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Study on the Efficacy of High-Throughput Real-Time Mass Spectrometry Detection of Exhaled Breath for Rapid Diagnosis of Pulmonary Tuberculosis

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Abstract: *Objective:* To evaluate the clinical efficacy of high-throughput real-time mass spectrometry detection technology for exhaled breath in the rapid diagnosis of pulmonary tuberculosis (PTB), providing a novel technological support for early screening and diagnosis of PTB. *Methods:* A total of 120 PTB patients admitted to a hospital from January 2023 to June 2024 were selected as the case group, and 150 healthy individuals and patients with non-tuberculous pulmonary diseases during the same period were selected as the control group. Exhaled breath samples were collected from all study subjects, and the types and concentrations of volatile organic compounds (VOCs) in the samples were detected using a high-throughput real-time mass spectrometer. A diagnostic model was constructed using machine learning algorithms, and core indicators such as diagnostic sensitivity, specificity, and area under the curve (AUC) of this technology were analyzed and compared with the efficacy of traditional sputum smear examination, sputum culture, and GeneXpert MTB/RIF detection. *Results:* The diagnostic sensitivity of the high-throughput real-time mass spectrometry diagnostic model for exhaled breath in diagnosing PTB was 92.5%, the specificity was 94.0%, and the AUC was 0.978, which were significantly higher than those of sputum smear examination (sensitivity 58.3%, specificity 90.0%, AUC 0.741). Compared with GeneXpert technology, its specificity was comparable (94.0% vs 93.3%), and the detection time was shortened to less than 15 minutes. The model achieved an accuracy of 91.3% in distinguishing PTB from other pulmonary diseases and was not affected by demographic factors such as age and gender. *Conclusion:* High-throughput real-time mass spectrometry detection technology for exhaled breath has the advantages of being non-invasive, rapid, highly sensitive, and highly specific, and holds significant clinical application value in the rapid diagnosis and large-scale screening of PTB, warranting further promotion.

Keywords: Tuberculosis; Exhaled breath detection; High-throughput real-time mass spectrometry; Volatile organic compounds; Rapid diagnosis

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1. Introduction

Tuberculosis, a chronic respiratory infectious disease caused by *Mycobacterium tuberculosis* infection, has become the leading infectious cause of death globally. According to the World Health Organization (WHO) Global Tuberculosis Report 2024, there were 10.8 million new tuberculosis cases and 1.25 million deaths worldwide in 2023, with China accounting for 6.8% of the global case count, indicating a severe epidemic prevention and control situation [1]. Early and accurate diagnosis is crucial for controlling the spread of tuberculosis and improving patient prognosis. However, traditional diagnostic methods have significant limitations: sputum smear microscopy has a sensitivity of only 30–50%, leading to a high risk of missed diagnoses; sputum culture, considered the "gold standard", requires a detection period of 2–8 weeks, failing to meet the demand for rapid diagnosis; although the GeneXpert MTB/RIF technology can shorten detection time, it relies on specialized equipment and sputum samples, making it difficult to popularize in resource-limited areas and subject to a certain false-negative rate [2].

In recent years, exhaled breath detection technology has become a research hotspot in the field of infectious disease diagnosis due to its non-invasive and convenient characteristics. Volatile organic compounds (VOCs) in exhaled breath, serving as "biological fingerprints" of metabolic status, undergo characteristic changes in tuberculosis patients due to the metabolic activities of *Mycobacterium tuberculosis* and host immune responses. Existing studies have shown that exhaled breath detection technology based on gas chromatography-mass spectrometry (GC-MS) can achieve a diagnostic sensitivity for tuberculosis of 82.6–91.7%.

However, the complexity of the operation and long detection period of this technology limit its clinical application and promotion [3]. High-throughput real-time mass spectrometry technology, with its advantages of rapid detection and high resolution, exhibits tremendous potential in the diagnosis of respiratory diseases. This study aims to systematically evaluate the efficacy of exhaled breath high-throughput real-time mass spectrometry detection for the rapid diagnosis of tuberculosis and provide a novel diagnostic technology solution for clinical practice. The research results are now reported as follows.

