



Robotic-assisted navigation system for preoperative lung nodule localization: a pilot study

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Background: Preoperative percutaneous computed tomography (CT)-guided localization of pulmonary nodules plays a pivotal role in the diagnosis and treatment of early-stage lung cancer. However, conventional manual localization techniques have inherent limitations in achieving a high degree of accuracy. Consequently, a novel robotic-assisted navigation system was developed to attain precise localization of small lung nodules. This study aims to investigate the accuracy and safety of this system in clinical applications.

Methods: Patients with peripheral solitary pulmonary nodules measuring less than 20 mm were enrolled. The robotic-assisted navigation system generated a three-dimensional (3D) model based on the patient's CT images, determining the optimal puncture path. The robotic arm then accurately located the nodule and, following percutaneous puncture, indocyanine green (ICG) was injected. The primary outcome measure was the accuracy of pulmonary nodule localization, while secondary outcomes included the complication rate, procedural duration, and total radiation exposure.

Results: A total of 33 nodules were successfully localized using the robotic-assisted navigation system and resected through video-assisted thoracoscopic surgery (VATS). The first-pass success rate was 100%, with a median deviation of 6.1 mm [interquartile range (IQR), 2.5–7.2 mm] between the localizer and the nodule. The median localization time was 25.0 minutes, and the single and cumulative exam dose-length products (DLP) were 534.0 and 1491.0 mGy·cm, respectively. Notably, no observable complications were reported during the procedures.

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Conclusions: The innovative robotic-assisted navigation system demonstrated satisfactory accuracy and holds promise for improving the percutaneous localization of lung nodules. This method represents a safe and viable alternative to traditional CT-guided manual localization techniques.

Keywords: Robotic-assisted; navigation system; lung nodule; preoperative localization; video-assisted thoracoscopic surgery (VATS)

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Introduction

The increasing utilization of low-dose computed tomography (CT) has led to the detection of a higher number of asymptomatic solitary pulmonary nodules in clinical settings, raising concerns for both patients and clinicians (1). Video-assisted thoracoscopic surgery (VATS) is commonly employed for the diagnosis and treatment of suspected malignant nodules through excisional biopsy. However, the manual palpation of the lung parenchyma to locate the target nodule is a time-consuming and

challenging process. Localization of small and non-solid nodules, particularly those lacking pleural indentation or discoloration, during VATS via palpation poses significant difficulties. Accurate preoperative localization of lung nodules is crucial for improving surgical success rates (2). Conversely, an imprecise localization or mislocalization of pulmonary nodules may result in positive margins or unsuccessful nodule resection. This, in turn, contributes to extended surgical durations, increased radiation exposure, elevated occurrence of adverse events, and the potential necessity for secondary surgical interventions (3).

The key challenge in percutaneous pulmonary nodule localization lies in achieving accurate placement of the localizer in close proximity to the target nodule. Despite advancements in imaging techniques, localization accuracy can be affected by factors such as challenging puncture routes, respiratory motion, slight patient movements, and changes in target location due to mechanical pressure (4). To enhance localization accuracy, several techniques have been developed for preoperative or intraoperative localization of target pulmonary nodules, including CT-guided hook wire localization, microcoil localization, claw-suture localization, and electromagnetic navigational bronchoscopy localization (3,5-9). However, the accuracy of these methods heavily relies on the experience and expertise of the operator, and even experienced operators may require iterative adjustments to accurately guide the localizer for nodule localization.

In recent years, innovative technologies have emerged to improve trajectory planning and localization accuracy, such as three-dimensional (3D) printed navigational templates, interventional robot systems, and augmented reality (AR) navigation-guided localization techniques (10-12). In a previous animal study, we introduced a novel surgical navigation puncture robot that enables percutaneous robotic-assisted nodule localization through CT-based 3D reconstruction and optoelectronic navigation (13). Our

Highlight box

Key findings

- A novel robotic-assisted navigation system achieved a 100% first-pass success rate in precisely localizing pulmonary nodules <20 mm, with a median deviation of 6.1 mm from the target point. The procedure's median duration was 25.0 minutes, and the procedure involved low radiation exposure, ensuring patient safety. Additionally, no observable complications were reported during the procedures, further establishing the system's safety and efficacy.

What is known and what is new?

- Preoperative localization of pulmonary nodules is a critical step in ensuring the precision of diagnosis and treatment. Traditional manual localization methods, while widely practiced, encounter inherent limitations that compromise the attainment of a high level of accuracy.
- The innovative robotic-assisted navigation system provides an advanced and effective solution for preoperative pulmonary nodule localization, ensuring precise accuracy, minimal complications, and reduced radiation exposure.

