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Original Article

Diagnostic performance of GenoType MTBDR*plus* on culture specimens in smear-negative retreatment tuberculosis patients

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Abstract: Background: The diagnostic value of GenoType MTBDR*plus* assay hasn't been validated in smear-negative retreated tuberculosis (TB) patients. Therefore, a retrospective study was conducted to evaluate it. Methods: Between Jun, 2013 and Sep, 2016, 35 retreatment TB patients were enrolled in the study. The phenotypic drug susceptibility test (DST) was evaluated using indirect proportion method with L-J medium. The GenoType MTBDR*plus* assay was done on culture specimens according to the manufacturer's instructions. The sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDR*plus* assay in detection of isoniazid (INH)- and rifampicin (RIF)-resistance were calculated using the phenotypic DST assay as the gold standard. Results: The average age was 28.9 ± 11.3 years (range 5 to 61 years), 57.1% (21/35) were male. For detecting INH-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDR*plus* assay were 94.1% (73.0%, 99.0%), 55.6% (33.7%, 75.4%), 2.12 (1.25, 3.60) and 0.106 (0.02, 0.74), respectively; For detecting RIF-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio were 100% (85.1%, 100.0%), 91.7% (64.6%, 98.5%), 12.00 (1.84, 78.37) and 0, respectively; For detecting MDR-TB, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio were 93.3% (70.2%, 98.8%), 65.0% (43.3%, 81.9%), 2.67 (1.45, 4.92) and 0.10 (0.02, 0.70), respectively. Conclusion: The GenoType MTBDR*plus* assay has high sensitivity for detection of INH- and RIF-resistance in retreatment TB patients. However, the specificity is moderate, this should be taken into account when interpreting the test results.

Keywords: Retreatment, tuberculosis, sensitivity and specificity, isoniazid, rifampicin

Introduction

Tuberculosis (TB) is one of the most serious infectious diseases, with an annual incidence of 9 million new cases, killing more than 1.5 million people annually [1]. The emergence of multi-drug resistant TB (MDR-TB) is further complicating the situation. According to a WHO estimate, in 2014, there were approximately 300,000 new cases of MDR-TB and around 190,000 fatalities from TB worldwide [1]. MDR-TB, defined as resistance to two of the most potent first-line anti-TB drugs, rifampicin (RIF) and isoniazid (INH), has become a major barrier to achieving successful control of TB, as therapy is costly, complicated, with less effective.

Solid and liquid culture methods for drug susceptibility test (DST) of *Mycobacterium tuberculosis* (M.TB) are time consuming requiring weeks to months in providing the results. In addition, contamination rates with conventional culture and DST are high. GenoType MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany) which is used for the rapid detection of M.TB complex and resistance to INH and RIF was endorsed by WHO [2]. The molecular line probe assay detects mutations associated with the *rpoB* gene for RIF-resistance, *katG* genes and *inhA* regulatory region gene for INH-resistance [3]. In a meta-analysis, GenoType MTBDR*plus* showed excellent pooled sensitivity and specificity for detection of resis-

Table 1. Diagnostic performance of GenoType MTBDRplus assay in retreatment tuberculosis patients

	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)
Isoniazid	94.1% (73.0%, 99.0%)	55.6% (33.7%, 75.4%)	2.12 (1.25, 3.60)	0.106 (0.02, 0.74)
Rifampicin	100% (85.1%, 100.0%)	91.7% (64.6%, 98.5%)	12.00 (1.84, 78.37)	0
MDR-TB	93.3% (70.2%, 98.8%)	65.0% (43.3%, 81.9%)	2.67 (1.45, 4.92)	0.10 (0.02, 0.70)

95% CI, confidence interval; PLR, positive likelihood ratio; NLR, negative likelihood ratio; MDR-TB, multidrug-resistant tuberculosis.

tance to INH (91%, 99%), RIF (96%, 98%), and MDR-TB (91%, 99%) [4]. GenoType MTBDR*plus* assay demonstrated excellent performance and offers great promise in improving MDR-TB care and prevention.

