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A novel fluorescent lung-marking technique using the photodynamic diagnosis endoscope system and vitamin B2

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Key question

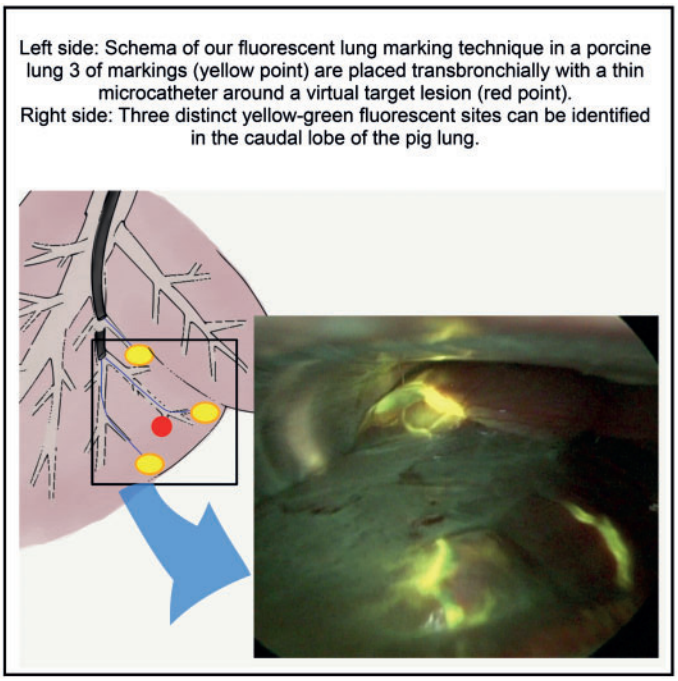
What technique is ideal for locating small, non-palpable lung nodules?

Key finding(s)

Three distinct yellow-green fluorescent sites can help visualize the planned area of pulmonary resection.

Take-home message

Our transbronchial fluorescent lung-marking technique using vitamin B2 can be used to locate small pulmonary nodules.



Abstract

OBJECTIVES: For small pulmonary nodules that are unidentifiable by palpation or in endoscopic surgeries wherein palpation is not feasible, visualizing their location is necessary when performing pulmonary sublobar resection procedures, such as wedge resection or segmentectomy. We invented a new transbronchial lung-marking technique using the photodynamic diagnosis endoscope system and vitamin B2 and examined its feasibility and safety via porcine studies.

METHODS: We established the marking procedure in pigs and examined the marking clarity and size, fluorescence intensity and duration and possible complications. In another study, sublobar resection for virtual target lesions was performed in pigs based on the fluorescent markings. The procedure duration, marking visibility, surgical margin from the lesions and technique-related complications were assessed.

RESULTS: All 36 markings in 6 pigs were identifiable and were widely distributed over the right lung. The median diameter and fluorescence intensity at 60 min after marking were 6.0 (5.5–6.7) mm and 137.5 (122–168), respectively. All 18 markings for the 6 virtual target lesions (3 markings for each target) were clearly identified, and all target lesions were found in the resected specimens. The median

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duration per marking was 244 (194–255) seconds. The shortest median surgical margin from a target lesion was 11.5 (9.3–13.5) mm. No procedure-related complications were observed.

CONCLUSIONS: This novel transbronchial fluorescent lung-marking technique was useful and safe in sublobar resections for small non-palpable pulmonary lesions.

Keywords: Tumour localization • Lung marking • Transbronchial • Fluorescent technique • Vitamin B2

ABBREVIATIONS

CT	Computed tomography
GGO	Ground-glass opacity
MFI	Median fluorescence intensity
PDD	Photodynamic diagnosis
VAL-MAP	Virtual-assisted lung mapping
3D	3-Dimensional

INTRODUCTION

Advances in high-resolution computed tomography (CT) have facilitated small pulmonary nodule detection [1]. Occasionally, sublobar pulmonary resection procedures, such as segmentectomy or wedge resection, can be indicated for these nodules. Accurate nodule identification is required to successfully perform these procedures. However, thoracoscopic surgery has a limitation of requiring palpation for finding nodules. Furthermore, an adenocarcinoma in situ or minimally invasive adenocarcinoma with a ground-glass opacity (GGO) finding on CT cannot be palpated or visualized frequently, even when the lesions are located just beneath the visceral pleura. Therefore, several techniques have been proposed for the localization of such small nodules or GGO lesions [2–6].

However, there is currently no well-established technique available, and the choice of method is dependent on each surgeon's preference. Techniques such as percutaneous CT-guided marking using hook wires [2] and CT-guided lipiodol marking [6] require puncturing of the lung parenchyma. Such puncturing may lead to several complications, including pneumothorax, haemothorax, intrapulmonary haemorrhage and air embolism. Air embolism after percutaneous puncture is extremely rare but can be fatal.

Lung nodule localization is a supplementary and supportive procedure for pulmonary resections; it must be safe and accurate for nodule detection. Accordingly, the following underlying principles are required for an ideal localization procedure: A transbronchial lung-marking technique is desirable to avoid various complications related to lung puncture, the markers must be theoretically non-toxic and these prominent safety features make it possible to perform multiple markings for each pulmonary lesion, allowing more accurate tumour localization.

