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Original Article

Study on the value of molecular biology combined with liquid MGIT culture method in clinical examination of mycobacterium tuberculosis

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Abstract: Objective: To investigate the clinical value of molecular biology (xpert MTB/RIF) combined with a liquid mycobacterium culture tube (MGIT) in the clinical examination of Mycobacterium tuberculosis (MTB). Methods: A total of 782 patients with suspected pulmonary tuberculosis who came to our hospital from February 2018 to February 2020 were selected as the research subjects. Sputum samples of all patients were taken in the morning, and BACTEC MGIT culture and xpert MTB/RIF, Lowenstein-Jenden (L-J) culture and acid fast staining microscopy were used respectively to detect the sputum samples. The positive samples were cultured, and the bacteria identification and liquid drug sensitivity test were carried out. We also analyzed the value of xpert MTB/RIF combined with MGIT culture method in the clinical testing of MTB. Results: (1) Among the 782 suspected patients, 405 cases were diagnosed with pulmonary tuberculosis and 377 cases were non tuberculosis patients. The sensitivity of smear microscopy was 17.28% (70/405), the specificity was 98.94% (373/377), and the average time was 3.05 h. The sensitivity of L-J culture test was 20.49% (83/405), the specificity was 97.61% (368/377), and the average time was 28.58 h. The sensitivity of MGIT culture test was 38.02% (154/405), the specificity was 96.82% (365/377), and the average time was 11.23 h. The sensitivity of xpert MTB/RIF test was 36.54% (148/405), the specificity was 99.46% (375/377), and the average time was 2.03 h. The sensitivity of MTB/RIF + MGIT test was 42.47% (172/405), the specificity was 96.02% (362/377), and the average time was 11.29 h. The sensitivity of MTB/RIF combined with MGIT culture was significantly higher than that of smear microscopy and L-J culture (χ^2=61.31, 45.33, P<0.001). (2) The average culture time of 13 smear negative xpert MTB/RIF negative specimens was 17.02 days. The average culture time of 82 smear negative xpert MTB/RIF positive samples was 14.12 days. The average culture time of 77 smear positive xpert MTB/RIF positive samples was 7.45 days. (3) The sensitivity of Xpert MTB/RIF test for rifampicin resistance is 100% (11/11), and the specificity is 91.14% (144/158). Conclusion: The method of molecular biology combined with MGIT has a high sensitivity and specificity for clinical diagnosis of MTB.

Keywords: Mycobacterium tuberculosis, clinical examination, molecular biology, liquid MGIT culture method

Introduction

Currently, the focus of tuberculosis prevention and treatment in China is to raise the accurate and positive detection rate of tuberculosis patients [1-3]. To date, the most effective testing method for tuberculosis is to take samples such as sputum for mycobacterium tuberculosis (MTB) test. At present, the most common bacteriological diagnosis methods in tuberculosis laboratories are traditional drug susceptibility testing, with Lowenstein-Jenden (L-J) medium and smear microscopy [4-6]. However, due to the time-consuming property of traditional drug sensitivity experiments and L-J culture experiments, and lower sensitivity of smear microscopy, delayed or missed diagnosis often occur. To our knowledge, the mycobacteria growth indicator tube (MGIT) culture method can quickly cultivate MTB. Compared with the LJ culture method, the liquid MGIT culture method can increase the positive rate and reduce the culture time. It can implement a first-line and second-line anti-tuberculosis drugs sensitivity experiment [7-9]. The emergence of molecular biological testing methods provides a new treatment approach for tuberculosis early diagnosis and treatment [10]. PCR

real-time fluorescence quantitative experiment (Xpert MTB/RIF) is a common analytical biological test method. In this study, we mainly explore the clinical value of the molecular biology combined with liquid MGIT culture method in the clinical testing of MTB, with an aim to provide better references for the diagnosis of tuberculosis.

Materials and methods

General information

We selected 782 patients with suspected tuberculosis who came to our hospital for treatment from February 2018 to February 2020 as the research subjects, including patients who received first and repeat treatment. Tuberculosis patients met WS288-2008 (diagnostic criteria for tuberculosis). Inclusion criteria: aged 18-90 years; patients with clinically diagnosed pulmonary tuberculosis but negative sputum smear 3 times and negative sputum culture 1 time; patients who had no effect after 2-weeks of regular anti-inflammatory treatment; patients who underwent a percutaneous lung puncture biopsy with a non-calcified and non-vascular lesion (lesion maximum diameter ≥5 mm) shown by CT images; patients who gave informed consent and signed the informed consent. Exclusion criteria: patients who could not control their cough or did not cooperate with the examination; patients with a bleeding tendency or coagulopathy; patients with severe pulmonary hypertension; patients with poor cardio-pulmonary function; patients who refused to undergo a lung biopsy; patients with severe hearing impairment or mental disorders; and patients with one-sided pneumonectomy. According to clinical symptoms, laboratory tests and chest imaging, 405 of the 782 suspected patients were diagnosed with tuberculosis, including 256 males and 149 females, with an average age of (56.78±4.52) years. The study was approved by the hospital ethics committee and the patients and family members provided consent forms.

