Clinical Study Protocol

Clinical Utility of Needle-Based Confocal Laser Endomicroscopy for Peripheral Pulmonary Lesions

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1.Title

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2. Background

Peripheral pulmonary lesions (PPLs) are focal pulmonary opacities detected on imaging that cannot be directly visualized with conventional flexible bronchoscopy.[1-2] With the widespread adoption of low-dose computed tomography (LDCT) for lung cancer screening, the detection of pulmonary nodules has increased substantially,[3] and more than 70% of these lesions are PPLs.[4] Some PPLs have malignant potential: malignant tumors account for approximately 18% of lesions measuring 0.8-2 cm and approximately 50% of lesions larger than 2 cm.[5] The National Lung Screening Trial (NLST) demonstrated that early detection of PPLs can reduce lung cancer-specific mortality by approximately 20%.[6] Accurate early diagnosis of PPLs therefore has clear clinical value.

Current diagnostic approaches for PPLs include bronchoscopy with tissue sampling and computed tomography-guided transthoracic needle aspiration (CT-TTNA). The diagnostic yield of conventional bronchoscopy is limited and strongly affected by lesion size: approximately 14% for PPLs measuring 2 cm or less and approximately 31% for lesions larger than 2 cm.[7] Although the diagnostic yield of CT-TTNA may exceed 50%, the procedure carries greater risks.[8-11] Pneumothorax may occur in up to 45% of patients, and approximately 15% may require chest tube drainage.[12] Radial probe endobronchial ultrasound (r-EBUS)-guided bronchoscopy has a diagnostic yield of approximately 69%-73% for PPLs,[13-14] which is substantially higher than that of conventional transbronchial lung biopsy.[15] However, the r-EBUS probe must be removed before biopsy, preventing real-time confirmation of the biopsy instrument position and creating a risk of off-target sampling. Navigation bronchoscopy, including virtual, electromagnetic, and robotic navigation, can guide biopsy instruments to PPLs in real time and may improve diagnostic yield, particularly for lesions without a bronchus sign or those that are difficult to reach. Its accuracy may nevertheless be affected by intraprocedural lung displacement, and the technology involves high equipment and consumable costs, a substantial learning curve, and procedural risks.[16-17] Video-assisted thoracoscopic surgery (VATS) offers very high diagnostic accuracy for PPLs (>95%), but requires general anesthesia and hospitalization, places greater demands on cardiopulmonary reserve, and is considerably more costly than transbronchial biopsy.[18] A minimally invasive, precise, safe, and diagnostically effective approach is therefore needed.

Confocal laser endomicroscopy (CLE) is an emerging optical biopsy technology.[19] When integrated with endoscopic platforms, CLE provides high-resolution, real-time, in vivo imaging of living tissue and can reveal cellular and subcellular morphology. By

depicting microscopic features such as glandular architecture, cellular morphology, and vascular networks, it enables immediate in vivo assessment and may facilitate early diagnosis of respiratory disease. Several clinical studies have demonstrated the potential value of CLE in evaluating PPLs. In 2017, a case study first applied needle-based confocal laser endomicroscopy (nCLE) to real-time in vivo imaging of peripheral pulmonary nodules. nCLE clearly distinguished the microscopic features of pulmonary adenocarcinoma and tuberculosis and supported lesion localization and targeted biopsy.[20] A study of 20 patients with suspected peripheral pulmonary nodules used robotic bronchoscopy combined with nCLE; nCLE helped guide needle placement and optimize the sampling site, with an overall diagnostic yield of 80%.[21] In a prospective study of 26 patients with suspected peripheral lung cancer, nCLE-guided needle biopsy was performed after r-EBUS localization. nCLE achieved 95% accuracy for detecting malignant airway or pulmonary parenchymal lesions, with good interobserver agreement (kappa = 0.78).[22] Another prospective study enrolled 15 patients with PPLs suspicious for malignancy and introduced cryoprobes and nCLE probes in parallel after r-EBUS localization. Simultaneously guided cryobiopsy was successfully completed in 11 patients without serious complications; 91% of nCLE images were of good quality, and the tool-in-lesion signal agreed with histopathology in 82% of cases.[23]

This study will integrate nCLE with r-EBUS, with navigation bronchoscopy used when required, for the evaluation of PPLs. r-EBUS and the available navigation platform will first be used for macroscopic lesion localization and route planning. nCLE will then provide real-time, high-resolution in vivo cellular imaging at the intended biopsy site to support lesion characterization and localization of an optimal sampling position. Using histopathology as the principal reference standard, supplemented by 6-month clinical follow-up when initial pathology is benign or nondiagnostic, the study will evaluate the diagnostic performance of nCLE for distinguishing malignant from benign PPLs and assess its feasibility and safety.

