Methylene Blue Staining: A New Technique for Identifying Intersegmental Planes in Anatomic Segmentectomy

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Background. Pulmonary segmentectomy is being increasingly used to resect small lung nodules; however, identifying the intersegmental plane is difficult. We describe a new methylene blue staining technique that we developed to identify the intersegmental planes in anatomic segmentectomy using video-assisted thoracic surgery (VATS) or thoracotomy and to evaluate its feasibility and safety.

Methods. Between October 2013 and December 2013, 14 consecutive patients with lung disease underwent anatomic segmentectomy at our institution (10 VATS, 4 conventional thoracotomy). Methylene blue 0.1% (20 mL) was slowly injected into the bronchus of the target pulmonary segments using an intravenous needle after division of the artery, vein, and bronchus of the target segments, and the boundaries were detected, followed by anatomic segmentectomy.

Results. The staining took only 3 min. The target pulmonary segments stained blue, allowing for the clear identification of the intersegmental plane on both the surface and in the lung parenchyma, and all operations were successfully completed. Staining did not affect pathologic examination of the resected specimens. The fluid that drained from the chest tube and the patients’ sputum, urine, and feces were not blue. There were no perioperative deaths or major complications.

Conclusions. To our knowledge, this study is the first to report a safe and feasible methylene blue staining method for identifying the lung segment borders that does not require any special equipment. More importantly, this method can clearly detect the intersegmental planes on the pleural surface and within the lung parenchyma, enabling thoracic surgeons to accurately perform anatomic segmentectomy.


With the development and widespread application of new imaging examinations such as computed tomography, small lung nodules can be identified easily. Studies have shown that segmentectomy is the primary method for the treatment of small-sized stage IA lung cancer [1, 2]. For pulmonary nodules with diameters of 2 cm or less, video-assisted thoracic surgery (VATS) segmentectomy has similar survival rates and long-term prognosis compared with lobectomy, but it has the advantages of less trauma and greater preservation of healthy lung tissue [1–3]. Because there is no natural anatomic interface such as an interlobar fissure between the lung segments, identification of the intersegmental plane is one of the most difficult aspects of anatomic segmentectomy. Studies have reported the segment inflation method and intravenous or target segmental bronchus injection of indocyanine green (ICG) to help with identification [4–6], but the imprecise positioning of VATS and the special equipment required along with the complicated manipulations involved in segmentectomy hamper the use of these methods. Therefore, it is necessary to identify a safe and feasible method that will allow precise positioning for identifying the intersegmental plane. We developed a new methylene blue staining technique for intersegmental plane identification. First, we stained each lung segment in vitro to prove the feasibility of this method, and then we performed 14 segmentectomies with VATS or thoracotomy using this method to identify the intersegmental planes. The study was approved by the institutional review board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Informed consent was obtained. The following section details our new staining method.

Material and Methods

Methylene Blue Preparation
Methylene blue (Ji Chuan Pharmaceutical, Jiangsu, China; drug approval number H32024827, China Food and Drug Administration) is commonly used in clinical settings. To prepare the methylene blue, 2 mL of 1% methylene blue was drawn using an ordinary syringe,
and normal saline was added to achieve a final volume of 20 mL, whereby 0.1% methylene blue was obtained. The 50-mL dispensing syringe was obtained from Medical Devices (Zhejiang, China; Executive Standard: YY/T 0821-2010; registration certificate of medical products approval no. 2013 2150061; Zhejiang Food and Drug Administration). The intravenous infusion needle (0.7 × 25 mm) was obtained from Ling Yang Medical Devices (Zhejiang, China; Executive Standard: GB 18671-2002; registration certificate of medical products approval no. 2010 3150911; State Food and Drug Administration).

