STUDY PROTOCOL

WITH

STATISTICAL ANALYSIS PLAN

Official Title:

Deep Learning-Based Intraoperative Dual-Tracer Video Analysis of Sentinel Lymph Node Mapping for Metastasis Prediction in Clinically Node-Negative Papillary Thyroid Carcinoma: A Prospective Cohort Study

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1. PROTOCOL SUMMARY

Study Title	Deep Learning-Based Intraoperative Dual-Tracer Video Analysis of Sentinel Lymph Node Mapping for Metastasis Prediction in cN0 Papillary Thyroid Carcinoma
Study Type	Prospective Observational Cohort Study
Registry Number	NCT06871956
Study Population	Adults (≥18 years) with clinically node-negative papillary thyroid carcinoma (cN0-PTC)
Sample Size	336 participants (112 per group × 3 groups)
Study Groups	1) ICG Group, 2) CNs Group, 3) ICG+CNs (Dual-Tracer) Group
Primary Outcomes	SLN detection rate, SLNM status, SeLNM, NsLNM
Study Duration	April 2024 - October 2024 (enrollment); 12 months follow-up
Study Sites	The First Affiliated Hospital of Chongqing Medical University, Chongqing, China
Principal Investigator	Professor Xinliang Su, MD, PhD

2. INTRODUCTION AND BACKGROUND

2.1 Disease Background

Papillary thyroid carcinoma (PTC) is among the fastest-growing endocrine malignancies worldwide. Despite excellent overall prognosis, lymph node metastasis (LNM) remains a central clinical challenge. Even in patients classified as clinically node-negative (cN0) by routine preoperative ultrasonography and cross-sectional imaging, 30% to 80% may harbor occult LNM that is not detected before surgery.

This staging gap creates a significant clinical dilemma: extensive lymph node dissection may increase morbidity (including hypoparathyroidism and recurrent laryngeal nerve injury), while limited dissection risks leaving metastatic disease behind, potentially contributing to disease recurrence. A reliable intraoperative strategy that improves detection and risk stratification of occult nodal disease is urgently needed to support patient-specific, precision lymph node management in PTC.

2.2 Sentinel Lymph Node Mapping in PTC

Sentinel lymph node (SLN) mapping has been investigated to tailor the extent of lymph node dissection by identifying first-echelon nodes along patient-specific thyroid lymphatic drainage. The concept is based on the principle that if cancer spreads through the lymphatic system, it will reach these sentinel nodes first.

However, SLN-based approaches in PTC have shown variable performance, partly because thyroid lymphatic pathways are complex and difficult to visualize in vivo. No single tracer provides both precise SLN localization and robust information on lymphatic flow dynamics:

- Radiotracer methods can be accurate but require nuclear-medicine infrastructure, increase cost, and raise concerns about workflow and contamination.
- Superparamagnetic iron oxide techniques depend on specialized equipment and can be affected by intraoperative signal interference.
- Carbon nanoparticles (CNs) provide durable visual staining yet offer limited dynamic information.
- Indocyanine green (ICG) enables near-infrared fluorescence imaging of lymphatic flow but can diffuse, suffer from background contamination, and has limited penetration depth.

2.3 Rationale for Dual-Tracer Approach

To address these limitations, we employ a dual-tracer strategy combining CNs and ICG, leveraging sustained visual staining with spatiotemporal near-infrared fluorescence (NIRF) signals during intraoperative SLN mapping. This combination offers several advantages:

- ICG enables real-time, dynamic monitoring of lymphatic flow and aggregation, allowing complete visualization of lymphatic drainage pathways.
- CNs provide long-lasting black staining that facilitates identification and resection of lymph nodes.
- The combined approach compensates for individual tracer limitations and improves sentinel node identification accuracy.

2.4 Role of Deep Learning

Deep learning (DL) is well suited to extract high-dimensional spatiotemporal patterns from surgical videos and to integrate them with clinicopathological variables, potentially revealing predictive signals beyond what is apparent to the human eye. By applying explainable artificial intelligence (XAI) methods such as SHAP (SHapley Additive exPlanations), we can identify influential features and provide interpretable outputs to support intraoperative decision-making.