In this experimental study, we invented a novel fluorescent pulmonary marking technique using the photodynamic diagnosis (PDD) endoscope system and vitamin B2 and evaluated its technical feasibility and safety.

MATERIALS AND METHODS

The study was performed in accordance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and 'Guide for the Care and Use of Laboratory Animals' of Kanazawa University (approval number: AP432941).

Photodynamic diagnosis endoscope system™

In our technique, the key instrument was the PDD endoscope system that contains a powerful light source for fluorescence excitation and a special fluorescence-sensing endoscope. We used the D-Light™ system as the excitation light source and TRICAM™ camera as the fluorescence-sensing endoscope (KARL STORZ GmbH & Co., Tuttlingen, Germany). The D-Light system emits light with a wavelength ranging from 375 to 450 nm. The fluorescence-capable TRICAM camera has a modified wavelength-dependent sensitivity, thereby achieving sensitivity for fluorescence detection greater than that of a standard camera. Additionally, this PDD endoscope system can perform not only fluorescence imaging but also normal light imaging.

Fluorophore

Vitamin B2, a water-soluble vitamin, is theoretically non-allergic and non-toxic [7] and was used as a fluorophore in this lung-marking technique. We previously reported the feasibility and safety of using the fluorescence properties of vitamin B2 for pulmonary sentinel node navigation and pulmonary segment identification [8, 9]. It emits a strong yellow-green fluorescence when exposed to light with a wavelength of ~400 nm. In humans, it undergoes conversion to flavin mononucleotide and flavin adenine dinucleotide via the action of cellular enzymes, with flavin adenine dinucleotide sodium being the major metabolite. Therefore, flavin adenine dinucleotide sodium (Flavitan injection™, Toa Eiyo Ltd, Tokyo, Japan) was used herein.

Bronchoscope and microcatheter

A thin mobile bronchoscope (OLYMPUS MAF TYPE GM™; Olympus Corp., Tokyo, Japan) that can access the peripheral airways and an extremely delicate, soft-tip microcatheter (Estream 2marks™; Toray Medical Co., Ltd, Chiba, Japan), primarily used in cerebrovascular interventional treatments, were used.

Pulmonary marking technique and procedure

After the thin bronchoscope reached the peripheral airways, a microcatheter was inserted through the bronchoscope. Thereafter, the microcatheter tip was positioned just underneath the target lesion on the visceral pleura. Fifty-fold diluted vitamin B2 solution (0.1 ml) was then slowly injected through the catheter (Fig. 1A). A marked lesion was identified as a yellow-green fluorescent spot on the visceral pleura by the PDD endoscope system (Fig. 1B).

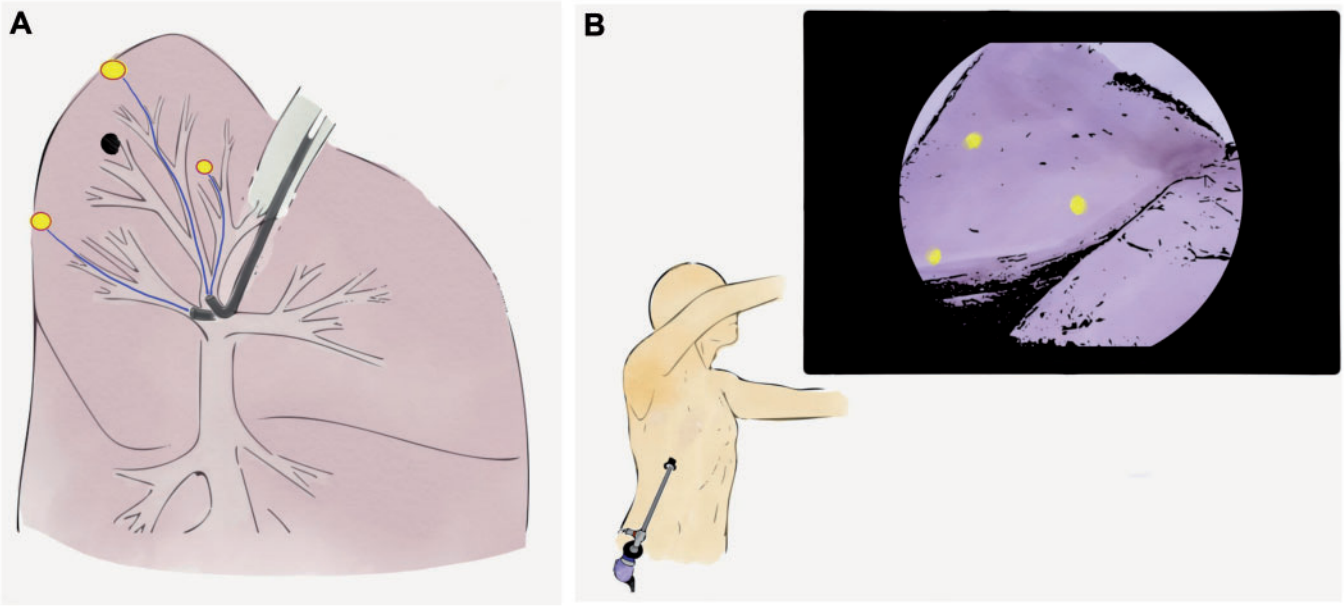

Figure 1: Illustrative schemas of our fluorescent lung-marking technique. (**A**) Three transbronchial lung markings were performed for a target lesion using a thin bronchoscope and soft tip microcatheter. (**B**) Three well-defined fluorescent markings can be identified using the photodynamic diagnosis endoscope system.

