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***Original Article*** / *Özgün Makale*

# Preclinical study of peripheral parenchymal lymphatic drainage in lung

*Akciğerde periferik parankimal lenfatik drenajın klinik öncesi çalışması*

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***ABSTRACT***

***Background:*** This study aims to determine the regularity of sentinel lymph nodes of peripheral lung parenchyma and the lymphatic drainage between adjacent pulmonary segments in experimental animals.

***Methods:*** Thoracotomy was performed on 12 experimental Guizhou miniature pigs, and 1 mL methylene blue was injected into the superior segment of the lower lobe (S6) and the anterior segment of the upper lobe (S3), successively, to observe lymphatic drainage, in sentinel lymph nodes and the lymphatic drainage between adjacent segments.

***Results:*** A total of 161 lymphatic vessels were observed in 48 pulmonary segments, with an average of 3.4 lymphatic vessels per segment: RS6 (superficial 1.0±0.61, deep 2.5±1.00), RS3 (superficial 1.0±0.51, deep 2.0±1.07), LS6 (superficial 3.0±0.42, deep 1.0±0.38), LS3 (superficial 1.0±0.43, deep 2.0±0.62). There were significantly more lymphatic vessels in deep plexus than in superficial ($p<0.01$). As for sentinel lymph nodes, LS6 drained to the hilar, subcarinal and 4L lymph nodes; RS6 drained to the hilar and subcarinal lymph nodes; LS3 drained to the hilar and 4L lymph nodes; and RS3 drained to the hilar and 4R lymph nodes. In addition, methylene blue could drain from peripheral lung tissue of S3 and S6 directly to mediastinal lymph nodes through superficial plexuses. Lymphatic drainage regularity of S3 and S6 to adjacent pulmonary segments were also observed. The R6 rarely drained to the basal segment, while R3 could possibly drain to the posterior segment.

***Conclusion:*** The regularity of peripheral pulmonary parenchymal lymphatic drainage in experimental animals can provide a basis for the management of lymph nodes in pulmonary segmentectomy in humans, to a certain extent.

***Keywords:*** Experimental animal, preclinical study, pulmonary lymphatic drainage, segmentectomy, sentinel lymph node.

***ÖZ***

***Amaç:*** Bu çalışmada periferik akciğer parenkiminin sentinel lenf nodlarının düzenliliği ve deney hayvanlarında komşu pulmoner segmentler arasındaki lenfatik akıntı belirlendi.

***Çalışma planı:*** On iki deney hayvanı olan minyatür Guizhou domuzlarında torakotomi gerçekleştirildi ve sentinel lenf nodlarındaki lenfatik akıntıyı ve komşu segmentler arasındaki lenfatik akıntıyı gözlemlemek için ardı ardına alt lobun (S6) üst katmanına ve üst lobun (S3) ön katmanına 1 mL'lik metilen mavi enjekte edildi.

***Bulgular:*** Katman başına ortalama 3.4 lenfatik damar olacak şekilde 48 pulmoner katmanda toplam 161 lenfatik damarın olduğu gözlemlendi: RS6 (yüzeysel 1.0±0.61, derin 2.5±1.00), RS3 (yüzeysel 1.0±0.51, derin 2.0±1.07), LS6 (yüzeysel 3.0±0.42, derin 1.0±0.38), LS3 (yüzeysel 1.0±0.43, derin 2.0±0.62). Derin damar ağında yüzeysel olanda bulunandan anlamlı düzeyde daha fazla lenfatik damar vardı ($p<0.01$). Sentinel lenf nodları açısından hilar, subkarinal ve 4L lenf nodlarına LS6 drene olurken, hilar ve subkarinal lenf nodlarına RS6, hilar ve 4L lenf düğümlerine LS3 ve hilar ve 4R lenf düğümlerine RS3 drene oldu. İlave olarak, metilen mavi, S3 ve S6 periferik akciğer dokusundan yüzeysel damar ağları yoluyla doğrudan mediastinal lenf nodlarına drene olabildi. S3 ve S6'nın komşu pulmoner katmanlarına giden lenfatik akıntıda bir düzenliliğinin de bulunduğu gözlendi. R3 muhtemelen arka segmente drene olurken, R6 bazal katmana seyrek olarak drene oldu.

