mellitus in contrast with the patients without TB (4.76%, $P = .016$). All patients were HIV-uninfected. Demographic and clinical characteristics are shown in Table 1.

Performance of Xpert Ultra in Pleural TB Diagnosis

We first evaluated Xpert Ultra testing of pleural fluid among the definite and probable pleural TB cases, which were defined without referring Xpert Ultra results. The direct head-to-head performance comparison for *M tuberculosis* detection showed that Xpert Ultra (44.23%, 92 of 208) produced a higher sensitivity than culture (26.44%, 55 of 208, $P < .001$), Xpert (19.23%, 40 of 208, $P < .001$), and smear (1.44%, three of 208, $P < .001$) (Table 2). Xpert Ultra was also more sensitive than Xpert among pleural effusion culture-positive cases (83.64% [46 of 55] vs 50.91% [28 of 55], $P < .001$). The specificities of smear, culture, Xpert, and Xpert Ultra were 100% (84 of 84), 100% (84 of 84), 98.67% (83 of 84), and 98.67% (83 of 84), respectively. One malignant pleural tumor case was misdiagnosed as pleural TB by both Xpert and Xpert Ultra.

The value of concomitant use of Xpert Ultra plus culture was also considered. Xpert Ultra plus culture (48.56%) demonstrated incremental sensitivity compared with Xpert Ultra (44.23%, $P = .376$); however, the difference was not significant.

At first, 68 patients yielded positive outcomes with their pleural fluid samples by any of the following: smear, culture, or Xpert testing. Among them, three were positive by smear, 55 were positive by culture, and 40 were positive by Xpert. After integrating Xpert Ultra outcomes, a total of 102 patients were defined as pleural fluid bacteriologically positive cases. Among these 102 patients, 34 were detected by Xpert Ultra assay compared with zero, eight, and one from smear, culture, and Xpert assay, respectively (Fig 2). When Xpert Ultra outcomes were also integrated into the composite reference standard, 18 of the 91 (19.78%) probable pleural TB cases were reclassified as definite cases, and the percentage of patients with definite pleural TB showed an obvious increase from 56.25% (117 of 208) to 64.90% (135 of 208).

Factors Linked to Positive Xpert Ultra

Compared with the Xpert Ultra-negative effusions, the Xpert Ultra-positive effusions had a higher adenosine

TABLE 1] Demographic and Clinical Characteristics of the Study Participants

Characteristic	Total (N = 292)	Pleural TB (n = 208)	Non-TB (n = 84)	*P* Value
Age, median (range), y	45 (15-89)	34 (15-89)	52 (15-86)	< .001[a]
Sex, No. of males/females	197/95	149/59	48/36	.017[a]
Pleural effusion test				
Adenosine deaminase, units/L	43.76 ± 30.40	52.86 ± 23.87	19.79 ± 32.79	< .001[a]
WBC count, /μL	4,311 ± 12,882	4,156 ± 12,796	4,808 ± 13,239	.722
Lymphocyte, %	78.11 ± 26.67	80.12 ± 26.41	71.71 ± 26.70	.026[a]
Protein, g/dL	46.76 ± 9.62	48.24 ± 8.11	43.10 ± 11.89	< .001[a]
Glucose, mg/dL	4.98 ± 2.47	4.85 ± 2.43	5.29 ± 2.55	.161
Lactate dehydrogenase, units/L	591.58 ± 913.70	613.35 ± 75.41	537.68 ± 1,227.70	.523
Underlying disease				
Diabetes mellitus	35 (11.99)	31 (14.90)	4 (4.76)	.016[a]
Hypertension	38 (13.01)	22 (10.58)	16 (19.05)	.051
Chronic kidney disease	3 (1.03)	3 (1.44)	0 (0)	.269
Autoimmune disease	3 (1.03)	2 (0.96)	1 (1.19)	.861
Effusion site				
Right	149 (51.03)	111 (53.37)	38 (45.24)	.209
Left	108 (36.99)	75 (36.06)	33 (39.29)	.605
Bilateral	35 (11.99)	22 (10.58)	13 (15.48)	.243

Values are No. (%), mean ± SD, or as otherwise indicated.
[a]Statistically significant ($P < .05$) when compared with pleural TB.