What is the implication, and what should change now?

- The robotic-assisted navigation system has shown promising results for precise and safe lung nodule localization. It could lead to improved procedures, reduced discomfort, and shorter operative times. This innovative technology could be a secure and feasible approach for pulmonary nodule localization.

animal study demonstrated a significant improvement in localization accuracy using this method, enabling smaller and more precise resections (14). In this prospective pilot study, we aim to evaluate the feasibility and safety of this novel robotic-assisted navigation system in clinical practice. We present this article in accordance with the TREND reporting checklist (available at <https://tldr.amegroups.com/article/view/10.21037/tlcr-23-493/rc>).

Methods

Patient selection

This study, carried out at the First Affiliated Hospital of Guangzhou Medical University from January 2021 to February 2021, involved a collaborative assessment by thoracic surgeons and radiologists. Their evaluation aimed to identify eligible patients necessitating diagnostic lung wedge resection through VATS. The inclusion criteria were as follows: (I) presence of solitary pulmonary nodules with a maximum diameter less than 20 mm; (II) morphological characteristics of the target nodule exhibiting mixed nodule or pure ground glass opacity (pGGO) on CT scan; (III) the minimum distance from the outer edge of the nodule to the nearest pleural surface was greater than 10 mm. Exclusion criteria encompassed the following: (I) pulmonary nodules situated in the scapular region that impeded percutaneous localization; (II) pulmonary nodules located in close proximity to the mediastinum or major vessels of the heart; (III) patients with severe comorbidities, advanced disease, or deemed unsuitable for surgical intervention.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study received approval from the Institutional Review Board of the First Affiliated Hospital of Guangzhou Medical University [No. EC-2020-093(QX)], and written informed consent was obtained from all participating patients.

Robotic-assisted navigation system

For preoperative CT-guided lung nodule localization, we utilized a commercially available robotic-assisted navigation system (TH-S1) obtained from TrueHealth Medical Technology Co. Ltd. in Hengqin, China. This system holds approval from the National Medical Products Administration (NMPA) as a class III medical device. The system comprises a photoelectric navigation system, a surgical planning system, and a robotic arm positioning

and puncture system (*Figure 1*), specifically designed for interventional procedures. The operational principle of the robotic-assisted navigation system for preoperative lung nodule localization is as follows:

- (I) The patient's preoperative CT scan is imported into the surgical planning system, enabling the reconstruction of a comprehensive 3D model encompassing the pulmonary nodules, vessels, bronchi, bone structures, and skin.
- (II) The 3D model is automatically registered with the patient's position information obtained through the photoelectric navigation system, ensuring accurate alignment.
- (III) Based on the nodule's location within the 3D model, the surgical planning system defines the puncture path. Subsequently, this planned path is transformed and registered within the real surgical space.
- (IV) The robotic arm positioning and puncture system precisely positions the puncture path, including the puncture site, needle direction, and depth, within the surgical space.
- (V) A puncture needle is then inserted manually, facilitating the placement of the marker for intraoperative localization.

Localization procedures

CT scan and 3D reconstruction

On the day of surgery, preoperative localization was conducted by an interventional radiologist and a surgeon in the radiology department. To ensure patient immobility during the procedure, a reusable immobilizer was utilized. The physician affixed a positioning tracker to the patient's chest wall within the intended region. Subsequently, an initial CT scan was acquired and loaded into the robotic computer interface. The CT data underwent reconstruction to generate a comprehensive 3D model encompassing pulmonary nodules, vessels, bronchi, bone structures, and skin, aiding in navigation planning (*Figure 2*). All CT data were saved in Digital Imaging and Communications in Medicine (DICOM) format and subsequently transferred to the Hisense computer-assisted surgery system (Hisense, Qingdao, China).