***Sonuç:*** Deney hayvanlarındaki periferik pulmoner parankimal lenfatik akıntı düzenliliği, büyük ölçüde, insanlardaki pulmoner segmentektomideki lenf nodlarının yönetilmesinde bir zemin oluşturabilir.

***Anahtar sözcükler:*** Deney hayvanı, ön klinik çalışması, pulmoner lenfatik akıntı segmentektomi, sentinel lenf nodu.

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Lung cancer is the most common malignancy in the world, with the highest mortality rate.[1] With the popularization of low-dose computed tomography (CT) screening, increasing numbers of early lung cancer have been detected;[2] however, surgery is still the main course of treatment. Although lobectomy has always been the gold standard for surgical treatment of lung cancer, recent studies have shown that in early-stage lung cancer, anatomic pulmonary segmentectomy can achieve the same long-term efficacy as lobectomy, and is conducive to the preservation of postoperative lung function.[3,4]

One of the key techniques of pulmonary segmentectomy is the management of lymph nodes. The clinical practice guidelines of the National Comprehensive Cancer Network (NCCN) for non-small cell lung cancer (NSCLC) lists pulmonary segmentectomy as one of the standard surgical methods for early lung cancer,[5] which can only be selected if there is no lymph node metastasis. However, resection of all hilar- and lobar-related mediastinal lymph nodes for frozen pathological examination not only requires a large amount of work and expense, but is also very difficult in practice. Muraoka et al.[6] proposed that intraoperative lymph node sampling of sentinel lymph nodes (SLNs) could maximize the discovery of metastatic lymph nodes, while reducing the range of lymph node sampling or dissection, the occurrence of complications and the associated cost. Therefore, SLN can be used as an indication for pulmonary segmentectomy.

In addition, whether lymph nodes in the retained segment should be dissected or not has not been determined, which is technically difficult to excise and may lead to injury of bronchi and blood vessels. Therefore, the study of peripheral lymphatic drainage between adjacent segments may provide a theoretical basis for the management of lymph nodes in segmentectomy.

In the present study, pigs were chosen as the experimental animal model for its similarity to humans in anatomy and physiology.[7] Besides, the hilar and mediastinal lymph nodes in pigs are easy to identify. The S3 and S6 were selected in this study, since S3 is associated with a high incidence of lung cancer,[8] and the resection of S6 is common in clinical practice and is technically feasible. We aimed to determine the regularity of SLNs of peripheral lung parenchyma and the lymphatic drainage between adjacent pulmonary segments in a pig model.

## MATERIALS AND METHODS

### Experimental animals

A total of 12 Guizhou miniature pigs (Kexing, China) of both sexes, weighing 35 to 40 kg were included.

### Experimental operation

*Anesthesia*

General anesthesia with a double cavity endotracheal intubation was performed. Anesthesia was induced with 3 mL of Zoletil 50 (Virbac, Carros, France), and maintained with 2% isoflurane inhalation (Hebei Yipin Pharmacy Co., Ltd., Hebei, China). Pigs were orotracheally intubated with a 28F dual-lumen endotracheal tube. Vital signs, such as body temperature, blood pressure, heart rate, electrocardiogram and blood oxygen saturation, were monitored during the operation.

*Operative process*

After thoracotomy, the hilar and mediastinal lymph nodes were explored, and their distribution and color were observed as preoperative controls.

The boundaries of injected segments were determined by segmental bronchial ventilation. One mL of methylene blue (a ratio of 2 mL/20 mg; Sigma, America) was injected into the peripheral lung parenchyma (1 cm subpleural) of S6 and S3, respectively. After the injection, the injection site was ligated with a suture to avoid methylene blue leakage, which could influence subsequent observations. After injection, staining of lymph vessels and lymph nodes was observed. Lymphatic drainage routes were determined by two senior thoracic surgeons synergistically, according to the following criteria:

*a*. *Superficial plexus:* Methylene blue-dyed lymphatic vessels were emitted directly from the injection site, running under the visceral pleura and draining directly to hilar or mediastinal lymph nodes.

*b*. *Deep plexus:* Methylene blue-dyed lymphatic vessels were only visible under the pleura at the root of the lung and then drained to the hilar or mediastinal lymph nodes.