Animal care

Adult pigs [median weight 30 (27.5–32) kg] were used and anaesthetized via intramuscular injection of ketamine hydrochloride (10 mg/kg; Sankyo Co., Ltd, Tokyo, Japan). A standard endobronchial tube (24 Fr; Fuji Systems Co., Tokyo, Japan) was introduced via tracheostomy. Maintenance anaesthesia was performed with inhalation of halothane (Takada Pharma Co., Ltd, Osaka, Japan) and intravenous injection of vecuronium (MSD, Tokyo, Japan).

Phase 1 experiments

Under general anaesthesia, the animals were placed in the left lateral decubitus position, and right thoracotomy was performed. Crystal violet was used only to make 1-cm virtual target lesions on the pleural surface of the right lung. The virtual lesions were randomly placed in the cranial and caudal lobes of the pig lung [10]. Those lesions were identified by direct vision, and fluorescent markings were applied around the lesions according to the marking procedure described above. Technically, it is essential to check whether the microcatheter tip is positioned just underneath the pleura of an intended marking site. In phase 1, it can be confirmed with a morphological change in the lung surface, such as a small elevation, because the entire right lung was fully exposed via open thoracotomy. The total number of identifiable fluorescent markings for each virtual target lesion was counted, and the success rate of this marking was calculated as the number of identifiable markings per total number of marking procedures. The diameter and fluorescent intensity of all identifiable markings were measured. The fluorescence intensity was measured using a region of interest analysis software (Hamamatsu Photonics, Hamamatsu, Japan), which analyses the brightness value of the region of interest in images. Adjacent non-fluorescent sites were also measured simultaneously as controls. To evaluate the fluorescent marking duration, we measured the fluorescent intensity of the marked sites every 15 min for an hour after injection. Intraoperative complications accompanying the procedure, such as bronchial and lung parenchymal injuries, airway haemorrhage and respiratory status changes, were evaluated as measures of safety assessment of the procedure.

Phase 2 experiments

Similar to the phase 1 experiments, the pigs were placed in the left lateral decubitus position under general anaesthesia. Under fluoroscopic imaging (ARCADIS Avantic^{TM}; Siemens, Munich, Germany), a virtual target lesion (Fig. 2A; yellow circle) was made via percutaneous injection of 0.5 ml barium sulphate to the right lung without thoracotomy, and the placed target lesion location was analysed 3-dimensionally. Three markings around the target lesion were then placed using our marking technique under fluoroscopic guidance (Fig. 2A–C; white arrow). In phase 2, we carefully observed the shape of the microcatheter tip under fluoroscopy to bring the tip into an appropriate position. Bending or flipping of the tip around the visceral pleura was considered as a typical finding while the microcatheter tip reached the visceral pleura. The catheter was pulled back until the tip was straightened beneath the visceral pleura, and the vitamin B2 solution was then injected. After the placement of the 3 markings, the PDD endoscope system was inserted to the right chest cavity through a 10-mm port to observe the marked lesions. Pulmonary resection for the virtual target lesions was performed using electric cauterization guided by the observed fluorescent markings. Similar to the phase 1 experiments, data on the marking procedure success rate, marking size and fluorescence intensity and duration were collected. Additionally, the time needed for the marking procedure per target and surgical margin from the target lesions in the resected specimens were evaluated. Further, intraoperative airway and lung damage, airway haemorrhage and respiratory status changes were assessed for safety evaluation.