Navigation planning and robotic-assisted positioning puncture

The reconstructed 3D model was imported into the surgical planning system, and registration between the 3D model and the patient's actual position was accomplished by

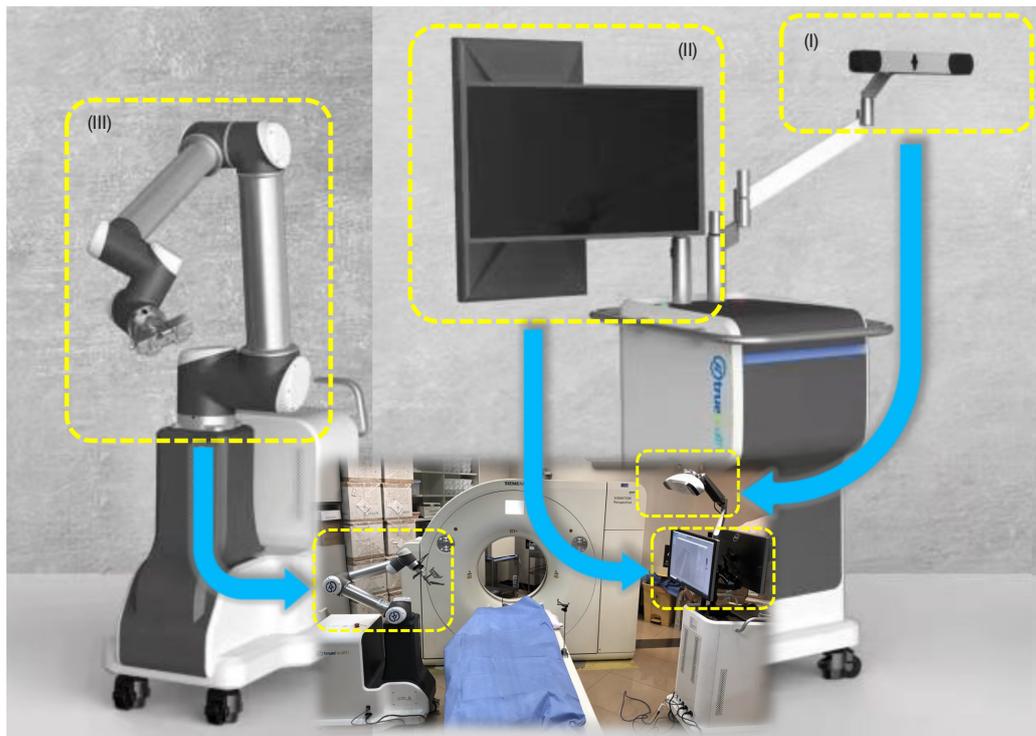


Figure 1 The robotic-assisted navigation system. (I) The photoelectric navigation system used to track the position of the robotic arm and the patient; (II) the surgical planning system employed for the precise planning of the needle insertion path; (III) the robotic arm positioning and puncture system responsible for accurate positioning of the needle holder and assisting with needle insertion.

utilizing CT image coordinates and real spatial coordinates of the positioning tracker. Surgeons defined the target and skin entry points through the robotic computer interface, and the needle trajectory was planned accordingly. The robotic arm precisely positioned a specialized needle holder in accordance with the planned trajectory. Subsequently, local anesthesia was administered, and indocyanine green (ICG) was introduced during a deep inspiration phase for all patients. The operator, assisted by the robotic arm, inserted the needle to a depth automatically measured and displayed on the screen (*Figure 3*).

CT scan to verify accuracy

A subsequent CT scan encompassing the entire needle path was conducted to confirm the proper insertion of the introducer needle. In cases where a deviation of more than 2 cm was identified, it was recorded as a failure of the robotic-assisted method, and conventional CT-guided localization was employed to locate the target lung nodule. After confirming the appropriate placement of the needle, the positioning marker was inserted, and the needle was

subsequently removed. Finally, a CT scan was performed to detect any associated complications such as pneumothorax or hemorrhage. The accuracy of nodule localization was assessed by measuring the deviation from the localizer to the target point (*Figure 4*). Procedural time, from the moment the patient was positioned on the CT scanner examination bed to the confirmation of localizer placement via the second CT scan, was recorded. A comprehensive description of the procedure is presented in *Video 1*.

Outcomes

The primary endpoint of the study was the accuracy of pulmonary nodule localization. A procedure was considered successful when the localization met the following criteria: (I) the distance between the localizer and the target point was less than 10 mm; (II) there was no instance of localizer displacement from the initial localization procedure to the surgical operation; and (III) there were no device-associated errors throughout the entirety of the procedure. Secondary endpoints encompassed the assessment of perioperative