2. Materials and methods

2.1. Study subjects

A total of 120 tuberculosis patients who sought treatment in the Respiratory Medicine and Infectious Diseases Departments of a tertiary grade-A hospital from January 2023 to June 2024 were selected as the case group. All patients met the criteria of the "WS 288-2017 Diagnosis of Pulmonary Tuberculosis". Among them, there were 85 newly-treated patients and 35 retreated patients; 68 males and 52 females; aged between 18 and 75 years old, with an average age of (42.3 ± 12.5) years [4].

During the same period, 150 cases were selected as the control group, including 80 healthy individuals undergoing physical examinations (with no history of pulmonary diseases and negative tuberculin skin tests) and 70 patients with non-tuberculous pulmonary diseases (including 25 cases of pneumonia, 20 cases of chronic obstructive pulmonary disease, 15 cases of bronchiectasis, and 10 cases of lung cancer); 82 males and 68 females; aged between 20 and 72 years old, with an average age of (40.8 ± 11.6) years.

Exclusion criteria include the patients with severe liver and kidney dysfunction, malignant tumors, or immune system diseases; those who had used anti-tuberculosis drugs or antibiotics within the past month; and those who were unable to cooperate with exhaled breath sample collection. This study was approved by the hospital's Ethics Committee (Ethics Approval Number: 2022-123), and all study subjects signed informed consent forms (refer **Table 1**).

Table 1. Comparison of baseline data of study subjects

Characteristic	Case group (n = 120)	Control group (n = 150)	Statistic	*p*-value
Gender (Male/Female, n)	68/52	82/68	$\chi^2 = 0.124$	0.724
Age (years, Mean ± SD)	42.3 ± 12.5	40.8 ± 11.6	$t = 0.987$	0.324
Smoking history (Yes/No, n)	45/75	50/100	$\chi^2 = 0.356$	0.550
Disease duration (months, Median [IQR])	3.5 (1.0—8.0)	-	-	-
Disease type (n)	-	Healthy: 80		
	-	Pneumonia: 25		
	-	COPD: 20		
	-	Bronchiectasis: 15		
	-	Lung Cancer: 10		

2.2. Main instruments and reagents

High-throughput real-time mass spectrometer for exhaled breath (Model: HRMS-2023, produced by a certain biotechnology company); GeneXpert MTB/RIF detector (produced by Cepheid, USA); *Mycobacterium tuberculosis* culture kit (produced by a biotechnology company in Hangzhou); disposable exhaled breath collection bags (made of inert materials, with a volume of 500 mL).

2.3. Sample collection and detection

2.3.1. Exhaled breath sample collection

After fasting for 12 hours, the study subjects rested for 15 minutes in a quiet environment. They were instructed to seal their mouths and noses with a disposable mask, take a deep breath, and then exhale slowly into the collection bag until approximately 300 mL of exhaled breath was collected. The bag was then sealed and immediately sent for testing. The detection was completed within 2 hours after sample collection.

2.3.2. Mass spectrometry detection process

Connect the collection bag to the sample inlet of the high-throughput real-time mass spectrometer, and set the sample injection temperature to 40°C, the ion source temperature to 200°C, the scanning range to m/z 20–350, and the scanning interval to 0.2.

The instrument automatically collects VOCs ion signals, performs data preprocessing through built-in software to remove background noise and interference signals, and extracts characteristic ion peaks.

2.3.3. Traditional detection methods

All patients in the case group and the suspected case control group simultaneously underwent sputum smear acid-fast staining, sputum *Mycobacterium* tuberculosis culture, and GeneXpert MTB/RIF testing. The operations were strictly conducted according to the reagent kit instructions and clinical laboratory standard procedures [5].

2.4. Data analysis

Data analysis was performed using SPSS 26.0 statistical software and Python 3.9. Measurement data conforming to a normal distribution were expressed as ($\bar{x} \pm s$), and comparisons between groups were made using the *t*-test.

Measurement data not conforming to a normal distribution were expressed as median (quartile), and comparisons between groups were made using the rank-sum test. Count data were expressed as cases (%), and comparisons between groups were made using the χ^2 test.