Lymph node nomenclature followed the methods of Riquet et al.[9] and Khullar et al.[10] and Tumor, Node, Metastasis (TNM) staging followed the of 8$^{th}$ staging criteria.[11]

If the staining was insufficient, 1 mL methylene blue was re-injected into the original site. In addition,

30 min were allowed to pass after the first site injection to ensure that the associated lymph nodes were dyed as deeply as possible. During this time, the staining of lymphatic vessels gradually decreased, thereby reducing the interference with the observation of lymphatic drainage at the second injection point.

The chest was closed and indwelling drainage tubes were used, until the same operation was completed on the other side of the chest. Then, a thoracotomy was performed again on both sides of the chest (according to the sequence of injection) to remove the hilar and mediastinal lymph nodes.

**Specimen collection**

*Experimental animal euthanasia*

Pentobarbital was given under deep anesthesia, and potassium chloride (1 to 2 mg/kg, Hushi, Shanghai, China) was given intravenously.

Both lungs were removed and preserved at -20°C for dissection.

*Postoperative pulmonary lymph dissection*

The intersegmental boundary was determined again by segmental bronchial ventilation, thereby confirming the accuracy of the intraoperative intersegmental boundary. Each pulmonary segment was separated along the intersegmental boundary.

*Order of lymph node dissection*

Hilar lymph nodes → interlobar lymph nodes → segmental lymph nodes. The dissection of segmental lymph nodes started from the non-injected segments, in the following sequence: right upper lobe S2 → S1 → S3, left upper lobe S4/5 v S2 → S1 → S3, and lower lobe S7/8/9/10 → S6.

*Lung segment lymphatic drainage*

Judgments were made by two senior thoracic surgeons, respectively. There were no obvious segmental lymph nodes observed in the pigs, but the localized methylene blue-dyed area could be seen (Figure 1), which was clearly separated from the injected area, and there were lymphatic vessels draining to those areas. These areas were identified as lymphoid tissue.

*Specimen preservation*

Lymph nodes and lymphoid tissues were resected and placed on white filter paper to determine whether there were dyed or not. After this was recorded, they were fixed in 10% formalin.

**Statistical analysis**

Statistical analysis was performed using the IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). Student's t-test was used for numerical data and the chi-square test was used for categorical data, and differences were considered statistically significant if $p<0.05$.

## RESULTS

### Gross anatomy

The right lung of pigs consists of four lobes: upper lobe, middle lobe, lower lobe and secondary lobe. The upper lobe bronchus arises directly from the lower part of trachea. The left lung consists of two lobes, the upper lobe and lower lobe. Incomplete fissures are usually observed between the lingual segment (LS4 and LS5) and the proper segment (LS1, LS2 and LS3) of upper lobe. Lobe fissures of the experimental animals were always fully developed.

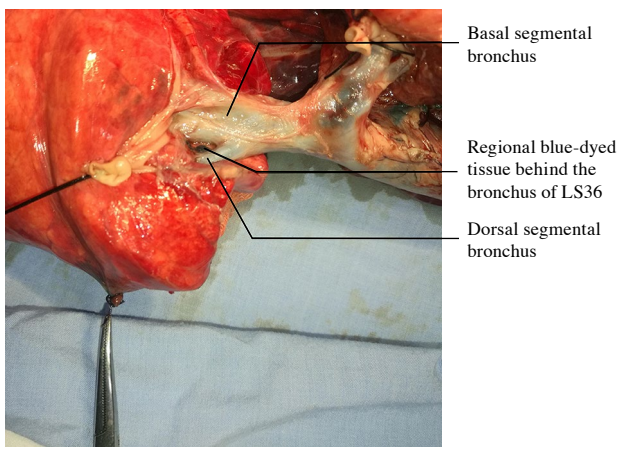


**Figure 1.** Dorsal segment of lower lobe after injection, localized segmental methylene blue-dyed tissue could be observed.