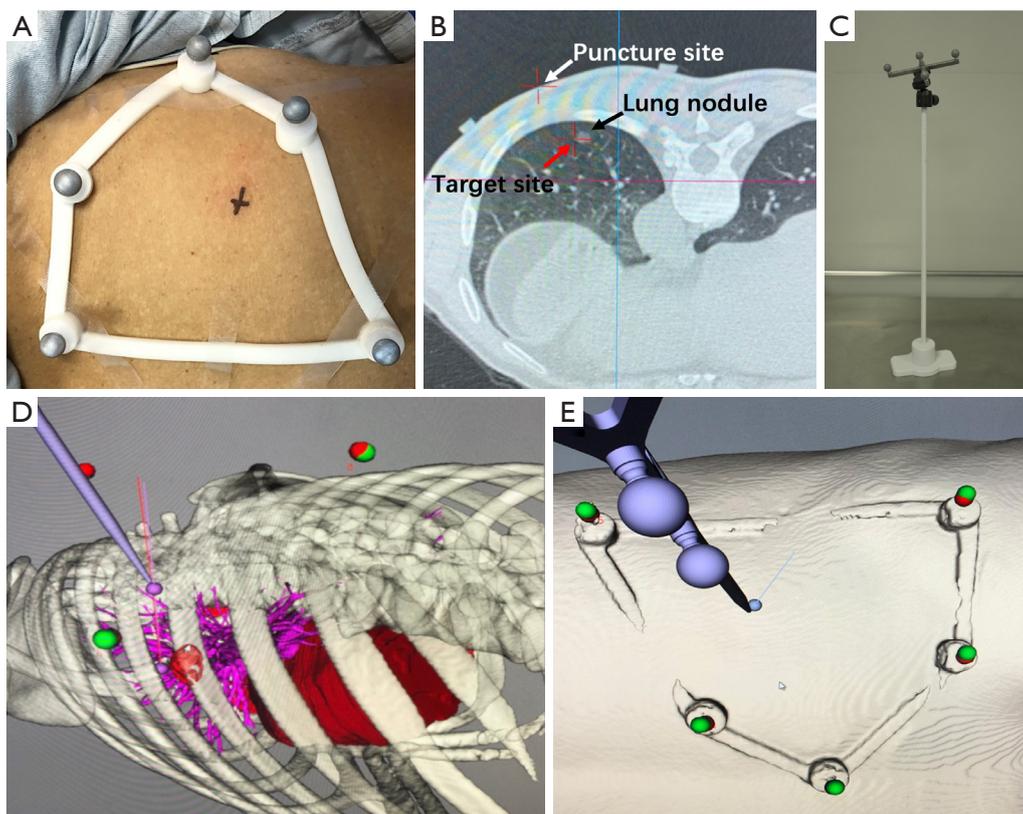


Figure 2 CT scan and 3D reconstruction of the lung. (A) Placement of the positioning tracker on the patient's chest wall; (B) planning of the needle trajectory based on the initial CT scan; (C) use of the fiducial tracker as the reference for precise positioning; (D) 3D reconstruction of the thoracic structure; (E) 3D verification of the puncture site. The white arrow indicates the puncture site; the black arrow indicates the target nodule; and the red arrow indicates the target site. CT, computed tomography; 3D, three-dimensional.

