Preoperative electromagnetic navigation bronchoscopy-guided one-stage multiple-dye localization for resection of subsolid nodules: A single-center pilot study

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Abstract
Background: Electromagnetic navigation bronchoscopy (ENB)-guided transbronchial dye marking and video-assisted thoracoscopic surgery (VATS) is an emerging technique that enables successful resection of multiple small subsolid pulmonary nodules. The aim of this study was to evaluate the accuracy and safety of preoperative ENB-guided transbronchial multiple dye localization for VATS resection of subsolid pulmonary nodules.

Methods: As a single-center pilot study, we recruited patients with at least two small or subsolid pulmonary nodules. Multiple-dye localization was performed by intraoperative ENB-guided transbronchial injection of an indigo carmine dye. The patients underwent VATS for sublobar resection immediately after localization. The accuracy of ENB-guided dye marking was checked.

Results: ENB-guided one-stage multiple dye localization was conducted for 18 pulmonary nodules in seven patients between September 2018 and December 2019. The mean diameter of the pulmonary nodules was 9.3 mm (range, 4–18) and the mean distance from the pleura to pulmonary nodule was 6 mm (range, 1–17 mm). ENB-guided transbronchial multiple dye localization was successfully performed in 94.4% (17/18), and the accuracy of ENB-guided dye marking was 88.2% (15/17). When two nodules were not seen in intraoperative fields, anatomical sublobar resection was performed. There was no conversion to thoracotomy and operative mortalities. Among the seven patients, only one patient showed mild intrabronchial bleeding but stopped spontaneously. The changes in lung function after multiple wedge resections (−1.6% to 24.8%) were tolerable level.

Conclusions: ENB-guided one-stage transbronchial dye localization showed accurate and safe intraoperative identification of multiple subsolid pulmonary nodules. A large scale prospective clinical study is warranted.

KEYWORDS
dye localization, electromagnetic navigation bronchoscopy, multiple subsolid pulmonary nodules

INTRODUCTION
The incidence of lung nodules is rising due to the increasing tendency of screening for lung cancer using chest computed tomography (CT). Two large prospective randomized controlled trials, the National Lung Screening Trial (NLST) and NELSON study, showed that early detection of lung cancer by low dose CT (LDCT) screening leads to a significant reduction in mortality from lung cancer and a significant shift in early-stage malignancies at the time of diagnosis.1,2
Thus, there is a growing need for appropriate surgical resection of early-stage lung cancers detected upon screening. Moreover, unlike in the past, adenocarcinoma has been reported to be the most frequent histopathological type of lung cancer.\(^3,^4\) It commonly presents with a subsolid shape on CT scans and sometimes manifests as a multifocal pattern.\(^5,^7\)

Several prior studies showed that video-assisted thoracoscopic surgery (VATS) biopsy for small subsolid nodules is challenging due to their impalpability.\(^8,^9\) Therefore, several techniques have been implemented to localize the subsolid nodules in the operating room. The most widely used technique is CT-guided transthoracic localization with hook wire. However, the technique has a limitation in that localization of multiple lesions is impractical due to the associated complications, such as pneumothorax or hemothorax.\(^10\) Therefore, there is a need for techniques that allow preoperative localization at multiple sites in patients with multiple small or subsolid nodules suspected of early lung cancer.

Nowadays, Electromagnetic navigation bronchoscopy (ENB) is an emerging bronchoscopic technology.\(^11\) It converts the CT image of a tracheobronchial airway tree into a three-dimensional virtual map and uses an electromagnetic tracking system to guide a steerable probe to approach peripheral lung lesions.\(^12\) According to previous studies, promising outcomes have been reported in terms of the accuracy and safety of ENB-guided transbronchial needle aspiration or biopsy.\(^13,^14\) It has also been applied as a useful preoperative localization tool for precise and successful VATS resection by transbronchial dye marking to target lung lesions these days.\(^8,^15\) Furthermore, this technique enables injection of dye to multiple target lesions for preoperative localization with a single procedure; it confers the advantage of allowing the simultaneous surgical resection on multiple lung nodules with a one-stage operation. Nonetheless, most previous studies have reported results for patients with only a single lung nodule, and data on the simultaneous resection of multiple lung nodules using ENB-guided localization are scarce.\(^13,^15\)

In this study, we aimed to validate the accuracy and safety of preoperative ENB-guided transbronchial dye localization for VATS resection of multiple subsolid pulmonary nodules suspected of lung cancer. Furthermore, we also investigated follow-up results regarding changes in lung function after resections of multiple lung nodules.