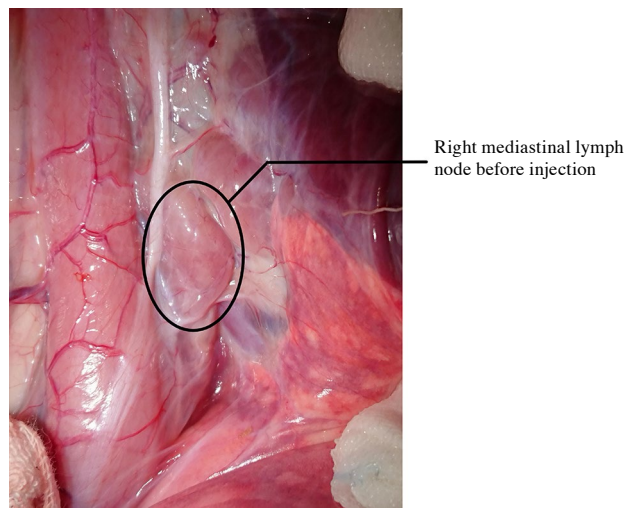


**Figure 2.** Right mediastinal lymph node before injection.

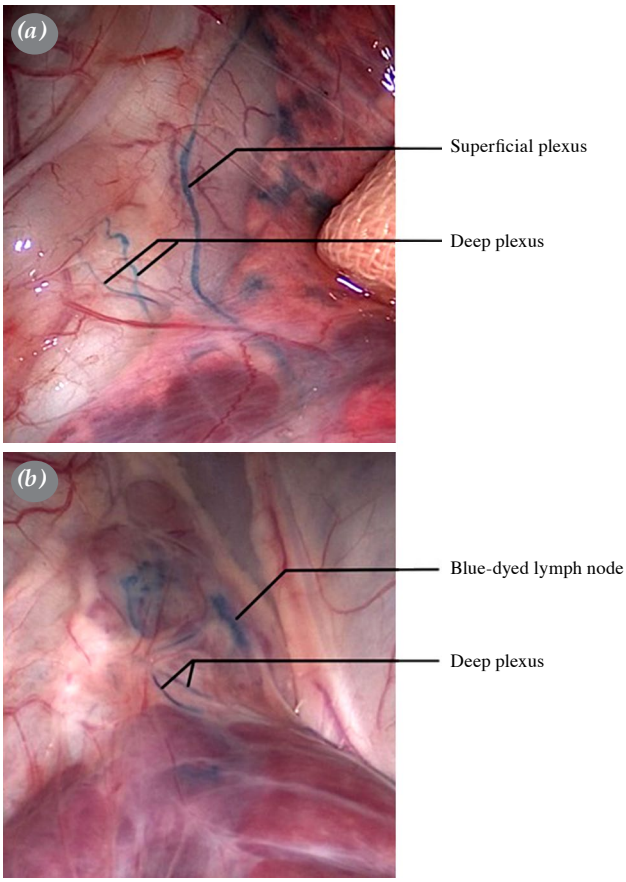


**Figure 3. (a)** Dorsal segment of right lower lobe after injection, both superficial and deep plexus lymphatics can be seen, located behind the hilum of the lung. **(b)** Anterior segment of right upper lobe after injection, methylene blue-dyed lymphatic vessels (above the hilum) can be seen, which drained directly to hilum or 4R lymph nodes.

### Lymphatic system

The intrathoracic lymphatic system was well developed in experimental pigs. Enlarged hilar and mediastinal lymph nodes could be observed, which were pink and obviously contrasted with methylene blue (Figure 2). Direct segmental bronchial ventilation was used during operation to ensure the accuracy of pulmonary segmental boundary, and the results were satisfactory.

### Observed results in the right side of the chest

*Lymphatic drainage of the superior segment of the right lower lobe (RS6)*

Methylene blue-dyed lymphatic vessels of the superficial and deep plexus could be seen after injection in RS6, located behind the hilum of the lung (Figure 3a), draining to hilar or subcarinal lymph nodes, respectively. The lymph nodes gathering those lymphatic vessels were also methylene blue-dyed and the color gradually deepened. No obvious lymph nodes were found at the bronchial root of RS6, but there was a limited methylene blue-dyed area with dyed lymphatic vessels connected to it.

Among 12 animals, there were 12 superficial plexus lymphatic vessels observed in total, which drained to the hilar and 4R lymph node, while no superficial plexus lymphatic vessel was observed in two animals among them. There were a total of 30 deep plexus lymphatic vessels, which drained to subcarinal or other mediastinal lymph nodes, with four deep lymphatic vessels at most in one case (Table 1).