3. Study Objective

The primary objective is to evaluate the diagnostic performance and clinical utility of nCLE combined with r-EBUS for PPLs during bronchoscopy, with navigation used as needed. The study will focus on the feasibility, accuracy, and safety of nCLE for distinguishing malignant from benign PPLs, using histopathology as the principal reference standard and follow-up confirmation when appropriate.

4. Study Design

4.1. Type of study design

This is a prospective, multicenter, observational study.

4.2. Sample size

This is an exploratory diagnostic study. A previous study[24] reported an accuracy of 91.7% and sensitivity of 88.1% for nCLE identification of granulomatous lesions, and an accuracy of 75.0% and sensitivity of 93.8% for nCLE identification of malignancy. On this basis, with a two-sided significance level of 0.05 and power of 80%, the required number of histopathologically confirmed PPLs was estimated using a diagnostic study sample-size formula. At least 90 patients with histopathologically confirmed PPLs are required, including no fewer than 30 patients in each of the following categories: granulomatous lesions, malignant lesions, and benign non-granulomatous lesions. Allowing for an estimated 10% attrition, procedural failure, or nonevaluable imaging rate, the planned total enrollment is 100 patients. This sample size is intended to support the primary objective of estimating the diagnostic performance of nCLE for etiologic classification of PPLs and to provide data for exploratory subgroup analyses.

Site	**Site Principal Investigator**	**Telephone**	**Planned enrollment**
The First Affiliated Hospital of Ningbo University	Zhongbo Chen	13777125910	34
Shanghai Chest Hospital	Jiayuan Sun	18017321598	33
Anhui Chest Hospital	Fei Tang	13505696936	33

4.3. Rationale for indications

All participants will meet the routine clinical indications for bronchoscopy and biopsy. Study procedures will follow authoritative domestic and international guidance, including the Chinese Technical Specifications for Diagnostic Bronchoscopy (2021 Edition) and the British Thoracic Society Guideline for Diagnostic Flexible Bronchoscopy in Adults.

5. Study Procedures

(1). Assess whether the patient meets the study eligibility criteria.

(2). Before study participation, the investigator will explain the study, including its potential risks and benefits, to the patient or his/her authorized representative in clear and understandable language. The patient or authorized representative and the investigator will sign and date the informed consent form.

(3). Record demographic information.

(4). Record intra-procedural data and capture images for subsequent color-difference analysis and visibility scoring.

(5). Follow up for adverse reactions and collect pathological results after the procedure.

6. Participant Selection

6.1. Inclusion criteria

(1) Age 18 years or older.

(2) A peripheral pulmonary lesion identified on chest CT.

(3) Bronchoscopic biopsy considered clinically necessary by the treating physician to establish a pathologic diagnosis.

(4) Willingness to undergo r-EBUS and nCLE examinations.

(5) Voluntary participation with written informed consent.

6.2. Exclusion criteria

(1) A contraindication to bronchoscopy, including severe cardiopulmonary disease, coagulation disorder, failure to discontinue anticoagulant therapy or anticoagulant medication for at least 5 days before the procedure, poor tolerance of anesthesia, psychiatric illness, or severe neurosis.

(2) Pregnancy or breastfeeding.

(3) Known allergy to fluorescein sodium.

(4) Coagulation disorder.

(5) A known allergic disorder, history of asthma, hepatic failure, renal failure, or another contraindication to fluorescein sodium injection.

(6) Continued use of a systemic beta-blocker on the day of the procedure, or inability to discontinue the beta-blocker safely as assessed by the investigator and anesthesiologist.

(7) Inability to cooperate with the study for any reason, or any other condition that the investigator considers unsuitable for enrollment.

6.3. Criteria for study discontinuation

(1) Participants may discontinue study participation at any time without prejudice. If. a participant discontinues the study, the reason will be recorded in the electronic case report form (eCRF).

(2) The sponsor or regulatory authority terminates the study.

7. Study Methods and Technical Route

7.1. Preoperative preparation

At admission, a detailed medical history will be obtained. Evaluations will include a complete blood count, liver and renal function tests, serum electrolytes, coagulation testing, arterial blood gas analysis, hepatitis B serology, syphilis and HIV antibody testing, electrocardiography, chest CT, and other clinically indicated examinations.

7.2. Patient preparation

The purpose, risks, and possible complications of the examination will be explained in detail, and written informed consent will be obtained. The general procedure will be described before bronchoscopy to reduce anxiety, facilitate stable blood pressure and heart rate, and support cooperation. Participants will fast from food and fluids for 4 hours before the procedure. Intravenous access will be established, and energy-containing intravenous fluids may be administered when clinically necessary until completion of the procedure.

7.3. Procedure

All bronchoscopic biopsies will be performed by chief physicians or equivalently senior bronchoscopists with more than 10 years of experience at the respiratory endoscopy center of each participating institution.