A diagnostic model for exhaled breath VOCs was constructed using the random forest algorithm, and the receiver operating characteristic (ROC) curve was plotted to calculate sensitivity, specificity, AUC, and the 95% confidence interval (95% CI). A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Analysis of exhaled breath VOCs characteristics

Through high-throughput real-time mass spectrometry detection, a total of 32 differentially expressed VOCs were identified. Among them, the concentrations of 18 VOCs, including ortho-cymene, naphthalene, and 1-methylcyclohexane, were significantly higher in the case group than in the control group.

While the concentrations of 14 VOCs, including cyclohexanone and nonanal, were significantly lower in the case group than in the control group ($p < 0.05$). Ten core characteristic VOCs (**Table 2**) were selected based on the random forest algorithm to construct a tuberculosis diagnostic model.

Table 2. Core characteristic VOCs for tuberculosis diagnosis

No.	VOC name	Molecular weight	Case group concentration (ng/L, $\bar{x} \pm s$)	Control group concentration (ng/L, $\bar{x} \pm s$)	Fold change	p-value
1	o-Cymene	134	45.6 ± 12.3	12.8 ± 5.6	3.57	< 0.001
2	Naphthalene	128	38.9 ± 10.5	10.2 ± 4.8	3.81	< 0.001
3	1-Methylcyclohexane	98	32.4 ± 9.6	8.5 ± 3.2	3.81	< 0.001
4	2-Butyl-1-octanol	172	28.7 ± 8.9	7.3 ± 2.9	3.93	< 0.001
5	Decane	142	25.3 ± 7.8	6.9 ± 2.5	3.67	< 0.001
6	Cyclohexanone	98	8.2 ± 3.1	25.6 ± 9.2	0.32	< 0.001
7	Nonanal	142	6.5 ± 2.8	22.4 ± 8.5	0.29	< 0.001
8	Toluene	92	18.7 ± 6.5	10.3 ± 4.2	1.82	0.002
9	p-Xylene	106	15.4 ± 5.8	8.6 ± 3.5	1.79	0.003
10	Butane*	72	12.8 ± 4.9	5.7 ± 2.1	2.25	0.001

3.2. Evaluation of diagnostic model efficacy

The diagnostic model based on high-throughput real-time mass spectrometry of exhaled breath demonstrated a sensitivity of 92.5% (95% CI: 87.2–96.1%) and a specificity of 94.0% (95% CI: 89.3–97.1%) for tuberculosis diagnosis, with an AUC of 0.978 (95% CI: 0.965–0.991).

In subgroup analysis, the model showed a sensitivity of 94.1% for newly diagnosed tuberculosis patients and 88.6% for retreated patients. The diagnostic accuracy was 95.2% for individuals under 30 years old and 91.8% for those aged 30 or older.

The sensitivity for female patients (93.8%) was slightly higher than that for male patients (91.5%), but the difference was not statistically significant ($p > 0.05$).

Table 3. Comparison of diagnostic efficacy of different diagnostic methods

Diagnostic method	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	Accuracy (%)	AUC (95% CI)	Turnaround time
Exhaled breath High-throughput Real-time mass spectrometry	92.5 (87.2–6.1)	94.0 (89.3–97.1)	93.3	0.978 (0.965–0.991)	< 15 min
Sputum smear microscopy	58.3 (50.1–66.5)	90.0 (84.5–94.1)	76.2	0.741 (0.698–0.784)	2–4 h
Sputum culture	80.0 (72.5–86.3)	100.0 (Reference)	91.5	0.900 (0.872–0.928)	2–8 h
GeneXpert MTB/RIF	89.2 (82.8–94.0)	93.3 (88.5–96.7)	91.6	0.945 (0.925–0.965)	2 h

4. Differential diagnostic efficacy for other pulmonary diseases

When distinguishing tuberculosis from non-tuberculous pulmonary diseases, the model achieved a diagnostic accuracy of 91.3%. Specifically, it showed a sensitivity of 90.5% and a specificity of 88.0% for pneumonia, a sensitivity of 92.0% and a specificity of 90.0% for chronic obstructive pulmonary disease, and a sensitivity of 89.0% and a specificity of 92.0% for lung cancer, with an AUC of 0.962 (95% CI: 0.948–0.976).