**Staining of Lung Segments In Vitro**

Sick lungs excised by thoracotomy were stained to evaluate the feasibility of our methylene blue staining method. The artery, vein, and bronchus of the target segments were isolated first. Methylene blue was injected through the segmental artery, vein, or bronchus in different cases, and the results were compared. Injection through the arteries seemed to have the same effect as that through the bronchus, but the arteries were difficult to inject because of their small diameters and soft walls. Injection through the target segmental vein was not a good option, because there were countless venous variations and too many ramus communicans between the segments. Ultimately, the target segmental bronchus was selected for methylene blue injection. A 50-mL syringe was used to inject 20 mL of 0.1% methylene blue into the bronchus; the process was easy and the staining effects were good. Eighteen staining tests of the lung segments (i.e., each segment of both lungs) were included in this experiment.

Pictures were captured every 5 min to evaluate whether the methylene blue would diffuse into the adjacent segments. Observations were made for at least 30 min before lung segment avulsion and the target segmental and adjacent segmental bronchi were used as the stress points to separate them forcibly using tissue forceps and to avulse the segments completely. The segments stained well, and each segment’s boundaries were clearly visible, with no dispersion into the pleural surface or lung parenchyma 30 min after injection (Fig 1). Thus, this methylene blue staining method was subsequently applied in our in vivo study.

**Clinical Data**

Between October and December 2013, 14 patients with lung disease underwent anatomic segmentectomy with VATS or thoracotomy at the Thoracic Department of Tongji Hospital. None of the patients were allergic to methylene blue. Ten patients underwent anatomic segmentectomy via VATS. Segmentectomy regions included 2 left S1 + 2 + 3 segments (lingula-sparing segment of left upper lobe), 1 right S6 segment (the superior segment of the right lower lobe), 1 right S10 segment (posterior basal segment of the right lower lobe), 1 left S7 (medial basal) and S8 (anterior basal) segment, 3 right S2 segments (the posterior segment of the right upper lobe), 1 right S1 segment (the apical segment of the right upper lobe), 1 left S9 (lateral basal) segment, and 1 S10 (posterior basal) segments. The other four patients underwent anatomic pulmonary segmentectomy with thoracotomy, and their resected segments included 1 left S7 (medial basal) and S8 (anterior basal) segment, 2 left lingual segments, and 1 left S3 segment (anterior segment of the left upper lobe). Among the 14 patients (10 male and 4 female), there were 12 cases of small tumors (diameter ≤ 2 cm), 1 case of benign lung disease, and 1 case of bronchiectasis. The patients’ clinical data are shown in Table 1.

**Operative Procedure**

The anesthesia method, body position, and surgical incisions of pulmonary segmentectomy in our study were the same as those of conventional pulmonary lobectomy and posterolateral thoracotomy used in thoracic surgery procedures. Four thoracic ports were used for thoracoscopy: the camera port (10 mm) was located at the seventh intercostal space on the midaxillary line, the ports (20 mm) for the surgeon were located at the third and fifth intercostal spaces on the anterior axillary line, and the port (20 mm) for the surgical assistant was located at the seventh intercostal space on the scapular line. During surgery, the veins, arteries, and bronchus of the target segments were first dissociated. With the use of linear staplers to divide the veins and arteries, the bronchus was then divided after sufficient suction of the bronchial mucus. Thus, the target segment was connected with other lung tissue only through the intersegmental boundaries. In open surgery, the intravenous infusion

![Fig 1. Methylene blue was injected into the segmental bronchus, and pictures were captured every 5 min for comparison. After the injection of methylene blue, the anterior segment of the left upper lobe turned blue, while the adjacent segment remained normal. (A) Five minutes later. (B) Thirty minutes later.](image-url)
The target pulmonary segments stained blue immediately after the injection of methylene blue through the segmental bronchus. Clear boundaries were visible between the blue-colored target pulmonary segments and the adjacent unstained segments. On the pleural surface and within the