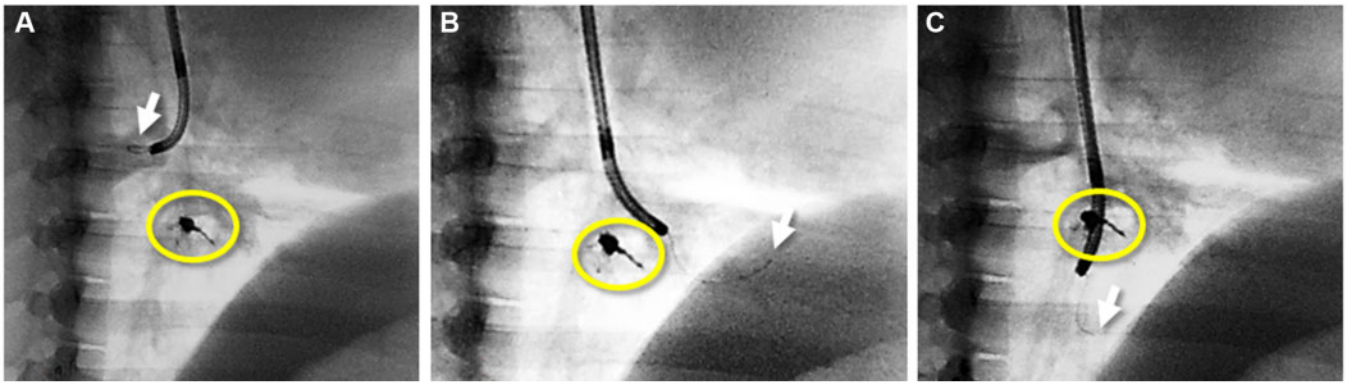

Figure 2: Fluoroscopic findings in the phase 2 experiments. Yellow circle: virtual target lesion made via barium sulphate injection; white arrow: microcatheter tip. (**A**) Flipping of the microcatheter tip around the visceral pleura. (**B**, **C**) Bending of the microcatheter tip around the visceral pleura. These are the typical findings while the microcatheter tip reaches the visceral pleura.

Statistical analysis

All data were expressed as medians with interquartile ranges. Comparative analyses were performed using the Mann–Whitney *U*-test at each time point using the StatMate software (StatMate V for Windows, Version 5, Released 2013; ATMS Co., Ltd, Tokyo, Japan). Statistical significance was considered at *P*-values <0.05 for all analyses. Correction for multiple comparisons was not built into the analyses.

RESULTS

A summary of the data from the phase 1 experiments performed in 6 pigs is shown in Table 1. Two virtual target lesions were placed in each pig: 1 in the cranial lobe of the right lung and the other in the caudal lobe. A total of 36 markings were performed. Each marking was identifiable with a bright yellow-green fluorescence for more than an hour after the marking procedure (Fig. 3A and B). The marking number and location were as follows: 6 in the cranial lobe, 18 in the lateral area of the caudal lobe and 12 in the dorsal area of the caudal lobe; this indicated that the markings could be made anywhere in the entire right lung. There were minimal time-dependent changes in the median marking diameter (mm): 5.6 (5.1–6.2) immediately after injection, 5.4 (5.0–6.3) at 15 min, 5.6 (5.1–6.4) at 30 min, 5.8 (5.3–6.3) at 45 min and 6.0 (5.5–6.7) at 60 min. The median fluorescence intensity (MFI) of the fluorescent sites at 0, 15, 30, 45 and 60 min after injection was 149.5 (135.5–162.3), 145.5 (163–138), 154 (145.3–168), 150 (134–167) and 137.5 (122–168), respectively. Conversely, the MFI of the adjacent non-fluorescent sites was 44 (39.3–50), 42 (40–50), 45 (39.8–52), 45.5 (36–53.3) and 43.5 (37–51.8), respectively. The MFI of all fluorescent sites was significantly higher than that of the adjacent non-fluorescent sites at any time point (Fig. 4A). No obvious complications were observed in both intraoperative and follow-up periods.

A summary of the data from the phase 2 experiments performed in 6 pigs is shown in Table 2. A total of 18 markings for 6 virtual target lesions were performed; all 6 target lesions were then resected with electric cauterization guided by the fluorescent markings. The bright yellow-green fluorescence of all markings was observed using the PDD endoscope (Fig. 3C). The median marking diameter was 6.2 (5.7–6.7) mm immediately after injection and 6.5 (5.3–7.5) mm before pulmonary resection.

Table 1: Phase 1 outcome measures

Number of identified marking	36/36
Marking site	
C	6
L	18
D	12
Size of marking (mm)	
0 min	5.6 (5.1–6.2)
15 min	5.4 (5.0–6.3)
30 min	5.6 (5.1–6.4)
45 min	5.8 (5.3–6.3)
60 min	6.0 (5.5–6.7)

The size of marking is expressed as medians with interquartile ranges.
C: cranial lobe; D: dorsal area of the caudal lobe; L: lateral area of the caudal lobe; 0, 15, 30, 45, 60 min: after injection of vitamin B2.

The median time required for making an individual marking was 244 (194–255) s, and that for the entire marking procedure (3 markings) was 17 (16.3–18) min. All virtual target lesions were found in the resected specimens, demonstrating the effectiveness of the marking procedure. The surgical margin was evaluated as the shortest distance between the target lesion and resection stump in each resected specimen. The median surgical margin in the phase 2 experiments was 11.5 (9.3–13.5) mm. The MFI and safety assessment findings in the phase 2 experiments were found to be similar to those observed in the phase 1 experiments. The MFI of the fluorescent sites was 160 (120–179) at 0 min, 161 (140.5–176) at 15 min, 133 (128–162) at 30 min, 139.5 (124.3–179.8) at 45 min and 168 (148–180) at 60 min after injection. The MFI of the adjacent non-fluorescent sites was 46 (40–50), 44.5 (39.3–48.8), 44.5 (37.8–52), 45.5 (41.3–48) and 45 (41–49.5), respectively. The MFI of all fluorescent sites was significantly higher than that of the adjacent non-fluorescent sites (Fig. 4B). Additionally, no complications related to the marking procedure were observed in both intraoperative and follow-up periods.

