Localization of Pulmonary Nodules Using Navigation Bronchoscope and a Near-Infrared Fluorescence Thoracoscope

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Background. Video-assisted thoracoscopic wedge resection of multiple small, non-visible, and nonpalpable pulmonary nodules is a clinical challenge. We propose an ultra-minimally invasive technique for localization of pulmonary nodules using the electromagnetic navigation bronchoscope (ENB)-guided transbronchial indocyanine green (ICG) injection and intraoperative fluorescence detection with a near-infrared (NIR) fluorescence thoracoscope.

Methods. Fluorescence properties of ICG topically injected into the lung parenchyma were determined using a resected porcine lung. The combination of ENB-guided ICG injection and NIR fluorescence detection was tested using a live porcine model. An electromagnetic sensor integrated flexible bronchoscope was geometrically registered to the three-dimensional chest computed tomographic image data by way of a real-time electromagnetic tracking system. The ICG mixed with iopamidol was injected into the pulmonary nodules by ENB guidance; ICG fluorescence was visualized by a near-infrared (NIR) thoracoscope.

Results. The ICG existing under 24-mm depth of inflated lung was detectable by the NIR fluorescence thoracoscope. The size of the fluorescence spot made by 0.1 mL of ICG was 10.4 ± 2.2 mm. An ICG or iopamidol spot remained at the injected point of the lung for more than 6 hours in vivo. The ICG fluorescence spot injected into the pulmonary nodule with ENB guidance was identified at the pulmonary nodule with the NIR thoracoscope.

Conclusions. The ENB-guided transbronchial ICG injection and intraoperative NIR thoracoscopic detection is a feasible method to localize multiple pulmonary nodules.

Lung cancer is the leading cause of cancer death in the Western world, accounting for 28% of all cancer deaths, which is more than breast, prostate, and colon. Combined with the application of computed tomography (CT) to lung cancer screening, sub-centimeter pulmonary nodules are frequently detected [1–4]. A recent clinical trial suggested that annual low-dose CT screening increased the incidence of lung cancer cases and reduced mortality from lung cancer, as compared with radiography [5]. Early diagnosis of lung cancer can lead to more treatment options, less invasive surgery, and a higher survival rate.

Because it is difficult to characterize subcentimeter lung nodules with noninvasive imaging techniques alone [3, 6], tissue diagnosis is required for those patients to determine the staging and treatment strategies. A CT-guided fine-needle aspiration or fluoroscopy-guided transbronchial biopsy can occasionally lead to sampling errors and excisional biopsy using video-assisted thoracoscopic surgery (VATS) is performed instead. The VATS procedure has been known to be ideal for nodule resection because it results in minimal lung volume loss and little postoperative morbidity and mortality [7–9]. However, localizing the small sized pulmonary nodules during VATS is challenging when there is no change in visceral pleura [10, 11]. Inadequate nodule localization might lead to a prolonged operative time while searching for the nodule or a conversion to an unplanned open thoracotomy [8, 10, 11].

Preoperative localization techniques have been introduced as a method of improving the success rates of VATS and to prevent unwanted thoracotomy [10, 11]. Micro-coil, hook wire, or colored dye is implanted...
adjacent to the objective pulmonary nodule by a CT-guided percutaneous approach in advance of surgery and is visualized by X-ray fluoroscopy or thoracoscopy during surgery [12, 13]. Percutaneous injection courses pneumothorax which may be minor, but does prevent the repeated procedure in order to localize multiple nodules.

In this article we propose a truly minimally invasive technique that is characterized by non-X-ray infrared fluorescence of indocyanine green (ICG) that is topically injected transbronchially nearby the pulmonary nodules by ENB guidance, without injuring the visceral pleura.

The purpose of the current study is to develop a minimally invasive technique to localize small sized multiple pulmonary nodules to assist video-assisted thoracic surgery. The technique is characterized by the transbronchial ICG injection and intraoperative fluorescence detection by a near-infrared (NIR) thoracoscope.

Material and Methods

In Vitro and Ex Vivo Study

FLUORESCENCE INTENSITY OF ICG IN LUNG TISSUE. Indocyanine green has a binding ability of 98% to plasma proteins; 80% to globulins and 20% to alpha-lipoprotein and albumin [14]. In exciting with 780 to 820 nm of infrared light, ICG emits fluorescence of the peak wavelength of 810 nm in water and approximately 830 nm in blood. The fluorescence of different dilutions of ICG mixed with albumin was imaged and semiquantified by the Maestro imaging system (Cambridge Research & Instrumentation, Inc, Woburn, MA) in vitro. Each 100 mL of ICG and albumin mix was applied in a 96-well plate (Fig 1A), and then the dose-dependent change of the fluorescence intensity was plotted (Fig 1B). The fluorescence level of ICG with or without albumin in lung tissue was examined using ex vivo lung tissue (Fig 1C). The ICG was dropped on the excised porcine lung tissue, in which the fluorescence intensities were measured by the Maestro imaging system (Fig 1C, Da,b).

