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# The *Mycobacterium tuberculosis* specific antigen MPT64 in BALF has potential diagnostic value in the diagnosis of pulmonary tuberculosis

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**Background:** Recent research on the use of the MPT64 antigen of *Mycobacterium tuberculosis* (MTB) in tuberculosis diagnosis has intensified. However, its detection in bronchoalveolar lavage fluid (BALF) has not been previously documented. This study aims to fill that gap.

**Methods:** We included a total of 176 patients, divided into a pulmonary tuberculosis (PTB) group of 104 cases and a non-tuberculosis (Non-TB) group of 72 cases as the control group. The PTB group includes 59 with bacteriologically confirmed PTB (BC-PTB) and 45 with clinically diagnosed PTB with negative pathogens (CD-PTB). The concentrations of MPT64 antigens were detected by enzyme-linked immunosorbent assay (ELISA). Optimal cut-off values were determined by receiver operating characteristic (ROC) curves to evaluate antigen diagnostic capability for active PTB, compared with acid-fast bacilli (AFB) and Xpert MTB/RIF.

**Results:** Xpert MTB/RIF Ct values and MPT64 concentration show significant negative correlation ( $R = -0.719$ ,  $P < 0.0001$ ), AFB and MPT64 concentration show significant positive correlation ( $R = 0.777$ ,  $P < 0.0001$ ). MPT64 showed a sensitivity of 59.62% (95% CI: 50.01–68.54%) in 104 cases, significantly higher than AFB (26.92%,  $P < 0.001$ ) and slightly higher than Xpert MTB/RIF (56.73%,  $P = 0.673$ ). Its specificity was 88.89% (95% CI: 79.58–94.26%), lower than both AFB and Xpert MTB/RIF (100%,  $P = 0.006$ ). Sensitivities for BC-PTB and CD-PTB were 64.41% (95% CI: 51.66–75.40%) and 53.33% (95% CI: 39.08–67.06%), respectively,  $P = 0.314$ .

**Conclusion:** The use of ELISA to detect the MPT64 in BALF may serve as an important supplementary diagnostic method for pulmonary tuberculosis, particularly for bacterial-negative pulmonary tuberculosis.

## KEYWORDS

BALF, enzyme-linked immunosorbent assay, MPT64, tuberculosis, tuberculosis antigen

## Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*MTB*) that poses a serious threat to human health. The World Health Organization's Global Tuberculosis Report 2024 (1) indicates that in 2023 there were 10.8 million new tuberculosis cases worldwide, with 1.25 million deaths, making tuberculosis the world's deadliest infectious disease. Early diagnosis of active tuberculosis is critical for effective treatment and prevention of disease transmission. Pathogenic testing remains the cornerstone for the diagnosis of pulmonary tuberculosis. Currently, clinical methods include acid-fast bacilli (AFB) staining, detection of *MTB* nucleic acids, and *MTB* culture. Although AFB staining is convenient, its positive rate is low and it cannot reliably differentiate between *MTB* and *non-tuberculous mycobacteria*. Xpert MTB/RIF, which detects *MTB* nucleic acid with higher sensitivity (2) and can assess rifampicin resistance, requires sophisticated equipment and skilled operators, limiting its use in underdeveloped areas. Moreover, the detection of dead *MTB* DNA fragments poses challenges in evaluating bacterial activity, TB lesion activity, and treatment follow-up (3, 4). *MTB* culture, while the gold standard, is time-consuming due to the slow growth of the organism and requires biosafety Level II laboratories, making it impractical in resource-limited settings (5–7). Therefore, a new, rapid, and simple complementary method for tuberculosis diagnosis is warranted.

Recent research on *MTB*-specific antigens has revealed that secreted proteins provide direct evidence of active *MTB* infection and may help distinguish current from past infection (8). MPT64 is a 24-kDa protein secreted by *MTB* during its active growth phase, this antigen is absent in *non-tuberculous mycobacteria* and the BCG strain, which lacks the RD2 region (9, 10). Has been extensively used to identify culture-positive *MTB* through immunohistochemistry and immunocytochemistry (11). Owing to its excellent specificity, detection of the MPT64 antigen aids in identifying viable *MTB*. Literature indicates that MPT64 antigen detection has significant diagnostic value in various extrapulmonary specimens, as confirmed by cohort-based diagnostic accuracy studies (12–14).

Although sputum examination is a commonly used method for tuberculosis diagnosis, its limitations—such as atypical clinical symptoms in some patients, insufficient sputum samples, and low bacterial loads—can delay diagnosis and treatment, especially in bacterial-negative cases (15, 16). In contrast, BALF directly targets lesions in the lower respiratory tract, thereby significantly improving the detection rate of pulmonary pathogens and garnering increased attention in clinical practice (17). Several studies have demonstrated that the diagnostic sensitivity of BALF is superior to that of sputum for AFB staining, PCR, and *MTB* culture (17, 18). For liquid samples, ELISA is considered the preferred method because of its ease of operation, rapidity, and minimal equipment and operator requirements. Therefore, this study aimed to use ELISA to detect the *MTB* antigen MPT64 in BALF and evaluate its potential value in diagnosing pulmonary tuberculosis. To date, no study has reported on the use of BALF to detect the *MTB*-specific antigen MPT64, this study is the first to detect, thereby addressing a gap in the existing literature.