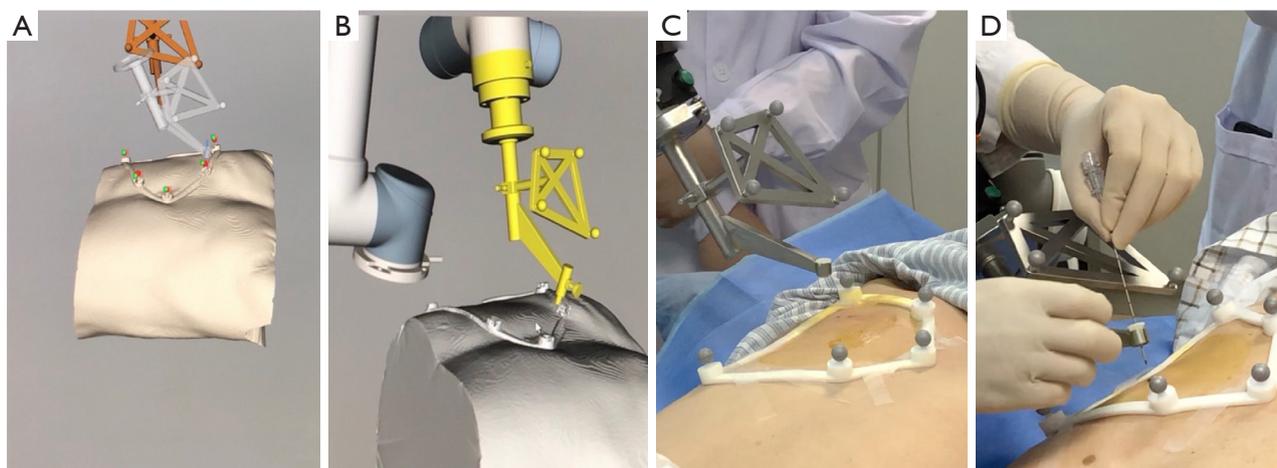


Figure 3 Navigation planning and robotic-assisted positioning puncture process. (A) Registration of the 3D model to the patient's precise position; (B) motion planning and simulation of the robotic arm to ensure avoidance of any obstructions during the procedure; (C) precise positioning of the needle holder. The robotic arm automatically positions the needle holder in alignment with the planned trajectory; (D) needle insertion guided by the robotic-assisted system. 3D, three-dimensional.

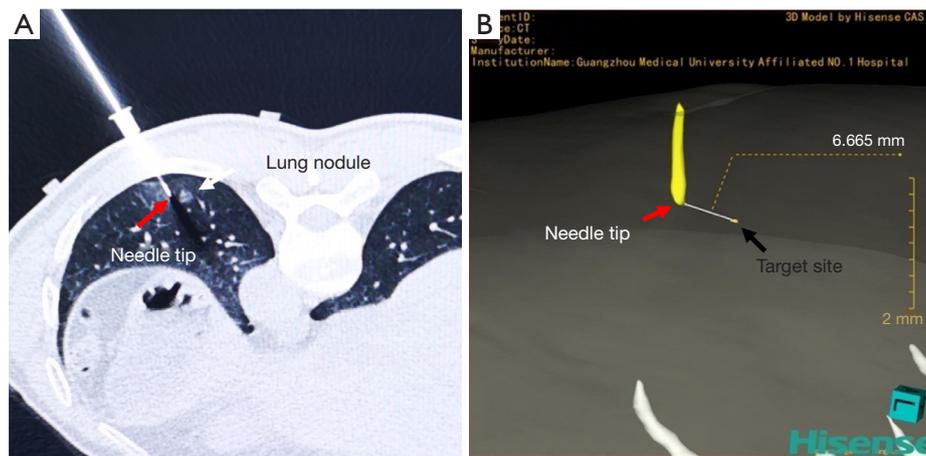
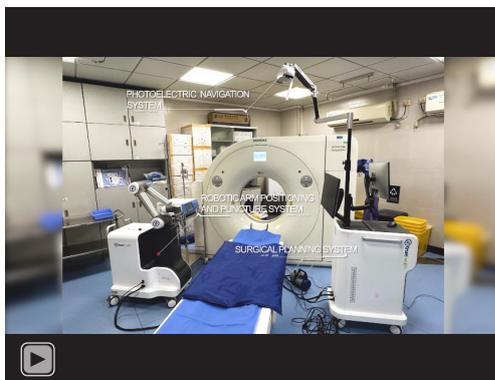


Figure 4 Evaluation of the precision of robotic-assisted lung nodule localization through CT scanning. (A) CT scan obtained after needle insertion; (B) measurement of the distance between the localizer and the target site. The white arrow indicates the target nodule; the black arrow indicates the target site; and the red arrow indicates the needle tip. CT, computed tomography.



Video 1 Lung nodule localization using the robotic-assisted navigation system. The video shows the step-by-step procedure of needle insertion employing the robotic-assisted system for precise lung nodule localization.

complications, procedural duration, and cumulative radiation exposure.

Statistical analysis

Patient characteristics were summarized using means with standard deviations for normally distributed continuous variables, and medians with interquartile ranges (IQRs) for non-normally distributed continuous variables. Categorical variables were presented as numbers with percentages. For comparisons of clinical characteristics between groups, Student's *t*-test or the Mann-Whitney *U*-test was applied

for continuous variables, while Pearson's Chi-square test or Fisher's exact test was used for categorical variables. The Spearman rank correlation test was employed to assess correlations between variables, with the corresponding Spearman correlation coefficient (*R*s) reported. Statistical significance was defined as a *P* value less than 0.05. All statistical analyses were performed using SPSS software (version 26.0; IBM SPSS Inc., Armonk, NY, USA).