METHODS

Study population

This study was a retrospective, single-center pilot study on patients aged 18–80 years, who could medically tolerate general anesthesia. We selected patients who had two or more subsolid pulmonary nodules in the ipsilateral lung, and at least one of the nodules was highly suspected of early malignancy. The exclusion criteria in this study were patients who were intolerant of general anesthesia, who had severe cardiopulmonary disease, pregnant women, and patients who did not consent to the study. All participants provided their written informed consent. The Institutional Review Board of the Asan Medical Center approved this study (approval no. 2020-1009).

Preoperative electromagnetic navigation bronchoscopy-guided one-stage multiple-dye localization

The enrolled patients underwent an enhanced chest CT, following the inspiration/expiration protocol, according to the recommendation of the manufacturer (Veran Medical Technologies) to reconstruct a 3D bronchoscopy map before resection. One physician planned preoperative bronchoscopy with the SPiN system and SPiN planning software (Veran Medical Technologies). Primarily, we tried to directly target the nodules. However, if direct access to the target nodules was not possible, such as when they were far from the pleural surface or without a bronchus sign, we performed dye marking to the pleura, which was the nearest site from the target lesions.

The patients underwent general anesthesia and ENB-guided dye marking was performed just before surgical incision in the operating room. Indigo carmine (1 ml) was injected into each target using a working catheter under an electromagnetic guide in a one-stage method; subsequently, 0.9% normal saline (1 ml) was injected into the lumen of the bronchoscopy and in the needle for dye flushing. After sufficient localization, VATS for sublobar resection was immediately conducted for surgical removal of lung nodules and intraoperative evaluation of dye marking (Figure 1). All subjects underwent baseline pulmonary function tests before surgery and postoperative pulmonary function tests 6–12 months after surgery.

Measurement of accuracy and safety of ENB guide dye localization

The primary endpoint in this study was the accuracy of preoperative ENB-guided dye localization. We defined whether the dye localizations were visible or distinguishable on lung surface in intraoperative fields. The secondary endpoints were success rate to reach the target lesions using ENB-guided technique, the safety profile of ENB-guided dye localization, successful resection rate of lung nodules, the extent of resection, pathologic findings of resected lung nodules, and changes in postoperative pulmonary functions compared with baseline values.

Statistical analysis

Data obtained from the participants were used to compare the demographic and baseline values among patients. Descriptive analyses were performed for the primary and secondary endpoints; for the analysis of the difference between a group of successful operations and failed operations, the \(t\)-test was conducted for continuous variables and
Pearson’s chi-square test for categorical variables. All statistical analyses results were considered significant if the p-value was less than 0.05. Statistical analyses were performed using IBM SPSS version 26.0 (IBM Corporation).

RESULTS

Baseline characteristics of the patients and nodules

Between September 2018 and December 2019, a total of seven patients with 18 lesions underwent ENB-guided one-stage dye localization. The baseline characteristics of the patients and nodules are described in Table 1. The ages of the enrolled subjects ranged from 41 to 66 (median age 53 years), and three subjects (42.8%) were male. Spirometry was performed on all subjects, the average values of forced expiratory volume in the first second and forced volume vital capacity were 90.7% and 89.7%, respectively. The number of lung nodules was three in four patients and two in three patients. The mean diameter of the pulmonary nodules was 9.3 mm (range, 4–18) and the mean distance from the pleura to pulmonary nodule was 6 mm (range, 1–17 mm). Of the 18 lung nodules, five (27.8%) were located in the right lung and 13 (72.2%) were located in the left lung. There were seven nodules (38.9%) located in the upper lobes, and 11 nodules (61.1%) in the lower lobes.

FIGURE 1 ENB-guided one-stage multiple dye localization case. (a) A ground-glass opacity (GGO) nodule in the anterior segment of the LUL. (b) A GGO nodule in the lingular division of the LUL. (c) A GGO nodule of LLL superior segment; (a, b, c-1) chest CT image; (a, b, c-2) the navigation screen during the transbronchial approaches; (a, b, c-3) the lung surface in intraoperative fields after transbronchial dye injection using indigo carmine; (a, b, c-4) the resected specimen stained by indigo carmine; (a, b, c-5) the photomicrographs of the resected tissue (X400)
Results of electromagnetic navigation bronchoscopy-guided one-stage multiple-dye localization