TISSUE PENETRATION PROPERTY OF ICG FLUORESCENCE DETECTED BY THE NIR FLUORESCENCE THORACOSCOPE. One hundred microliters of ICG (1.5 × 10⁻¹ [mg/mL]) and albumin (2.0 g/dL) mixture, exhibiting high fluorescence intensity (shown in Fig 1); was mixed with 5% agar to make a solid droplet (Fig 2). One millimeter thick sheets of the deflated porcine lung were created by cutting the tissue with Cryotome (Thermo Scientific Cryotome FE/FSE; Thermo Fisher Scientific Inc, Walthman, MA), and overlaid on the droplet. The ICG fluorescence penetrating the lung tissue was captured by the Novadaq SPY scope (Model SC8100; Bonita springs, FL), which has a 10-mm diameter with a 30-cm length in a straight, rigid scope. The optical system has a 0 degree view angle, and a 70 degree field of view, transmitting both visible light and 808 nm ± 5 nm NIR laser. The laser power is classified as class 3R at the tip of the scope as per the International Electro-Technical Commission IEC60825-1. Pulse duration is 17 ms in the maximum setting at 20 pulses per second. We also performed the same sequence of experiments with a D-light P thoracoscope (KARL STORZ GmbH & Co, Tuttinglen, Germany). The actual thickness of the lung sheet in a normally inflated state was estimated by the data obtained from CT images of both inflated and deflated lungs (Lotus Micro CT, General Electric, New York, NY) (Fig 2D).

In Vivo Study

TEMPORAL SPREAD OF ICG IN LUNG PARENCHYMA. Under general anesthesia, ICG was directly injected into the lung parenchyma of live porcine (n = 3) by transthoracic approach, and the animals were kept anesthetized for 6 hours. After euthanasia, the geographical spread of ICG fluorescence in lung parenchyma was examined in the cross sections of the lung.

ELECTROMAGNETIC NAVIGATION BRONCHOSCOPY GUIDED ICG INJECTION AND INTRAOPERATIVE LOCALIZATION. We created a subcentimeter artificial pulmonary nodule as the objective “pulmonary nodule” by topically injecting 0.3 mL of 5% agar containing iopamidol into the lung parenchyma of live porcine (n = 3). The injection of ICG adjacent to the pulmonary nodule was achieved by the prototype electromagnetic navigation bronchoscopy (ENB) system developed by the authors at the University Health Network (Fig 3A-D). A chest CT was taken for each live porcine to obtain a three-dimensional CT map of the thorax using a prototype mobile cone-beam CT (CBCT, PowerMobil; Siemens AG, Munich, Germany) [15-17]. This imaging system demonstrated capability of submillimeter spatial resolution and soft-tissue visibility at low radiation doses (~ 0.35 mSv) [18]. The intraoperative CBCT acquisition completed in 60 seconds with 200 projections over the C-arm orbit (~178 degrees) that encompass field of view 20 × 20 × 15 cm² sufficiency for chest imaging and navigation purposes. An acquired 3D image has an isotropic 0.8 mm³ voxels size and the volume of 256 × 256 × 192.

Electromagnetic devices were used to track the coordinates of the tip of the flexible endoscope [19]. The cylindrical electromagnetic sensor (5-mm long, 0.8-mm diameter) (Aurora, Northern Digital, Waterloo, Ontario, Canada) provides 6 degrees of freedom (x, y, z, pitch, yaw, roll). The sensor was integrated in the tip of the bronchoscope, which was assembled by Olympus Medical Systems, Tokyo, Japan, by modifying the model BF-MP160F (Fig 3B). Bronchoscopic images were registered to the 3D-CBCT images using software developed in-house for image-guided surgical procedures [20, 21]. The software provides image data processing and visualization, tool tracking, and rigid image registration algorithms necessary for the bronchoscopic registration. The software presents orthogonal (axial, coronal, sagittal) views from the perspective of the tracking device plus real and virtual endoscopic views, where the virtual view is based on the camera position defined by the tracking device. Each of these viewing windows can provide for the overlaying of multiple layers of image data with varying degrees of transparency. The bronchoscopic path was manually mapped on the CBCT image by selecting points in the image starting from the tumor location and
proceeding through the bronchus to the trachea. The operator manipulated the bronchoscope looking at both real time white light bronchoscopic image and the navigation image reconstructed using cone-beam CT data at the same time. The mapped path was also displayed on the real and virtual images as a guide to the clinician (Fig 3D).

