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Diagnostic performance of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) in bronchoalveolar lavage fluid for pulmonary tuberculosis in HIV-infected patients

Mei Zhang [a], Hongwei Zhang [b], Benyong Yan [a], Meixin Ren [a], Wen Wang [a], Tong Zhang [a,*]

[a] Department of Infectious Diseases and Medical Immunology, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China
[b] Clinic of Center for Infection, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

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ABSTRACT

Introduction: Pulmonary tuberculosis (PTB) remains a significant health concern, particularly in individuals infected with human immunodeficiency virus (HIV) who are more susceptible to developing active TB disease. Early and accurate diagnosis of TB is crucial for effective treatment and prevention of transmission. This study aims to evaluate the potential of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) analysis of bronchoalveolar lavage fluid (BALF) for diagnosis of suspected PTB in HIV-infected patients.

Methods: This retrospective study recruited 60 HIV-infected patients with suspected PTB presenting with respiratory symptoms and abnormal chest radiographs between January 2022 and June 2023. BALF samples were collected and subjected to analysis using MALDI-TOF MS, GeneXpert, acid-fast bacilli (AFB) smear and culture. And their diagnostic performance was compared.

Results: The sensitivity of MALDI-TOFMS for diagnosing PTB was 83.3 %, which was better than that of smear 11.9 %, culture 40.5 % or Xpert38.1 % (all $p < 0.01$). The area under the curve (AUC) value of MALDI-TOFMS was 0.889, which was better than that of smear 0.532, culture 0.675 or Xpert 0.690 (all $p < 0.01$). The katG315 and rpoB-RRDR 511 mutations were detected by the MALDI-TOFMS in two patients.

Conclusion: Nucleotide MALDI-TOFMS has a good clinical performance for rapid diagnosis of PTB from BALF samples in HIV infected patients, and detects mutations of TB simultaneously.

1. Introduction

Tuberculosis presents a significant global public health challenge. According to a report from the World Health Organization (WHO)[1], approximately 10.6 million individuals worldwide contracted TB in 2022, with an estimated 410,000 developing multidrug-resistant or rifampicin-resistant TB (MDR/RR-TB). The 2023 Global TB Report further emphasizes China's ongoing struggle with TB, documenting around 748,000 cases in 2022. China ranks third globally in TB burden, following India with 2.82 million cases and Indonesia with 1.06 million cases. Primarily affecting the lungs, there were 6.2 million reported cases of pulmonary TB worldwide in 2022.

The interaction between HIV and TB is intricate and has significant consequences for both diseases. HIV weakens the immune system, making individuals more vulnerable to TB infection and increasing the likelihood of developing active TB. TB is a leading cause of death among people living with HIV.

The diagnosis of TB in HIV-positive individuals can be challenging. Classic diagnostic tests for TB include AFB smears microscopy and mycobacterial culture. AFB microscopy is a widely used, rapid, and reasonable method for TB detection, but it has poor sensitivity and confusion with nontuberculous Mycobacteria (NTM) [2].Though culture testing remains the gold standard for TB diagnosis, it is limited by a low-sensitivity and time-consuming procedure (2–6 weeks) and is not routinely available [3,4].

Fortunately, there have been advancements in TB diagnostics that can help address this problem. One potential solution that has shown promise is the use of molecular-based tests, such as nucleic acid amplification tests (NAATs). These tests target specific regions of the TB genome and can rapidly detect the presence of the bacterium. One

* Corresponding author.
E-mail address: zt_doc@ccmu.edu.cn (T. Zhang).

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widely used NAAT is the GeneXpert MTB/RIF assay, which provides results within 2 h and can simultaneously detect TB and rifampicin resistance. This test has been endorsed by the WHO and has been implemented in many developing countries [5].

MALDI-TOF MS is a new type of soft ionized biomass spectrum rapidly developed in recent years, which has been widely used in the analysis of nucleic acids, proteins, and other biological macromolecules. It is a powerful tool in the diagnosis of PTB with rapid turn-around time, simple workflow, and low cost [6–8].