## Materials and methods

### Study design and participants

This retrospective study enrolled 176 patients who underwent fiber bronchoscopy and alveolar lavage at the Affiliated Hospital of North Sichuan Medical College from May 2021 to July 2023. Indications for fiber bronchoscopy examination (meeting one or more criteria): (1) Patients with suspected symptoms of tuberculosis, such as fever, night sweats, cough, sputum production, and weight loss, at all; (2) Patients with lesions on lung X-rays or CT scans.

Among the collected cases, 104 patients diagnosed with pulmonary tuberculosis were selected as the study subjects (pulmonary tuberculosis group, PTB). The diagnostic criteria for tuberculosis were based on the Health Industry Standard of the People's Republic of China WS288–2017 (19). This includes, (1) 59 cases of bacteriologically confirmed pulmonary TB (BC-PTB): Two specimens of BALF were AFB stained or Xpert MTB/RIF positive; (2) 45 were clinically diagnosed pulmonary tuberculosis (CD-PTB): The Xpert MTB/RIF and AFB staining of specimens are both negative, and other lung diseases are excluded after differentiation, while meeting one or more of the following criteria: Patients with lesions on lung X-rays or CT scans and suspected clinical manifestations of pulmonary tuberculosis; The lung X-ray or CT shows lesions and the interferon- $\gamma$  release assay (IGRA) result is positive; Fibrobronchial examination reveals lesions in the trachea or bronchi; Antituberculosis therapy is effect.

As the control group, 72 patients had no history of tuberculosis, and the IGRA result was negative (non-tuberculosis group, Non-TB). The pulmonary lesions were excluded from tuberculosis, and no tuberculosis was diagnosed after follow-up.

## Methods

### Collection and preservation of BALF

After obtaining informed consent, BALF collection strictly adhered to technical guidelines provided by the European Respiratory Society (20, 21) and standard operating procedures. Alveolar lavage involved three lavages using sterile saline, each with a volume of 20–60 mL, totaling 100–300 mL. Fluid recovery was conducted at a negative pressure of -3.3 to -13.3 kPa after each lavage, achieving a recovery rate of 40–70%. The collected fluid was immediately centrifuged at 500 g for 15 min to pellet cellular debris. The cell-free supernatant was then carefully aliquoted into sterile frozen tubes pretreated with silicone oil, and immediately stored at -20°C. To prevent sample degradation, each sample underwent a single freeze-thaw cycle, with thawing performed overnight at 4°C before use.

### Determination of optimal sample dilution

Determine the optimal sample dilution using chessboard titration method. Select three samples with the highest content of acid fast bacilli (smear results from +++ to +++++), and pre-test four dilution ratios of 1:1, 1:5, 1:10, and 1:20. Determine the optimal dilution by calculating the absorbance ratio (P/N value) between the positive sample and the negative control. He

experimental results showed that the undiluted sample had the highest P/N value, so subsequent tests were conducted using the original liquid sample.

## ELISA detection of MPT64

The human *Mycobacterium tuberculosis* ELISA Kit (Camilo Biological Company, Nanjing, China) was used according to the manufacturer's instructions. The thawed BALF was centrifuged at 4°C for 10 min, and the supernatant was used for testing. After equilibrating the kit to room temperature, the freeze-dried human MPT64 standard was reconstituted with 1.0 mL of standard dilution solution to produce a stock solution. This stock solution was serially diluted to obtain concentrations of 100, 50, 25, 12.5, and 6.3%, followed by further dilutions to 3.1, 1.5, and 0%. Standard wells and sample wells were then loaded (100 µL per well), and the plate was sealed and incubated for 90 min at 37°C. After washing twice, 100 µL of biotinylated human MPT64 antibody working solution was added, the plate was resealed and incubated at 37°C for 60 min. The plate was washed three times, and 100 µL of enzyme conjugate working solution was added to all wells (except blank wells). The plate was resealed and incubated at 37°C in the dark for 30 min. Following five washes, 100 µL of TBM color development working solution was added; incubation at 37°C continued until a clear color gradient was observed in the standard wells, after which 100 µL of stop solution was added immediately with mixing. The optical density (OD) values were measured using a Perlong microplate reader (Beijing). The average OD value of duplicate wells was used to construct a standard curve, ensuring that the coefficient of variation (CV) between wells was less than 10%. The OD value of the blank wells was subtracted from each standard well's OD value.