The results of ENB-guided one-stage multiple-dye localization are described in Table 2. Of the 18 nodules, 13, three, and two nodules were of ground-glass opacity, solid, and part solid, respectively. We performed localization using indigo carmine in all cases. ENB-guided transbronchial multiple-dye localization was successfully performed in 94.4% (17/18) cases, and there was only one case that failed to approach the target lesion, which was located in the superior segment of the left lower lobe. The
The accuracy of ENB-guided dye marking was 88.2% (15/17). Two nodules were invisible on lung surface in intraoperative fields; anatomical sublobar resections were performed to resect them. Final diagnosis was adenocarcinoma in 12 (66.7%), ameloblastoma in three (16.7%), and benign in three (16.7%) cases. VATS was performed successfully in all patients (100%). While the initial surgical approaches were wedge resections, surgical extents were extended to lobectomy in four nodules and segmentectomy in three nodules, because the results of the intraoperative frozen biopsy were confirmed as invasive cancer.

Safety profile and adverse events of ENB-guided dye localization

There was no conversion to thoracotomy and operative mortalities. Among the seven patients, there were no major complications, including moderate to severe bleeding or pneumothorax during ENB-guided dye localization. Only one patient showed mild intrabronchial bleeding, but it stopped spontaneously.

Changes in postoperative pulmonary function

Table 3 shows the results of the changes between baseline and postoperative pulmonary function. The range of baseline FEV1 was 2.35–3.67 L and the range of postoperative FEV1 was 2.02–3.55 L. FEV1 decreased by 1.6%–24.8% after the surgery and the average loss of FEV1 was 0.30 L (10.7%). The ranges of baseline and postoperative FVC were 2.82–4.75 L and 2.61–4.61 L, respectively.

DISCUSSION

In this retrospective pilot study, we successfully performed ENB-guided one-stage multiple-dye localization in 94.4% (17/18) cases, and the accuracy of dye marking was 88.2% (15/17). No major complications or procedure-related deaths were reported, and only mild intrabronchial bleeding was reported in one case (5.6%). Despite the inclusion of lobectomy or segmentectomy cases, the reductions of postoperative lung functions after multiple wedge resections were at a tolerable level.

The most widely used technique is CT-guided, hookwire localization that was considered successful in 84%–97.6% of cases. Other techniques with CT-guided percutaneous localization, including placement of a microcoil, lipiodol, and injection of dye, had success rates of 93%–100%. Nonetheless, the most common complications of CT-guided percutaneous marking were pneumothorax and intrapulmonary bleeding, and hemothorax and

| Cases | Location of lung nodules | Nodule characteristics | Type of dye used | Localization results | Extent of resection | Pathological finding |
|-------|-------------------------|-----------------------|------------------|---------------------|---------------------|---------------------|
| Case 1 | LLL posterior           | GGO                   | indigo carmine   | Success             | Segmentectomy       | Invasive ADC        |
|       | LUL anterior            | GGO                   |                  |                     | Wedge resection     | AIS                 |
|       | LLL superior            | GGO                   |                  | Inaccessible        | Wedge resection     | AIS                 |
| Case 2 | Lingular superior       | GGO                   | indigo carmine   | Success             | Segmentectomy       | Invasive ADC        |
|       | LLL superior            | GGO                   |                  | Success             | Wedge resection     | AIS                 |
|       | LUL anterior            | GGO                   |                  | Success             | Wedge resection     | AIS                 |
| Case 3 | RLL laterobasal         | Solid                 | indigo carmine   | Success             | Wedge resection     | Ameloblastoma       |
|       | RLL anterobasal         | Solid                 | invisible        | Wedge resection     | Ameloblastoma       |
|       | RLL mediobasal          | Solid                 |                  | Success             | Wedge resection     | Ameloblastoma       |
| Case 4 | Lingular superior       | GGO                   | indigo carmine   | Success             | Wedge resection     | Focal interstitial fibrosis |
|       | LLL anterobasal         | GGO                   |                  | Success             | Wedge resection     | Focal interstitial fibrosis |
| Case 5 | LLL laterobasal         | GGO                   | indigo carmine   | Success             | Lobectomy           | Minimally invasive ADC |
|       | LLL superior            | GGO                   |                  | Success             | Lobectomy           | Invasive ADC        |
|       | LLL posterbasal         | GGO                   |                  | invisible           | Lobectomy           | Minimally invasive ADC |
| Case 6 | RUL anterior            | Part solid            | indigo carmine   | Success             | Lobectomy           | Invasive ADC        |
|       | RLL superior            | GGO                   |                  | Success             | Wedge resection     | Minimally invasive ADC |
| Case 7 | LUL apical              | GGO                   | indigo carmine   | Success             | Wedge resection     | AAH                 |
|       | LUL anterior            | Part solid            |                  | Success             | Segmentectomy       | Invasive ADC        |

