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Clinical Value of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration Specimens Combined with DNA Methylation Detection in the Diagnosis of Lung Cancer

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Abstract: *Objective*: To explore the application value of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) specimens combined with DNA methylation detection in the clinical diagnosis of lung cancer, compare the diagnostic efficacy of single detection methods, and provide objective evidence for early and accurate diagnosis of lung cancer. *Methods*: A total of 120 patients with suspected pulmonary malignancies admitted to our hospital from December 2024 to December 2025 were selected. All patients underwent EBUS-TBNA to obtain biopsy specimens, and routine pathological cytological examination, individual detection of SHOX2 and RASSF1A gene methylation, and combined detection of both methods were performed. Using postoperative pathology or long-term follow-up results as the gold standard, the sensitivity, specificity, accuracy, positive predictive value, negative predictive value, and area under the ROC curve (AUC) of each detection method were calculated, and diagnostic differences were compared. *Results*: Among the 120 patients, 76 cases (63.33%) were diagnosed with lung cancer and 44 cases (36.67%) with benign lesions according to the gold standard. The sensitivity, specificity, and accuracy of routine cytological examination by EBUS-TBNA were 72.37%, 84.09%, and 76.67%, respectively; for individual DNA methylation detection, they were 78.95%, 86.36%, and 81.67%, respectively; for combined detection, they were 93.42%, 95.45%, and 94.17%, respectively. All diagnostic indicators of combined detection were significantly superior to those of single detection methods ($P < 0.05$). The area under the ROC curve for combined detection was 0.980, significantly higher than that for individual cytological detection (0.908) and individual methylation detection (0.934). *Conclusion*: EBUS-TBNA specimens combined with DNA methylation detection can significantly improve the sensitivity and accuracy of lung cancer diagnosis, overcome the limitations of routine cytological examination, and are particularly suitable for difficult cases such as mediastinal lymphadenopathy and peripheral pulmonary nodules, demonstrating high clinical promotion value.

Keywords: Endobronchial ultrasound-guided transbronchial needle aspiration; DNA methylation; Lung cancer; Diagnostic efficacy; SHOX2 gene; RASSF1A gene

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1. Introduction

Lung cancer is one of the most common malignant tumors with a high global incidence and mortality rate. In China, due to the increasing trend of its incidence population year by year and the relatively concealed early symptoms, many patients are diagnosed at an advanced stage, missing the best opportunity for surgical treatment, with a 5-year survival rate below 20% [1]. Early and accurate diagnosis is the core link in improving the prognosis and survival rate of lung cancer patients. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), as a minimally invasive interventional diagnostic technique, can accurately obtain specimens from mediastinal lymph nodes, hilar, and peripheral lung lesions, and is currently a commonly used method for lung cancer staging and pathological diagnosis [2]. However, in clinical practice, due to factors such as insufficient specimen collection, atypical cell morphology, and differences in the experience of pathologists in interpreting slides, conventional cytological examination has a certain rate of missed diagnoses, especially for lesions with low differentiation and atypical hyperplasia, and the diagnostic sensitivity needs to be improved. DNA methylation, as a key epigenetic alteration occurring early in tumorigenesis, exhibits highly specific hypermethylation expression during the development of lung cancer. Among them, SHOX2 (Short Stature Homeobox 2) and RASSF1A (Ras Association Domain Family 1A) are currently recognized as specific methylation markers for lung cancer diagnosis, and their promoter region hypermethylation can serve as a molecular indicator for early lung cancer diagnosis, not strictly limited by cell morphology or specimen collection volume, and can compensate for the deficiencies of conventional pathological examination [3,4]. Therefore, this study selected 120 patients with suspected lung malignancies and analyzed the efficacy of conventional cytological examination combined with DNA methylation detection in the early diagnosis of lung cancer based on biopsy specimens obtained by EBUS-TBNA, laying the foundation for further improving minimally invasive diagnosis and treatment plans for lung cancer.

