Effect of Xpert MTB/RIF on the treatment of multi-drug-resistant or rifampicin-resistant tuberculosis screened out from re-treatment pulmonary tuberculosis patients, a prospective cohort study

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Background: Re-treatment pulmonary tuberculosis (PTB) has a high risk of being multi-drug- or rifampicin-resistant tuberculosis (MDR/RR-TB). The Xpert MTB/RIF assay possesses high efficacy for the evaluation of rifampicin resistance. The aim of the present study was to assess the benefit of the Xpert MTB/RIF assay in the screening and treatment of MDR/RR-TB in re-treatment PTB patients.

Methods: Patients with suspected re-treatment PTB were prospectively enrolled and divided into Xpert MTB/RIF and mycobacterial tuberculosis (MTB) culture groups. No Xpert MTB/RIF assay was carried out in the MTB culture group. The diagnostic performance and turn-around time (TAT) of MDR/RR-TB detection and the culture results of MDR/RR-TB patients following two-month chemotherapy in two groups were calculated and compared.

Results: Using phenotypic DST as a reference standard, the positive predictive value of Xpert MTB/RIF for the detection of RR-TB and MDR-TB among re-treatment PTB patients was 90.72% and 77.32%, respectively. The Xpert MTB/RIF group had a significantly shorter interval for the initiation of anti-MDR/RR-TB treatment {1 [1–1] *vs.* 52 [47–57] days, P<0.0001}; and following two-month chemotherapy, the percentage of positive culture MDR/RR-TB patients in the Xpert MTB/RIF group was significantly reduced (24.18% *vs.* 50%, P=0.0003).

Conclusions: Xpert MTB/RIF can accurately screen MDR/RR-TB among re-treatment PTB patients, reducing both the turn-around time for therapy initiation and the percentage of positive culture MDR/RR-TB patients following two-month chemotherapy. This is not only beneficial for treatment but also for reducing MDR-TB transmission. We recommend that re-treatment PTB patients receive anti-RR/TB chemotherapy following a positive RFP resistance result in the Xpert MTB/RIF assay.

Keywords: Xpert MTB/RIF assay; multi-drug-resistant tuberculosis (MDR-TB); re-treatment PTB; diagnosis

Introduction

Re-treatment pulmonary tuberculosis (PTB) is defined as patients who have previously been treated with chemotherapy for a period of one month or longer, including relapse, failure, and return after loss of follow-up during treatment. The World Health Organization (WHO) recommends that re-treatment PTB patients be treated with the category II regimen, which consists of 5 standard drugs (1); however, the effective therapeutic rate of this regimen varies from 50% to 90% depending on the region and re-treatment type (2,3). A high rate of drug resistance has been found in patients with re-treatment PTB, which is associated with poor outcomes (3,4).

According to the 2015 Global TB report, 21% of re-treatment patients had multi-drug- or rifampicin-resistant TB (MDR/RR-TB) in comparison with 3.9% of new cases (5). A Chinese study showed that MDR-TB was present in 4.8% and 26.3% of new and re-treatment TB cases, respectively (6). Updated WHO guidelines recommend that drug susceptibility testing (DST) can be performed in all re-treatment TB patients, and individualized anti-TB treatments, even MDR-TB regimens, should be given based on the local prevalence of drug resistance and DST results (7). However, traditional methods for mycobacterial culture and DST are complex and time-consuming, prolonging diagnosis and increasing the risk of improper treatment and transmission of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains (8). Therefore, rapid screening of MDR/RR-TB among re-treatment patients is important for the administration of appropriate regimens. Gene Xpert MTB/RIF® (Cepheid, Sunnyvale, CA, USA) is a quick and simple molecular test for the detection of rifampicin-resistant (RR) TB (9), which is the representative MDR-TB, since resistance to rifampicin (RFP) typically correlates with resistance to isoniazid (INH) (10). Therefore, Xpert MTB/RIF has been endorsed by the WHO as an essential diagnostic tool for patients in any re-treatment category (11). Although a large number of studies have evaluated the usefulness of Xpert MTB/RIF in diagnosing PTB patients (12), limited data are available regarding the impact of Xpert MTB/RIF on treatment for re-treatment PTB patients (13).

The present study aimed to determine the advantages and benefits of the Xpert MTB/RIF assay in the treatment of MDR/RR-TB screened out from re-treatment PTB cases, and to build treatment patterns for MDR/RR-TB based on testing Xpert MTB/RIF in clinic practice.