It can be challenging to diagnose PTB bacteriologically in patients who have negative sputum smears or produce little sputum [9]. However, bronchoscopy can be a valuable tool for these patients. It allows for the collection of appropriate samples from the lower respiratory tract while thoroughly examining all accessible airways. In this study, we evaluated the diagnostic performances of different test methods for PTB diagnosis using BALF samples in HIV-infected patients.

2. Methods

2.1. Study sites and population

From January 2022 to June 2023, 60 patients were enrolled at the Department of Infectious Diseases and Medical Immunology in Beijing Youan Hospital, which is one of the National Reference Centers for TB/HIV co-infection. All patients were HIV positive and suspected of PTB based on respiratory symptoms and abnormal chest imaging. Enrolled patients received no antituberculosis treatment in the past and had to meet one of the two criteria: (1) having either no sputum or being unable to produce or expectorate sputum, or (2) testing negative for sputum/acid-fast bacilli smear and M. tb culture on at least two occasions. Fiberoptic Bronchoscopy is performed, if the patients have no contraindications and agreed to the examination. BALF samples were collected from an abnormal area of the lungs as seen on the radiographs and were analyzed for the presence of M. tuberculosis using AFB Smear, culture, Xpert® MTB/RIF, and MALDI-TOF MS. The protocol of the present study was approved by the Ethics Committee of Beijing Youan Hospital, Capital Medical University. To ensure patient privacy, all data were anonymized, and our reporting adheres to the STARD criteria [10]. All the samples were processed at the Beijing Key Laboratory for HIV/AIDS Research.

2.2. AFB smear and culture assays

All BALF samples were routinely tested by AFB smear (Zhuhai BASO Biotechnology, China) and culture. At least three BALF smears were prepared on clean glass slides and were subjected to acid-fast staining using an acid-fast staining kit (Y-S Biotechnology, Shanghai, China) according to the manufacturer's instructions. The culture was tested using the BACTECTM MGITTM 960 system (Becton Dickinson Diagnostic Systems, Sparks, MD). All tests were carried out at the Beijing Key Laboratory for HIV/AIDS Research.

2.3. Molecular test Xpert® MTB/RIF

Molecular test Xpert® MTB/RIF (Xpert) was performed on BALF samples. The samples were processed on the day of collection and 1 mL was tested in an Xpert cartridge according to the manufacturer's instructions. For MTB/RIF testing, 1.4 mL of MTB/RIF sample reagent was added to 0·7 mL of the BALF and subsequently processed according to the manufacturer's instructions. Rapid molecular assays were conducted using the GeneXpert® System (Cepheid, Sunnyvale, CA, USA), with all protocols provided by Cepheid company.

2.4. MALDI-TOFMS

MassArray was utilized for the identification of MTB and the detection of MTB resistance gene mutation sites. After the BALF is inactivated, a PCR reaction is conducted using a 5 μL solution containing buffer, dUTP/dNTP Mix, specific primers, and DNA template. Upon completion of amplification, the Shrimp Alkaline Phosphatase (SAP) reaction is employed to eliminate excess unincorporated dNTPs and dephosphorylate the remaining dNTPs. Subsequently, an extension reaction is carried out to facilitate the detection of specific nucleotide variations. Following the single-base extension reaction, PCR products are processed for detection and analysis using the MassARRAY system. The MTBDR panel is also utilized, employing commercial probes to detect mutations in certain resistance-associated genes, including rpoB (codons 511, 513, 516, 522, 526, 531, and 533), katG (codon 315), and inhA (promoter −15 nucleotide).

2.4. Diagnosis of PTB

Diagnosis of PTB was based on the "WS288-2017 Diagnostic Criteria for Pulmonary Tuberculosis"[11]. To clarify, patients were initially enrolled without prior antituberculosis treatment history to ensure a treatment-naive cohort for the study. Subsequently, PTB diagnosis was confirmed based on etiology evidence or the response to antituberculous therapy, along with corroborating imaging evidence. This approach was taken to ensure the accurate identification and inclusion of PTB cases in the study.

2.5. Statistical analysis

SPSS 16.0 statistical software was used to perform statistical analysis on the data. The sensitivity, specificity, and predictive values were calculated with 95 % confidence intervals (CIs). The receiver operating characteristic (ROC) curve was generated using graphpadPrism8.0 to assess the diagnostic efficacy of various detection methods, with the AUC serving as the evaluation metric. The Delong method was employed for comparative analysis. A statistically significant difference was indicated by a p-value of less than 0.05.