2. Materials and methods

2.1. General information

A total of 120 patients with suspected lung malignancies admitted to the Department of Respiratory and Critical Care Medicine at our hospital from December 2024 to December 2025 were selected. Inclusion criteria: (1) Chest CT indicated pulmonary space-occupying lesions and mediastinal lymphadenopathy (short diameter ≥ 1.0 cm), with a high clinical suspicion of malignancy; (2) No contraindications to bronchoscopy, able to tolerate EBUS-TBNA; (3) Patients and their families provided informed consent and signed the informed consent form; (4) Complete clinical data, with postoperative pathology or follow-up for more than 12 months allowing for a definitive final diagnosis. Exclusion criteria: (1) History of other malignancies; (2) Severe heart, lung, liver, or renal failure; (3) Coagulopathy; (4) Recent receipt of radiotherapy, chemotherapy, or targeted therapy; (5) Inability to complete testing due to unqualified specimen collection.

Among the 120 patients, there were 72 males and 48 females; aged 42 to 78 years old, with an average age of (61.25 ± 7.36) years old; lesion locations: 56 cases of central lung cancer, 42 cases of peripheral lung cancer, and 22 cases of isolated mediastinal lymphadenopathy; 78 cases with a smoking history and 42 cases without a smoking history.

2.2. Methods

2.2.1. EBUS-TBNA specimen collection

All patients were operated on by experienced respiratory interventional physicians using an EBUS-530T ultrasonic bronchoscope. Preoperative complete blood count, coagulation function, and electrocardiogram examinations were performed. Local anesthesia combined with intravenous sedation was used. The target lymph nodes or pulmonary lesions were located by ultrasound, and a 22-G ultrasonic dedicated puncture needle was used for needle aspiration biopsy. Each target site was punctured 2-3 times. The obtained specimens were divided into two portions: one portion was immediately sent to the pathology department for conventional cytological smear, HE staining, and immunohistochemical examination; the other portion was placed in a sterile EDTA anticoagulant tube and stored at -80°C for DNA methylation detection to avoid specimen degradation.

2.2.2. DNA methylation detection

The methylation status of the SHOX2 and RASSF1A genes in the specimens was detected using methylation-specific PCR (MS-PCR) according to the reagent kit instructions. The steps were as follows: (1) DNA extraction: Genomic DNA was extracted from the biopsy specimens using a DNA extraction kit, and the DNA concentration and purity were detected to ensure sample qualification; (2) Bisulfite conversion: The extracted DNA was modified with bisulfite to convert unmethylated cytosine to uracil, while methylated cytosine remained unchanged; (3) PCR amplification: A PCR reaction system was configured, and methylated and unmethylated primers were set up for gene amplification; (4) Result interpretation: The amplification products were subjected to agarose gel electrophoresis, and the presence of specific methylated bands was judged as methylation-positive. Either positive result was judged as DNA methylation-positive.

2.2.3. Diagnostic grouping and gold standard

All patients underwent conventional cytological examination by EBUS-TBNA, DNA methylation detection alone, and the combination of the two. The combined detection criterion: Either method being positive was judged as combined detection-positive, and both being negative was judged as combined detection-negative. The postoperative surgical pathology results, puncture tissue pathology results, or clinical follow-up for more than 12 months (imaging review, clinical symptom resolution) were used as the diagnostic gold standard, dividing patients into a lung cancer group and a benign lung lesion group. Benign lesions included inflammatory pseudotumors, tuberculosis, lymph node hyperplasia, and chronic inflammation.

2.3. Observation indicators

Using the gold standard as a reference, the diagnostic efficacy indicators of the three detection methods were calculated: sensitivity, specificity, accuracy, positive predictive value, and negative predictive value. The calculation formulas were as follows:

(1) Sensitivity = Number of true positives / Total number of lung cancer cases × 100%;

(2) Specificity = Number of true negatives / Total number of benign lesions × 100%;

(3) Accuracy = (Number of true positives + Number of true negatives) / Total number of cases × 100%;

(4) Positive predictive value = Number of true positives / (Number of true positives + Number of false positives) × 100%;

(5) Negative predictive value = Number of true negatives / (Number of true negatives + Number of false

negatives) × 100%.