## Conventional laboratory examinations

The collected bronchoalveolar lavage fluid was routinely sent to the hospital laboratory for AFB and Xpert MTB/RIF testing, and the results were reviewed by two experienced technicians. The grading of AFB smear positivity followed the standards provided by the Health Industry Standard of the People's Republic of China WS288–2017 (19), recorded as negative, (+), (++) and (+++). The Xpert MTB/RIF results were used to detect *MTB* and rifampicin resistance, with the minimum Ct value (threshold cycle number) of all detected probes recorded for each Xpert MTB/RIF test.  $15 \leq Ct \leq 18.9$  indicates a high detection of *MTB*,  $19 \leq Ct \leq 24.9$  indicates a moderate detection of *MTB*,  $25 \leq Ct \leq 28.9$  indicates a low detection of *MTB*, and  $29 \leq Ct \leq 32$  indicates a very low detection of *MTB*,  $Ct > 32$  indicators a negative.

## Statistical analysis

The standard curve was constructed and analyzed using ELISACalc. SPSS25 software was employed for statistical analysis. The relationship between the OD values and PTB status was evaluated using receiver operating characteristic (ROC) curve analysis. For tuberculosis diagnosis, the optimal ELISA cutoff value was determined by maximizing the Youden index. Spearman rank correlation coefficient to evaluate the correlation between two sets of non-parametric data. Categorical variables were compared using

the Pearson chi-square test or Fisher's exact test, with a significance threshold set at  $P < 0.05$ .

## Results

### Clinical data

The basic characteristics of the patients are summarized in Table 1. Ages ranged from 13 to 85 years. Males constituted 75.3% (123/176) of the sample, and cough was the most common symptom, observed in 69.3% (122/176) of cases. Among PTB patients, diabetes was the most common comorbidity, present in 11.5% (12/104) of cases.

### Correlation analysis of MPT64 concentration with Xpert MTB/RIF and AFB

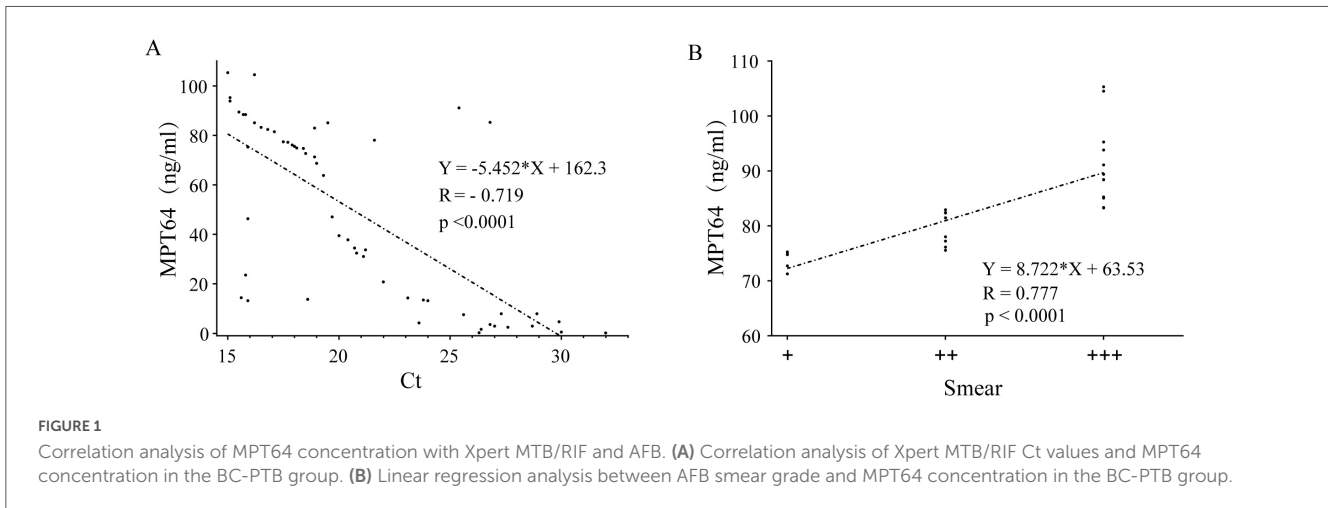
In the BC-PTB group, we observed a strong, statistically significant negative correlation between Xpert MTB/RIF Ct values and MPT64 concentration ( $R = -0.719$ ,  $P < 0.0001$ ) (Figure 1A), this indicates that lower Ct values are strongly associated with higher levels of the MPT64 antigen.

The linear regression reveals a strong, positive, and statistically highly significant correlation between the AFB smear grade and the MPT64 concentration ( $R = 0.777$ ,  $P < 0.0001$ ) (Figure 1B). This indicates that the higher the AFB smear grade, the higher the concentration of MPT64.

TABLE 1 Basic characteristics of participants ( $n = 176$ ).