Methods

Study design

From June 2017 to June 2018, we prospectively enrolled consecutive re-treatment PTB suspects at Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China. The re-treatment PTB diagnostic criteria were in accordance with the WHO guidelines and relied on information regarding clinical symptoms, X-ray, sputum test, histological observations, and lack of improvement following treatment with broad-spectrum antibiotics (14). Patients with known MDR-TB or HIV infection were excluded.

Patients were randomly assigned to two groups: the Xpert MTB/RIF group (in which patients were tested using the Xpert MTB/RIF assay and mycobacterial culture) and the Mycobacterial tuberculosis (MTB) culture group (in which patients were tested by mycobacterial culture only). In accordance with routine practice, sputum or bronchial alveolar lavage fluid (BALF) samples were collected. Specimens were subjected to routine acid-fast bacilli smear microscopy, mycobacterial culture, identification and drug susceptibility testing (DST) in authorized TB laboratories in Shanghai Pulmonary Hospital. Following positive rifampicin resistance results in the Xpert MTB/RIF group, anti-MDR-TB regimens were initiated. In the MTB culture group, patients underwent anti-MDR/RR-TB chemotherapy when the DST results indicated resistance to RFP with/without INH. All data associated with the treatment of patients were recorded, including: identification number; age; gender; results of the Xpert MTB/RIF assay, smear microscopy, culture, and DST; date of sputum or BALF sample collection and results; treatment regimens; and date of chemotherapy initiation. The diagnostic and treatment process for the present study is shown in *Figure 1*. Xpert MTB/RIF assay was performed only once for patients in Xpert MTB/RIF group.

All participants provided written informed consent. The study was approved by the Institutional Review Board of Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China (Approval No. K16-298).

Definitions

The case definitions are in accordance with the National TB and WHO guidelines (1). "New cases" are defined as PTB patients who have never been given anti-TB drugs or have taken anti-TB drugs for a period of less than one month.
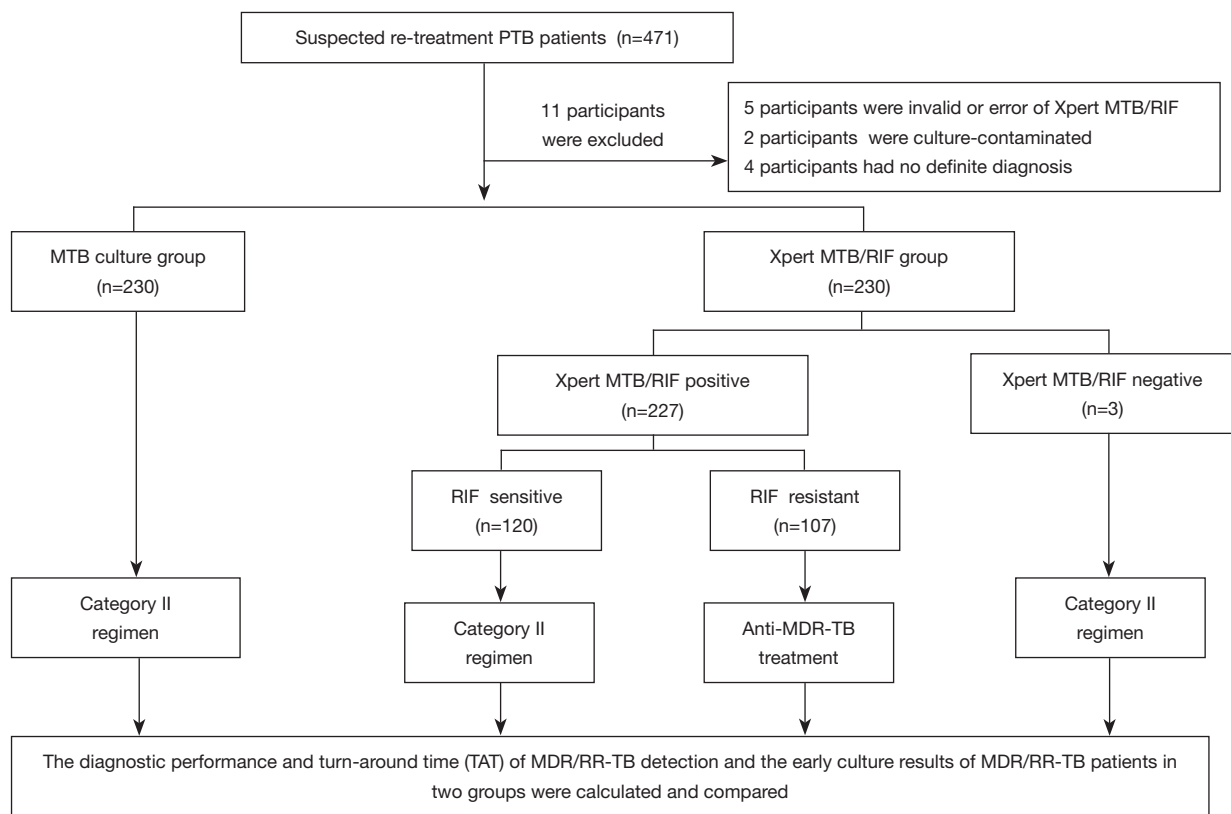
Figure 1 Flow chart of the present study. Among 471 re-treatment PTB suspects, 11 were excluded as a result of invalid Xpert MTB/RIF, culture contamination, or obscure diagnosis. Finally, 460 were included in the analysis. Category II refers to the re-treatment regimen with first-line drugs (2SHREZ/1HREZ/5HRE); Anti-MDR-TB treatment refers to the regimen with second-line drugs (6AmLfxZPtoCsPa/18LfxZPtoCsPa). S, streptomycin; H, isoniazid; R rifampicin; E, ethambutol; Z, pyrazinamide; Am, amikacin; Lfx, levofloxacin; Pto, prothionamide; Cs, cycloserine; Pa, pasiniazid.