DISCUSSION

Herein, we report the development of a new transbronchial fluorescent lung-marking technique using the PDD endoscope system and vitamin B2 as the fluorophore. Furthermore, we

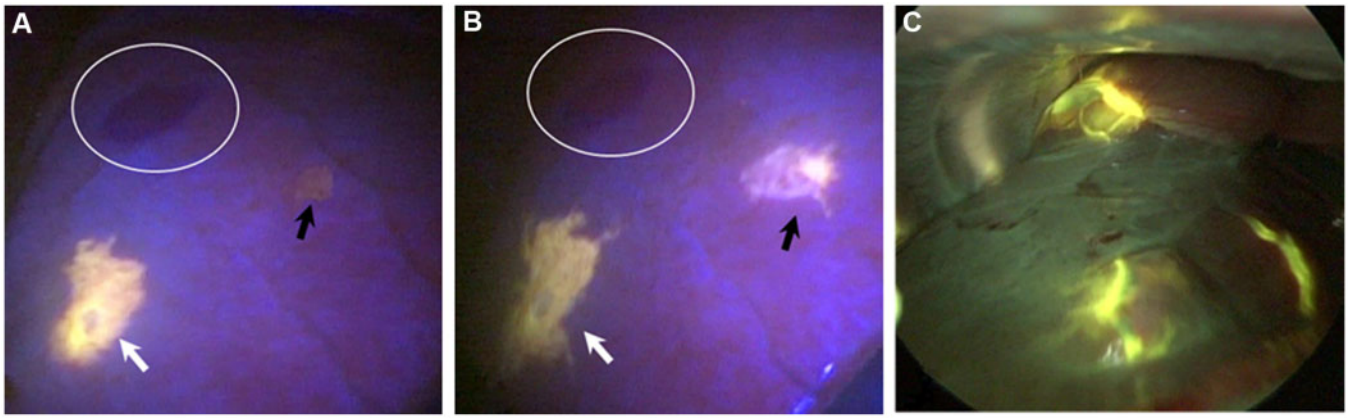

Figure 3: Fluorescent observation of the marking sites using the photodynamic diagnosis endoscope system. (**A**) Immediately after injection in phase 1. (**B**) At 30 min after injection in phase 1. The virtual target lesion painted by crystal violet is observed in the white circle. White arrow: the fluorescent site has minimal time-dependent changes in size and intensity. Black arrow: the fluorescent intensity of the marking site increased after 30 min. (**C**) At 60 min after injection in phase 2. Three distinct yellow-green fluorescent sites can be observed in the caudal lobe of the pig lung.

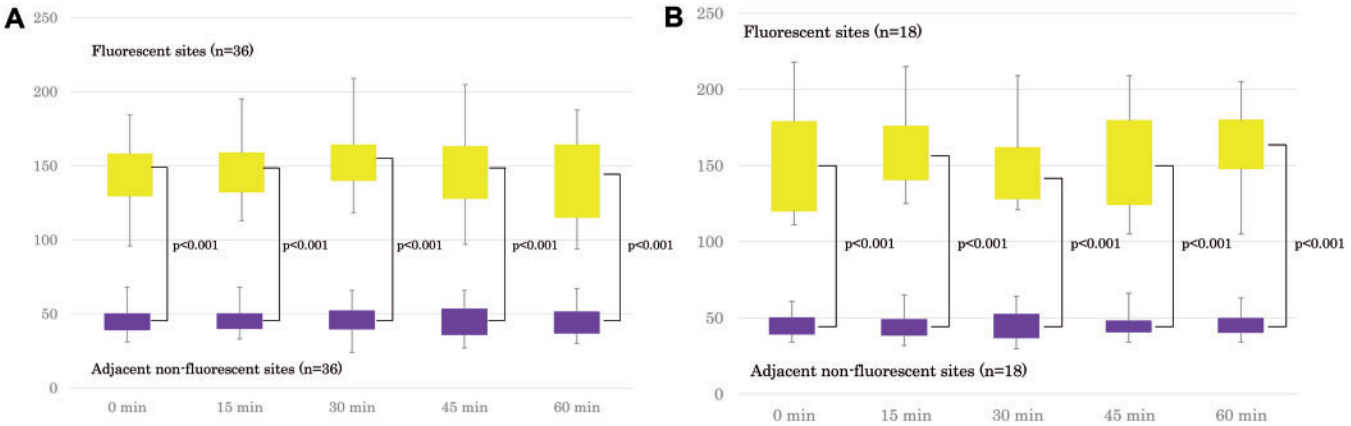

Figure 4: (**A**) Comparison of the fluorescence intensities of the fluorescent markings and unmarked area in the phase 1 experiments. The fluorescence intensities were measured every 15 min up to an hour after injection. (**B**) Comparison of the fluorescence intensities of the fluorescent markings and unmarked area in the phase 2 experiments. The fluorescence intensities were measured every 15 min up to an hour after injection.