Methods

Reagents and equipment: p-nitrobenzoic acid (PNB) and thiophene-2-carboxylic hydrazine (TCH) identification medium were purchased from ThermoFisher Scientific; acidic LJ medium and modified LJ medium were purchased from TOPBIO; Ziehl-neelsen stain solution was purchased from Xiamen Haifei Biotechnology Co., Ltd.; the mycobacterial species identification kit and supporting equipment were purchased from Chengdu Boao Jingxin; the BACTEC MGIT 320 mycobacterial analysis system and supporting MGIT culture tube were purchased from BD; GeneXpert MTB/RIF detection system and supporting kits were purchased from Cepheid.

Sample collection and smear acid-fast staining microscopy: In the morning, 3 sputum samples of all patients were collected. Then smear acid-fast staining was applied to each specimen. The procedure was as follows; Mixing of the 3 specimens, then extraction of 1 ml for Xpert MTB/RIF test, and 2 ml for L-J culture and MGIT culture. The acid-fast staining experiment of sputum smear was carried out in strict accordance with relevant experimental requirements.

L-J culture, MGIT culture and drug sensitivity experiment: After 2 ml of sputum specimens were treated with NALC-NaOH, they were respectively inoculated into two acidic L-J mediums, each 0.1 ml, and cultured at a constant temperature of 37°C. The remaining sputum sample was washed with phosphate buffer solution and 0.5 ml was extracted into the MGIT culture tube, then the bacteria inhibitor and nutrients were added, and it was put in the BACTEC MGIT 320 incubator for 42 d. Then the culture-positive specimens were subjected to a first-line anti-tuberculosis drug sensitivity test.

Identification of strains: The MGIT culture-positive specimens confirmed by acid-fast staining were identified by TCH and PNB identification medium. The DNA microarray chip method was used to identify non-tuberculous mycobacteria. This experiment was completed by the Shandong Center for Disease Control and Prevention.

Xpert MTB/RIF inspection: One ml of sputum and 2 ml specimen conditioning fluid were mixed to make them fully liquefied. Then 2 ml of the processed sample was extracted and added to the reaction box and put into the GeneXpert detection module. The cycle threshold (Ct) of the probe was used to calculate the result.

Sequencing of the core region of ropB gene: Upstream primer: 5'-CTTGCACGAGGGTCAGAC-

Table 1. Analysis of smear microscopic examination results

	Positive	Negative	Total
Tuberculosis group	70	335	405
Non tuberculosis group	4	373	377
Total	74	708	782

Table 2. Analysis of L-J culture results

	Positive	Negative	Total
Tuberculosis group	83	322	405
Non tuberculosis group	9	368	377
Total	92	690	782

Table 3. Analysis of MGIT results

	Positive	Negative	Total
Tuberculosis group	154	251	405
Non tuberculosis group	12	365	377
Total	166	616	782

Table 4. Analysis of xpert MTB/RIF results

	Positive	Negative	Total
Tuberculosis group	148	257	405
Non tuberculosis group	2	375	377
Total	150	632	782

CA-3' and the downstream primer: 5'-ATCTCGTCGCTAACCACGCC-3'. The primer synthesis and sequencing were completed by Shenggong Bioengineering. The sequencing experiments and analysis were performed using the BLAST query tool on the NCBI website.

Statistical analysis

All data analysis was done by SPSS20.0. The count data were expressed as [n (%)], and analyzed by χ^2 test. A *P* value of <0.05 was regarded as statistically significant.

Results

Analysis of smear microscopic examination results

By smear microscopic examination, there were 70 positive cases and 335 negative cases in tuberculosis patients; while there were 4 positive cases and 373 negative cases in tuberculosis patients, with the sensitivity of 17.28% and the specificity of 98.94%. As shown in **Table 1**.

Table 5. Analysis of MTB/RIF + MGIT results

	Positive	Negative	Total
Tuberculosis group	172	233	405
Non tuberculosis group	15	362	377
Total	187	595	782

Analysis of L-J culture results

By L-J culture examination, there were 83 positive cases and 9 negative cases of tuberculosis; while there were 322 positive cases and 368 negative cases non-tuberculosis patients, with the sensitivity of 20.49% and the specificity of 97.61%. As shown in **Table 2**.