7.3.1 Preprocedural Preparation

(1) Anesthesia: The participant will be placed supine, and heart rate, blood pressure, oxygen saturation, and other vital signs will be monitored. After adequate intravenous anesthesia administered by an anesthesiologist, an endotracheal tube or laryngeal mask airway will be placed.

(2) Hypersensitivity test: Before the examination, 1 mL of 2% fluorescein sodium solution will be administered slowly by intravenous injection, followed by observation for 15-20 minutes. Subsequent procedures will proceed only if no hypersensitivity reaction, such as nausea, vomiting, or urticaria, occurs.

(3) CT data acquisition and navigation route planning: When navigation is planned, preprocedural high-resolution chest CT images in DICOM format will be imported into an available navigation bronchoscopy system, such as LungPoint, Archimedes, or Monarch. Lobar segmentation, three-dimensional reconstruction of the bronchial tree, and target lesion marking will be performed. The system will plan an optimal biopsy route from the main bronchus to the target and generate a virtual navigation view for intraprocedural reference.

7.3.2 Intraprocedural Navigation and Combined Imaging

The participant will undergo routine bronchoscopy under general anesthesia or deep sedation. When a navigation platform is used, the virtual navigation view will be registered with the bronchoscopic image, and the bronchoscope and guide sheath will be advanced along the planned route to the vicinity of the target lesion. An r-EBUS probe will be introduced through the guide sheath to determine the spatial relationship between the probe and the lesion. Localization will be considered successful only when the ultrasound image clearly shows a concentric position, with the probe inside the lesion, or an eccentric position, with the probe at the lesion margin. After recording the ultrasound features, the guide-sheath depth will be marked and the r-EBUS probe removed. If the lesion cannot be clearly visualized by r-EBUS or localization is

unsuccessful, the localization failure will be recorded, nCLE imaging will not be performed, and the case will be excluded from subsequent nCLE diagnostic analyses, thereby minimizing the influence of localization failure on image quality.

An nCLE probe will then be introduced through the same guide sheath. After an intravenous bolus of 200 mg fluorescein sodium diluted in 8 mL normal saline, the probe will be placed in gentle contact with the lesion. A 1.4-mm probe will be preferred. If the 1.4-mm probe cannot pass because of an acute target-bronchus angle or marked curvature of the working channel or guide sheath, a 0.8-mm probe may be used. Repeated forceful advancement against resistance is prohibited. Laser scanning will begin when the nCLE probe reaches the intended position. If the image indicates that the probe is outside the lesion or in contact with tissue lacking diagnostically meaningful structure, such as a blurred, structureless fluorescent signal, the guide sheath will be adjusted by slow advancement or withdrawal or by gentle, small-angle rotation until diagnostically meaningful cellular features are obtained, such as inflammatory-cell infiltration or irregularly arranged atypical cells. The guide-sheath depth will then be fixed, the nCLE features recorded, and the location marked so that tissue sampling corresponds to the imaged site.

After nCLE imaging, the probe will be removed and tissue sampling performed sequentially through the same guide sheath. Sampling may include brushing, in which a cytology brush is advanced through the guide sheath and moved back and forth over the lesion surface before smear preparation; cryobiopsy, in which a 1.1-mm cryoprobe is introduced and activated for 3-6 seconds before rapid withdrawal with the specimen; and supplemental forceps biopsy when necessary. All specimens will undergo histopathologic assessment. Histopathology will serve as the principal reference standard.

7.3.3 Follow-up and Confirmation of the Reference Diagnosis

(1) Definitive diagnosis group: If the procedures establish a diagnosis of malignancy, study follow-up for diagnostic confirmation will end and the final pathologic diagnosis will be recorded.

(2) Benign or nondiagnostic group: If pathology suggests a benign lesion or does not establish a diagnosis, the participant will undergo 6 months of clinical follow-up. Changes in the lesion after treatment will be assessed dynamically, including improvement, stability, or progression. If the lesion continues to enlarge or develops features suspicious for malignancy, surgical resection will be recommended, and postoperative histopathology will be used as the final reference diagnosis.

7.3.4 Postprocedural Care

After the examination, the participant will be observed in the recovery area for at least 30 minutes. Vital signs and adverse reactions such as nausea, vomiting, and rash will be monitored. Participants will be encouraged to drink water to facilitate urinary

elimination of fluorescein sodium. Any allergic or other serious adverse reaction will be treated promptly according to the emergency response plan. After return to the ward, electrocardiographic monitoring will continue for 4 hours. Chest radiography will be performed in any participant who develops chest tightness, dyspnea, or reduced oxygen saturation within 12 hours after the procedure to assess for pneumothorax or other complications.