An ROC curve was drawn to clarify the assignment rules for the test variable and the state variable, and the area under the curve (AUC) and 95% confidence interval (CI) were calculated. The χ^2 test was used to compare the differences in AUC among different detection methods to judge their overall diagnostic value. The closer the AUC is to 1, the higher the diagnostic value.

2.4. Statistical methods

Data analysis was performed using SPSS 26.0 statistical software. Count data were expressed as the number of cases (%), and comparisons between groups were made using the χ^2 test. Measurement data were expressed as mean ± standard deviation (SD). ROC curve analysis was used to evaluate diagnostic efficacy. $P < 0.05$ was considered statistically significant, and all data were retained to two decimal places.

3. Results

3.1. Final diagnostic results

Among the 120 suspected patients confirmed by the gold standard, 76 cases (63.33%) were diagnosed with lung cancer, including 68 cases of non-small cell lung cancer (42 cases of adenocarcinoma, 22 cases of squamous cell carcinoma, and 4 cases of large cell carcinoma), and 8 cases of small cell lung cancer; 44 cases (36.67%) were diagnosed with benign lung lesions, including 12 cases of inflammatory pseudotumor of the lung, 10 cases of tuberculosis, 16 cases of reactive hyperplasia of mediastinal lymph nodes, and 6 cases of chronic bronchitis.

3.2. Comparison of diagnostic results among different detection methods

For EBUS-TBNA conventional cytology examination: there were 55 true positive cases, 7 false positive cases, 37 true negative cases, and 21 false negative cases; for DNA methylation testing alone: there were 60 true positive cases, 6 false positive cases, 38 true negative cases, and 16 false negative cases; for combined testing: there were 71 true positive cases, 2 false positive cases, 42 true negative cases, and 5 false negative cases. Specific diagnostic data are shown in **Table 1**.

Table 1. Diagnostic results of lung cancer by different detection methods

Detection Method	True Positive (Cases)	False Positive (Cases)	True Negative (Cases)	False Negative (Cases)
Conventional Cytology Examination	55	7	37	21
DNA Methylation Detection	60	6	38	16
Combined Detection	71	2	42	5

3.3. Comparison of diagnostic efficacy among different detection methods

The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of the combined detection method were all significantly higher than those of conventional cytological examination and DNA methylation detection alone ($P < 0.001$). Specific diagnostic efficacy indicators are presented in **Table 2**.

Table 2. Comparison of diagnostic efficacy indicators among different detection methods

Detection Method	Sensitivity(%)	Specificity(%)	Accuracy(%)	Positive Predictive Value(%)	Negative Predictive Value(%)
Routine Cytology Examination	72.37	84.09	76.67	88.71	63.79
DNA Methylation Test	78.95	86.36	81.67	90.91	70.37
Combined Test	93.42	95.45	94.17	97.26	89.36

3.4. ROC curve analysis

The area under the curve (AUC) for conventional cytological examination was 0.908 (95% confidence interval [CI]: 0.854–0.962), for DNA methylation testing it was 0.934 (95% CI: 0.888–0.981), and for the combined detection method it was 0.980 (95% CI: 0.954–1.000). The AUC for the combined detection method was significantly higher than that for the single detection methods, with statistically significant differences ($\chi^2_1 = 82.589$, $\chi^2_2 = 94.429$, both $P < 0.001$), indicating that the combined detection method offers the best overall diagnostic value. See **Figure 1**.

