The First Affiliated Hospital of Ningbo University

Clinical Study Protocol

Red Dichromatic Imaging for Improving Post-Biopsy Bronchoscopic Field Visibility

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1.Title

Red Dichromatic Imaging for Improving Post-Biopsy Bronchoscopic Field Visibility

2. Background

Bronchoscopic biopsy is a key method for obtaining pathological specimens from abnormal endobronchial lesions. It is commonly used for sampling and assessment of endobronchial neoplasms, mucosal abnormalities, airway stenosis, and other airway lesions of unclear etiology. [1] Whether the underlying lesion is malignant tumor, inflammatory disease, granulation tissue proliferation, or another benign condition, post-biopsy local bleeding and blood coverage may compromise visualization of the procedural field. Although most bleeding is mild, in the immediate post-biopsy setting local blood coverage can reduce the visual distinction among the biopsy wound, the lesion surface, and the surrounding mucosa, thereby affecting subsequent sampling localization and procedural continuity. [2, 3]

Current management of bleeding after bronchoscopic biopsy mainly includes local vasoactive medications and, when necessary, balloon tamponade. These approaches can control obvious active bleeding in most circumstances, but their ability to improve procedural field clarity remains limited. In scenarios where additional tissue sampling or further intervention is still required, residual blood, adherent clots, or persistent oozing from a vascular lesion or fragile wound surface can make it difficult to maintain a clear view. [4, 5]

Red dichromatic imaging (RDI) is an optical image-enhancement technology recently introduced by Olympus. Its principle is based on multispectral light of relatively longer wavelengths, mainly including green light at 520–550 nm, amber light at 595–610 nm, and red light at 620–640 nm. [6] By exploiting differences in hemoglobin absorption and scattering at different wavelengths, RDI enhances luminance and chromatic contrast and thereby improves the visual distinction among areas with different degrees of bleeding. [7, 8]

Since its introduction, RDI has been widely used in gastrointestinal endoscopy, particularly for intra-procedural bleeding management and hemostasis visualization. Previous studies have shown that RDI can significantly improve visibility after bleeding during endoscopic procedures, assisting operators in more rapidly identifying bleeding sources and vascular courses and thereby improving procedural safety.

However, systematic application and evaluation of RDI in respiratory endoscopy remain limited. Therefore, this study focuses on the immediate procedural field after the first bronchoscopic biopsy and before any field-clearing or hemostatic intervention. Using a multicenter, prospective, observational design, this study will compare the proportion of procedural fields judged suitable for direct continuation of subsequent biopsy or operation under RDI versus white-light imaging (WLI). The

study will also compare the objective composite color difference and subjective visual discriminability score between the post-biopsy bleeding area and the surrounding blood-covered background under the two imaging modes, and will explore the potential value of RDI in subsequent sampling localization, procedural continuity, and safety.

3. Objective

The objective of this study is to evaluate whether RDI, compared with WLI, can improve visualization of the procedural field after the first bronchoscopic biopsy and increase the proportion of cases in which the operator or image evaluator judges that subsequent biopsy or operation can be continued directly. By combining subjective visibility scoring, objective composite color difference, and safety outcomes, this study will further assess the potential value of RDI for subsequent sampling localization and procedure.

4. Study Design

4.1. Type of study design

This is a multicenter, prospective, observational study.

4.2. Sample size

The primary endpoint is whether the procedural field after the first biopsy is judged suitable for direct continuation of the next biopsy or operation under different imaging modes. This endpoint is a paired binary variable measured in the same patient under WLI and RDI; therefore, the sample size is estimated using the McNemar test.

A preliminary single-center study included 50 patients. In that cohort, 21 patients were judged suitable for direct continuation under WLI and 31 under RDI. Ten patients were judged to require field improvement under WLI but suitable for direct continuation under RDI, whereas no discordant cases in the opposite direction were observed.

Considering that the preliminary study was a small single-center study and that differences among centers, operators, and image-acquisition conditions in a multicenter study may reduce the observed effect, this study uses a conservative effect assumption for sample size estimation. Assuming $p10 = 0.15$ and $p01 = 0.05$, corresponding to an approximate 10% net decision improvement with RDI compared with WLI, and setting a two-sided alpha of 0.05 and power of 90%, the sample size was estimated using PASS software. Allowing for approximately 20% of cases with unacceptable image quality, incomplete WLI/RDI paired analysis, or missing data, approximately 206 patients will be enrolled.