TABLE 2] Performance of Different Methods for Pleural TB Diagnosis

Patient Group[a]	Smear	Culture	Xpert	Xpert Ultra	Culture + Xpert Ultra
Definite pleural TB					
Sensitivity	3/117 (2.56)[b]	55/117 (47.01)[c]	40/117 (34.19)[b]	74/117 (63.25)	101/117 (86.32)[b]
Specificity	84/84 (100)	84/84 (100)	83/84 (98.81)	83/84 (98.81)	83/84 (98.81)
PPV	3/3 (100)	55/55 (100)	40/41 (97.56)	74/75 (98.67)	101/102 (99.02)
NPV	84/198 (42.42)[b]	84/146 (57.53)	83/160 (51.88)[c]	83/126 (65.87)	83/99 (83.84)[c]
Probable pleural TB					
Sensitivity	…	…	…	18/91 (19.78)	…
Specificity	…	…	…	83/84 (98.81)	…
PPV	…	…	…	18/19 (94.74)	…
NPV	…	…	…	83/156 (53.21)	…
Pleural TB total					
Sensitivity	3/208 (1.44)[b]	55/208 (26.44)[b]	40/208 (19.23)[b]	92/208 (44.23)	101/208 (48.56)
Specificity	84/84 (100)	84/84 (100)	83/84 (98.81)	83/84 (98.81)	83/84 (98.81)
PPV	3/3 (100)	55/55 (100)	40/41 (97.56)	92/93 (98.92)	101/102 (99.02)
NPV	84/289 (29.07)[c]	84/237 (35.44)	83/251 (33.07)	83/199 (41.71)	83/190 (43.68)

Values are No./total No. (%). NPV = negative predictive value; PPV = positive predictive value; Xpert = Xpert MTB/RIF; Xpert Ultra = Xpert MTB/RIF Ultra.
[a]Patients were classified according to composite reference standard criteria that does not include Xpert Ultra results.
[b]Statistically significant ($P < .001$) when compared with Xpert Ultra.
[c]Statistically significant ($P < .05$) when compared with Xpert Ultra.

deaminase level (46.15 ± 15.87 units/L vs 61.33 ± 29.10 units/L, $P = .046$) and a lower glucose level (5.42 ± 2.04 mg/dL vs 4.12 ± 2.69 mg/dL, $P = .019$) (Table 3). The probability of a positive effusion Xpert Ultra was also associated with the effusion bacterial load (OR, 8.894; 95% CI, 3.793-20.851) (Table 3).

Performance of Xpert Ultra in RIF Resistance Detection

Fifty-five participants had effusion culture-positive outcomes and phenotypic DST results. Among those, Xpert Ultra provided interpretable RIF resistance detection results for 36 participants and 10 trace results, whereas Xpert provided interpretable results for 27 participants. Comparison among 26 cases with phenotypic DST outcomes and eligible Xpert and Xpert Ultra results was also performed. Both Xpert Ultra and Xpert correctly identified all of the five RIF-resistant cases and 21 RIF-sensitive cases defined by phenotypic DST. Therefore, the sensitivity and specificity for both Xpert Ultra and Xpert were 100%, respectively. Besides, among 153 patients with effusion culture-negative plural TB, Xpert Ultra provided interpretable RIF resistance detection results for 17 patients including two RIF-resistant cases, whereas the counterpart for Xpert was 12 patients and one RIF-resistant case.

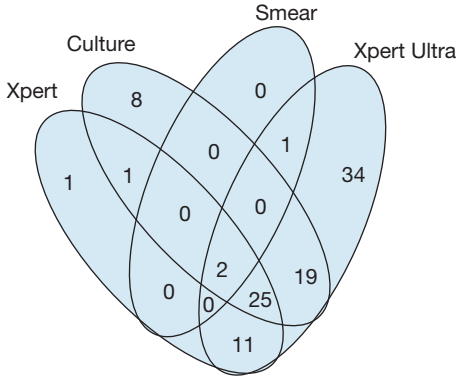

Figure 2 – *Venn diagram of the overlap among different diagnostics for pleural fluid testing. See Figure 1 legend for expansion of abbreviations.*

Mean Time to Positivity

The semiquantitative readout for positive Xpert Ultra samples was as follows: 5.43% (five of 92) for medium, 14.13% (13 of 92) for low, 38.04% (35 of 92) for very low, and 42.39% (39 of 92) for trace. No specimen produced a high category result. Regarding the time required for culture-positive growth (Fig 3), significant differences were found when specimens from the categories not detected (32.33 ± 14.64 days, $P = .013$), trace (27.30 ± 10.58 days, $P = .011$), and very low (23.50 ± 7.00 days, $P = .009$) were compared with those from the medium category (13.25 ± 5.56 days). The comparison between the not detected, trace, very low, and low (23.00 ± 7.31 days) categories presented minor but not statistically significant differences (all $P > .05$).