	PTB ( $n = 104$ )	Non-TB ( $n = 72$ )
Age (median)	44 (13–78)	58.5 (16–85)
<b>Gender</b>		
Male (%)	75 (72.1)	48 (66.7)
Female (%)	29 (27.9)	24 (33.3)
Prior history of tuberculosis (%)	1 (1.0)	0
<b>Symptom</b>		
Cough (%)	68 (65.3)	54 (75)
Fever (%)	14 (13.4)	23 (31.9)
Marasmus (%)	19 (18.2)	11 (15.2)
Hot flash and night sweats (%)	27 (25.9)	5 (6.9)
Asymptomatic (%)	18 (17.3)	12 (16.6)
<b>Major comorbidities</b>		
Diabetes mellitus (%)	12 (11.5)	9 (12.5)
Hypertension (%)	2 (1.9)	9 (12.5)
HIV (%)	0	4 (5.6)
Lung tumor (%)	3 (2.9)	8 (11.1)

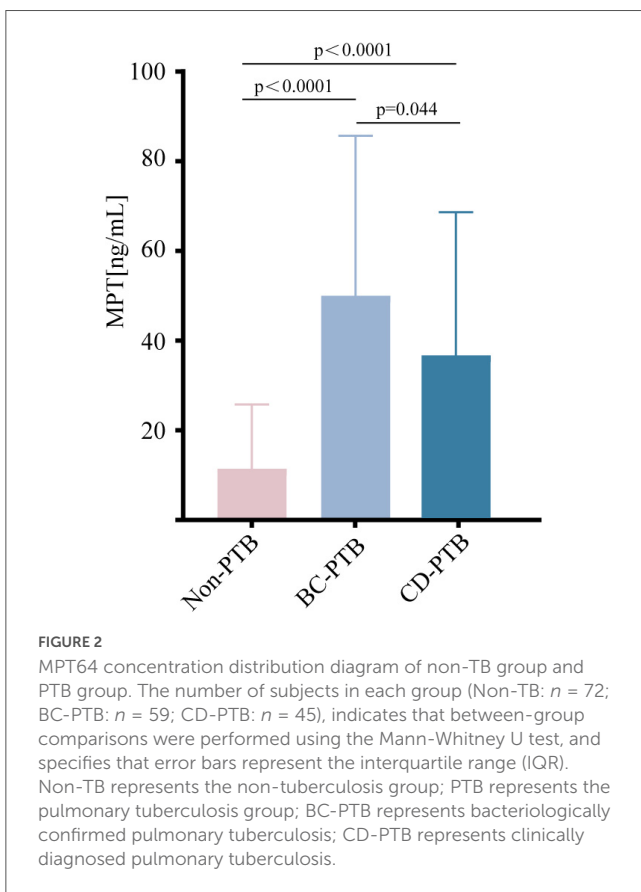
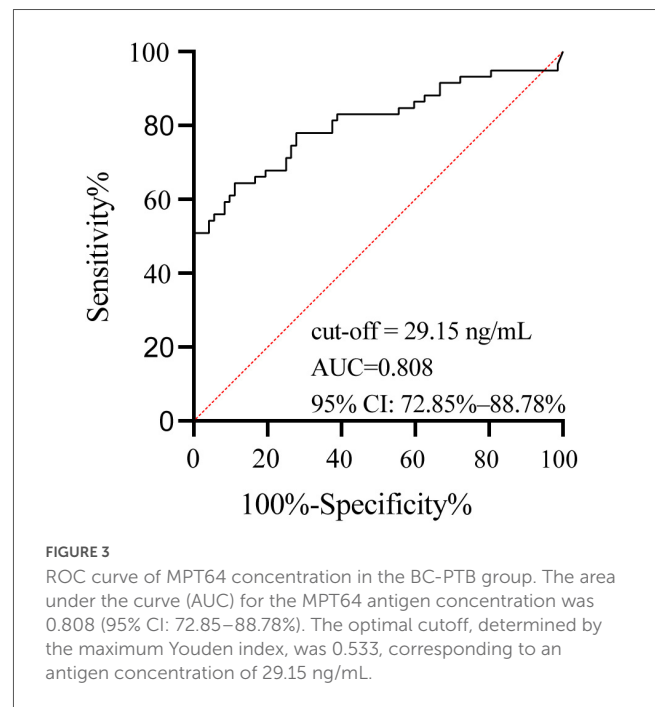
PTB, pulmonary tuberculosis group; Non-TB, non-tuberculosis group.



### BALF MPT64 concentrations

Based on the ELISA standard curve, the protein concentration in each sample was calculated. The analysis demonstrated that the concentration of MPT64 in the non-TB group was significantly lower than in the BC-PTB and CD-PTB groups ( $P < 0.0001$ ). However, MPT64 levels were higher in BC-PTB than CD-PTB groups ( $P = 0.044$ ) (Figure 2).