Table 1. Patient Characteristics and Target Segments

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Tumor Size (mm)</th>
<th>Target Segment</th>
<th>VATS</th>
<th>Histology</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>Male</td>
<td>15</td>
<td>Left: S1+2+3</td>
<td>Yes</td>
<td>Adenocarcinoma</td>
<td>IA</td>
</tr>
<tr>
<td>2</td>
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<td>Male</td>
<td>10</td>
<td>Left: S7+8</td>
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<td>Squamous cell</td>
<td>IA</td>
</tr>
<tr>
<td>3</td>
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<td>Male</td>
<td>10</td>
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<td>Yes</td>
<td>Adenocarcinoma</td>
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<tr>
<td>4</td>
<td>60</td>
<td>Female</td>
<td>16</td>
<td>Left: S4+5</td>
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<td>Squamous cell</td>
<td>IA</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>Female</td>
<td>15</td>
<td>Left: S4+5</td>
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<td>6</td>
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<td>Male</td>
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<tr>
<td>7</td>
<td>32</td>
<td>Male</td>
<td>18</td>
<td>Right: S7+8</td>
<td>Yes</td>
<td>BOOP</td>
<td>...</td>
</tr>
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<td>8</td>
<td>48</td>
<td>Male</td>
<td>13</td>
<td>Right: S2</td>
<td>Yes</td>
<td>SCLC</td>
<td>IA</td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>Female</td>
<td>15</td>
<td>Right: S2</td>
<td>Yes</td>
<td>Adenocarcinoma</td>
<td>IA</td>
</tr>
<tr>
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<td>16</td>
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</tr>
<tr>
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<td>Left: S1+2+3</td>
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</tr>
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<td>14</td>
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<td>15</td>
<td>Left: S9+10</td>
<td>Yes</td>
<td>Bronchiectasis</td>
<td>...</td>
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</tbody>
</table>

BOOP = bronchiolitis obliterans with organizing pneumonia; S1 = apical; S2 = posterior; S3 = anterior; S4 = superior; S5 = inferior; S6 = superior; S7 = medial basal; S8 = anterior basal; VATS = video-assisted thoracic surgery; SCLC = small cell lung cancer; S9 = lateral basal; S10 = posterior basal;
lung parenchyma as well, these boundaries could be clearly identified (Figs 2B, 2D). The entire staining process was 2–4 min (mean, 3 min). Linear staplers were used to resect the target segment along the boundary. All surgeries were completed successfully in the 10 male and 4 female patients. The patient and tumor characteristics are described in Table 1. The methylene blue staining method had no effect on the pathologic examination of the resected specimen. No perioperative deaths or major complications occurred. No blue fluids or bubbles spilled out of the chest tube. The mean drainage duration was 2.5 days (range, 1–4 days). No pulmonary infection, hemoptysis, blue sputum, blue urine, or blue feces were seen in these patients. The mean hospital stay was 8.5 days (range, 7–10 days).

Comment

Thoracoscopic segmentectomy, a popular new technique in thoracic surgery, is mainly used in the treatment of small pulmonary nodules and benign lung lesions confined to the segment, and its safety and efficacy are well recognized [1, 2]. Thoracoscopic segmentectomy is also a minimally invasive technique that boasts a small surgical incision and resection range; it represents significant advancement in the technology and concepts of minimally invasive lung surgery. For patients with small (≤2 cm) peripheral stage IA non-small cell lung cancer or benign lesions confined to a single segment, segmentectomy is a safe option, and anatomic segmentectomy may become a more prominent procedure for small lung cancer [1, 2, 3]. However, identifying the intersegmental planes is the most critical technical component that widely limits the application of this method.

There is no obvious anatomic boundary between the lung segments; therefore, precise resection of the target segment is difficult. A number of previous methods have been reported for identifying the intersegmental plane [4–8]. However, there are shortcomings to determining the boundary between lung segments. The inflation method has been used; it involves deflating the target segment by clamping its relevant bronchus to create a boundary between the deflated target segment and inflated adjacent segment. The inflation-to-deflation transitional zone is then marked using an electrotome on the lung surface. Okada and colleagues [7, 8] reported a method of intraoperative bronchoscopy involving localization and selective targeting of high-frequency ventilation (40 Hz, 2 kg/cm²). This method could draw a boundary between the deflated normal segment and the inflated target segment. However, both methods have some disadvantages. For example, they can distinguish only the boundary on the pleural surface of the lung instead of the parenchyma; furthermore, they require an anesthetist and intraoperative bronchoscopy to ensure success.