Among 12 animals, lymphatic drainage of deep plexus to the bronchus of RS6 was observed in seven cases, and no lymphatic drainage was observed from RS6 to the bronchi of the medial basal segment (RS7), anterior basal segment (RS8), lateral basal segment (RS9) or posterior basal segment (RS10) (Table 2).

*Lymphatic drainage of the anterior segment of the right upper lobe (RS3)*

Methylene blue-dyed lymphatic vessels (above the pulmonary hilum) could be seen after injection in RS3, which drained directly to the hilum or 4R lymph nodes (Figure 3b). No obvious lymph nodes were observed at the bronchial root of RS3, but there was a localized methylene blue-dyed area with dyed lymphatic vessels connected to it.

**Table 1. Superficial and deep plexus lymphatic vessels of observed pulmonary segments**

| | RS6 (12 cases) | | RS3 (12 cases) | | LS6 (12 cases) | | LS3 (12 cases) | | *Total* (n=48) |
|---|---|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | n | Mean±SD | n | Mean±SD | n |
| Superficial plexus | 12 | 1.0±0.61 | 7 | 1.0±0.51 | 14 | 1.0±0.38 | 12 | 1.0±0.43 | 45 |
| Deep plexus | 30 | 2.5±1.00 | 28 | 2.0±1.07 | 37 | 3.0±0.42 | 21 | 2.0±0.62 | 116 |
| Total | 42 | | 35 | | 51 | | 33 | | 161 |

SD: Standard deviation.

**Table 2. Deep plexus lymphatic drainage of observed pulmonary segments**

| | RS6 | | | LS6 | | | RS3 | | | LS3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experimental animal number | S7/8 | S9/10 | S6 | S7 | S9/10 | S6 | S1 | S2 | S3 | S1 | S2 | S3 | S4/5 |
| No. 1 | - | - | + | - | - | + | - | - | + | - | - | + | - |
| No. 2 | - | - | - | - | - | + | - | - | + | + | - | - | - |
| No. 3 | - | - | - | - | - | - | - | + | - | - | - | + | - |
| No. 4 | - | - | - | - | - | + | + | - | + | - | - | + | - |
| No. 5 | - | - | + | - | - | + | - | + | - | - | + | - | - |
| No. 6 | - | - | + | - | - | + | - | - | + | - | - | + | - |
| No. 7 | - | - | - | - | - | + | - | - | + | - | - | + | - |
| No. 8 | - | - | + | + | - | + | - | + | + | - | - | + | - |
| No. 9 | - | - | + | - | - | - | - | + | - | - | + | + | - |
| No. 10 | - | - | + | - | - | + | - | - | + | - | - | - | - |
| No. 11 | - | - | - | - | - | - | - | - | + | - | - | - | - |
| No. 12 | - | - | + | - | - | + | - | - | - | - | + | + | - |
| *Total* (cases) | 0 | 0 | 7 | 1 | 0 | 9 | 1 | 4 | 8 | 1 | 3 | 8 | 0 |

+ Represents observed; – Represents NOT observed deep plexus lymphatic drainage in the corresponding segment.

**Table 3. Sentinel lymph nodes location**

| | | Hilar SLN | Mediastinal SLN | |
|---|---|---|---|---|
| Injection site | Number of cases | No. 10 lymph nodes | No. 4 lymph nodes | No. 7 lymph nodes |
| RS6 | 12 | 7 | 0 | 5 |
| RS3 | 12 | 1 | 11 | 0 |
| LS6 | 12 | 3 | 2 | 7 |
| LS3 | 12 | 2 | 10 | 0 |
| *Total* | 48 | 13 | 23 | 12 |

SLN: Sentinel lymph nodes.

There were a total of seven superficial plexus lymphatic vessels observed in 12 animals, and seven of the 12 animals had superficial plexus lymphatic vessels observed. Among 12 animals, there were a total of 28 deep plexus lymphatic vessels observed, with five vessels at most in one case (Table 1).

Among 12 cases, eight had deep plexus drainage to the anterior segment (RS3) bronchus, four to the posterior segment (RS2) bronchus, and one to the apical segment (RS1) bronchus (Table 2).

**Observed result in the left side of the chest**

Hilar, subcarinal, 4L and 5 (located below the aortic arch and azygos vein) lymph nodes were observed and were pink in color.