3. Results

3.1. Characteristics of study population

Demographic and clinical features of the study subjects were shown in Table 1. Out of the 60 patients included, 54were male and 6were female, with a median age of 37.5 years. Nearly half of them had typical TB symptoms, such as fever and cough. Their median CD4 count was 43cells/mm^3 and median viral load was 73400copies/ml.

According to the final clinical diagnosis, 42 cases (70.0 %, 42/60) were diagnosed with PTB, while 18 cases (30.0 %, 18/60) were diagnosed with non-TB diseases. These non-TB diseases included pulmonary fungal infection in 9 cases (15.0 %, 9/60), bacterial pneumonia in 3 cases (5.0 %, 3/60), NTM lung disease in 3 cases (5.0 %, 3/60), pulmonary aspergillosis in 2 cases (3.3 %, 2/60), and pulmonary abscess in 1 cases (1.7 %, 1/60) (Table 1).

3.2. The performances of different assays in PTB diagnosis

The diagnostic specificities of AFB smear, culture, Xpert, and MALDI-TOFMS were all over 90.0 %. Their sensitivities were 11.9 %, 40.5 %, 38.1 %, and 83.3 %, respectively. Their positive predictive values were 83.3 %, 94.4 %, 100.0 %, and 97.2 %, respectively. Their negative predictive values were 31.5 %, 40.5 %, 40.9 %, and 70.8 %, respectively (Table 2).

Additionally, the ROC curve was used to evaluate the diagnostic performances of AFB smear, culture, Xpert, and MALDI-TOFMS. Using clinical diagnosis as a reference, the AUC of AFB smear, culture, Xpert, and MALDI-TOFMS was 0.532, 0.675, 0.690, and 0.889, respectively. The MALDI-TOF MS assay had the highest AUC value and was

Table 1
Demographic and clinical characteristics of 60 HIV infected patients with suspected pulmonary TB.

Characteristics	Total(n = 60)	TB(n = 42)	Non-TB(n = 18)
Age, years (Mean ± SD)	37.50 ± 10.6	34.5 ± 10.6	42.0 ± 10.7
Male	54 (90.0 %)	36(85.7 %)	18(100 %)
Chest symptoms (present)			
Cough	29 (48.3 %)	21(50.0 %)	8(44.4 %)
Fever	28 (46.7 %)	18(42.8 %)	10(55.6 %)
Hemoptysis	3 (5.0 %)	3(7.1 %)	0(0.0 %)
Weight loss	6 (10.0 %)	4(9.5 %)	2(1.1 %)
Night sweat	3 (5.0 %)	2(4.7 %)	1(5.5 %)
Laboratory examination result			
AFB smear positive	6(10.0 %)	5 (8.3 %)	1(5.5 %)
Culture positive	18(30.0 %)	17 (28.3 %)	1(5.5 %)
Xpert MTB/RIF positive	16(26.7 %)	16 (26.7 %)	0(0.0 %)
MALDI-TOF MS positive	36(60.0 %)	35 (58.3 %)	1(5.5 %)
Non-TB patients type			
Pulmonary fungal infection	9 (15.0 %)	/	9 (15.0 %)
Bacterial pneumonia	3 (5.0 %)	/	3 (5.0 %)
NTM lung disease	3 (5.0 %)	/	3 (5.0 %)
Pulmonary aspergillosis	2 (3.3 %)	/	2 (3.3 %)
Pulmonary abscess	1 (1.7 %)	/	1 (1.7 %)
CD4 (cells/μl)[median (range)]	42.5 (7.8, 147.8)	56.5(10.3, 156.0)	14.5(3.3, 119.0)
HIV RNA (copies/ml) [median(range)]	73,364 (149.88, 383794.0)	97952(319.0, 383784.0)	18685.5(51.5, 685495.5)

significantly superior to the other three methods (Z = 3.851, 2.542, and 2.424, all $p < 0.01$) (Fig. 1).