Misaki and colleagues [6] reported the intravenous injection of 3.0 mg/kg ICG after ligation of the dominant pulmonary artery of the target segment, and Sekine and colleagues [5] intraoperatively injected the target bronchus with a fivefold dilution of 20–30 mL ICG. Both methods involved infrared thoracoscopy for identifying the boundaries; however, the former method requires the intravenous injection of ICG, which requires a complex operation, and is not applicable in patients with poor liver function or allergies. These methods also require special dyes and equipment that are not readily available. Kenji Suzuki and colleagues [4] identified the boundaries by slowly injecting ICG into the target bronchus through an intravenous cannula to stain the target segments green. This method is performed in thoracoscopically assisted open surgery, and it requires special dyes. Using ICG to perform segmentectomy does not always create obvious staining boundaries under thoracoscopic observation [5]; methylene blue, however, is suitable for open surgery and can be used easily under thoracoscopic guidance.

The safety of methylene blue is well recognized. It can be used in the human body for sentinel lymph node localization [9, 10], ureter staining [11], and lung lesion localization in thoracic surgery [12, 13]. Sandhya and colleagues [14] suggested that methylene blue could be applied to lymphatic mapping in pregnancy-associated breast cancer with minimal fetal risk.

To our knowledge, we have proved for the first time the feasibility of methylene blue for staining the lungs in vitro. Furthermore, we have demonstrated the successful use of methylene blue in surgery as well. Post-operative observation revealed that neither the drainage fluid from the chest tube nor the patients’ sputum, urine, or feces were stained blue, indicating that methylene blue did not diffuse into the blood. However, even if that did happen, methylene blue would exit the body through urine without being metabolized [14]. The dose of methylene blue was 20 mg or less, which is in the safe range. Before injection, we divided and ligated the veins, arteries, and bronchus of the target segment. In fact, after all these procedures were performed, no immediate connection between the target segments and the body was observed. Thus, injection through the target segmental bronchus was safe and reliable.

Five points are critical for successful methylene blue staining of target segmental bronchi. First, especially for patients with bronchiectasis or lung inflammation, sufficient suction of the sputum before methylene blue injection is important and can prevent incomplete target segment staining caused by sputum blockage. Second, when the intravenous infusion needle is pierced, its strength, depth, and direction should be noted in case penetration of the contralateral bronchial wall or sub-segmental bronchus occurs. Third, the injection time of methylene blue is 1–2 min, and its injection speed should not be too fast, in order to prevent spillover into the surgical field or spreading through the Kohn pores, which would mislead the surgeon into resecting too much functional lung tissue. Fourth, if there is a feeling of resistance, the intravenous infusion needle should be reinserted. Fifth, approximately 20 mL of 0.1% methylene blue is injected. Too small a dose will not completely stain the target segments, whereas too high a dose will make resection and specimen extraction in thoracoscopic surgery difficult. Methylene blue injection into the target segmental bronchus cannot be used in patients with a completely blocked
segmental or subsegmental bronchus. For these patients, injection through the target arteries has the same effect.

The rapid development of VATS lobectomy has widened the indications for and improved the accuracy of segmentectomy, which is a minimally invasive technique that features a small surgical incision and resection range. The most difficult aspect of complete segment resection is determining the boundary between segments, and our new technique of methylene blue staining solves this problem. In vitro and surgical testing both proved that methylene blue staining could be used for accurate identification of the boundaries of the segments in the pleural surface and lung parenchyma. This safe and feasible technique has high efficacy, is easy to perform, and does not require special stains or equipment. Furthermore, it is applicable for the treatment of segmentectomy and is suitable for patients with emphysema or poor liver function. This methylene blue method should enable thoracic surgeons to perform segmentectomy simply and precisely, and its widespread adoption may be worthwhile, especially in developing countries.

References