Analysis of MGIT results

By MGIT examination, there were 154 positive cases and 12 negative cases in tuberculosis patients; while there were 251 positive cases and 365 negative cases in non-tuberculosis patients, with the sensitivity of 38.02% and the specificity of 96.82%. As shown in **Table 3**.

Analysis of xpert MTB/RIF results

By xpert MTB/RIF examination, there were 148 positive cases and 257 negative cases in tuberculosis patients; while there were 2 positive cases and 375 negative cases in non-tuberculosis patients, with the sensitivity of 36.54% and the specificity of 99.46%. As shown in **Table 4**.

Analysis of MTB/RIF + MGIT results

By MTB/RIF + MGIT examination, there were 148 positive cases and 2 negative cases in tuberculosis patients; while there were 257 positive cases and 375 negative cases in non-tuberculous patients, with the sensitivity of 42.47% and the specificity of 96.02%. As shown in **Tables 5** and **6**.

The correlation between MGIT culture and Xpert MTB/RIF test Ct value

Among the 158 specimens that were positive for both MGIT culture and Xpert MTB/RIF test, there was no marked correlation between the MGIT culture time and the Ct value of Xpert MTB/RIF test (r^2=0.46, P<0.01).

Table 6. Comparison of sensitivity, specificity and average culture time of different MTB testing methods

	Sensitivity (%)	Specificity (%)
Smear microscopic examination	17.28	98.94
L-J culture	20.49	97.61
MGIT culture	38.02	96.82
Xpert MTB/RIF	36.54	99.46
Xpert MTB/RIF + MGIT	42.47	96.02
χ^2	95.65	12.57
P	<0.001	0.0014

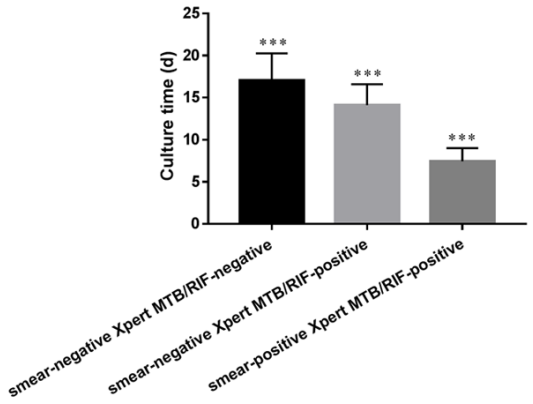

Figure 1. Analysis of MGIT culture time of different specimens. ***indicated P<0.001 when compared with others.

Analysis of non-tuberculous mycobacterium inspection

The 14 cases of non-tuberculous mycobacteria were tested in the 377 non-tuberculous patients, and all of them were negative by Xpert MTB/RIF test. The 14 cases of non-tuberculous mycobacteria were typed using DNA microarray method, and the results showed that 7 strains were intracellular, 2 strains were mycobacterium abscesses, 4 strains were mycobacterium avium, and 1 strain was mycobacterium kansas.

Analysis of MGIT culture time of different specimens

The average culture time of the 13 smear-negative Xpert MTB/RIF-negative specimens was (17.02±3.25) d. The time for the 82 smear-negative Xpert MTB/RIF-positive specimens was (14.12±2.47) d. The time for the 77 smear-positive Xpert MTB/RIF-positive specimens was (7.45±1.57) d. As shown in **Figure 1**.

Culture time and results of liquid MGIT test for first-line anti-tuberculosis drugs and Xpert MTB/RIF for rifampicin resistance

The liquid drug sensitivity method tested the resistance of 158 strains of MTB to different first-line anti-tuberculosis drugs, respectively, and found that streptomycin was 12.03% (19/158), isoniazid 13.29% (21/158), rifampicin 6.96% (11/158), and ethambutol 5.70% (9/158). Taking the liquid drug sensitivity method as the gold standard, the Xpert MTB/RIF test had a sensitivity of rifampicin resistance (11/11) and a specificity of 91.14% (144/158). Using ropB gene sequencing to test the 3 strains that were sensitive to rifampicin and Xpert MTB/RIF test resistant strains, no gene mutations were found. As shown in **Table 7**.