The ROC curve was constructed for the PTB group (Figure 3). The area under the curve (AUC) for the MPT64 antigen concentration was 0.808 (95% CI: 72.85–88.78%). The optimal



cutoff, determined by the maximum Youden index, was 0.533, corresponding to an antigen concentration of 29.15 ng/mL. This cutoff was then used to calculate the sensitivity and specificity of MPT64 detection in all tuberculosis groups (Table 2).

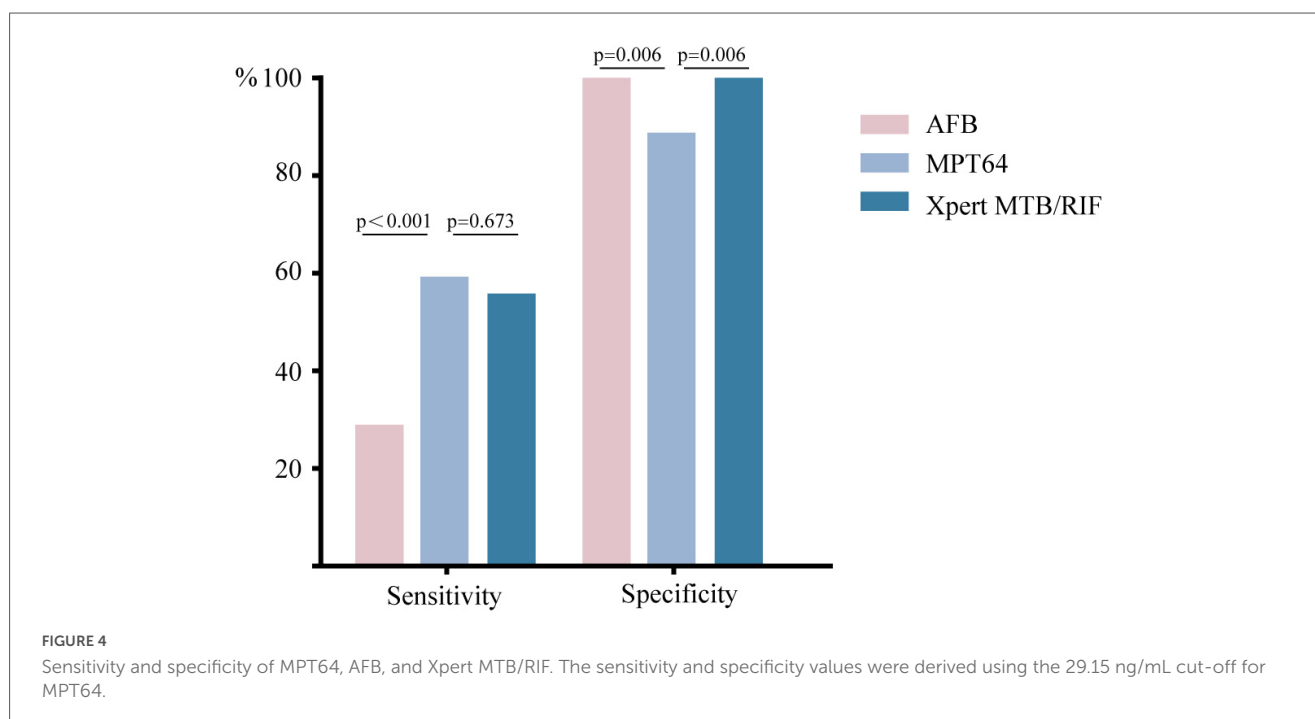
### Comparison of MPT64 with AFB staining and Xpert MTB/RIF

As shown in Table 2, Compared with AFB, the sensitivity of MPT64 detection increased by 32.7% ( $P < 0.001$ ), which was statistically significant. In comparison with Xpert MTB/RIF, the sensitivity increased by 2.89% ( $P = 0.673$ ), which was not statistically significant. In terms of specificity, MPT64 detection was 11.11% lower than that of both AFB and Xpert MTB/RIF, and this difference was statistically significant (Figure 4).

TABLE 2 Diagnostic value of MPT64, AFB, and Xpert MTB/RIF for tuberculosis (n = 176).

		PTB (n=104)	Non-TB (n=72)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AFB	+	28	0	26.92 (18.45–35.39)	100 (93.75–100)	100 (87.93–100)	48.65 (40.69–56.60)
	-	76	72				
Xpert MTB/RIF	+	59	0	56.73 (47.45–66.20)	100 (93.75–100)	100 (93.88–100)	61.54 (52.72–70.35)
	-	45	72				
MPT64	+	62	8	59.62 (50.20–69.13)	88.89 (79.32–96.14)	88.57 (79.04–94.10)	60.38 (51.07–69.69)
	-	42	64				

AFB, acid-fast staining; Xpert MTB/RIF, MTB complex rifampicin resistance gene detection; MPT64, tuberculosis specific antigen MPT64; PTB, pulmonary tuberculosis group; Non-TB, non-tuberculosis group; PPV, positive predictive value; NPV, negative predictive value.