TABLE 3] Risk Factors for Xpert Ultra Positivity in Patients Diagnosed With Pleural TB

Characteristic	Xpert Ultra		Univariate Analysis		Multivariate Analysis	
	Positive (n = 92)	Negative (n = 116)	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age, median (range), y	34 (15-89)	34 (15-86)	0.994 (0.979-1.009)	.408	…	…
Sex, No. of males/females	72/20	77/39	1.823 (0.974-3.415)	.061	…	…
Pulmonary parenchymal lesions	55 (59.78)	56 (48.28)	1.359 (0.783-2.357)	.275	1.098 (0.568-2.121)	.781
Loculated pleural effusion	25/84 (29.76)	15/103 (14.56)	1.699 (1.002-2.883)	.049[a]	1.402 (0.736-2.609)	.304
Pleural effusion ADA, units/L	61.33 ± 29.10	46.15 ± 15.87	4.316 (2.018-9.228)	< .001[a]	2.341 (1.017-5.387)	.046[a]
Pleural effusion WBC count, /μL	5,352 ± 1,9047	3,208 ± 2,378	1.027 (0.959-1.099)	.454	…	…
Pleural effusion lymphocyte, %	72.34 ± 31.93	86.28 ± 19.03	0.979 (0.967-0.990)	< .001[a]	0.99 (0.976-1.004)	.153
Pleural effusion protein, g/dL	49.28 ± 8.58	47.43 ± 7.71	1.029 (0.993-1.066)	.112		
Pleural effusion glucose, mg/dL	4.12 ± 2.69	5.42 ± 2.04	0.757 (0.650-0.881)	< .001[a]	0.814 (0.685-0.967)	.019[a]
Positive effusion *Mycobacterium tuberculosis* culture	46 (50.00)	9 (7.76)	11.889 (5.376-26.290)	< .001[a]	8.894 (3.793-20.851)	< .001[a]
Positive sputum *M tuberculosis* culture	24 (26.09)	22 (18.97)	1.508 (0.782-2.910)	.221	…	…

Values are No. (%), mean ± SD, No./total No. (%), or as otherwise indicated. ADA = adenosine deaminase. See Table 2 legend for expansion of other abbreviation.
[a]Statistically significant ($P < .05$) when compared with Xpert Ultra positive.

Discussion

Existing tests for the diagnosis of pleural TB have major limitations in terms of accuracy, time to diagnosis, and drug resistance testing. In most cases, they also require special expertise for sample acquisition and interpretation of the results. Therefore, alternative tests have long been sought.

In this head-to-head cohort study, the diagnostic performance of Xpert Ultra was evaluated in comparison with culture and Xpert. Xpert Ultra (44.23%) demonstrated higher sensitivity than both culture (26.44%, $P < .001$) and Xpert (19.23%, $P < .001$) for pleural TB diagnosis. This is in line with a recent publication, where 47.6% (10 of 21) of smear-negative culture-positive pleural fluids were detected by Xpert Ultra.[14] Our results also demonstrated that Xpert was less sensitive than culture, whereas Xpert Ultra was superior to culture for pleural TB diagnosis. The limit of detection of automated mycobacterial liquid culture is about 10 to 50 CFUs/mL.[20] Xpert Ultra has an eightfold lower analytical limit of detection than Xpert (15.6 CFUs/mL vs 1,112.6 CFUs/mL).[11] This could be the main reason for the improved sensitivity of Xpert Ultra for paucibacillary TB diagnosis. In our study, Xpert Ultra detected 54 additional pleural TB cases in contrast with Xpert, which led to the percentage increase of patients with definite pleural TB from 56.25% (117 of 208) to 64.90% (135 of 208). In clinical practice, a rapid confirmed pleural TB diagnosis will speed up the initiation of appropriate treatment.