Results

A total of 48 patients with pulmonary nodules scheduled for thoroscopic resection were initially screened, and after exclusions and patient refusals, 33 patients were enrolled in the study (Figure S1). The baseline characteristics of the patients and targeted nodules are presented in Table 1. The study population predominantly consisted of female participants (27/33), with a median age of 50 years and a median chest wall thickness of 34.0 mm. The majority of nodules were classified as pGGO (29/33) with a median density of -633.0 Hounsfield units (HU) while the remaining 4 were mixed nodules. The distribution of nodules among the lung lobes was fairly equal, with 8 (24.2%) in the right upper lobe, 6 (18.2%) in the right lower lobe, 9 (27.3%) in the left upper lobe, and 10 (30.3%) in the left lower lobe. The median nodule diameter was 9.0 mm (IQR, 7.5–10.0 mm), and the median distance from the outer edge of the nodule to the pleural surface was 10.4 mm (IQR, 3.3–15.5 mm).

Table 1 Patient preoperative demographic and clinical information

Characteristics	Total (n=33)
Age (years), median [IQR]	50 [38–62]
Sex, n (%)	
Male	6 (18.2)
Female	27 (81.8)
Chest wall thickness (mm), median [IQR]	34.0 [29.1–42.4]
Nodule size (mm), median [IQR]	9.0 [7.5–10.0]
Nodule density (HU), median [IQR]	–633.0 [–708.0 to –556.0]
Distance from the outer edge of nodule and pleura (mm), median [IQR]	10.4 [3.3–15.5]
Location, n (%)	
RUL	8 (24.2)
RLL	6 (18.2)
LUL	9 (27.3)
LLL	10 (30.3)

IQR, interquartile range; HU, Hounsfield units; RUL, right upper lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe.

All 33 nodules were successfully localized using the robot-assisted navigation system, resulting in a first-pass success rate of 100%. The median deviation between the localizer and the target point was 6.1 mm (IQR, 2.5–7.2 mm) (Table 2). The deviation of the localizer was found to have a significant correlation with nodule density ($R_s = 0.40$, $P = 0.021$), but not with chest wall thickness ($R_s = -0.07$, $P = 0.681$), nodule size ($R_s = 0.25$, $P = 0.161$), distance from the outer edge of the nodule to the pleura ($R_s = 0.09$, $P = 0.603$), or decubitus position. The median deviation for nodules located in the upper lobe and lower lobe was 5.5 and 6.9 mm, respectively, but no significant difference was observed between the two groups ($P = 0.471$).

Regarding the secondary outcome, the median duration of the localization procedure was 25.0 minutes (IQR, 18.0–29.0 minutes). The median values for the single and cumulative exam dose-length products (DLP) were 534.0 mGy·cm (IQR, 374.0–645.0 mGy·cm) and 1,491.0 mGy·cm (IQR, 1,210.0–1,781.5 mGy·cm), respectively (Table 2). Importantly, with respect to localization-related complications, asymptomatic pneumothoraxes were observed in four patients on the CT scan, which did not require further intervention. No

Table 2 Characteristics of nodule localization and assessment of the accuracy

Variables	Total (n=33)
Procedural duration (min), median [IQR]	25.0 [18.0–29.0]
Decubitus position, n (%)	
Supine	7 (21.2)
Prone	17 (51.5)
Lateral	9 (27.3)
Single DLP (mGy·cm), median [IQR]	534.0 [374.0–645.0]
Cumulative DLP (mGy·cm), median [IQR]	1,491.0 [1,210.0–1,781.5]
Distance between localizer and the target point (mm), median [IQR]	
All nodules	6.1 [2.5–7.2]
RUL + LUL	5.5 [3.0–7.0]
RLL + LLL	6.9 [2.2–8.3]

IQR, interquartile range; DLP, dose-length product; RUL, right upper lobe; LUL, left upper lobe; RLL, right lower lobe; LLL, left lower lobe.

other complications occurred during the robotic-assisted localization procedure.

Following the successful localization of pulmonary nodules within the CT room, all patients were transferred to the operating room. The median time interval between nodule localization and surgery was 70 minutes (IQR, 45–120 minutes). During this phase, all patients underwent wedge resection by VATS with the guidance of ICG, and no instances of ICG diffusion or leakage in the thoracic cavity were observed. The median operating time for the wedge resection procedure was 50 minutes (IQR, 40–65 minutes).