3. STUDY OBJECTIVES AND HYPOTHESES

3.1 Primary Objectives

1. To compare the sentinel lymph node detection rate among three tracer methods: ICG alone, CNs alone, and ICG+CNs dual-tracer combination.
2. To evaluate and compare the diagnostic performance (sensitivity, specificity, PPV, NPV) of each tracer method for predicting central lymph node metastasis.
3. To develop and validate deep learning models using multimodal features (spatiotemporal video features + clinical variables) for predicting second-echelon lymph node metastasis (SeLNM) and non-sentinel lymph node metastasis (NsLNM).

3.2 Secondary Objectives

- To identify the optimal deep learning architecture for lymph node metastasis prediction.
- To determine the most important predictive features using SHAP analysis.
- To assess the clinical utility of AI-assisted intraoperative decision support.
- To evaluate safety outcomes including surgical complications and tracer-related adverse events.

3.3 Hypotheses

Primary Hypothesis: The ICG+CNs dual-tracer combination will demonstrate superior sentinel lymph node detection rate compared to either tracer alone.

Secondary Hypothesis: Time-series fluorescence-flow signatures, combined with spatial structural cues and clinicopathological factors, will improve prediction of SeLNM and NsLNM compared with SLN status alone.

4. STUDY DESIGN

4.1 Study Type and Design

This is a prospective, single-center, three-arm observational cohort study. Participants will be allocated to one of three groups based on the sentinel lymph node mapping technique used during their thyroid surgery.

4.2 Study Groups

Group 1 - ICG Group (n=112): Sentinel lymph node mapping using indocyanine green alone with near-infrared fluorescence imaging.

Group 2 - CNs Group (n=112): Sentinel lymph node mapping using carbon nanoparticles alone with visual identification.

Group 3 - ICG+CNs Group (n=112): Sentinel lymph node mapping using combined dual-tracer technique with both NIRF imaging and visual identification. This group will also undergo deep learning video analysis.

4.3 Study Timeline

- Ethics Approval: October 16, 2023
- Enrollment Period: April 2024 - October 2024
- Follow-up Period: 12 months post-surgery
- Data Analysis and Reporting: After completion of enrollment

4.4 Study Setting

The study will be conducted at the Department of Breast and Thyroid Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China. This is a tertiary referral center with expertise in thyroid cancer surgery and advanced surgical imaging techniques.

5. STUDY POPULATION

5.1 Target Population

Adults diagnosed with clinically node-negative papillary thyroid carcinoma (cN0-PTC) who are scheduled for surgical treatment.

5.2 Inclusion Criteria

1. Age ≥18 years at the time of enrollment
2. Histologically confirmed papillary thyroid carcinoma by preoperative fine-needle aspiration biopsy
3. Clinically node-negative (cN0) status confirmed by preoperative imaging (ultrasound and/or cross-sectional imaging showing no evidence of lymph node metastasis)
4. Scheduled to undergo thyroid surgery with simultaneous central lymph node dissection
5. Willing and able to provide written informed consent
6. Complete preoperative clinical data available

5.3 Exclusion Criteria

1. History of previous neck surgery
2. History of external beam radiotherapy to the head and neck region
3. Diagnosis of thyroid malignancy other than papillary thyroid carcinoma
4. Known allergy or hypersensitivity to indocyanine green (ICG), iodine, or carbon nanoparticles
5. Severe hepatic insufficiency (ICG is metabolized by the liver)
6. Pregnancy or breastfeeding
7. Incomplete medical records or missing essential preoperative data
8. Refusal to undergo dual-tracer imaging procedure
9. Participation in another interventional clinical trial that may interfere with the current study

5.4 Sample Size Justification

Sample size was calculated based on the following assumptions:

- Expected sensitivity of ICG+CNs combination: 85%
- Expected sensitivity of single tracer: 50%
- Expected specificity: 90%
- Expected prevalence of lymph node metastasis: 50%
- Significance level (α): 0.05 (two-sided)
- Statistical power (1-β): 0.80

Using the formula for comparing two proportions, the minimum required sample size is 335 participants. To account for potential dropouts (approximately 10%), we plan to enroll 336 participants (112 per group).