Discussion

At present, the infection and treatment of tuberculosis is one of the key issues that has received much attention in clinical medicine, and it remains a major public health concern in China [11-13]. The gold standard for diagnosis of tuberculosis requires mycobacterial culture, yet the positive rate of traditional laboratory diagnostic methods such as smear acid-fast staining method is low, which fails to meet the needs of clinical diagnosis [14-16]. In this study, we used different methods to detect Mycobacterium tuberculosis in sputum samples. The results found that the sensitivity of MTB/RIF combined with MGIT culture method was 42.47%, significantly higher than the sensitivity of smear microscopy (17.28%) and LJ culture. The single experiment time of Xpert MTB/RIF was 2.03 h, the sensitivity was 36.54%, specificity was 99.46%, and the test results of 14 cases of nontuberculous mycobacteria were negative. Relevant studies have confirmed that the sensitivity and specificity of the Xpert MTB/RIF method for detecting Mycobacterium tuberculosis in sputum specimens are 94.5% (69/73) and 93.6% (73/78) respectively, which are consistent with the results of this study. MGIT culture took 11.23 days, being 17.35 days faster than L-J culture. In our study, the Xpert MTB/RIF combined with MGIT culture method could quickly detect MTB

Table 7. Culture time and results of MGIT of first-line anti-tuberculosis drugs and Xpert MTB/RIF to rifampicin resistance

Methods	streptomycin		isoniazide		rifampicin		ethambutol		Average culture time
	Strains	drug resistance rate	Strains	drug resistance rate	Strains	drug resistance rate	Strains	drug resistance rate	
MGIT	19	12.03%	21	13.29%	11	6.96%	9	5.70%	8.35 d
Xpert MTB/RIF	-	-	-	-	14	8.86%	-	-	2 h
χ^2						0.391			
P						0.532			

in sputum samples, and the sensitivity was higher than smear microscopy and L-J culture.

Among the 782 specimens, the MGIT culture method and Xpert MTB/RIF tests were positive in 158 cases. We analyzed the relationship between MGIT culture time and Xpert MTB/RIF test, and found that the shorter the culture time, the greater the number of MTB in the specimen despite that theoretically MGIT culture time and Ct value are in direct proportion. Then, we analyzed the results of Xpert MTB/RIF and smeared microscopy, and found that the shortest culture time was smear-positive Xpert MTB/RIF-positive specimens, with an average culture time of 7.45 days, followed by smear-negative Xpert MTB/RIF positive, with the average culture time of 14.12 days. These test results together demonstrated that the sensitivity of MGIT culture is high due to the small amount of tuberculosis bacteria in the specimen, resulting in an average culture time of 17.02 days. Furthermore, we also found that under the condition that Xpert MTB/RIF combined with MGIT was used to culture test specimens, and Xpert MTB/RIF was negative and the culture time >10 days, or both results were positive, the probability of detecting MTB was greater. Whereas when Xpert MTB/RIF was negative and 3 d ≤ culture time ≤ 7 d, the probability of detecting nontuberculous mycobacteria is higher.

Based on the liquid drug susceptibility method, the analysis of the 158 strains of MTB revealed that the Xpert MTB/RIF detected all rifampicin-resistant bacteria (11/11) and 91.14% (144/158) rifampin-sensitive bacteria. Relevant studies have confirmed that Xpert detection is significantly better than acid-fast staining, MGIT liquid rapid culture, and fluorescent quantitative PCR in the diagnosis of tuberculous pleurisy, which is of great help to the early diagnosis of tuberculous pleurisy; simultaneously, Xpert technology yielded a favorable detection capability consistent with the results of tuberculosis culture drug susceptibility test in rifampicin-resistant strains. After ropB gene sequencing was used to examine the three specimens, no genetic mutation was found, and the result was rifampicin-sensitive bacteria. The reason may be attributed to the fact that Xpert MTB/RIF uses patient sputum samples, while gene sequencing uses positive cultures, which may lead to different dominant bacterial groups in the two types of samples [17-19]. According to a study conducted by Dhammapal et al. [20], the erroneous results of rifampicin may be related to the amount of MTB in sputum samples. In this study, it is shown that patients with suspected MDRTB can use Xpert MTB/RIF test for initial screening, and liquid MGIT culture method can be used for drug sensitivity testing to ensure the sensitivity of first-line anti-tuberculosis drugs and resistance to rifampicin. The Xpert MTB/RIF test has shown excellent performance in the diagnosis of tuberculosis. The latest research confirms that the Xpert MTB/RIF test has a sensitivity of 90.4% (95% CI: 89.2%-91.4%) and specificity of 98.4% (95% CI: 98.0%-98.7%) for the diagnosis of tuberculosis [21]. Using Xpert MTB/RIF test for rifampicin-resistant patients can also increase the second-line drug sensitivity test. This study confirms that molecular biology combined with MGIT culture method has better diagnostic value. However, its contamination risk is high, the equipment required for testing is expensive, and it is difficult to popularize in primary hospitals, which restricts its application [22, 23].

To conclude, molecular biology combined with the MGIT culture technique can serve as a preferable option for the diagnosis of MTB, with high sensitivity and specificity.

Disclosure of conflict of interest

None.

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