Site	Site Principal Investigator	Telephone	Planned enrollment
The First Affiliated Hospital of Ningbo University	Zhongbo Chen	13777125910	36
Shanghai Chest Hospital	Ying Li	15821959854	36
Anhui Chest Hospital	Fei Tang	13505696936	36
Huzhou Central Hospital	Jiayou Luo	13757071890	28
The Second Hospital of Jiaxing	Na Li	18858334316	28
Zhoushan Hospital	Haifeng Li	13666589830	21
Lanxi Hospital of Traditional Chinese Medicine	Xin Shi	15356893371	21

4.3. Rationale for indications

All participants will meet the routine clinical indications for bronchoscopy and bronchoscopic biopsy. Study procedures will be conducted in accordance with relevant domestic and international guidelines, including the Chinese Technical Specification 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. Study Procedures

6.1. Inclusion criteria

a. Age ≥18 years.

b. Visible abnormal endobronchial lesion under bronchoscopy, including endobronchial neoplasm, mucosal abnormality, airway stenosis, or other lesion requiring biopsy to determine its nature.

c. Provision of informed consent to participate in this clinical study and signing of the informed consent form.

6.2. Exclusion criteria

a. Presence of contraindications to bronchoscopy, such as severe cardiopulmonary disease, coagulation dysfunction (including patients receiving anticoagulant therapy and/or anticoagulant drugs who have not discontinued these drugs for more than 5 days before the examination), poor tolerance of anesthesia, psychiatric disease, or severe neurosis.

b. Intra-procedural decrease in SpO_2 or inability to tolerate bronchoscopy.

c. Obvious airway bleeding observed during bronchoscopy that may increase the risk of worsening the patient's condition.

d. Images that are obviously blurred or out of focus, or that contain severe reflection, bubbles, or instrument obstruction, making subsequent image analysis impossible.

e. Inability to cooperate with the study for any reason, or any other condition that the investigator considers unsuitable for study participation.

6.3. Criteria for study discontinuation

a. 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).

b. The sponsor or regulatory authority terminates the study.

7. Study Methods and Technical Route

7.1. Preoperative preparation

At admission, the patient's medical history will be carefully obtained. Routine preoperative assessments will be completed, including complete blood count, liver function, renal function, electrolytes, coagulation function, blood gas analysis, hepatitis B serology, syphilis and HIV antibody testing, electrocardiography, and chest CT, as clinically indicated.

7.2. Patient preparation

The purpose, risks, and possible complications of the procedure will be explained to the patient in detail, and informed consent will be obtained. The general procedure will also be introduced before the examination to reduce anxiety, help stabilize blood pressure and heart rate, and facilitate cooperation. The patient will fast from food and water for 4 hours before the procedure. An intravenous line will be established, and energy-containing fluids may be supplemented as needed until the end of the examination.

7.3. Procedure

All bronchoscopic biopsies will be performed by respiratory endoscopists with substantial experience in bronchoscopic biopsy at each participating center, and, when necessary, under the guidance of senior physicians. The patient will be placed in the supine position, and heart rate, blood pressure, oxygen saturation, and other vital signs will be monitored. After adequate intravenous anesthesia by an anesthesiologist, endotracheal intubation or laryngeal mask airway placement will be performed.

A comprehensive examination of the bronchial lumen will first be performed under WLI. AFI may be used, where available, to assist identification of abnormal regions. For visible endobronchial neoplasms, mucosal abnormalities, airway stenosis, or other lesions requiring biopsy, the operator will initially determine the target lesion and assessment range. After the target lesion is identified, high-resolution screenshots under WLI and RDI should be obtained; AFI images may be additionally captured according to actual conditions. During image acquisition, the target lesion should be placed as close to the center of the field as possible, while retaining sufficient surrounding bronchial mucosal background.

Before the first biopsy, 1–2 mL of diluted epinephrine solution should in principle be sprayed locally onto the target lesion (1 mg epinephrine added to 10 mL normal saline). If epinephrine is not used because of the patient’s condition, lesion characteristics, or the operator’s safety judgment, the specific reason should be recorded. When the biopsy forceps are about to sample the lesion, a high-resolution screenshot should be captured to record the relative spatial relationship among the forceps, the target lesion, and surrounding airway structures, which will serve as a reference for localization of analytical regions during subsequent image analysis.

The first biopsy will be completed under WLI, and a standard tissue sample will be obtained. Immediately after completion of the first biopsy, before suction, irrigation, additional local medication, or any other field-clearing or hemostatic intervention, images will be obtained under both WLI and RDI. The two imaging modes should maintain, as far as possible, similar observation distance, angle, and field of view, and the images should be captured consecutively.

After the first biopsy, two independent investigators will judge whether the procedural field under WLI and RDI is suitable for direct continuation of subsequent biopsy or operation. If both WLI and RDI are judged not suitable for direct continuation, the operator will perform one field-clearing or hemostatic intervention as clinically needed. Such interventions may include suction, irrigation combined with suction, cold saline, local epinephrine, or other necessary measures. After this single intervention and before the next biopsy or further treatment, additional WLI and RDI images will be collected, and the specific intervention and whether the field under

each imaging mode has recovered sufficiently for continued operation will be recorded.

7.4. Postoperative observation

Routine electrocardiographic monitoring will be performed for 4 hours after the procedure. For any patient with suspected chest tightness, dyspnea, or decreased oxygen saturation within 12 hours after the procedure, a chest X-ray will be performed to evaluate pneumothorax and other complications.