*Lymphatic drainage of the superior segment of the left lower lobe (LS6)*

Methylene blue-dyed lymphatic vessels of superficial and deep plexus could be observed after injection in LS6, located behind the hilum (Figure 4a), which drained to the subcarinal, 4L and 5 lymph nodes, respectively. The lymph nodes gathering those lymphatic vessels were also methylene blue-dyed, and the color gradually deepened. No obvious lymph nodes were found in the bronchial root of LS6, but there was a localized methylene blue-dyed area with dyed lymphatic vessels connected to it.

There were total of 14 superficial lymphatic vessels observed. In total, 37 deep plexus lymphatic

vessels were observed, with five vessels at most in one case (Table 1).

Nine animals had drainage to the bronchi of LS6, and one had drainage to the medioanterior basal segment (LS7+LS8) bronchi. However, no drainage to the lateral basal segment (LS9) bronchi or posterior basal segment (LS10) bronchi was observed (Table 2).

*Lymphatic drainage of the anterior segment of the left upper lobe (LS3)*

Methylene blue-dyed lymphatic vessels could be observed after injection in LS3, located above and behind the pulmonary hilum, draining into the hilar, 4L and 5 lymph nodes, respectively (Figure 4b). Those lymph nodes were also methylene blue-dyed, and the color gradually deepened. No obvious lymph nodes were found at the bronchial root of LS3, but there was a limited methylene blue-dyed area with dyed lymphatic vessels connected to it.

Among 12 cases, there were a total of 12 superficial lymphatic vessels observed, and in one case, superficial vessels were not observed. In addition, 21 deep plexus lymphatic vessels in total were observed, with three vessels at most in one case (Table 1).

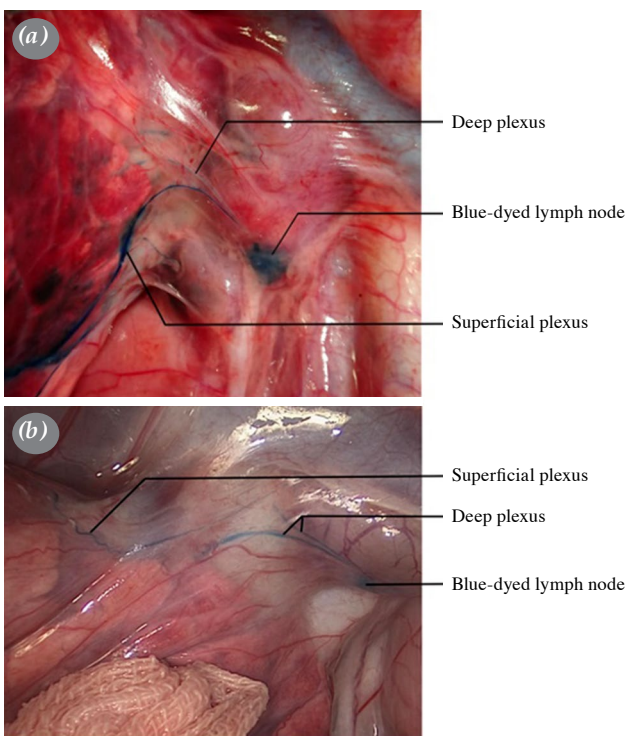


**Figure 4. (a)** Dorsal segment of left lower lobe after injection, both superficial and deep plexus which drained to subcarinal, 4L and No. 5 lymph nodes can be seen locating behind the hilum. **(b)** Anterior segment of left upper lobe after injection, methylene blue-dyed lymphatic vessels can be observed (above and behind the hilum of the lung), which draining to the hilar, 4L and No. 5 lymph nodes.

Lymphatic drainage from the peripheral parenchyma of LS3 to the bronchi of LS3 was observed in eight of 12 animals, and drainage to the apicoposterior segment (LS1+LS2) bronchi was observed in three cases (Table 2).

In four cases in which the operation was started on the left side, the subcarinal lymph nodes were all methylene blue-dyed while performing the thoracotomy on the right side. Among those cases, methylene blue-dyed mediastinal posterior lymph nodes were seen in two cases. No mediastinal lymph node drainage beyond the subcarinal lymph node was observed in the group that received the operation on the right side first (Table 1).