"Re-treatment cases" include: (I) relapse (following prior completion of TB treatment); (II) treatment interruption (lost to follow-up during a prior treatment course); and (III) treatment failure (acid-fast bacilli sputum smear-positivity after a five-month treatment course or longer). "Multi-drug-resistant tuberculosis (MDR-TB)" refers to TB that is resistant to at least isoniazid and rifampicin. "Extensively drug-resistant tuberculosis (XDR-TB)" refers to TB that is resistant to at least isoniazid and rifampicin (like MDR-TB), any fluoroquinolone, and at least one of three second-line injectable anti-TB drugs, i.e., capreomycin, kanamycin, or amikacin.

Bacterial culture, DST, and Xpert MTB/RIF assays

Specimens were processed in N-acetyl-L-cysteine-NaOH-Na citrate, neutralized in phosphate-buffered saline (PBS), and centrifuged at 3,000 g for 15 min. The sediments were subsequently resuspended in 2 mL PBS and smears were stained with auramine O to detect acid-fast bacilli (AFB).

An aliquot of the processed specimen was placed in a mycobacterial growth indicator tube (MGIT) and bacterial culture was performed using the BD BACTEC™ MGIT™ 960 Mycobacteria Culture System (Becton Dickinson and Company, Allschwil, Switzerland). Incubation of cultures was carried out for 6 weeks or until signaled by the respective instrument as positive. Culture-based drug susceptibility testing (DST) was performed using the MODS assay (TB MODS Kit™, Hardy Diagnostics, Santa Maria, CA USA), following the manufacturer's protocol.

The remaining specimens were subjected to the Xpert MTB/RIF assay, according to the manufacturer's instructions (Cepheid, Sunnyvale, CA, USA). Specimens were transferred into a single-use disposable cartridge

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and placed in the GeneXpert™ Dx module. The semi-quantitative system automatically interpreted all results in less than 2 h, as very low, low, medium, or high. The output of rifampicin resistance was: detected, not detected, or indeterminate.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated (plus 95% confidence intervals (CIs)) as compared with DST to evaluate the efficacy of Xpert MTB/RIF for the detection of MDR/RR-TB among re-treatment PTB patients. Clinical data consisted of the median and interquartile range (IQR) for age and time variables, and mean ± standard deviation (SD) for other continuous variables. χ^2 test was employed for comparison of categorical variables, and an independent t-test and Mann-Whitney U test were used for continuous variables. P<0.05 was considered statistically significant. Statistical analysis was performed using SPSS 24.0 (IBM, Armonk, New York, USA).