Using ENB, 0.1 mL of a mixture of ICG and iopamidol (ICG for 1.5 × 10^{-1} mg/mL and iopamidol for 150 mg/mL) was injected by a transbronchial needle aspiration (Olympus Medical Systems) to the pulmonary nodule (Fig 3E). The position of the injected ICG marker should be confirmed by CT scan after ENB injection in order to perform VATS wedge resection considering the relative positional relation of the target and the marker (Fig 3F). After 6 hours the lung was examined by SPY thoracoscope (Fig 3G). The accuracy of the localization technique was examined for the resected lung (Fig 3H).

**Humane Animal Care**

This study was conducted under the approval of the animal care committee at the University Health Network. Humane care was provided throughout all animal experiments according to the 1996 Guide for the Care and Use of Laboratory Animals recommended by the US National Institutes of Health.

**Results**

**In Vitro and Ex Vivo Study**

The ICG exhibited a maximum fluorescence of 830 nm infrared light at a concentration of 1.5 × 10^{-1} (mg/mL) with the presence of 2.0 g/dL of albumin (Figs 1A, 1B). The different concentrations of albumin were mixed to 1.5 × 10^{-1} g/L of ICG in vitro (Fig 1D). The level of...
fluorescence increased with a logarithmic curve (Fig 1D). When ICG was dropped onto the sliced porcine lung tissue (Fig 1C), ICG alone ($1.5 \times 10^{-3}$ mg/mL) showed an increased fluorescence that is equivalent to that of ICG with 2.0 g/dL of albumin (Fig 1D(a)). When ICG was mixed with 2.0 g/dL of albumin beforehand and dropped onto the lung parenchyma the fluorescence intensity was equivalent to that of ICG with 4.0 g/dL of albumin (Fig 1D(b)).

Tissue penetration properties of ICG fluorescence was investigated using the sliced porcine lung captured by either the SPY scope (Figs 2A, 2B) and Storz D-light P system (Fig 2C). The ICG fluorescence was barely detectable when 12.0-mm thick of deflated lung slice was placed on a ICG agar droplet (Figs 2B, 2C). In order to be scattered when passing through the lung tissue, fluorescence was observed larger than the original droplet size. The results of CT scans of both inflated and deflated porcine lung suggested that a 4.84-mm-thick deflated lung was equivalent to 9.93 mm of the inflated lung (Fig 2D). Considering that the thickness of the deflated lung was about half that of the expanded lung, the fluorescence located at 24 mm in lung parenchyma could be detectable when deflated to 12-mm thick.
Fig 3. Electromagnetic navigation bronchoscope (ENB) guided indocyanine green (ICG) injection and intraoperative thoracoscopic detection of ICG fluorescence. (A) A 0.8-mm electromagnetic (EM) sensor. (B) The sensor was integrated in the tip of a thin bronchoscope (red arrow). (1) The accessory channel, (2) light guide, and (3) eye of the bronchoscope. (C) The ENB system displays the positional information of the bronchoscope on the multidimensional computed tomographic (CT) images. (D) Three-dimensional view displays the path to the target (green line) and the position of the bronchoscope (pink line). (E) The real bronchoscopic image shows bronchoscopic needle (yellow asterisk). (F) CT images show the agar target (yellow arrow) and injected ICG/iopamidol mixture (red arrow). (G) SPY thoracoscope examination on 6 hours after ICG injection (WL = white light image; FL = fluorescence image). (H) The cross section of the lung demonstrated that the center of the pulmonary nodule (yellow asterisk) and the center of ICG fluorescence (red asterisk).
In Vivo Study

The size of the ICG fluorescent spot on the surface of the lung was examined by an in vivo porcine model. A 0.1 mL ICG made a fluorescence spot of 10.4 ± 2.2 mm in size in the cross section of the lung and was significantly smaller than a 0.2 mL ICG fluorescence spot of 19.6 ± 6.2 mm. When studying time course changes in the size of the spot of the pleural surface of ICG over time, the surface spot size changed from 6.6 ± 5.7 to 7.8 ± 3.8 (mm) over 6 hours (photograph not shown). The ENB-guided transbronchial ICG and iopamidol injection was performed in 3 porcine models (Fig 3). After a 6-hour interval, the SPY thoracoscope successfully detected ICG fluorescence as a single spot (Fig 3G). The cross-section of the resected lung showed that ICG fluorescence was observed as a single spot overlapping the pulmonary nodule (Fig 3H). The center of the ICG fluorescence spot (the red asterisk) was observed within 3.3 ± 0.95 mm distance from the center of the agar target (yellow asterisk).