6. STUDY INTERVENTIONS AND PROCEDURES

6.1 Preoperative Assessment

All participants will undergo standard preoperative evaluation including:

- Complete medical history and physical examination
- Thyroid ultrasound with detailed lymph node assessment
- Fine-needle aspiration biopsy for cytological confirmation
- Genetic testing for common mutations (BRAF, etc.)
- Standard laboratory tests (complete blood count, thyroid function, liver function)
- Preoperative photography and documentation

6.2 Tracer Preparation and Administration

6.2.1 ICG Group

- Preparation: ICG powder (25 mg) dissolved in 10 ml sterile water to achieve concentration of 2.5 mg/ml
- Administration: 0.2 ml of ICG solution injected at multiple points around the thyroid tumor under real-time ultrasound guidance
- Visualization: Near-infrared fluorescence imaging system (excitation 750-800 nm, emission 820 nm)

6.2.2 CNs Group

- Preparation: Carbon nanoparticle suspension at commercial concentration (50 mg/ml)
- Administration: 0.2 ml of CNs suspension injected at multiple points around the thyroid tumor under ultrasound guidance
- Visualization: Direct visual identification of black-stained lymph nodes

6.2.3 ICG+CNs Group (Dual-Tracer)

- Preparation: 0.1 ml ICG solution (2.5 mg/ml) mixed with 0.1 ml CNs suspension (50 mg/ml) to form 0.2 ml dual-tracer composite
- Administration: Multi-point stereotactic injection under ultrasound guidance
- Visualization: Combined NIRF imaging and visual black staining identification
- Video recording: Minimum 5 minutes at 1920×1080 resolution, 29.97 fps

6.3 Surgical Procedure

All participants will undergo standard thyroid surgery following institutional protocols:

- Identification and separate submission of sentinel lymph node for pathological examination
- Ipsilateral thyroid lobectomy or total thyroidectomy as clinically indicated
- Ipsilateral central lymph node dissection (Level VI)
- Intraoperative frozen section analysis of sentinel lymph node

- Extended dissection (lateral compartment, contralateral central) based on frozen section findings

Surgical decisions will follow standard protocols and will NOT be influenced by deep learning predictions (which are performed retrospectively).

6.4 Video Recording Protocol (ICG+CNs Group)

- Equipment: Near-infrared fluorescence imaging system (Mingde Pharmaceutical, Henan, China)
- Recording parameters: 1920×1080 resolution, 29.97 frames per second
- Duration: From tracer injection until secondary lymph node fluorescence disappears (minimum 5 minutes)
- Content: Complete SLN visualization process including lymphatic flow dynamics
- Storage: Digital files exported via USB for subsequent analysis

6.5 Pathological Examination

- Intraoperative frozen section: Sentinel lymph node and representative lymph nodes
- Postoperative formalin-fixed paraffin-embedded (FFPE) processing for definitive diagnosis
- Independent review by three pathologists
- Final diagnosis based on paraffin section results (gold standard)
- Documentation of metastasis location, size, and extent

7. OUTCOME MEASURES

7.1 Primary Outcomes

7.1.1 Sentinel Lymph Node Detection Rate

Definition: Proportion of participants with successfully identified sentinel lymph nodes using each tracer method.

Measurement: Number with identified SLN / Total number in group × 100%

Time Frame: Intraoperative (Day 0)

7.1.2 Sentinel Lymph Node Metastasis (SLNM) Status

Definition: Presence or absence of cancer metastasis in the sentinel lymph node.

Classification: Positive (macrometastasis or micrometastasis) or Negative

Gold Standard: Final histopathological examination (paraffin section)

Time Frame: Within 7 days after surgery

7.1.3 Second-Echelon Lymph Node Metastasis (SeLNM)

Definition: Presence or absence of cancer metastasis in second-echelon lymph nodes (beyond the sentinel node in the drainage pathway).

Primary prediction target for deep learning models in ICG+CNs group.

Time Frame: Within 7 days after surgery

7.1.4 Non-Sentinel Lymph Node Metastasis (NsLNM)

Definition: Presence or absence of cancer metastasis in any lymph node other than the sentinel lymph node.