8. Research endpoints

8.1. Primary endpoint

The primary endpoint is the comparison of the proportion of cases in which the procedural field after the first biopsy is judged suitable for direct continuation of biopsy or operation under WLI versus RDI.

Direct continuation of subsequent biopsy or operation is defined as a procedural field in which, after the first biopsy and without suction, irrigation, additional local hemostatic medication, or any other field processing, the biopsy wound, lesion surface, or lesion boundary remains identifiable, and the operator or image evaluator judges that the next biopsy or subsequent operation can be continued based on that field.

Not suitable for direct continuation is defined as inability to identify the biopsy wound, lesion surface, or lesion boundary because of blood coverage, clot, active bleeding, or blurred visualization, requiring prior suction, irrigation, or hemostatic treatment.

8.2. Secondary endpoints

8.2.1 Objective color difference between the post-biopsy bleeding area and the surrounding blood-covered area under RDI and WLI. Color difference will be quantified using the CIE 1976 L*a*b* color space (CIELAB). High-resolution static frames with matched composition under RDI and WLI will be selected, with a unified resolution of 1080p and consistent illumination as far as possible.

For each case, three regions of interest (ROIs) will be selected in the post-biopsy bleeding area and in the surrounding blood-covered abnormal mucosal lesion/background area. Each ROI will be a 10 × 10 pixel matrix. ROI selection will be performed using the sampling tool/ROI Manager in ImageJ (NIH, USA). The mean L*, a*, and b* values of each ROI will be recorded. Differences between the bleeding area and the surrounding abnormal mucosal lesion/background area will be calculated as ΔL, Δa, and Δb, and the total color difference ΔE will be calculated according to the CIELAB definition:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

8.2.2 Difference in visual discriminability score between the post-biopsy bleeding area and the surrounding blood-covered area after the first biopsy:

1. The two areas are almost indistinguishable.
2. The two areas have limited distinction and can only be barely distinguished after careful observation.
3. The two areas are basically distinguishable and can be clearly differentiated after observation.
4. The two areas are clearly distinguishable and easy to identify.

8.2.3 Exploratory analysis of color-difference measures and clinical decisions in predefined subgroups.

8.2.4 Exploratory analysis, among cases judged not suitable for direct continuation under both WLI and RDI, of whether the procedural field becomes suitable for continued biopsy or operation under WLI and RDI after one field-clearing or hemostatic intervention.

9. Efficacy Evaluation Criteria

Not applicable.

10. Observation of Adverse Events

If any adverse event or serious adverse event occurs, the treatment plan will be adjusted according to relevant clinical routines, and the patient's participation in the study will naturally be discontinued when appropriate.

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

1. Bronchospasm or asthma attack.
2. Airway mucosal injury and bleeding.
3. Arrhythmia.
4. Postoperative chills or fever.
5. Pneumothorax and bleeding.
6. Allergy to anesthetic drugs.
7. Failure to achieve the diagnostic purpose, such as a biopsy pathology report inconsistent with the clinically suspected condition.

10.2. Risk prevention

1. Routine examinations should be performed before the procedure and contraindications carefully excluded.
2. The procedure should be standardized and performed gently.
3. If adverse reactions or complications occur, the study physician will provide appropriate management according to the specific situation.

11. Quality Control and Quality Assurance

Bronchoscopy will be performed by well-trained physicians participating in this study with more than 10 years of experience in bronchoscopy. The physician responsible for the patient's inpatient care will observe and record symptoms before and after treatment. Study team members designated by the research team will be responsible for data supervision, management, and statistical analysis.

12. Statistical Analysis

All statistical analyses will be performed using IBM SPSS Statistics 27 (SPSS Inc., USA). Categorical variables will be presented as counts and percentages. Continuous variables will be tested for normality using the Shapiro–Wilk test. Normally distributed variables will be presented as mean ± standard deviation, and non-normally distributed variables will be presented as median and interquartile range.

The primary endpoint is paired binary data and will be compared using the McNemar test. For secondary endpoints, objective composite color difference ΔE and color-channel differences under WLI and RDI will be compared using a paired t-test or Wilcoxon signed-rank test according to the distribution of paired differences. Subjective visibility score is ordinal and will be compared using the Wilcoxon signed-rank test. Reliability of objective color-difference measures will be assessed using the intraclass correlation coefficient (ICC), and reliability of subjective scoring will be assessed using weighted kappa. All statistical tests will be two-sided, and $P < 0.05$ will be considered statistically significant.

13. Ethical Considerations

This clinical study will be conducted in accordance with the Declaration of Helsinki of the World Medical Association and other applicable regulations. Before study initiation, the study protocol must be approved by the ethics committee. During the study, participant privacy and data confidentiality will be protected.

14. Study Duration

July 2026 to December 2026.

15. References

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