### Diagnostic value of MPT64 in the BC-PTB and CD-PTB groups

Among the 59 BC-PTB cases, 38 tested positive for the MPT64 antigen, yielding a sensitivity of 64.41% (95% CI: 51.66–75.40%). In the 45 CD-PTB cases, 24 tested positive, resulting in a sensitivity of 53.33% (95% CI: 39.08–67.06%). The difference in sensitivity between these two groups was not statistically significant ( $P = 0.314$ ) (Table 3).

TABLE 3 Sensitivity of MPT64 in the BC-PTB and CD-PTB groups.

	MPT64 +	MPT64 -	Sensitivity (%)	P
BC-PTB (n=59)	38	21	64.41 (51.66–75.40%)	0.314
CD-PTB (n=45)	24	21	53.33 (39.08–67.06%)	

BC-PTB, bacteriologically confirmed PTB; CD-PTB, clinically diagnosed PTB.

### Discussion

To further elucidate the relationship between mycobacterial load and MPT64 antigen levels, we employed two clinically relevant surrogate measures: semi-quantitative AFB smear grading and Xpert MTB/RIF Ct values. Our analysis revealed that lower Ct values and higher smear grades were associated with a corresponding rise in MPT64 concentration. These strong correlations indicate that MPT64 levels directly reflect the mycobacterial burden in the lungs, validating its role as a biologically relevant marker and supporting its potential as a diagnostic tool for tuberculosis.

The results of this study demonstrate that the sensitivity of MPT64 detection in the PTB group (59.62%) is significantly higher than that of AFB staining and marginally higher than that of Xpert MTB/RIF. Although the specificity of MPT64 (88.89%) did not reach the 100% observed with AFB staining and Xpert MTB/RIF, its ability to reflect *MTB* activity via secretory properties offers unique

advantages. These findings suggest that MPT64 detection could serve as an effective supplementary tool for the etiological diagnosis of tuberculosis. Previous studies have reported sensitivities of 88.00–100% and specificities of 96.4–100% for MPT64 detection using ultrasensitive ELISA on MTB cultures (22–24). The lower sensitivity and specificity observed in this study may be attributed to the lower bacterial load in BALF compared to culture media and the presence of proteinaceous substances in BALF that may lead to false-positive results. Furthermore, differences in detection methods might also account for these discrepancies, suggesting that enhancements in detection techniques could further improve sensitivity.

Comparing the sensitivity of MPT64 in BC-PTB and CD-PTB groups, there was no statistically significant difference ( $P = 0.314$ ), suggesting that MPT64 antigen detection is less dependent on bacterial load than microbial methods, it has potential diagnostic value in the diagnosis of bacterial negative pulmonary tuberculosis. The complex interaction between *MTB* and the host, particularly regarding the mechanisms of bacterial protein secretion, remains only partially understood. Current research indicates that *MTB* employs a specialized secretory system to transfer specific proteins (24–26). These secreted proteins are usually smaller than the bacteria itself, and some of them can spread through the gaps or cells in the lung tissue to reach a distance from the bacterial body; the other part of the antigen may bind to the host's immune cells and be transported to other parts (27–29). Previous studies have shown that *MTB* antigens are more diffusely distributed in lung tissues than the bacteria themselves (30, 31), suggesting that antigen detection may be more sensitive than direct bacterial detection. This supports the potential of using MPT64 detection in BALF as an auxiliary diagnostic method, particularly for bacterial-negative pulmonary tuberculosis. Given its simplicity, rapidity, cost-effectiveness, and minimal infrastructure requirements, the ELISA-based detection of MPT64 could be implemented widely, including in primary care settings.

There are some limitations to this article, including the lack of a healthy control group, as bronchoscopy is an invasive procedure and routine examinations are not recommended for healthy individuals; In addition, this study is a single center study with a relatively small sample size. In the future, a multi-center study can be conducted to increase the sample size to verify the critical value and standardize the protocol.

Diabetes is a common complication, which may have a potential impact on our core findings. Because the sample size is too small, no additional grouping of diabetes patients is conducted in this study. We will collect a larger sample size in the future.

## Conclusion

ELISA-based detection of the *MTB*-specific antigen MPT64 in BALF shows promise as an important supplementary diagnostic method for pulmonary tuberculosis, particularly in cases of bacterial-negative tuberculosis.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

JS: Funding acquisition, Writing – review & editing, Data curation, Formal analysis, Writing – original draft, Methodology. WL: Writing – review & editing, Validation, Methodology, Software, Investigation. SF: Software, Writing – review & editing, Investigation, Validation. JJ: Formal analysis, Data curation, Resources, Writing – review & editing. FL: Supervision, Funding acquisition, Writing – review & editing.