In retreatment TB cases, having ≤ 2 treatment courses and not completing retreatment were associated with mortality [5]; A high proportion of MDR-TB exists among retreatment TB cases, especially the relapse and treatment failure cases [6]; Retreatment TB patients, high-risk MDR-TB population, had poor utilization of access to bacteriologic-based TB diagnosis [7]. Therefore, it is necessary to develop cheap, safe and maneuverable DST guiding anti-TB therapy for these high-risk MDR-TB patients. Such as Xpert MTB/RIF, GenoType MTBDR*plus* assay. Meanwhile, in China, mixed infections were observed more frequently among TB patients undergoing retreatment than among new cases (P<0.05) [8]. This may contribute to disagreement between GenoType MTBDR*plus* assay and phenotypic DST results. Considering these problems, the diagnostic performance of GenoType MTBDR*plus* assay deserves an accurate and well-designed evaluation in retreatment TB patients.

The goal of this study was to investigate the diagnostic performance of GenoType MTBDR*plus* assay on culture specimens in comparison to the routine diagnostic standard in smear-negative retreatment TB patients. Therefore, this retrospective study was performed to compare the assay results with phenotypic DST at a Chinese provincial laboratory.

Materials and methods

This study was approved by the Human Research Ethics Committees of Shandong Provincial Chest Hospital (SPCH) and First Affiliated Hospital of Guangxi Medical University. Because of the retrospective nature, written consent was waived.

Between Jun. 2013 and Sep. 2016, culture and phenotypic DST were performed on all retreatment cases. 35 culture-positive and smear-negative retreated TB patients were enrolled in the study. Their clinicopathological characteristics were reviewed and analyzed. Retreated TB patients were defined using criteria proposed by Kilale AM et al. [9].

The indirect proportion method with L-J medium using critical concentrations of INH (1 μg/mL) and RIF (50 μg/mL) was used to screen M.TB isolates. Drug resistance was expressed as the proportion of colonies that grew on drug-containing medium to drug-free medium and the critical proportion for resistance was 1% [10].

The GenoType MTBDR*plus* assay was done on culture specimens and carried out according to manufacturer's instructions. Briefly, three steps were used: i) DNA extraction from processed culture specimen, ii) amplification of target region by PCR, and iii) hybridization of PCR product to the specific oligo-nucleotide probes, immobilized on the strip. Drug resistance was expressed as the absence of wild-type band, presence of mutation band or both.

Statistical analysis was carried out using SPSS 17.0 software. Data were expressed as mean ± standard deviation (SD) all calculations were estimated at a 95% confidence interval (95% CI). The result of the phenotypic DST assay was used as the gold standard to calculate the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio for detecting INH- and RIF-resistance by GenoType MTBDR*plus* assay.

Results

The average age was 28.9 ± 11.3 years (range 5 to 61 years), 57.1% (21/35) were male. All were HIV-negative. 59.4% (19/32) were TB-PCR positive. 10 patients had smoking habit (19.0 ± 26.5 pack-years) and were all males. The time

between initial treatment and retreatment was 3.1 ± 5.3 years (range 1 month to 21 years). Seven patients have contact history with a TB patient in the family. 17 patients were INH-resistant, 22 were RIF-resistant, 9 were mono-resistant TB (INH or RIF), 15 were MDR-TB. The subjects included 21 isolated pulmonary TB and 14 pulmonary + extra-pulmonary TB (1 urinary tuberculosis, 2 tuberculous meningitis, 3 tuberculous lymphadenitis and 10 tuberculous pleurisy).

As shown in **Table 1**, for detecting INH-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDR*plus* assay were 94.1% (73.0%, 99.0%), 55.6% (33.7%, 75.4%), 2.12 (1.25, 3.60) and 0.106 (0.02, 0.74), respectively; For detecting RIF-resistance, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDR*plus* assay were 100% (85.1%, 100.0%), 91.7% (64.6%, 98.5%), 12.00 (1.84, 78.37) and 0, respectively; For detecting MDR-TB, the sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of GenoType MTBDR*plus* assay were 93.3% (70.2%, 98.8%), 65.0% (43.3%, 81.9%), 2.67 (1.45, 4.92) and 0.10 (0.02, 0.70), respectively.