5. Discussion

Rapid and early diagnosis of tuberculosis is a critical component of global tuberculosis control efforts. The limitations of traditional diagnostic methods have significantly hindered the progress of these efforts. Exhaled breath analysis, as a non-invasive and convenient diagnostic technique, reflects disease status by analyzing characteristic changes in volatile organic compounds (VOCs) and has been applied in the diagnosis of various respiratory diseases [6]. The tuberculosis diagnostic model developed in this study, based on high-throughput real-time mass spectrometry technology, exhibited excellent diagnostic efficacy, providing a new technological pathway for rapid tuberculosis diagnosis.

This study identified 10 core characteristic volatile organic compounds (VOCs), among which ortho-cymene, reported for the first time as a specific biomarker for tuberculosis, exhibited significantly elevated concentrations in the case group. This finding is consistent with the latest research results published in the Nature sub-journal Scientific Reports. Ortho-cymene not only effectively distinguishes active tuberculosis from healthy individuals but also holds potential value in differentiating drug-resistant tuberculosis [7]. The differential expression of VOCs such as naphthalene and 1-methylcyclohexane has also been validated by previous studies. The Phillips team discovered high expression levels of these substances in *Mycobacterium tuberculosis* cultures using gas chromatography-mass spectrometry (GC-MS) technology, with a clinical sample detection sensitivity of 82.6% [8]. This study further confirmed the reliability of these VOCs as biomarkers for tuberculosis diagnosis, providing targets for the development of subsequent diagnostic kits.

In terms of diagnostic performance, the model constructed in this study outperformed traditional sputum smear examinations in terms of sensitivity, specificity, and area under the curve (AUC) values. Compared to the GeneXpert technology, the detection time was reduced from 2 hours to less than 15 minutes, and it did not rely on sputum samples, offering advantages for patients unable to produce sputum. Compared to the BreaTB breathomics model reported in Health World (with a sensitivity of 91.7% and specificity of 93.0%), the performance of our model was slightly improved, possibly due to the higher detection resolution and more precise feature ion

extraction capabilities of high-throughput real-time mass spectrometry technology [9]. Additionally, the model maintained stable diagnostic performance across patients of different ages, genders, and treatment stages, indicating its broad clinical applicability.

In terms of differential diagnosis, the model achieved a 91.3% accuracy rate in distinguishing tuberculosis from common pulmonary diseases such as pneumonia and chronic obstructive pulmonary disease, addressing the challenge of differentiating early lesions in traditional imaging diagnosis. This advantage stems from the ability of high-throughput real-time mass spectrometry technology to capture subtle differences in VOC profiles across different disease states. Compared to single biomarker detection, the multi-feature VOCs combination model exhibits stronger discriminatory power. Meanwhile, the non-invasive nature of this technology makes it more suitable for large-scale population screening, particularly in resource-limited areas, where it can effectively improve tuberculosis detection rates and reduce disease transmission risks [10].

This study has certain limitations. Firstly, it employed a single-center design with a relatively limited sample size, which may introduce selection bias. Subsequent multi-center, large-sample studies are needed to validate the external validity of the model. Secondly, no specific analysis was conducted on patients with drug-resistant tuberculosis, and the differences in core volatile organic compounds (VOCs) between infections caused by drug-resistant and sensitive strains warrant further exploration. Finally, the high cost of detection instruments restricts their immediate adoption in primary healthcare facilities. In the future, it is necessary to optimize technical solutions and develop portable detection devices.

6. Conclusion

The high-throughput real-time mass spectrometry detection technology for exhaled breath enables rapid and accurate diagnosis of tuberculosis by analyzing characteristic VOCs spectra. It offers significant advantages, including high sensitivity, high specificity, and non-invasiveness, and holds substantial clinical value in early diagnosis, large-scale screening, and differential diagnosis of tuberculosis [11]. The widespread adoption of this technology is expected to overcome the limitations of traditional diagnostic methods and provide new technical support for global tuberculosis prevention and control [12]. Subsequent research should focus on multi-center validation, differentiation of drug-resistant tuberculosis, and miniaturization of detection devices to further expand its clinical applications.

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Disclosure statement

The authors declare no conflict of interest.

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