In this study, one 72-year-old man with malignant pleural tumor was misdiagnosed with pleural TB by both Xpert and Xpert Ultra, with very low category outcomes for both tests. Studies showed that false-positive Xpert Ultra results were recognized among patients with TB history, especially for the trace semiquantitative category.[11,15] To identify this as a true or false-positive result, an interferon gamma release assay using T-SPOT.TB (Oxford Immunotec) on pleural effusion and peripheral blood was performed. Both pleural effusion and peripheral blood produced positive outcomes (380 and 148 spot-forming cells per million mononuclear cells, respectively). Based on this information, we presume that this patient might have TB comorbidity or history of TB. It is possible for the patient to have both pleural tumor and pleural TB in a high TB burden country such as China. However, contamination or other unknown reasons are also possible.

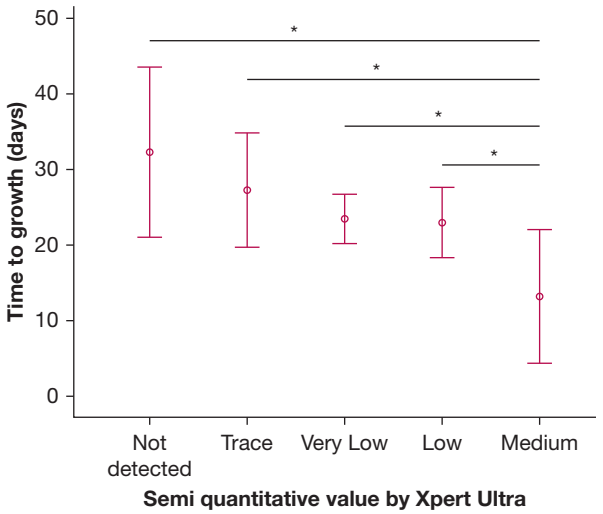

Figure 3 – *Turnaround time of positive culture for pleural fluid samples classified according to the semiquantitative value provided by Xpert MTB/RIF Ultra. *Statistically significant ($P < .05$).*

Multidrug-resistant TB is an increasing concern globally and directly threatens disease control efforts in many countries. In this study, Xpert Ultra detected all five multidrug-resistant cases defined by phenotypic DST. Moreover, 17 additional interpretable RIF DST results including two RIF-resistant outcomes were obtained by Xpert Ultra among patients with effusion culture-negative plural TB. The assay in our report, together with another report, manifested that Xpert Ultra is a more efficient approach to identify patients with drug resistance compared with other diagnostic tools.[12] Furthermore, conventional phenotypic DST methods take 2 to 3 months from sample collection to the production of outcomes, whereas Xpert and Xpert Ultra could detect drug resistance within a few hours, which dramatically diminishes the delay of appropriate treatment. Presumably, application of Xpert Ultra could shorten the whole course of medical care and decrease the risk of disabling sequelae caused by pleural TB. That Xpert Ultra shares the same diagnostic platform with Xpert can also facilitate its implementation.

The prospective multicenter design, the ≥ 12 months of follow-up step, and the large sample size were important strengths of this study, but its limitations should be noted. Although this is a prospective cohort study, we performed the Xpert Ultra assay with frozen samples. The stored pleural fluid samples may have hampered the detection of *M tuberculosis* by Xpert Ultra. Friedrich et al[21] showed a mean increase of the cycle threshold of 2.1 when comparing frozen and fresh sputum pellets by Xpert. However, Perez-Risco et al[14] showed that the duration of freezing of the smear-negative extrapulmonary samples had no significant effect on the culture.

In conclusion, Xpert Ultra outperforms culture and Xpert assay in diagnosis of pleural TB and its RIF resistance, which could speed up the appropriate treatment in clinical practice.

Acknowledgments

Author contributions: H. Huang, G. Wang, and X. C. contributed to the study concept and design, and all other authors reviewed the protocol and made contributions to study design. S. Wang, X. Y., Q. S., and G. J. collected the data. M. Huang, F. H., and Y. M. provided the specimens. S. Wang, X. Y., Q. S., and G. Wang participated in analysis and interpretation of the data. G. Wang drafted the first manuscript, and all other authors were involved in revising it critically for important intellectual content and have given final approval of the final version of the article before submission.

Financial/nonfinancial disclosures: None declared.

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

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