3.3. Detection of resistance

The katG315 and rpoB-RRDR 511 mutations were detected by the MALDI-TOFMS in two patients. Mutation of rpoB was also detected by the Xpert MTB/RIF assay.

4. Discussion

Patients with concurrent HIV and TB infections often exhibit weakened immune systems and atypically low bacterial loads in their sputum, complicating diagnosis through traditional methods such as sputum smear and culture, tuberculin skin tests, and IFN-γ release assays, which show reduced sensitivity in these individuals [12]. Autopsies in South Africa have found that nearly half of HIV-positive individuals had undiagnosed TB at the time of death [13]. Fiberoptic bronchoscopy lavage can enhance *Mycobacterium tuberculosis* detection by providing high-concentration samples from bronchial sites, thereby improving diagnostic accuracy. Our study evaluates the efficacy of various TB diagnostic tests using BALF.

Smear microscopy, despite its simplicity and efficiency, is limited by its sensitivity and the potential for non-specific results due to other mycobacteria [14]. In this particular study, the sensitivity of smear microscopy in detecting *tuberculosis* in bronchoalveolar lavage fluid was low when compared to positive culture, Xpert and MALDI-TOFMS techniques, a finding that aligns with previous studies [15,16].

Mycobacterium tuberculosis culture is currently the gold standard in the diagnosis of TB. Recent studies [15,17]have found that in patients suspected of having TB with negative sputum smears, the sensitivity of *Mycobacterium tuberculosis* culture from bronchoalveolar lavage ranged from 57.1 %-74.2 %. However, our study demonstrated a lower sensitivity of 40.5 % when using *Mycobacterium tuberculosis* culture from bronchoalveolar lavage in HIV-infected patients. A significant drawback of *tuberculosis* culture is its long culture cycle, which does not meet the demands of timely clinical diagnosis. In our study, the median time required for *Mycobacterium tuberculosis* culture was 22 days.

The introduction of nucleic acid amplification has significantly advanced TB diagnosis. In recent years, the World Health Organization has recommended molecular diagnostic methods like Xpert to enhance *tuberculosis* and drug-resistant *tuberculosis* detection[18]. Results from previous studies[19,20] showed that Xpert exhibited high sensitivity and specificity. Different studies have reported varying sensitivities of Xpert in detecting *Mycobacterium tuberculosis* in bronchoalveolar lavage fluid, ranging from 81 % to 97 %. Furthermore, Xpert can also be employed to confirm cases of culture-negative *tuberculosis* [15,21,22]. In our study, Xpert showed a sensitivity of 38.1 % when the final clinical diagnosis was used as the reference standard.

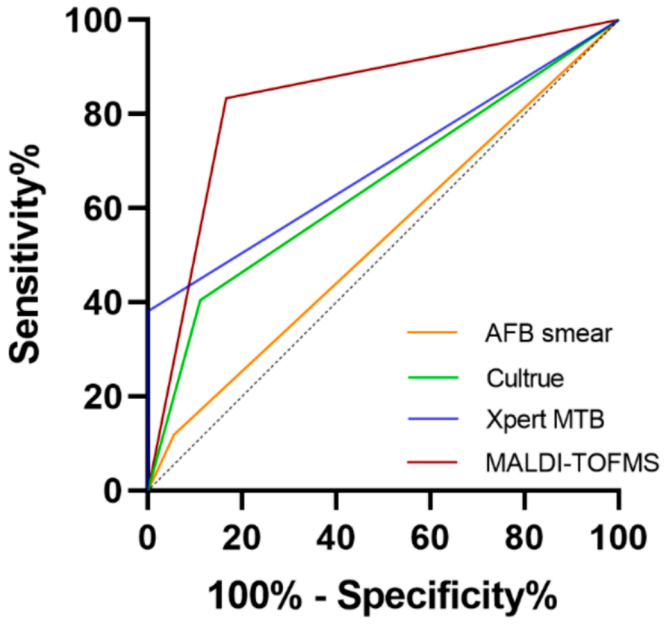

Fig. 1. The diagnostic accuracy of different detection techniques on BALF samples. The receiver operating characteristic (ROC) curves of MALDI-TOF MS, AFB smear, culture and Xpert using clinical diagnosis as reference.