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## Conflict of interest

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## References

- Sadovska D, Nodieva A, Pole I, ĩmsis J, Viksna A, Ozere I, et al. Advantages of analysing both pairwise SNV-distance and differing SNVs between *Mycobacterium tuberculosis* isolates for recurrent tuberculosis cause determination. *Microb Genom.* (2023) 9:000956. doi: 10.1099/mgen.0.000956
- Kohli M, Schiller I, Dendukuri N, Yao M, Dheda K, Denkinger CM, et al. Xpert MTB/RIF Ultra and Xpert MTB/RIF assays for extrapulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev.* (2021) 1:CD012768. doi: 10.1002/14651858.CD012768.pub3
- Lo CK, Purych D, Sekirov I, Khattra J, Hird TJ, Masud S. Evaluation of GeneXpert MTB/Rif Ultra assay performance on formalin-fixed paraffin-embedded tissues for *Mycobacterium tuberculosis* detection. *J Med Microbiol.* (2024) 73:001918. doi: 10.1099/jmm.0.001918
- Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme CC. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? *Eur Respir J.* (2016) 48:516–25. doi: 10.1183/13993003.00543-2016
- Adler H, Straub C, Frei R. Comparison of BacT/ALERT 3D, Lowenstein-Jensen medium and Middlebrook 7H10/7H11 biplate for recovering mycobacteria from clinical specimens. *Eur J Clin Microbiol.* (2005) 24:499–500. doi: 10.1007/s10096-005-1362-2
- Samra Z, Kaufman L, Bechor J, Bahar J. Comparative study of three culture systems for optimal recovery of mycobacteria from different clinical specimens. *Eur J Clin Microbiol.* (2000) 19:750–4. doi: 10.1007/s100960000369
- Naveen G, Peerapur BV. Comparison of the lowenstein-jensen medium, the middlebrook 7H10 medium and MB/BacT for the isolation of *Mycobacterium tuberculosis* (MTB) from clinical specimens. *J Clin Diagn Res.* (2012) 6:1704–9. doi: 10.7860/JCDR/2012/4603.2635
- Sheng G, Chu HQ, Liu DY, Sun ZG, et al. Progress in the identification of *Mycobacterium tuberculosis* antigenic proteins in clinical specimens. *Chin J Antituberc.* (2022) 44:1363–8. doi: 10.19982/j.issn.1000-6621.20220312
- Hasegawa N, Miura T, Ishii K, Yamaguchi K, Lindner TH, Merritt S, et al. New simple and rapid test for culture confirmation of *Mycobacterium tuberculosis* complex: a multicenter study. *J Clin Microbiol.* (2002) 40:908–12. doi: 10.1128/JCM.40.3.908-912.2002
- Harboe M, Nagai S, Patarroyo ME, Torres ML, Ramirez C, Cruz N. Properties of proteins MPB64, MPB70, and MPB80 of *Mycobacterium bovis* BCG. *Infect Immun.* (1986) 52:293–302. doi: 10.1128/iai.52.1.293-302.1986
- Wu X. Diagnosis for pulmonary tuberculosis WS 288-2017. *Electrom J Emerg Infect Dis.* (2018) 3:59–61. doi: 10.19983/j.issn.2096-8493.2024022
- Klech H, Pohl W. Technical recommendations and guidelines for bronchoalveolar lavage (BAL). Report of the European Society of Pneumology Task Group. *Eur Respir J.* (1989) 2:561–85. doi: 10.1183/09031936.93.02060561
- Jain P, Mehta A. Standardized guidelines for surveillance bronchoscopy reduce complications in lung transplant recipients. *J Bronchol.* (2004) 11:276. doi: 10.1097/00128594-200410000-00016
- Kim S, Can MH, Agizew TB, Auld AF, Balcels ME, Bjerrum S, et al. Factors associated with tuberculosis treatment initiation among bacteriologically negative individuals evaluated for tuberculosis: an individual patient data meta-analysis. *PLoS Med.* (2025) 22:e1004502. doi: 10.1371/journal.pmed.1004502
- Mase SR, Ramsay A, Ng V, Henry M, Hopewell PC, Cunningham J, et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *Int J Tuberc Lung Dis.* (2007) 11:485–95.
- Licata MA, Mencarini P, Mastrobattista A, Carli SM, Cerva C, Mosti S, et al. Clinical utility of induced sputum and bronchoalveolar lavage cultures in diagnosing nontuberculous mycobacterial pulmonary disease. *Pathogens.* (2024) 13:1064. doi: 10.3390/pathogens13121064