Results

Study population

A total of 471 re-treatment PTB suspects were enrolled in the present study (*Figure 1*). Among these, 11 were excluded as a result of invalid Xpert MTB/RIF, culture contamination, or obscure diagnosis. Finally, 460 were included in the analysis. Patients were divided into two groups: 230 cases in the Xpert MTB/RIF group and 230 cases in the MTB culture group. The patient demographic characteristics are shown in *Table 1*. In the Xpert MTB/RIF group, the median age was 44 years old [IQR 28–57], 159 (69.13%) were male, the average BMI (body mass index) was 21.05±2.82, and 32 cases (13.91%) were in combination with diabetes mellitus; while in the MTB culture group, the median age was 44 years old [IQR 28–58], 159 (69.13%) were male, the average BMI was 21.29±2.61, and 36 cases (15.65%) were in combination with diabetes mellitus. All patients were HIV-negative. In the Xpert MTB/RIF group, 170 patients (73.91%) were "relapse", 10 (4.35%) were "treatment interruption", and 50 (21.74%) were "treatment failure"; while in the MTB culture group, 160 patients (69.57%) were "relapse", 12 (5.22%) were "treatment interruption", and 58 (25.22%) were "treatment failure". The number of MDR/RR-TB cases was 91 (39.57%) in

the Xpert MTB/RIF group and 94 (40.87%) in the MTB culture group. According to the Xpert MTB/RIF assay: the number of patients with high-level results was 41 (17.83%), those with medium-level was 75 (32.61%), those with low-level was 90 (39.13%), those with very low-level was 21 (9.13%), and those with negative results was 3 (1.30%) (*Table 1*).

Diagnostic value of Xpert MTB/RIF for screening out MDR/RR-TB among re-treatment PTB patients

In the present study, a total of 157 cases with positive results from both Xpert MTB/RIF and MTB culture were included in the analysis. Conventional DST showed that there were 90 RR-TB patients among the 157 cases, and the Xpert MTB/RIF assay correctly detected 88 cases of these, with a sensitivity of 97.78%. A total of 13 patients (13/107; 12.15%) were resistant to RFP according to Xpert MTB/RIF and sensitive to isoniazid (INH) by phenotypic DST. Consequently, use of phenotypic DST as the reference standard showed that the PPV of Xpert MTB/RIF for the detection of RR-TB and MDR-TB among re-treatment PTB patients was 90.72% and 77.32%, respectively (*Table 2*).

Effect of the Xpert MTB/RIF assay on the treatment of MDR/RR-TB patients

The results of phenotypic DST were used as the reference standard for the initiation of anti-MDR-TB treatment regimens. In the present study, the turnaround time (TAT) from sample collection to the initiation of anti-MDR-TB treatment for MDR/RR-TB cases was significantly shorter in the Xpert MTB/RIF group, which was 1 [1–1] days *vs.* 52 [47–57] days in the MTB culture group (P<0.0001) (*Table 3*).

We compared the culture results between the two groups following two-month chemotherapy. The number of positive culture MDR/RR-TB patients in the Xpert MTB/RIF group was significantly reduced as compared with those in the MTB culture group (24.18% *vs.* 50.00%, P=0.0003) (*Table 4* and *Figure 2*).

Discussion

In the present prospective cohort study, it was demonstrated that a diagnostic and treatment process involving the rapid molecular Xpert MTB/RIF assay could quickly and accurately detected MDR/RR-TB among re-treatment

Table 1 Demographic characteristics of the study population

Characteristics	Xpert MTB/RIF group (n=230)	MTB culture group (n=230)	P value
Age, years, median [IQR]	44 [28–57]	44 [28–58]	0.6725
Sex, male, n (%)	159 (69.13)	159 (69.13)	1.0000
BMI (average ± SD)	21.05±2.82	21.29±2.61	0.0935
Comorbidity, diabetes mellitus, n (%)	32 (13.91)	36 (15.65)	0.5993
Typology of retreatment PTB, n (%)			
Cases of relapse	170 (73.91)	160 (69.57)	0.3004
Cases of treatment interruption	10 (4.35)	12 (5.22)	0.6621
Cases of treatment failure	50 (21.74)	58 (25.22)	0.3789
Proportion of RR-TB and MDR-TB	91 (39.57)	94 (40.87)	0.7754
Resistance profile based on phenotypic DST results			
RR-TB	15	17	0.7140
MDR-TB	35	36	0.8973
Pre-XDR-TB	25	27	0.7684
XDR	16	14	0.7057
MTB loads from quantitative results of Xpert MTB/RIF, n (%)			
High	41 (17.83)		
Medium	75 (32.61)		
Low	90 (39.13)		
Very low	21 (9.13)		
Negative	3 (1.30)		