Abbreviations: AAH, atypical adenomatous hyperplasia; ADC, adenocarcinoma; AIS, adenocarcinoma in situ; GGO, ground-glass opacity; ICG, indocyanine green; LLL, left lower lobe; LUL, left upper lobe; RLL, right lower lobe; RUL, right upper lobe.
air embolism occurred as rare complications. Some studies with CT-guided hook-wire insertion have described that pneumothorax, intrapulmonary bleeding, and hemothorax were confirmed in 5.9%–38%, 5.9%–27.1%, and 0%–0.6% of cases, respectively.\textsuperscript{10,16,22} Further, the occurrence of systemic complications, the most serious complication, has also been reported.\textsuperscript{24} In the present study, we found a satisfactory success rate, which was comparable to that of CT-guided localization methods. Furthermore, we observed no major complication aside from mild intrabronchial bleeding in only one case. Therefore, our study demonstrates that the ENB-guided transbronchial localization is as successful as CT-guided localization techniques and may be considered superior in terms of safety.

Bowling et al. recently reported that ENB-guided transbronchial dye marking procedure was regarded as sufficient for surgical resection in 91.3% cases in a large scale prospective cohort study.\textsuperscript{15} Cho et al. also used ENB-guided dye markings for 32 lesions in 25 patients to localize pulmonary nodules and achieved an 87.5% success rate.\textsuperscript{24} Meanwhile, ENB-guided transbronchial approach has a relatively low incidence rate of procedure-related complications.\textsuperscript{15,24–26} Most studies have reported no specific complications except mild intrabronchial bleeding; one large scale study of 1000 subjects described the occurrence of pneumothorax, bronchopulmonary hemorrhage, and respiratory failure in 4.9, 2.3, and 0.6% cases, respectively.\textsuperscript{13} Nonetheless, most existing studies on ENB-guided methods have conducted only for one or two pulmonary nodules. In our study, we performed ENB-guided one-stage localizations for two or more lung lesions, and confirmed the safe and successful outcomes of the procedure and surgical resection compared to previous reports.

It is generally known that there is a notable decline in FEV1 immediately after lobectomy, but it shows a gradual improvement over 3–6 months afterwards.\textsuperscript{27,28} Previous studies have reported that the mean FEV1 level decreased by 9%–17% following lobectomy in patients with lung cancer.\textsuperscript{29–31} The concern is that the resection of multiple lung nodules suspected of lung cancer may cause a substantial loss of lung function. To our knowledge, there is a lack of data on changes in lung function after localization and resection of the lung nodules, whereas, several previous studies have reported on the efficacy or safety of the ENB-guided localization technique. In the present study, we found that FEV1 decreased by 1.6%–24.8% after surgery, and the mean percentage of reduction in FEV1 was 10.7%. Despite the inclusion of patients who underwent lobectomy or segmentectomy, the changes in lung function following multiple wedge resections were not significant in our study. In particular, two patients with only multiple wedge resections (Cases 3, 4) showed minimal changes of postoperative FEV1.

Long et al.\textsuperscript{32} performed electromagnetic transthoracic nodule localization for 31 nodules in 30 patients. In that study, 94% of the nodules were successfully localized, and failure was reported in only two cases. Although this percutaneous transthoracic approach shows a good localization accuracy, it is associated with procedural-related complications, such as pneumothorax and bleeding. Meanwhile, the main difference between our study and the research above was in the method of approach to lesions. In our study with transbronchial approaches, there was a limitation that the localization failed in areas that were less accessible using bronchoscopy, such as those located in the superior segment of the left lower lobe (Case 1). Thus, if we apply both transthoracic and transbronchial routes at once, we can expect a better outcome by enabling localization in areas where bronchoscopy is inaccessible and localization of multiple lesions simultaneously with low complication rates.