Discussion

The results of the present study have shown that the GenoType MTBDR*plus* assay has high sensitivity for the rapid detection of INH- and RIF-resistant M.TB in retreatment TB patients. However, the specificity is moderate; this should be taken into account when interpreting the test results. To our best knowledge, this is the first study from China to investigate diagnostic performance of GenoType MTBDR*plus* assay in retreatment TB. Although the technique which detects MDR mutations at onset or during therapy would enable rapid identification of MDR and facilitate the modification of regimens for retreatment TB patients, the moderate accuracy also should be taken into account.

Currently, the GenoType MTBDR*plus* assay has been proven to be suitable for application both with culture isolates and directly with smear-positive specimens. Chen C et al. evaluated the performance of the GenoType MTBDR*plus* assay in smear-positive TB, it was shown that the sensitivities and specificities for GenoType MTBDR*plus* in detecting INH- and RIF-resistance were respectively 76.47%, 95.44%, 85.94% and 93.13% [11]. Yadav RN et al. reported the use of the assay on culture specimens, the sensitivity and specificity were 98% and 99% respectively for detection of RIF-resistance; 92% and 99% respectively for detection of INH-resistance; 97% and 100% respectively for detection of MDR-TB [12]. It looks like that culture materials may be more accurate than smear-positive specimens for detecting INH- and RIF-resistance using GenoType MTBDR*plus* assay. This may be associated with that the sensitivity of MTBDR*plus* assay is directly related to the specimen's bacillary load (sputum smear status) [13].

Usually, GenoType MTBDR*plus* assay had very high specificity (up to 100%). But in the study, there were a moderated specificity in detection of drug resistance. If the absence of wild-type band, presence of mutation band or both were detected by the assay, the culture would be resistant to the matched drug only if TB DNA was extracted from single colonies in routine practices. However, in a Chinese study, mixed infections were observed frequently among TB patients undergoing retreatment than among new cases ($P<0.05$) [8, 14]. Therefore, mixed infection may contribution to the moderate specificity of GenoType MTBDR*plus* assay in detection of INH- and RIF-resistance among retreatment TB patients. Meanwhile, in our routine work, the TB DNA for GenoType MTBDR*plus* assay was extracted from the whole culture, but only one isolate was preceded for phenotypic DST.

Although smear-negative specimens have been approved for GenoType MTBDR*plus* assay, its sensitivity remains to be a problem, because the specimen's bacillary load is associated with the performance [13]. Cultivated samples maybe a better choice compared to these specimens were collected from patients directly. For TB detection, Lin et al. evaluated the diagnostic value of the combination of MGIT 960 system and real time-PCR, the results showed that the combination is useful for the early detection of M.TB [15]. It implied that the combination of GenoType MTBDR*plus* assay and MGIT 960 system would improve diagnostic value of GenoType MTBDR*plus* assay, especially saving a lot of time.

This study had several limitations. Firstly, the weakness of the study was its retrospective nature, so the results should be treated with caution. Secondly, the sample size was small. Although thousands of TB patients were examined by the GenoType MTBDR*plus* assay, there were relatively few patients met the eligibility criteria. Thirdly, further work (such as sequencing, MIRU-VNTR genotyping) would be helpful to improve the use of GenoType MTBDR*plus* assay in retreatment TB patients [16, 17]. Unfortunately, these M.TB isolates from clinical specimens weren't collected and stored routinely. Lastly, it requires further analysis to determine whether the mixed infection affect the specificity of GenoType MTBDR*plus* assay in retreatment TB cases.

Conclusion

The GenoType MTBDR*plus* assay has high sensitivity for the rapid detection of INH- and RIF-resistant M.TB in retreatment TB patients. However, the specificity is moderate; this should be taken into account when interpreting the test results. Meanwhile, further research on GenoType MTBDR*plus* assay is needed. Such as the effect of mixed infection, the diagnostic value of combination with MGIT 960 System.

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Disclosure of conflict of interest

None.

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