Clinical data from the patients comprise the median and interquartile range (IQR) for age and time variables, and the mean and standard deviation (SD) for other continuous variables. The χ^2 test was used to compare categorical variables, and an independent t-test and the Mann-Whitney U test was used for continuous variables. P<0.05 was considered statistically significant. RR-TB, rifampicin-resistant tuberculosis; MDR-TB, multi-drug-resistant tuberculosis refers to the resistance to at least isoniazid and rifampicin. XDR-TB, extensively drug-resistant tuberculosis refers to the resistance to at least isoniazid and rifampicin (like MDR-TB), any fluoroquinolone, and at least one of three second-line injectable anti-TB drugs, i.e., capreomycin, kanamycin, or amikacin.

Table 2 Diagnostic value of the Xpert MTB/RIF assay for detecting MDR/RR-TB among re-treatment PTB patients

Item	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)
Detected RR-TB	88/90 (97.78, 92.20–99.73)	58/67 (86.57, 76.03–93.67)	88/97 (90.72, 83.12–95.67)	58/60 (96.67, 88.47–99.59)
Detected MDR-TB	75/75 (100, 95.20–100.00)	60/82 (73.17, 62.24–82.36)	75/97 (77.32, 67.70–85.21)	60/60 (100, 94.04–100.00)

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; RR-TB, rifampicin-resistant tuberculosis; MDR-TB, multi-drug-resistant tuberculosis refers to the resistance to at least isoniazid and rifampin.

Table 3 Comparison of the impact of the Xpert MTB/RIF assay on the turn-around time in MDR/RR-TB patients

Item	Xpert MTB/RIF group (n=91), median [IQR]	MTB culture group (n=94), median [IQR]	P value
TAT for MDR/RR-TB cases	1 [1–1]	52 [47–57]	<0.0001

In the present study, turn-around time (TAT) refers to the time from sample collection to the initiation of anti-MDR-TB treatment in MDR/RR-TB cases.

Table 4 Comparison of the culture results following two-month chemotherapy

Results of culture (at the end of two-month therapy)	Xpert MTB/RIF group (n=91), n (%)	MTB culture group (n=94), n (%)	P value
Positive	22 (24.18)	47 (50.00)	0.0003
Negative	37 (40.66)	23 (24.47)	0.0187
Non-sputum	22 (24.18)	14 (14.89)	0.1109
Loss to follow up	10 (10.99)	10 (10.64)	0.9388

The χ^2 test was used to compare categorical variables. P<0.05 was considered statistically significant.
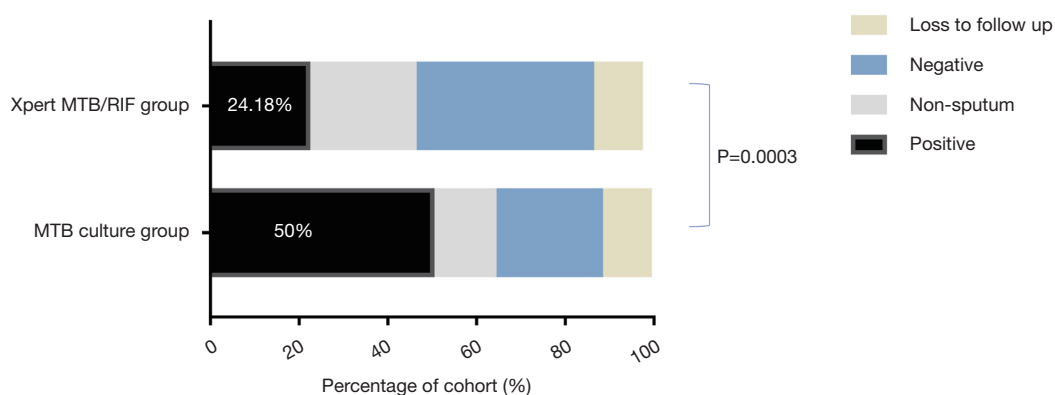

Figure 2 Comparison of the culture results following two-month chemotherapy. The χ^2 test was used to compare categorical variables. P<0.05 was considered statistically significant.

PTB patients, shortening the time to initiation of anti-MDR-TB treatment regimens for MDR/RR-TB patients, and reducing percentage of positive culture MDR/RR-TB patients following two-month chemotherapy.