In the current study, there were three lung lesions in one patient (Case 3) and the pathological results were confirmed as ameloblastoma. Ameloblastoma is an uncommon odontogenic tumor that mainly affects the mandible and maxilla.\textsuperscript{33} Although metastasis of ameloblastoma mainly involves the lung, metastasizing ameloblastomas are extremely rare.\textsuperscript{34,35} In Case 3 in our study, a 43-year-old man was first diagnosed with ameloblastoma in 2009 after mandible cyst resection. In 2018, multiple lung nodules suspected of pulmonary metastatic ameloblastoma were identified in chest CT. Video-assisted thoracoscopic wedge resection was performed through the CT-guided hook-wire localization method. Metastasizing ameloblastoma was finally confirmed in the pathology report. Since there was

| Cases | Baseline value (% of predicted value) | Postoperative value (% of predicted value) | Change of FEV1, L (% of change) |
|-------|------------------------------------|--------------------------------------------|--------------------------------|
|       | FVC, L | FEV1, L | FEV1/FVC | FVC, L | FEV1, L | FEV1/FVC |                                 |
| Case 1 | 2.82 (86) | 2.35 (87) | 83 | 2.61 (80) | 2.02 (75) | 77 | −0.33 (−14.0) |
| Case 2 | 3.34 (92) | 2.79 (96) | 83 | 3.16 (89) | 2.35 (83) | 74 | −0.44 (−15.8) |
| Case 3 | 4.75 (83) | 3.67 (81) | 77 | 4.61 (81) | 3.55 (80) | 77 | −0.12 (−3.3) |
| Case 4 | 3.20 (103) | 2.47 (106) | 77 | 3.13 (102) | 2.43 (105) | 78 | −0.04 (−1.6) |
| Case 5 | 2.99 (90) | 2.78 (98) | 93 | 2.35 (71) | 2.09 (74) | 89 | −0.69 (−24.8) |
| Case 6 | 3.12 (73) | 2.55 (77) | 82 | 2.83 (67) | 2.48 (77) | 88 | −0.07 (−2.7) |
| Case 7 | 4.60 (101) | 3.12 (90) | 68 | 4.49 (98) | 2.70 (78) | 60 | −0.42 (−13.5) |

Abbreviations: FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; L, liter.
no evidence of metastasis involving another area, a metastasectomy was performed for all resectable pulmonary nodules in a multidisciplinary approach. We decided to choose the ENB-guided transbronchial method for preoperative localization, considering that CT-guided percutaneous localization of multiple lung lesions was not possible and might have a high risk of complications; we obtained safe and successful outcomes. In addition, the changes in pulmonary functions after multiple wedge resections were at a tolerable level.

The present study has several limitations. First, because the study was conducted as a single-center, retrospective study with a small sample size of seven patients, the risk of selection bias cannot be excluded. However, our study is worthwhile in that it is a pilot study showing the clinical usefulness of transbronchial one-stage multiple-dye localization using an ENB-guided technique, paving the way for a large prospective clinical trial, and it shows satisfactory outcomes in safety and accuracy. Second, we mainly used the indigo carmine dye alone, which can sometimes be indistinguishable from pleural pigmentation in the operating field; we did not check the efficacy of different combinations of dye markers, such as indocyanine green (ICG), which can potentially overcome these limitations. In one case (Case 7), we performed dye marking using both indigo carmine and ICG, and it showed better visibility and distinguishability. Therefore, further studies are needed to evaluate the effectiveness of various combinations of dye markers and identify the most efficient method. Third, this analysis could not confirm a survival benefit or effect of preventing the lesions from progressing to invasive cancer depending on whether or not each lung nodule was resected. However, given that most atypical adenomatous hyperplasia (AAH) are likely to progress slowly over the years and have a high probability of developing into invasive cancer, surgical resection of all lesions at the first stage can offer the advantage of avoiding an increased risk of postoperative complications associated with aging or post-surgical adhesions. In addition, the fact that this study showed a tolerable result of postoperative lung function following multiple wedge resection also supports the idea that simultaneous resection may be a better treatment option. Lastly, this analysis did not provide data on the long-term prognosis in patients who had a resection of multiple lung nodules. Further research on whether simultaneous resection of all lesions in patients with multiple lung nodules can help to improve the long-term prognosis and survival rate is required.

In conclusion, ENB-guided transbronchial one-stage multiple-dye localization is an efficient and safe tool for guiding sublobar resection of multiple lung nodules. In addition, the postoperative decrease in lung function due to multiple pulmonary nodules was tolerable. A large scale prospective clinical study using ENB-guided one-stage multiple transbronchial dye localization is warranted.

CONFLICT OF INTEREST
Any authors have no competing interests to declare.

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