7.3.5 Histopathologic Examination

Histopathology is the principal reference standard. All specimens will be fixed in 10% formalin, submitted to the pathology department, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined histopathologically. Two experienced pulmonary pathologists, each with at least an intermediate professional title and more than 5 years of pulmonary pathology experience, will interpret the slides independently while blinded to the nCLE diagnosis. If their diagnoses agree, that diagnosis will be recorded. If they disagree, a third senior pathologist will review the slides and establish the final diagnosis.

7.3.6 Data Collection

Collected data will include demographics (age and sex); past and current medical history, including allergic disease and asthma; past and current treatment; clinical symptoms and lifestyle factors; physical examination findings; laboratory and imaging results; nCLE findings; lesion characteristics, including location and size; procedure-related information; postprocedural complications; medication treatment and adherence assessment; and adverse event and serious adverse event records.

8. Research endpoints

8.1. Primary endpoint

The primary outcome is the diagnostic performance of nCLE for distinguishing malignant from benign PPLs. Performance measures will include sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy.

An nCLE image will be considered suggestive of lung cancer when dark cellular aggregates are observed together with disruption or loss of the basement membrane. A granulomatous pattern will be defined as a uniform, brightly fluorescent compact aggregate surrounded by small granular inflammatory cells. An infectious pattern will be defined by sharply demarcated, granular hyperfluorescent punctate material within alveolar spaces together with large hyperfluorescent alveolar macrophages. For the primary binary analysis using the final reference diagnosis, nCLE images showing dark cellular aggregates, a malignant feature, will be classified as positive. Images showing a uniform bright lesion accompanied by inflammatory cells, a granulomatous feature,

or granular hyperfluorescent material accompanied by macrophages, an infectious feature, will be classified as negative.

Operators at each center may record a real-time interpretation and diagnostic impression. De-identified original videos and images must also be uploaded for independent central review and final nCLE interpretation.

8.2. Secondary endpoints

Secondary outcomes are safety and procedural feasibility measures, including but not limited to the incidence and severity of procedure-related complications and procedure duration.

9. Efficacy Evaluation Criteria

Not applicable.

10. Observation of Adverse Events

If any adverse event or serious adverse event occurs, treatment will be adjusted in accordance with routine clinical care, and the participant's involvement in the study will be discontinued when clinically appropriate.

10.1. Common intra-procedural and postoperative adverse reactions and risks of bronchoscopy

(1) Bronchospasm or an asthma attack.

(2) Airway mucosal injury and bleeding.

(3) Cardiac arrhythmia.

(4) Postprocedural chills or fever.

(5) Pneumothorax or bleeding.

(6) Allergic reaction to anesthetic agents or fluorescein sodium.

(7) Failure to establish the diagnosis, including disagreement between the biopsy pathology report and the clinical impression.

10.2. Risk prevention

(1) Complete routine preprocedural assessment and carefully exclude contraindications.

(2) Perform the procedure gently and in accordance with technical standards.

(3) Provide appropriate management according to the nature and severity of any adverse reaction or complication.

11. Quality Control and Quality Assurance

Bronchoscopy will be performed by well-trained physicians with more than 10 years of bronchoscopy experience. The physician responsible for the participant's inpatient care will observe and record symptoms before and after the procedure. Designated members

of the research team will be responsible for data oversight, data management, and statistical analysis.

12. Statistical Analysis

All statistical analyses will be performed using R version 4.4.1. Categorical variables will be reported as counts and percentages. Continuous variables will be assessed for normality using the Shapiro-Wilk test. Normally distributed variables will be summarized as mean and standard deviation, and nonnormally distributed variables as median and interquartile range. For paired data, such as paired assessments of nCLE image quality, a paired-samples t test will be used after normality has been confirmed.

nCLE interpretations will be compared with the final reference diagnosis and classified as true positive (TP: nCLE positive and pathology/reference diagnosis positive), false negative (FN: nCLE negative but pathology/reference diagnosis positive), false positive (FP: nCLE positive but pathology/reference diagnosis negative), or true negative (TN: nCLE negative and pathology/reference diagnosis negative). A 2 x 2 table will be constructed, and sensitivity, specificity, PPV, NPV, and accuracy with 95% confidence intervals will be estimated. Interobserver agreement for nCLE interpretation will be assessed using Cohen's kappa, with kappa >= 0.75 indicating good agreement. The significance level will be alpha = 0.05, and a two-sided $P < 0.05$ will be considered statistically significant.

13. Ethical Considerations

The study will be conducted in accordance with the World Medical Association Declaration of Helsinki and other applicable requirements. The clinical study will begin only after the protocol has been approved by the ethics committee. Participant privacy and data confidentiality will be protected throughout the study.

14. Study Duration

October 2026 to October 2027.

15. References

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