Among the 33 participants, 25 (75.8%) underwent wedge resection and lymph node sampling, 6 (18.2%) underwent segmentectomy and systemic lymph node dissection, and 2 (6.1%) underwent lobectomy and systemic lymph node dissection. The decision for lobectomy was made due to an intraoperative frozen section diagnosis of invasive adenocarcinoma (IA). Of the 33 nodules, 9 (27.3%) were diagnosed as adenocarcinoma *in situ* (AIS), 20 (60.6%) as minimally invasive adenocarcinoma (MIA), 2 (6.1%) as IA, 1 (3.0%) nodule was benign, and 1 (3.0%) nodule was bronchiolar adenoma. Frozen section analysis provided information on nodule size and margin distance, with a median diameter of 8.0 mm (IQR, 7.0–10.0 mm) and a

Table 3 Surgical approach and postoperative pathology

Variables	Total (n=33)
Surgical approach, n (%)	
Wedge resection	25 (75.8)
Segmentectomy	6 (18.2)
Lobectomy	2 (6.1)
Pathology, n (%)	
Benign	1 (3.0)
Bronchiolar adenoma	1 (3.0)
AIS	9 (27.3)
MIA	20 (60.6)
IA	2 (6.1)
Nodule size (mm), median [IQR] [†]	8.0 [7.0–10.0]
Margin distance (mm), median [IQR]	20.0 [16.0–21.0]

[†], nodule size was measured on specimen. AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; IA, invasive adenocarcinoma; IQR, interquartile range.

median margin distance of 20.0 mm (IQR, 16.0–21.0 mm). Importantly, the margin distance exceeded the diameter of the nodule in all resected specimens. Detailed surgical outcomes and pathological analysis results are summarized in *Table 3*.

Discussion

Percutaneous CT-guided lung nodule localization is a widely used approach for the diagnosis and treatment of suspected malignancies due to its simplicity and effectiveness. However, inaccurate localization can lead to repeated scans and puncture attempts (3). To address this issue, a novel robotic-assisted navigation system was developed to enhance localization accuracy and reduce radiation exposure. In this prospective clinical study, robotic-assisted lung nodule localization was successfully performed in 33 patients, leading to the successful resection of all nodules through VATS with oncologically safe margins. This study represents the first clinical validation of robotic-assisted navigation in thoracic surgery.

The median deviation between the localizer and the target point was 6.1 mm, with an impressive first-pass success rate of 100% and no significant complications. In a randomized clinical trial comparing a 3D-printed navigation template with traditional CT-guided needle insertion, the

average deviation in the CT-guided group was 9.6 mm (10), which exceeded the 6.1 mm observed in our robotic-guided group. Furthermore, the conventional CT-guided group had a much higher rate of insertion attempts, with 94% of cases requiring needle readjustments (10), whereas the robotic-guided system achieved a 100% first-pass success rate. This discrepancy can likely be attributed to the fact that the traditional CT-guided method often relies on the experience of the operator, necessitating iterative adjustments in needle depth and angle based on CT images. These frequent adjustments not only lead to patient discomfort but also increase the potential for associated complications.

The accuracy of the robotic-assisted navigation system was not influenced by nodule location, distance to the pleura, chest wall thickness, or patient positioning, demonstrating the stability of the robotic-assisted system. Notably, we observed a positive correlation between nodule density and deviation, indicating that higher-density nodules can be localized more accurately.

During the development process of the robotic-assisted navigation system, several factors influencing nodule localization accuracy were identified. Respiratory motion of the patient was found to be the most prominent factor, as navigation planning is based on a static image acquired during deep inspiration. The position of pulmonary nodules relative to the chest wall can shift with the movement of the diaphragm during respiration, particularly in lower lobe nodules. Failure to account for respiratory motion can result in inadvertent damage to adjacent tissues and organs during needle insertion.

To mitigate the negative impact of respiratory motion on positioning deviation, various measures were implemented in this study. Firstly, patients were trained to perform controlled breathing prior to nodule localization with the aid of a respiratory trainer. This served to ensure that the patient's respiratory motion remained stable during the entire procedure, and to reduce the interference of respiratory motion on positioning deviation. Secondly, dynamic visualization of respiratory motion information was developed to mitigate the adverse effects caused by respiratory motion during the nodule localization procedure. This technique, which uses synchronized respiratory signals to render images of tissue motion, is superior to conventional static methods in terms of interpretability of tissue motion (15). Additionally, ambulatory monitoring of respiration provides appropriate timing for puncture, thereby reducing puncture-related

complications.