Includes central and lateral compartment nodes (when dissected).

Time Frame: Within 7 days after surgery

7.2 Secondary Outcomes

- Sensitivity, Specificity, PPV, NPV of each tracer method
- Deep learning model performance metrics (AUC, accuracy, F1 score, Brier score)
- Number of sentinel lymph nodes identified per participant
- Total number of lymph nodes retrieved
- Feature importance rankings from SHAP analysis

7.3 Safety Outcomes

- Surgical complications (hypoparathyroidism, recurrent laryngeal nerve injury, bleeding, infection)
- Tracer-related adverse events (allergic reactions, injection site reactions)
- Time Frame: Within 30 days after surgery

8. DATA COLLECTION AND MANAGEMENT

8.1 Clinical Data Collection

A total of 32 clinical variables will be collected for each participant:

Demographics (3 variables): Age, sex, body mass index (BMI)

Ultrasound features (9 variables): Tumor size, tumor margin, calcification pattern, aspect ratio, internal echoes, homogeneity, intratumoral blood flow, peritumoral blood flow, TI-RADS classification

Pathological features (10 variables): Tumor location, laterality, multifocality, capsular invasion, extrathyroidal extension (ETE), Hashimoto's thyroiditis, T stage, SLN location, SLN metastasis status, number of positive SLNs

Genetic data: BRAF mutation status and other relevant mutations

8.2 Video Data Processing

For the ICG+CNs group:

- Extract 3-minute segment showing complete SLN visualization process
- Resample to 150 frames (5 fps) at 1920×1080 resolution
- Manual ROI annotation by two senior surgeons (>10 years experience)
- Generate binary mask images for each frame
- Resize to standard 512×512 dimensions

8.3 Feature Extraction

Spatial Features (2,048 dimensions):

- Deep image features from EfficientNet-B5 pre-trained model
- Grayscale characteristics
- Shape and morphological features
- Hu moment descriptors

Temporal Features (20 dimensions):

- Frame-to-frame difference features (4 dimensions)
- Optical flow features (3 dimensions)
- Fluorescence time-series features (20 dimensions)

Dimensionality Reduction: Principal Component Analysis (PCA) to 32 dimensions

8.4 Data Quality Assurance

- Double data entry for clinical variables
- Range checks and logical validation
- Regular data audits
- Secure electronic data storage with backup
- De-identification of all data before analysis

9. STATISTICAL ANALYSIS PLAN

9.1 Analysis Populations

Intention-to-Treat (ITT) Population: All enrolled participants who received the assigned tracer injection.

Per-Protocol (PP) Population: All participants who completed the study according to the protocol without major deviations.

Safety Population: All participants who received any study intervention.

9.2 Descriptive Statistics

- Continuous variables: Mean ± standard deviation (normal distribution) or median with interquartile range (non-normal distribution)
- Categorical variables: Frequencies and percentages
- Normality testing: Shapiro-Wilk test
- Baseline characteristics compared among three groups

9.3 Primary Outcome Analysis

9.3.1 SLN Detection Rate Comparison

- Chi-square test or Fisher's exact test for comparison among groups
- 95% confidence intervals for detection rates
- Pairwise comparisons with Bonferroni correction

9.3.2 Diagnostic Performance

For each tracer method, calculate:

- Sensitivity = TP / (TP + FN) × 100%
- Specificity = TN / (TN + FP) × 100%
- Positive Predictive Value (PPV) = TP / (TP + FP) × 100%
- Negative Predictive Value (NPV) = TN / (TN + FN) × 100%
- 95% confidence intervals using Wilson score method

9.4 Deep Learning Model Development

9.4.1 Model Architectures

Nine deep learning architectures will be developed and compared:

- Convolutional Neural Network (CNN)
- Long Short-Term Memory (LSTM)
- CNN + LSTM hybrid
- CNN + LSTM + Attention
- Transformer
- Crossformer
- 3D-CNN

- LSTM + Transformer hybrid
- LSTM + Crossformer hybrid

9.4.2 Model Training

- Loss function: Binary cross-entropy
- Optimizer: Adam
- Learning rate: Adaptive with decay
- Regularization: Dropout and gradient clipping
- Data augmentation: Rotation, scaling, noise injection
- Class imbalance handling: Weighted loss function and oversampling