- Gupta E, Agarwal P, Gupta A, Prakash P, Dasgupta A. Comparison of induced sputum and bronchoalveolar lavage fluid examination in the diagnosis of sputum negative pulmonary Tuberculosis. *Indian J Chest Dis Allied Sci.* (2022) 58:173–5. doi: 10.5005/ijcdas-58-3-173
- Khalil KF, Butt T. Diagnostic yield of Bronchoalveolar Lavage gene Xpert in smear-negative and sputum-scarce pulmonary tuberculosis. *JCPSP-J Coll Physic.* (2015) 25:115–8.
- Armstrong DT, Pretty L, D'Agostino K, Redhead-Harper R, Parrish N. Diagnostic accuracy of the abbot SD bioline MPT64 antigen test for identification of MTB complex in a U.S. Clinical Mycobacteriology Laboratory. *Heliyon.* (2024) 10:e30501. doi: 10.1016/j.heliyon.2024.e30501
- Tadele A, Beyene D, Hussein J, Gemechu T, Birhanu A, Mustafa T, et al. Immunocytochemical detection of *Mycobacterium tuberculosis* complex specific antigen, MPT64, improves diagnosis of tuberculous lymphadenitis and Tuberculous pleuritis. *BMC Infect Dis.* (2014) 14:585. doi: 10.1186/s12879-014-0585-1
- Baba K, Dyrhol-Riise AM, Sviland L, Langeland N, Hoosen AA, Wiker HG, et al. Rapid and specific diagnosis of Tuberculous pleuritis with immunohistochemistry by detecting *Mycobacterium tuberculosis* complex specific antigen MPT64 in patients from a HIV endemic area. *Appl Immunohisto Mol Morphol.* (2008) 16:554–61. doi: 10.1097/PAL.0b013e31816c3f79
- Arora J, Kumar G, Verma AK, Bhalla M, Sarin R, Myneedu VP. Utility of MPT64 antigen detection for rapid confirmation of *Mycobacterium tuberculosis* complex. *J Glob Infect Dis.* (2015) 7:66–9. doi: 10.4103/0974-777X.154443
- Raheem TY, Ojo O, Adenipekun EO, Olalekan AO, Oluwadun A, Iwalokun BA. Performance assessment of SD bioline TB MPT64 assay for the diagnosis of *Mycobacterium tuberculosis* complex in Lagos, Nigeria. *J Immunoass Immunoch.* (2021) 42:543–58. doi: 10.1080/15321819.2021.1911812
- Orikiriza P, Nyehangane D, Atwine D, Kisakyee JJ, Kassaza K, Amumpaire JM, et al. Evaluation of the SD bioline TB Ag MPT64 test for identification of *Mycobacterium tuberculosis* complex from liquid cultures in Southwestern Uganda. *Afr J Lab Med.* (2017) 6:383. doi: 10.4102/ajlm.v6i2.383
- Mehra A, Zahra A, Thompson V, Sirisaengtaksin N, Wells A, Porto M, et al. *Mycobacterium tuberculosis* type VII secreted effector EsxH targets host ESCRT to impair trafficking. *PLoS Pathog.* (2013) 9:e1003734. doi: 10.1371/journal.ppat.1003734
- Zulauf KE, Sullivan JT, Braunstein M. The SecA2 pathway of *Mycobacterium tuberculosis* exports effectors that work in concert to arrest phagosome and autophagosome maturation. *PLoS Pathog.* (2018) 14:e1007011. doi: 10.1371/journal.ppat.1007011
- Stamm CE, Pasko BL, Chaisavaneeyakorn S, Franco LH, Nair VR, Weigle BA, et al. Screening *Mycobacterium tuberculosis* secreted proteins identifies Mpt64 as a eukaryotic membrane-binding bacterial effector. *mSphere.* (2019) 4:e00354-19. doi: 10.1128/mSphere.00354-19
- Housden NG, Webby MN, Lowe ED, El-Baba TJ, Kaminska R, Redfield C, et al. Toxin import through the antibiotic efflux channel TolC. *Nat Commun.* (2021) 12:4625. doi: 10.1038/s41467-021-24930-y
- Simeone R, Bobard A, Lippmann J, Bitter W, Majlessi L, Brosch R, et al. Phagosomal rupture by *Mycobacterium tuberculosis* results in toxicity and host cell death. *PLoS Pathog.* (2012) 8:e1002507. doi: 10.1371/journal.ppat.1002507
- Che N, Qu Y, Zhang C, Zhang L, Zhang H. Double staining of bacilli and antigen Ag85B improves the accuracy of the pathological diagnosis of pulmonary tuberculosis. *J Clin Pathol.* (2016) 69:600–6. doi: 10.1136/clinpath-2015-203244
- Purohit MR, Sviland L, Wiker H, Mustafa T. Rapid and specific diagnosis of extrapulmonary tuberculosis by immunostaining of tissues and aspirates with Anti-MPT64. *Appl Immunohistochem Mol Morphol.* (2017) 25:282–8. doi: 10.1097/PAI.0000000000000300