The status of re-treatment PTB patients is more complicated as compared with newly diagnosed cases. Re-treatment patients have a history of chemotherapy, particularly if there has been previous-treatment interruption or failure, rendering it difficult to apply appropriate regimens. The WHO category II re-treatment regimen for all re-treatment PTB patients has had a low treatment success rate, which is unacceptable (15). Several studies have shown that fast molecular testing for diagnostic and treatment regimens can improve anti-TB treatment outcome (16,17). Individualized treatments based on drug sensitivity testing are more effective than category-based treatments (16).

Xpert MTB/RIF is an accurate and cost-effective automated PCR-based assay that detects *Mycobacterium tuberculosis* DNA and rifampicin resistance in less than 2 h. The sensitivity, specificity, PPV and NPV of Xpert MTB/RIF have been evaluated in suspected or newly diagnosed TB patients (11); however, few studies have examined the effects of the Xpert MTB/RIF assay on the treatment of patients with re-treatment PTB (18,19). Implementation of Xpert MTB/RIF in different health-care systems and settings would have different results, closely associated with its position in the clinical pathway and the way in which clinicians view the technology (20). Therefore, the present prospective study was carried out to determine the effect of

Xpert MTB/RIF on the treatment of MDR/RR-TB in re-treatment PTB patients.

Positive RFP resistance according to the Xpert MTB/RIF assay suggests MDR-TB (9), and anti-MDR-TB therapy has been recommended by WHO for re-treatment PTB patients with Xpert MTB/RIF positive resistance to rifampicin (RFP). In the present study, among the patients who were positive for RFP resistance in the Xpert MTB/RIF assay, 12.15% (13/107) were INH-susceptible by phenotypic DST. A treatment regimen including isoniazid is beneficial for these patients. These results are similar to those reported by two other retrospective studies, in which 6.3–6.9% of the patients who were RFP-resistant according to Xpert MTB/RIF were INH-susceptible by DST (13,21). The positive predictive value (PPV) of the Xpert MTB/RIF assay for RR-TB was 90.72%, while that for MDR-TB was 77.32%. Thus, it can be concluded that RFP-resistance in re-treatment PTB patients according to the Xpert MTB/RIF assay indicates that anti-RR-TB regimens rather than anti-MDR-TB regimens should be performed. Moreover, rapid and accurate diagnostic tools for the detection of INH-resistant *Mycobacterium tuberculosis* deserve further study (13).

MDR/RR-TB patients in the Xpert MTB/RIF group had a much shorter interval for initiation of anti-MDR/RR-TB treatment; and following two-month chemotherapy, the percentage of positive culture MDR/RR-TB patients in the Xpert MTB/RIF group was significantly reduced. MDR/RR-TB transmission is a serious threat to public health. Introduction of Xpert MTB/RIF among re-treatment PTB patients has the potential to reduce MDR/RR-TB transmission by shortening the time to the initiation of MDR/RR-TB regimens. Thus, implementing the Xpert MTB/RIF assay in the treatment of re-treatment PTB patients has essential value in the clinic and in public health.

There exist certain limitations in the present study. There were 70 patients with positive Xpert MTB/RIF results but their culture results were negative. According to the WHO recommendation, a repeat Xpert MTB/RIF test is needed for these patients (22,23); however, due to various restrictions, only once Xpert MTB/RIF test was carried out in the present study. The treatment plans for these patients were based on the results of the Xpert MTB/RIF assay, and further clinical follow-up evaluation would be needed to evaluate the validity of the regimen. Moreover, since the present study was conducted in a single specialized tuberculosis hospital, a higher proportion of patients presented with MDR-TB, which may have had an impact on the prevalence of drug resistance.

Conclusions

In conclusion, through this prospective clinical study, the advantage of Xpert MTB/RIF on the treatment of MDR/RR-TB screened out from re-treatment PTB patients was demonstrated. Application of the Xpert MTB/RIF assay in the diagnosis and treatment among re-treatment PTB patients can significantly reduce the turn-around time for the initiation of anti-MDR/RR-TB treatment, quickly reducing the proportion of patients with positive MDR/RR-TB cultures, which is not only beneficial for patient treatment but also for reducing MDR-TB transmission. We recommend that re-treatment PTB patients receive anti-RR-TB chemotherapy following positive RFP resistance according to Xpert MTB/RIF.

Acknowledgments

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All participants provided written informed consent. The study was approved by the Institutional Review Board of Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China (Approval No. K16-298).

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