Table 2
The diagnostic performance of AFB smear, culture, Xpert MTB/RIF, MALDI-TOF MS performed on BALF using the final clinical diagnosis as the reference standard.

Variables	Results	Clinical Diagnosis		Sensitivity (%) (95 %CI)	Specificity (%) (95 %CI)	Positive Predictive Value (%) (95 %CI)	Negative Predictive Value (%) (95 %CI)
		PTB (n = 42)	Non-PTB (n = 18)				
AFB smear	+	5	1	11.9(5.2–25.0)	94.4(74.2–99.7)	83.3(40.5–126.2)	31.5(18.7–44.3)
	−	17	37				
Culture	+	17	1	40.5(27.0–55.5)	94.4(74.2–99.7)	94.4(82.7–106.2)	40.5(25.0–56.0)
	−	25	17				
Xpert MTB	+	16	0	38.1(25.0–53.2)	100(82.4–100)	100(82.4–100)	40.9(25.8–56.0)
	−	26	18				
MALDI-TOF MS	+	35	1	83.3(69.4–91.7)	94.4(74.2–99.7)	97.2(91.6–102.9)	70.8(51.2–91.4)
	−	7	17				

The MALDI-TOFMS technology is a robust and versatile research platform that offers various functionalities, including protein and peptide detection, microbial identification, glycosylation analysis, as well as mutation, methylation, and CNV (copy number variation) analysis of genes. It possesses remarkable features such as high throughput, accuracy, speed, and cost-effectiveness [23–26]. Recent studies [26,27] showed that the sensitivity and specificity of nucleotide MALDI-TOFMS ranged from 82.5 % to 94.1 % and 71.4 % to 93.0 %, respectively. Consistent with previous studies, we found that the sensitivity and specificity was 83.3 % and 94.4 %, respectively. The MALDI-TOFMS achieved the highest AUC value. Our findings suggest that nucleotide MALDI-TOFMS detection has superior sensitivity and accuracy in diagnosing pulmonary *M. tuberculosis* complex.

Regarding drug resistance, the nucleotide MALDI-TOFMS assay has the capability to directly identify various mycobacterium species from clinical samples, while simultaneously detecting mutations in drug resistance-related genes [27]. This provides a foundation for rapid diagnosis and precise treatment of PTB. In our study, there were two cases of multi-drug resistant mutations (resistant to both INH and RFP) detected from 42 HIV infected patients, which was similar to a previous study in Beijing with a rate of 6.8 % in newly diagnosed TB patients [28].

In our setting, the cost of MALDI-TOF analysis is approximately 3500 RMB, while Xpert testing costs around 960 RMB. In terms of turnaround time, MALDI-TOF analysis typically requires about 3 h from sample processing to reporting. On the other hand, Xpert testing is usually completed in approximately 2 h. These estimates may vary depending on factors such as laboratory workload, sample volume, and instrument availability. However, these figures provide a general overview of the cost and time differences between the two methods in our setting.

Our study, however, has limitations: its retrospective single-center design may introduce bias and limit generalizability, and diagnoses were subjectively made by clinical experts. Also, the evaluation of drug-resistant mutations was constrained by the low incidence of such cases. Future research should expand to prospective multicenter studies to validate the MALDI-TOFMS assay's clinical diagnostic value for TB and its drug resistance.

5. Conclusions

The nucleotide MALDI-TOFMS assay demonstrates promising clinical performance in the rapid diagnosis of PTB from BALF samples in HIV-infected patients. This assay not only detects the presence of TB but also identifies any associated mutations. Implementing this technique in routine clinical practice has the potential to enhance patient outcomes, reduce transmission rates, and optimize healthcare resource utilization in HIV-infected individuals.

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Ethical statement

The study protocol was approved by the Ethics Committee of Beijing Youan Hospital, Capital Medical University. All subjects provided written informed consent.

CRediT authorship contribution statement

Mei Zhang: Writing – original draft, Methodology, Data curation. **Hongwei Zhang:** Writing – original draft, Data curation. **Benyong Yan:** Writing – review & editing, Methodology, Data curation. **Meixin Ren:** Supervision, Investigation. **Wen Wang:** Supervision, Investigation. **Tong Zhang:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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