In addition, the choice of a suitable marker is a critical consideration in lung nodule localization. Hook-wire localization, while historically prevalent, presents a notable risk of complications such as pneumothorax, wire migration, and even life-threatening air embolism (16). Methylene blue (MB) has been widely employed with good success rates, but its rapid diffusion and poor identification in severely anthracotic lungs (17), as well as its interference with histopathology assessment due to specimen staining, pose significant limitations. In contrast, ICG, a near-infrared (NIR) fluorescent dye, offers superior advantages. NIR imaging with ICG provides high tissue penetration and low autofluorescence, ensuring clear contrast between localized and non-localized areas (18). Furthermore, ICG does not disrupt the surgical field as it remains invisible without the use of an NIR imaging system. Consequently, our preference for ICG as a marker for localization stems from its enhanced visibility, tissue penetration, and compatibility with histopathology, making it a valuable tool in improving the precision and safety of surgical procedures.

Previous studies have demonstrated that the use of a robotic-assisted navigation system is associated with fewer postoperative complications, shorter procedural duration, and reduced radiation exposure (4,19,20). The technique presented in this study enables accurate transmission of CT-derived spatial information for guiding nodule localization, enhancing depth perception and spatial orientation of the target nodule and adjacent anatomical structures. This leads to more precise surgical performance, reducing lung injury resulting from repeated needle insertion attempts. Furthermore, the use of the robotic system simplifies the procedure, minimizing the risk of marker displacement and lung injury due to patient movement. Importantly, CT fluoroscopic guidance is not required throughout the procedure, eliminating additional radiation exposure for both patients and surgeons. Thus, this technique holds potential for simplifying and improving surgical procedures.

A potential limitation of this novel system may be the increased treatment costs. However, these initial costs could potentially translate into savings in terms of complication-related expenses. To comprehensively assess the economic implications and weigh the benefits against the costs, future health economics evidence is warranted to estimate the total medical expenditures associated with the application of this technology. This balanced evaluation of costs and benefits will be important in making informed decisions about the integration of robotic-assisted navigation systems into

clinical practice.

Despite the promising results, several limitations should be acknowledged. The primary limitation of this study is the small sample size, which restricts the generalizability of the findings. For instance, there might be yet insufficient evidence to identify patient populations that would experience the greatest benefit from preoperative percutaneous lung nodule localization with robotic-assisted navigation system. Additionally, the majority of nodules in this study were located within 20 mm of the pleura, and it remains uncertain whether this novel application is equally effective for nodules located deeper beneath the pleura. Moreover, the absence of a control group makes it difficult to draw definitive conclusions regarding the superiority of this technique. Therefore, future randomized clinical trials with larger sample sizes and rigorous designs are warranted to confirm the safety and efficacy of this approach.

Conclusions

In conclusion, our study has demonstrated the safety and feasibility of the novel robot-assisted navigation system for achieving precise percutaneous lung nodule localization. These findings provide a solid foundation for further investigation into the clinical application of this system. As such, a randomized clinical trial with a larger sample size and more rigorous design is currently underway to evaluate the superiority of the robot-assisted navigation system compared to traditional methods. The results of this trial will provide additional valuable insights into the potential benefits of this system and will guide its future implementation in clinical practice.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study received approval from the Institutional Review Board of the First Affiliated Hospital of Guangzhou Medical University [No. EC-2020-093(QX)], and written informed consent was obtained from all participating patients.

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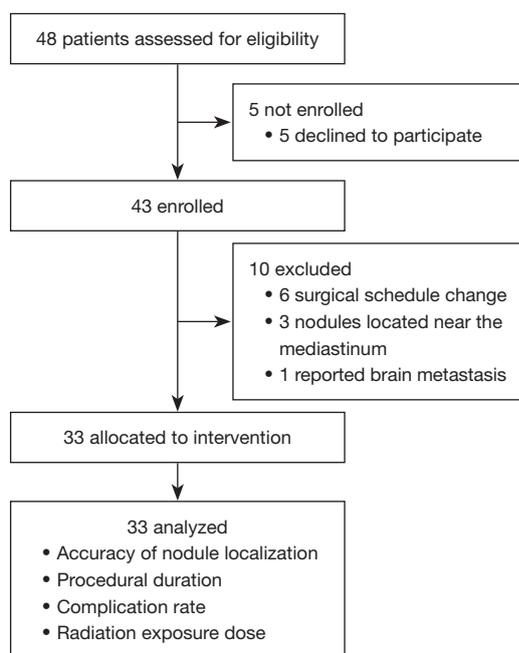


Figure S1 Flowchart of the study participants.