**Comparison of the number of lymphatic vessels in the superficial and deep plexus**

The total number of lymphatic vessels in 48 lung segments, including the superficial plexus and deep plexus, was 161, with an average of 3.4 lymphatic vessels in each lung segment (LS3, LS6, RS3 and RS6).

The RS6 (superficial 1.0±0.61, deep 2.5±1.00), RS3 (superficial 1.0±0.51, deep 2.0±1.07), LS6 (superficial 3.0±0.42, deep 1.0±0.38), LS3 (superficial 1.0±0.43, deep 2.0±0.62) (Table 1). The number of lymphatic vessels in the deep plexus was significantly higher than that in the shallow plexus, indicating a statistically significant difference ($p<0.01$).

**Observed results of SLN**

*SLN of S6*

In RS6, the superficial plexus drained directly to the subcarinal lymph nodes in five cases, and drained to hilar lymph nodes in the other seven cases.

In LS6, the superficial plexus could drain directly to the subcarinal and 4L lymph nodes through the subpleural lymphatic drainage. Of the 12 cases, nine cases drained to mediastinal lymph nodes, including seven cases to subcarinal lymph nodes and two cases to 4L lymph nodes. The superficial plexus in the other three cases first drained to hilar lymph node, and then to subcarinal lymph node.

*SLN of S3*

In LS3, the superficial plexus drained to the 4L lymph nodes directly in 10 cases. In the other two cases, the superficial plexus drained to the hilar lymph nodes first, and then to the mediastinal lymph nodes.

In RS3, the superficial plexus directly drainage to 4R lymph nodes in 11 cases, and drainage to hilum occurred first in one case (Table 3).

## DISCUSSION

The indications for intentional segmental resection according to NCCN guidelines[5] suggest that appropriate N1 and N2 lymph node sampling is required, and pulmonary segmentectomy can only be selected when the frozen pathology of those lymph nodes is negative. However, the dissection and pathological examination of all related N1 and N2 lymph nodes is technically impossible, which increases the cost and chance of injury. Under these circumstances, a detailed study of SLN could help to locate the appropriate N1 and N2 lymph node stations that needed to be dissected.

In the current study, we observed the regularity of SLN drainage of peripheral lung parenchyma of specific segments (S3 and S6). Therefore, according to the SLN theory, the pulmonary segmentectomy of RS6 can be used when subcarinal and hilar lymph nodes be selectively removed and frozen pathological examination results are negative. Similarly, the resection of LS6 can be used when subcarinal, 4L and hilar lymph nodes be selectively removed and pathological examination results are negative. The resection of RS3 or LS3 can be used when 4R or 4L lymph nodes and hilar lymph nodes be selectively removed and pathological examination results are negative.

The application of pulmonary segment-specific SLN technology can not only narrow the range of lymph node sampling or dissection and reduce the incidence of complications and the cost, but it can also maximize the detection of metastatic lymph nodes.[12] Muraoka et al.[6] confirmed that there was no statistically significant difference in the five-year survival rate, local tumor recurrence rate or distant metastasis rate while comparing regional lymph node dissection based on SLN and expanded lymph node dissection, suggesting that SLN may be an indicator for lymph node dissection during pulmonary segmentation resection. Besides, accurate SLN technique enables pathologists to use more sensitive methods, such as immunohistochemistry and PCR, to detect lymph node micro-metastasis, which may reduce the inadequacy of N staging caused by conventional lymph node pathological examination.[13]

In addition to the SLN results, we also found that peripheral lung tissue of S3 and S6 could directly drain to mediastinal lymph nodes through superficial plexuses (e.g., Figure 4a). This phenomenon was frequently observed in this study: in LS6, 9/12 cases; in RS6, 5/12 cases; in LS3, 10/12 cases, and in RS3, 11/12 cases. The high incidence of skip lymphatic drainage through superficial plexuses indicates that the lymphatic drainage through superficial plexuses may be the physiological basis for skip lymphatic metastasis.

The observed results of our study showed that there were significantly more vessels of the deep plexus than of the superficial plexus, suggesting that the peripheral pulmonary parenchymal lymphatic drainage still mainly depends on the deep plexus. However, we also found that peripheral parenchymal lymphatic drainage was able to drain directly to mediastinal lymph nodes through superficial plexus lymphatic vessels. These suggested that although superficial plexus lymphatic vessels are less numerous than deep plexus lymphatic vessels, they may be an important anatomical basis for the skip metastasis of lung cancer.