Table 2: Phase 2 outcome measures

Number of identified marking	18/18
Marking site	
L	13
D	5
Size of marking (mm)	
0 min	6.2 (5.7–6.7)
60 min	6.5 (5.3–7.5)
Duration of marking (s)	244 (194–255)
Surgical margin (mm)	11.5 (9.3–13.5)

The size and duration of marking and surgical margin are expressed as medians with interquartile ranges.

D: dorsal area of the caudal lobe; L: lateral area of the caudal lobe; 0 min: just after injection of vitamin B2; 60 min: 60 min after injection of vitamin B2.

examined its feasibility and safety via animal experiments using a porcine model. Its safety profile allowed multiple markings for each target lesion, and the markings were clearly identifiable for sufficient duration to perform pulmonary resection. Furthermore, the technique permitted precise resections of virtual target lesions with ideal surgical margins in the resected specimens. Consequently, its distinct advantages over currently used marking methods include its utility in sublobar resections for non-palpable nodules or in total endoscopic approaches that do not allow surgeons to perform palpation.

With the widespread adoption of CT screening and improvement of CT equipment itself, the opportunities for detecting small lung nodules have increased [1]. In some small nodule cases, especially those with GGO components, sublobar pulmonary resection procedures, such as segmentectomy and partial wedge resection, have been indicated instead of pulmonary lobectomy. Successful sublobar resections require accurate lung nodule identification. Intraoperative palpation is generally helpful in nodule detection; however, it is sometimes very challenging for tiny nodules, especially for pure GGO lesions. Additionally, thoracoscopic surgery does not allow finger palpation of nodules. Therefore, an effective technique for pulmonary nodule localization is necessary for such clinical scenarios.

Various nodule localization methods, i.e. lung-marking techniques, have been invented and reported [2–6]. Each of them has strengths and limitations. A commonly used lung-marking method involves placing a marker around a target lesion through a CT-guided percutaneous pulmonary puncture. A metallic wire, such as a hook wire, is one such well-known marker. This

technique is widely used because the markings can be performed using existing CT systems without the need for any specific equipment or technique; the wire materials are generally not expensive; and the procedure itself is comparatively simple. Conversely, this technique requires puncturing of the lung parenchyma, potentially leading to hazardous complications, including pneumothorax and air embolism. Ichinose *et al.* [2] observed complications in 500 hook-wire marking procedures. According to their report, pneumothorax was detected on CT scans in more than half of the cases immediately after the procedure, and 4.6% of those required invasive drainage. The incidence rate of pulmonary haematoma or haemoptysis was 10.3%, and the morbidity rate of the procedure was 15.1%. Although air embolism occurred much less frequently (0.15%) than did other puncture-related complications, it is a potentially serious and fatal complication leading to cerebral or myocardial infarction [11]. A microcoil is another available metallic marker [12]. It works essentially similar to the hook wire, although it is softer and thinner than the wire. Consequently, microcoils may be more suitable for localizing nodules deep inside the lung parenchyma than hook wires. However, they are slightly more expensive. As substitutes for metallic materials, dyes [13], contrast agents [4, 6] and radiotracers [14] have been reported as useful alternative markers. In all of these marking techniques, including markings with metallic materials, potential risks accompanying puncture of the lung parenchyma are theoretically unavoidable. These severe complications associated with supportive procedures, such as lung marking, are not commensurate with those of minimally invasive surgeries, such as thoracoscopic surgery or sublobar resection.

Alternative pulmonary marking techniques are based on bronchoscopic delivery of markers. The transbronchial lung-marking technique does not require puncturing of the pulmonary parenchyma, thereby avoiding potential complications. Several transbronchial lung-marking methods have been reported; procedure-related complications in these methods are indeed reported to be minimal. Essentially, the same markers used for markings with CT-guided lung puncture could also be used in the transbronchial techniques. However, transbronchial marker delivery is technically challenging, and the marking quality is dependent on the patients' bronchial anatomy. Therefore, various navigation tools, e.g. active CT imaging, 3-dimensional (3D)-virtual bronchoscopy and electromagnetic navigation, are used to assist with transbronchial marking [3, 5, 15–19].

Sato *et al.* [3, 19, 20] invented and reported one of the most popular and well-established transbronchial lung-marking techniques, i.e. virtual-assisted lung mapping (VAL-MAP). It entails 2–4 dye markings on the lung parenchyma surface via a bronchoscope prior to sublobar resection toadied by 3D virtual imaging. The efficacy and safety of VAL-MAP have been demonstrated in 100 consecutive cases in a single institution [20] and observed in a multicentre study in Japan [21]. In these reports, 99% of sublobar resections were performed successfully with VAL-MAP.

Ultrasonography or mobile CT using the O-arm imaging system is another tumour localization modality and uses even less invasive approaches. The feasibility and safety of these active intraoperative imaging tools have been described [22, 23]. Although these techniques are free from procedure-related complications, except for a small amount of radiation exposure with mobile CT, the lung nodule identification rate is ~70% with ultrasonography [22] and 88% with mobile CT [23]. These detection rates are not adequate, especially in unhealthy lungs. Kato *et al.* [23] reported that their 3D-CT simulation-based precise anatomical segmentectomy could resect undetectable lesions properly without any lung marking. However, the indication for these techniques should be considered very carefully. As previously mentioned, any complications during tumour localization procedures are not desirable, especially because those complications are avoidable when standard pulmonary lobectomy is performed. Similarly, inefficient tumour localization in sublobar resections is also unacceptable, as it may undermine successful oncologic resections.