Comment

The current study demonstrates that the application of ICG fluorescence dye can be used to localize small-sized pulmonary nodules during minimally invasive thoracic surgery. The excitation of ICG by a class 3 laser light and the deep tissue penetration properties of NIR emission light both contribute to the visualization of the small amount of diluted ICG staying in lung parenchyma. Unlike color dye detection by a white light endoscope, the specific wavelength of ICG fluorescence is always detectable regardless of any changes in color or texture of the visceral pleura, which occurs due to anthracosis or other underlying pulmonary diseases. Another group tested a CT-guided radiotracer (technetium labeled albumin mixed with iodinated contrast medium) injection and detection by a specially designed handheld probe. It is also detectable regardless of the color of lung surface [22], although the radiotracer does not provide real-time image intraoperatively.

The CT-guided percutaneous metallic material implantations methods such as the micro-coil or hook wire may cause pneumothorax, which complicates the localization of multiple nodules. In addition, when the metallic material is misplaced or has migrated, intraoperative X-ray fluoroscopy may be needed to identify and remove the piece of metal. The ENB-guided fiducial placement is often performed to aid stereotactic radiotherapy [23] and can be utilized for VATS localization purposes. The advantage would be the capability to localize multiple points without causing pneumothorax, the durability without spread or dilution after placement, and the ability to confirm accurate placement with aCT scan. However, the fiducial must be removed together with the pulmonary nodules during surgery while liquid markers such as methylene blue, radiotracer, iodine, barium, and ICG do not necessarily have to be removed. Liquid markers can be injected far from the site of the nodule and the surgeon is able to distinguish the location of the target pulmonary nodule by confirming the relative positional relationship between the pulmonary nodule and the liquid marker. This may be important in the ENB-guided transbronchial localization method because ENB-guided injection is limited by the anatomic structure of the bronchi. We demonstrate that the location of ICG and iopamidol can be confirmed by CT scan before surgery.

Thoracoscopic ultrasound can be another method to localize multiple pulmonary nodules [24]. The reported sensitivity of video-assisted thoracoscopic ultrasonography was 93%. However, the lung needs to be completely deflated and an echo probe needs to contact the surface of the lung at all times to obtain a clear ultrasound image.

There are several choices of clinically available ICG fluorescence that can be used for this technique. In our study, D-LIGHT ICG fluorescence endoscope (Karl Storz) showed the comparative sensitivity of ICG fluorescence compared with the SPY scope. Recently, a thoracoscope incorporating the SPY scope technology has been released in the market as PINPOINT endoscopic fluorescence imaging (Novadaq Technologies Inc). With PINPOINT’s ability to simultaneously display the fluorescence image and the white-light image together, the surgeon can recognize the fluorescence at all times during VATS.

For ENB technology, there are some clinically available products such as iLogic (superDimension Inc, Plymouth, MN) and SPiNDrive (Veran Medical Technologies). They were originally developed to increase the accuracy and safety of bronchoscopic biopsy for undiagnosed pulmonary nodules. To avoid the risk of injury to the visceral pleura and pneumothorax, the distance between the tip of the bronchoscopic needle and the visceral pleura should be carefully monitored.

Indocyanine green is approved by the US Food and Drug Administration as an intravenously injectable drug for indications and usage such as determining cardiac output, hepatic function, and liver blood flow, and for ophthalnic and cardiovascular angiography. Indocyanine green also has been used for sentinel lymph node detection of skin melanoma [25–27], breast cancer [28–31], gynecologic carcinoma [32], head and neck carcinoma [33], and lung cancer [34]. The ICG is considered to be safer than the FDA non-approved dye such as indigo carmine [35]. Further, our method requires only 0.1 mL of 16.7 times diluted ICG (1.5 × 10⁻¹ mg/dL), so that it does not adversely affect the pathologic examination.

Our method may be limitedly applied to cases with severe pulmonary emphysema in which bullae replace normal lung tissue. Indocyanine green may be injected into the alveolar space and diffuse more than expected.

The authors gratefully acknowledge Ms Sandra Lafrance for her technical assistance with animal surgical studies, and also Ms Lisa Di Di Diodato, Ms Debora Scollad, and Mr Trevor Do for technical assistance in the animal CT imaging studies.

References