9.4.3 Model Validation

10-fold stratified cross-validation will be used:

- Data split: 90% training, 10% testing in each fold
- Stratification ensures balanced outcome distribution
- Performance metrics averaged across all folds
- Standard deviation calculated to assess stability

9.5 Model Performance Metrics

The following metrics will be calculated for each model:

- Area Under the ROC Curve (AUC)
- Accuracy
- Sensitivity (Recall)
- Specificity
- Positive Predictive Value (Precision)
- Negative Predictive Value
- F1 Score
- False Positive Rate
- Lift
- Brier Score
- Kappa Coefficient
- Dice Coefficient

9.6 Model Comparison

- DeLong test for comparing AUC values between models
- Probability-based model ranking approach (PMRA)
- Win-probability heatmaps
- Wald test p-value comparisons
- Decision curve analysis for clinical utility
- Calibration curves for reliability assessment
- Learning curves for overfitting assessment

9.7 Model Interpretability (SHAP Analysis)

SHapley Additive exPlanations (SHAP) will be used to:

- Calculate feature importance scores
- Generate summary plots (bar charts, beeswarm plots)
- Perform cluster analysis of feature contributions
- Create decision plots for individual predictions
- Identify key predictive features for clinical interpretation

9.8 Missing Data Handling

- Complete case analysis for primary outcomes
- Multiple imputation for secondary analyses if missing data >5%
- Sensitivity analyses to assess impact of missing data

9.9 Software

- Python (version 3.9.21) for deep learning
- PyTorch (version 2.0) for model development
- Scikit-learn (version 1.2.2) for preprocessing and evaluation
- SPSS (version 21.0) for traditional statistical analyses
- SHAP library for model interpretability
- Significance level: $\alpha = 0.05$ (two-sided) for all tests

10. ETHICAL CONSIDERATIONS

10.1 Ethics Approval

This study has been approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University.

Approval Number: 2023-322

Approval Date: October 16, 2023

The study will be conducted in accordance with:

- Declaration of Helsinki (2013 revision)
- Good Clinical Practice (GCP) guidelines
- National Medical Products Administration (NMPA) regulations
- Applicable local laws and regulations

10.2 Informed Consent

- Written informed consent will be obtained from all participants before enrollment
- Consent process will include explanation of study purpose, procedures, risks, and benefits
- Participants will be informed of their right to withdraw at any time
- Consent forms will be available in Chinese
- Copy of signed consent will be provided to participants

10.3 Privacy and Confidentiality

- All participant data will be de-identified for analysis
- Data stored in secure, password-protected systems
- Access limited to authorized study personnel
- Video recordings stored separately from identifiable data
- Data retention according to institutional and regulatory requirements

10.4 Risk Assessment

This study poses minimal additional risk to participants because:

- Both tracers (ICG and CNs) are approved for clinical use
- The dual-tracer injection uses standard clinical techniques
- Video recording is non-invasive and does not affect surgical decisions
- Deep learning analysis is performed retrospectively
- All surgical decisions follow standard clinical protocols

Known risks of tracers:

- ICG: Allergic reactions (<0.05%), contraindicated in iodine allergy
- CNs: Local tissue staining, generally well-tolerated

11. DATA AND SAFETY MONITORING

11.1 Adverse Event Reporting

- All adverse events will be recorded and assessed for severity and relatedness
- Serious adverse events reported to Ethics Committee within 24 hours
- Regular safety reports submitted to Ethics Committee

11.2 Stopping Rules

The study may be terminated if:

- Unacceptable adverse event rate observed
- New safety information emerges that changes risk-benefit assessment
- Enrollment goals cannot be achieved
- Significant protocol violations occur

11.3 Protocol Amendments

- Any protocol modifications require Ethics Committee approval before implementation
- Participants will be re-consented if amendments affect their participation
- ClinicalTrials.gov record will be updated to reflect amendments

12. REFERENCES

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--- END OF STUDY PROTOCOL WITH STATISTICAL ANALYSIS PLAN ---