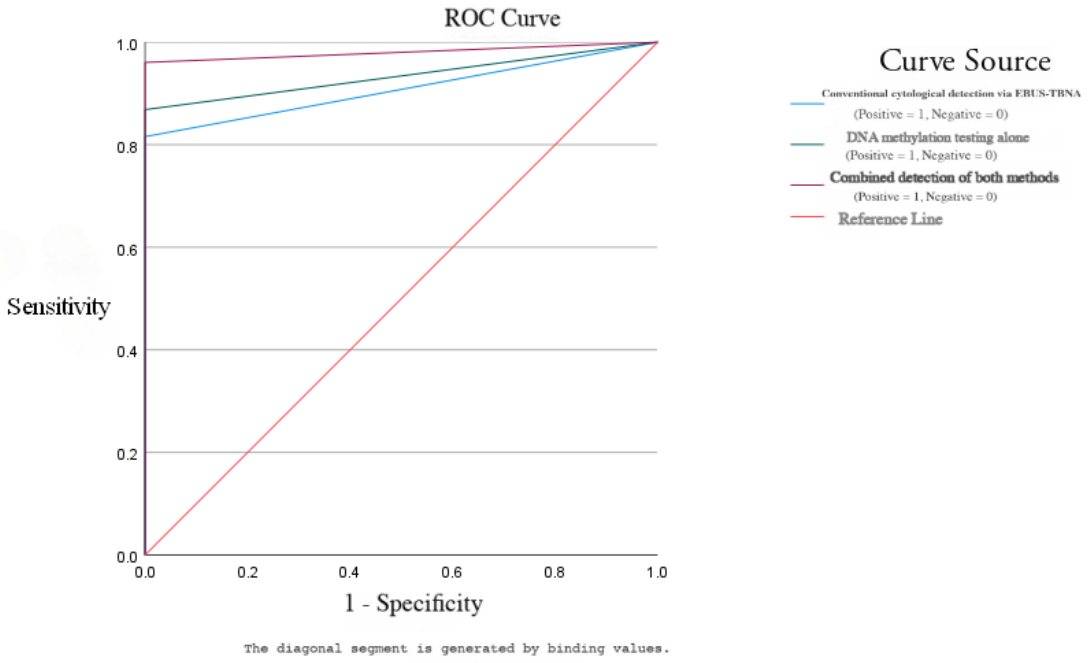

Figure 1. Schematic representation of ROC curves for different detection methods.

4. Discussion

In the early diagnosis of lung cancer, the EBUS-TBNA technique offers advantages such as no need for thoracotomy, minimal trauma, and high safety. It enables precise localization of lesions under ultrasound guidance, allowing for accurate acquisition of mediastinal lymph nodes and pulmonary lesions, thereby avoiding unnecessary risks associated with blind puncture. It is currently the preferred minimally invasive method for the diagnosis, treatment, and staging of lung cancer [5]. However, traditional cytological examination of EBUS-TBNA specimens presents several issues, such as a small sample size, dispersed tumor cells, atypical cell morphology, or the

presence of necrosis or inflammatory cell infiltration, which can easily lead to misdiagnosis or missed diagnosis by pathologists [6]. In this study, the sensitivity of conventional cytological examination was only 72.37%, with a relatively high missed diagnosis rate, consistent with previous research findings, indicating that relying solely on conventional cytological examination cannot meet the demands of precise clinical diagnosis.

DNA methylation, as an important biological behavior, offers advantages over traditional pathological detection methods, including high specificity, high detection sensitivity, and low specimen requirements. It can detect specific methylation changes in tumor cell specimens with minimal content, thereby effectively compensating for the deficiencies of traditional cytological detection methods. SHOX2 and RASSF1A represent the most lung cancer-specific methylation gene combination. SHOX2 gene hypermethylation is primarily observed in lung adenocarcinoma and squamous cell carcinoma, while RASSF1A gene hypermethylation is closely associated with lung cancer cell proliferation and invasion. Combined detection of these two genes can cover the majority of lung cancer pathological types and enhance the sensitivity of molecular diagnosis [7,8]. In this study, the sensitivity of DNA methylation testing alone was 78.95%, higher than that of conventional cytological examination, further confirming the advantages of methylation testing in the molecular diagnosis of lung cancer.

This study innovatively employed EBUS-TBNA specimens combined with DNA methylation testing. The results showed that the sensitivity of the combined detection method was significantly higher than that of single detection methods, with a false-negative rate of only 6.58%, substantially reducing the risk of missed diagnosis. Meanwhile, the specificity reached 95.45%, reducing false-positive misdiagnosis and avoiding overtreatment. ROC curve analysis revealed an AUC of 0.980 for the combined detection method, approaching the ideal diagnostic level, indicating that the combination of the two methods can achieve complementary advantages. Conventional cytological examination judges the nature of lesions from a cellular morphology perspective, while DNA methylation testing identifies tumor-specific changes at the molecular level [9]. Their combination can comprehensively improve the precise diagnosis of lung cancer, particularly for challenging cases that are difficult to diagnose using conventional cytological examination, such as those with small specimens, low differentiation, and atypical hyperplasia. Furthermore, EBUS-TBNA specimens can complete pathological examination and DNA methylation testing without the need for multiple puncture acquisitions, reducing patient pain and shortening diagnosis time, aligning with the clinical concept of minimally invasive and efficient diagnosis [10].