Our novel lung-marking method is a bronchoscopic pulmonary marking technique and conceptually similar to VAL-MAP. However, there are several distinct features in our technique in comparison with VAL-MAP. First, our technique uses a fluorescent agent as a marker in contrast to using a common dye (indigo carmine) in VAL-MAP. The bright yellow-green fluorescent marking of vitamin B2 emitted by the PDD endoscope system could be identifiable even in dark lungs in cases of anthracosis or fibrotic lungs with pleural thickening. Approximately 99% of sublobar resections have been successfully performed using VAL-MAP; however, ~10% of markings were unidentifiable. The unidentifiable markings were especially frequent in the lungs with abnormalities, such as anthracosis, emphysema and fibrosis. These challenges associated with imaging in abnormal lungs could be overcome by the strong fluorescence in our technique. Second, vitamin B2 is delivered just underneath the lung surface using a microcatheter with a thin bronchoscope, thereby permitting pinpoint markings without injury to the lung parenchyma. The mean marking diameter was <7 mm, which might be smaller than the markings of VAL-MAP. Regarding complications accompanying the marking procedure, several minor lung parenchymal injuries, including bulla formation or haematoma on the pleural surface, were observed in VAL-MAP. These complications could be related to the spraying of dye through a catheter with air injection. In our method, the use of an ultra-soft microcatheter and extremely slow and gentle injection of a minimum amount of vitamin B2 solution were crucial to avoid injuring the visceral pleura. Consequently, those complications were not observed herein, even in the very fragile pig lungs. Finally, the absolute safety profile of vitamin B2 makes it an ideal marker of choice. Vitamin B2 is theoretically devoid of risks of allergy or overdose. Therefore, it is superior to any other commonly used dyes, especially when used in conjunction with a PDD endoscope.

Limitations

Apart from the above-mentioned advantages of our marking technique, the limitations of our fluorescent technique and this study should also be considered. The study was only based on porcine experiments; therefore, the findings require confirmation in humans. However, it might be optimistic for our marking technique to be applied in human lungs. As previously mentioned, it is crucial to bring the microcatheter tip into an appropriate position around a target lesion. Herein, conventional fluoroscopy was only used for marking guidance owing to the limitation of our animal centre facilities. Conversely, various medical equipment to support endobronchial lung marking is available in human clinical settings. Endobronchial navigation tools, such as virtual bronchoscopy or electromagnetic navigation bronchoscopy, are useful for learning how microcatheters can reach an appropriate position. Further, using a hybrid operating theatre with cone-

beam CT may be an ideal strategy for our marking technique because surgery can be performed immediately after successful marking. Again, we demonstrated that our technique could make good-size markings safely with enough visibility everywhere even in pig lungs, which are anatomically more complex and fragile than human lungs. Therefore, our technique may allow ideal tumour localization in combination with existing useful equipment. Moreover, we have applied the same fluorescence technique to support anatomical pulmonary segmentectomy [15, 16] and introduced it in human clinical practice with satisfactory results.

Another limitation is that only 2-dimensional information could be obtained from the markings made on the lung surface. Naturally, multiple markings can be placed around a target with careful preoperative planning, as in VAL-MAP. Although those markings can depict the ideal resection extent, they cannot express the true target depth. Accordingly, integrating the markings with anatomical pulmonary segmentectomy, which can manage lesions deep inside the lungs, may address the need for 3D information.

CONCLUSION

Our fluorescent lung-marking technique using the PDD endoscope system and vitamin B2 makes accurate tumour localization feasible while offering the best safety profile. Further evaluation of this technique in a clinical comparative trial is now planned.

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Conflict of interest: none declared.

Author contributions

Nobuhiro Tanaka: Data curation; Formal analysis; Investigation; Methodology; Project administration; Visualization; Writing—original draft. **Ryuichi Waseda:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Visualization; Writing—review & editing. **Daisuke Saito:** Data curation. **Masahiro Ohsima:** Data curation. **Isao Matsumoto:** Validation; Writing—review & editing. **Hirofumi Takemura:** Project administration.