Although intentional segmental resection is mentioned in the NCCN guidelines,[5] the dissection or sampling of pulmonary segmental lymph nodes of no-tumor located adjacent segments is not required. Nomori et al.[14] found that 29% (12/42) of non-tumor located segments had SLN, while 47% (7/15) of non-tumor located segments had SLN when the tumor was located in S3, which was significantly higher than the rate of 17% (4/24, p=0.04) when the tumor was located in S6. They also found that SLN presented only at the non-neoplastic segments in three cases. Yamanaka et al.[15] studied the metastasis of SLN in 94 cases of small lung cancer and found segmental lymph node metastasis in 11 cases, among which five cases only had lymph node metastasis in the tumor located segment, one case only had lymph node metastasis in the non-tumor located segment and four cases had metastasis in both. Similar to the study by Riquet et al.,[9] no obvious lymph nodes were found between the segments in our study, but there were localized methylene blue-dyed areas at the root of the segmental bronchi with methylene blue-dyed lymphatic vessels connecting to it. These findings suggest that non-tumor located segments have the possibility of metastasizing, while the probability is different among each segment. The dissection of all segmental lymph nodes of adjacent pulmonary segments is also technically difficult, which may increase the risk of injury. Therefore, the study of lymphatic drainage among each pulmonary segment

may help us limit the scope of dissected lymph nodes of adjacent pulmonary segments.

Among the 24 cases of S6 in our study, drainage to the S6 bronchi was found in 16 cases, while drainage to the basal segment (S7, S8, S9 and S10) bronchi was found only in one case (Table 2). These results suggest that routine basal segment lymph node dissection may not be necessary during S6 segmentectomy, while hilar and mediastinal lymph nodes should still be dissected. On the contrary, the observed lymphatic drainage of S3 was different. In addition to drainage to the S3 bronchi, the deep plexus lymphatic vessels of S3 could also drain to the bronchi of S1 and S2, especially to S2 (7/24, Table 2). Therefore, during S3 segmentectomy, S2 bronchial lymph nodes should be dissected for frozen pathology. Briefly, whether the non-tumor segmental lymph nodes should be removed during segmentectomy may vary for different segments. Furthermore, the dissection of lymph nodes in reserved segments may be technically difficult, which may lead to injury of the bronchi and blood vessels. The regulation of pulmonary segmental lymph node drainage needs to be further explored by expanding the sample size, and only by doing so can personalize segmental lymph node management may be implemented.

Nonetheless, there are some limitations to this experiment. First, methylene blue was used to trace lymph nodes, so deep lymphatic drainage of lung tissue could not be clearly observed. Second, the bronchi of the pig's right upper lobe come from the trachea alone, the regularity of lymphatic drainage from the right upper lobe to the hilar and mediastinal lymph nodes may be different from that of human. Third, the sample size of our study was not big enough to show a stronger statistical significance, so it is necessary to expand the sample size for further study.

In conclusion, as a preclinical study, the regularity of lymphatic drainage of peripheral lung parenchyma and the distribution of SLN were preliminarily studied in S3 and S6. As for S3 segmentectomy, 4L or 4R and hilar lymph nodes should be dissected; as for S6 segmentectomy, hilar, subcarinal and 4L lymph nodes should be dissected. The observed result of SLN of S3 and S6 may help to narrow the range of lymph node sampling and guide the clinical practice. In addition, peripheral lung tissue of S3 and S6 can directly drain to mediastinal lymph nodes through superficial plexuses, which may be the physiological basis of skip lymphatic metastasis. Moreover, the basal segmental lymph node dissection may not be necessary during S6 segmentectomy, while S2 bronchial lymph node dissection should be performed during S3 segmentectomy. However, these conclusions need to be further studied by expanding the sample size, which may contribute to personalized segmental lymph node management in clinical practice.

**Ethics Committee Approval:** This study was approval by the Medical Ethics Committee of Beijing Chest Hospital Affiliated to Capital Medical University (2016 Ethical Review of Clinical Trail Recommended Project No. 014).

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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