This study had a sample size of 120 cases, which still has certain limitations: it was a single-center study with a relatively limited sample size, and subsequent multi-center, large-sample studies could be conducted for further validation. Additionally, stratified analysis based on different pathological subtypes and clinical stages of lung cancer was not performed, and future research could delve into the diagnostic differences of combined detection in different subtypes of lung cancer.

5. Conclusion

In summary, the combination of bronchoscopic ultrasound-guided needle aspiration biopsy specimens with SHOX2 and RASSF1A gene methylation testing can significantly improve the sensitivity, specificity, and accuracy of lung cancer diagnosis, effectively compensating for the deficiencies of conventional cytological examination in missed diagnosis. It represents a minimally invasive, precise, and efficient lung cancer diagnosis scheme suitable for clinical promotion and application, particularly for patients with suspected lung cancer who cannot be definitively diagnosed through conventional pathological examination, providing a reliable basis for early clinical

diagnosis and treatment plan formulation.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] Fan H, Deng S, Wang L, et al., 2025, Nrf2 Serves as a Biomarker and Therapeutic Target in Lung Cancer Based on Bibliometric Analysis and Molecular Mechanisms. Discover Oncology, 17(1): 171.

[2] Yang Y, Zhang Z, Liu P, et al., 2026, The Application Value of Endobronchial Ultrasound-Guided Transbronchial Mediastinal Cryobiopsy in the Diagnosis of Pulmonary Sarcoidosis: A Retrospective Comparative Study with Needle Aspiration Biopsy. Journal of Xuzhou Medical University, 46(1): 57–61.

[3] Ruan H, Wu D, Zhu C, et al., 2026, Detection of SHOX2 and RASSF1A Gene Methylation Assists in the Early Diagnosis of Lung Cancer in Three Cases. Chinese Journal of Clinical Laboratory Science, 44(1): 64–66.

[4] Cao R, Wang B, Lai X, et al., 2025, Correlation Study Between Traditional Chinese Medicine Susceptibility Constitution of Small Pulmonary Nodules and Methylation of SHOX2/RASSF1A/PTGER4 Genes. Chinese Journal of Information on Traditional Chinese Medicine, 32(8): 146–152.

[5] Cen L, Sui B, 2025, Research Progress of Fiberoptic Bronchoscopy in the Diagnosis, Staging, and Treatment of Lung Cancer. Chinese Journal of Medical Physics, 42(11): 1500–1506.

[6] Pan D, Ying J, Wu Q, et al., 2019, Comparative Application of Conventional Exfoliative Cytology and Liquid-Based Cytology with Bronchial Brushings in the Pathological Diagnosis of Lung Cancer. Zhejiang Practical Medicine, 24(2): 86–89.

[7] Qi Q, Su H, Hu J, et al., 2025, Diagnostic Value of Serum SHOX2, RASSF1A, and PTGER4 Methylation Levels for Benign and Malignant Solitary Pulmonary Nodules and Pathological Classification. Journal of Diagnostic Pathology, 32(8): 1010–1015.

[8] Wang M, Mukandais T, Nurziba A, et al., 2024, Predictive Value of a Nomogram Model Constructed by Combining SHOX2, RASSF1A, and CEA for the Occurrence of Lung Cancer. Journal of Xinjiang Medical University, 47(7): 996–1000 + 1007.

[9] Liu Y, 2023, DNA Methylation Testing Aids in Early Tumor Screening and Diagnosis. Theory and Practice of Diagnostics, 22(4): 393–401.

[10] He S, Tian J, Long L, et al., 2024, Lung Cancer Screening and Early Diagnosis: Progress from Imaging to Biomarkers. Chinese Journal of Cancer Prevention and Treatment, 16(1): 120–126.