Reviewer information

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REFERENCES

[1] Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, Fagerstrom RM *et al.* Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395–409.
[2] Ichinose J, Kohno T, Fujimori S, Harano T, Suzuki S. Efficacy and complications of computed tomography-guided hook wire localization. Ann Thorac Surg 2013;133:753–8.
[3] Sato M, Omasa M, Chen F, Sato T, Sonobe M, Date H *et al.* Use of virtual lung mapping (VAL-MAP), a bronchoscopic multisport dye-marking technique using virtual images, for precise navigation of thoracoscopic sublobar lung resection. J Thorac Cardiovasc Surg 2014;147:1813–19.
[4] Moon SW, Wang YP, Jo KH, Kwack MS, Kim SW, Jang HS *et al.* Fluoroscopy-aided thoracoscopic resection of pulmonary nodule localized with contrast media. Ann Thorac Surg 1999;68:1815–20.
[5] Kojima F, Sato T, Takahata H, Okada M, Sugiura T, Nakamura T *et al.* A novel surgical marking system for small peripheral lung nodules based on radio frequency identification technology: feasibility study in a canine model. J Thorac Cardiovasc Surg 2014;147:1384–9.
[6] Watanabe K, Nomori H, Ohtsuka T, Kaji M, Naruke T, Suemasu K. Usefulness and complications of computed tomography–guided lipiodol marking for fluoroscopy-assisted thoracoscopic resection of small pulmonary nodules: experience with 174 nodules. J Thorac Cardiovasc Surg 2006;132:320–4.
[7] Konings EJ; Committee on Food Nutrition. Water-soluble vitamins. J AOAC Int 2006;89:285–8.
[8] Waseda R, Oda M, Matsumoto I, Takizawa M, Suzuki M, Ohsima M *et al.* A novel fluorescence technique for identification of pulmonary segments by using the photodynamic diagnosis endoscope system: an experimental study in *ex vivo* porcine lung. J Thorac Cardiovasc Surg 2013;146:222–7.
[9] Matsumoto I, Ohta Y, Waseda R, Masaya T, Oda M, Watanabe G. Vitamin B2 as a tracer for intraoperative pulmonary sentinel node navigation surgery. Anticancer Res 2010;30:4109–14.
[10] Nakakuki S. Bronchial tree, lobular division and blood vessels of the pig lung. J Vet Med Sci 1994;56:685–9.
[11] Sakiyama S, Kondo K, Matsuoka H, Yoshida M, Miyoshi T, Yoshida S *et al.* Fatal air embolism during computed tomography–guided pulmonary marking with a hook-type marker. J Thorac Cardiovasc Surg 2003;126:1207–9.
[12] Bommart S, Bourdin A, Marin G, Berthet JP, Pujol JL, Serre I *et al.* Impact of preoperative marking coils on surgical and pathologic management of impalpable lung nodules. Ann Thorac Surg 2014;97:414–18.
[13] Galetta D, Bellomi M, Grana C, Spaggiari L. Radio-guided localization and resection of small or ill-defined pulmonary lesions. Ann Thorac Surg 2015;100:1175–80.
[14] Toba H, Kondo K, Miyoshi T, Kajiura K, Yoshida M, Kawakami Y *et al.* Fluoroscopy-assisted thoracoscopic resection after computed tomography-guided bronchoscopic metallic coil marking for small peripheral pulmonary lesions. Eur J Cardiothorac Surg 2013;44:126–32.
[15] Bolton WD, Howe H III, Stephenson JE. The utility of electromagnetic navigational bronchoscopy as a localization tool for robotic resection of small pulmonary nodules. Ann Thorac Surg 2014;98:471–5.
[16] Awais O, Reidy MR, Mehta K, Bianco V, Gooding WE, Schuchert MJ *et al.* Electromagnetic navigation bronchoscopy-guided dye marking for thoracoscopic resection of pulmonary nodules. Ann Thorac Surg 2016;102:223–9.
[17] Marino KA, Sullivan JL, Weksler B. Electromagnetic navigation bronchoscopy for identifying lung nodules for thoracoscopic resection. Ann Thorac Surg 2016;102:454–7.
[18] Luo K, Lin Y, Lin X, Yu X, Wen J, Xi K *et al.* Localization of peripheral pulmonary lesions to aid surgical resection: a novel approach for electromagnetic navigation bronchoscopic dye marking. Eur J Cardiothorac Surg 2017;52:516–21.
[19] Sato M, Yamada T, Menju T, Aoyama A, Sato T, Chen F *et al.* Virtual-assisted lung mapping: outcome of 100 consecutive cases in a single institute. Eur J Cardiothorac Surg 2015;47:e131–9.
[20] Sato M, Kuwata T, Yamanashi K, Kitamura A, Misawa K, Imashimizu K *et al.* Safety and reproducibility of virtual-assisted lung mapping: a multicentre study in Japan. Eur J Cardiothorac Surg 2017;51:861–8.
[21] Sortini D, Feo C, Maravegias K, Carcoforo P, Pozza E, Liboni A *et al.* Intrathoracoscopic localization techniques. Surg Endosc 2006;20:1341–7.
[22] Ohtaka K, Takahashi Y, Kaga K, Senmaru N, Kotani Y, Matsui Y. Video-assisted thoracoscopic surgery using mobile computed tomography: new method for locating of small lung nodules. J Cardiothorac Surg 2014;9:110.
[23] Kato H, Oizumi H, Suzuki J, Hamada A, Watarai H, Sadahiro M. Thoracoscopic anatomical lung segmentectomy using 3D computed tomography simulation without tumour markings for non-palpable and non-visualized small lung nodules. Interact CardioVasc Thorac Surg 2017;25:434–41.