Feasibility of the Detection of the Sentinel Lymph Node in Peripheral Non-small Cell Lung Cancer With Radioisotopic and Blue Dye Techniques*

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Study objectives: The objective of this study was to evaluate the feasibility of the sentinel lymph node (SLN) biopsy in peripheral clinically stage I or II non-small cell lung cancer (NSCLC) using $^{99m}$Tc colloid and a hand-held gamma detection probe, associated with a blue dye technique.

Design: Prospective study.

Setting: Royal Brompton Hospital, London, UK; and Hôpital Nord, Saint Etienne, France.

Methods: After thoracotomy, a total of 2 mL patent blue dye mixed with 1,600 μCi $^{99m}$Tc-albumin or $^{99m}$Tc-colloid was injected into each quadrant of lung tissue immediately surrounding the tumor. Routine lymphadenectomy was carried out. The first lymph nodes to stain blue or radioactive, if any, were considered SLNs.

Results: Twenty-four patients were evaluated. We successfully identified 17 SLNs in 13 patients (detection rate, 54.2%). Mean time from injection to identification of SLNs was 18 min (range, 5 to 30 min). In nine cases, the SLN was blue and radioactive, in six cases only blue, and in two cases only radioactive. The pathologic status of the SLN reflected the pathologic status of other nodes of the routine lymphadenectomy except one case of false-negative SLN (14%). Four SLNs were in N2 stations (23.5%).

Conclusions: The sentinel node mapping in NSCLC with blue dye and radioisotopic techniques is feasible, but the detection rate has to be improved. This technique is an accurate method of identifying the first node draining a tumor, although it is not yet sufficiently sensitive to have a role in reducing the extent of nodal dissection.

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Key words: intraoperative detection; lung cancer; sentinel lymph node

Abbreviations: ILL = left lower lobe; LUL = left upper lobe; NSCLC = non-small cell lung cancer; RLL = right lower lobe; RUL = right upper lobe; SLN = sentinel lymph node

In developed countries, non-small cell lung cancer (NSCLC) remains the leading cause of cancer mortality.¹ Early stages of disease enjoy favorable survival rates following surgery: 5-year actuarial survival is from 50 to 80% for stage I, from 24 to 45% for stage II, and 22 to 50% for T3N0.²⁻⁵ However, the ranges for 5-year survival are wide, which may in part be explained by variations in the manner and extent of nodal dissection.⁶ It is known that adequate surgical dissection of the regional lymphatics improves treatment results, but how far this lymph node dissection is directly therapeutic is a source of controversy.⁷⁻⁹ Our knowledge of the mechanisms and pathway of lymphatic tumor spread is rather limited and based on statistical evaluation. Different teams have described the percentage of stations involved depending of the location of the primary tumor,¹⁰⁻¹² but what the thoracic surgeon wants to know during the operation is the individual risk of lymphatic spread in order to achieve selective lymphadenectomy. Three potential reasons could explain the high rate of recurrence for T1N0 NSCLC (20 to 50%): first, an inadequacy of the

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lymphadenectomy, in other words the lymph nodes draining the tumor are either not or incompletely removed; second, a pathologic failure in that the appropriate lymph nodes were removed, but routine histologic evaluation failed to unveil microscopic disease; finally, there may be hematologic or pleural dissemination. The sentinel lymph node (SLN) is the first node draining a tumor. As shown for early stage in malignant melanoma and breast cancer, the SLN is the first site affected in cases of metastatic dissemination. Verification of the SLN concept in melanoma has allowed the identification of patients who may benefit from lymphadenectomy, and prevented unnecessary lymphadenectomy in those with no affected SLN. Moreover, when the pathologist focused on a single node, up to 8.4% of the patients had a SLN containing micrometastasis. In future, the SLN technique may be the solution for adequate surgical resection and pathologic evaluation in NSCLC. The intraoperative knowledge of tumor lymphatic drainage could help the surgeon to perform a better lymphadenectomy. Moreover, SLN biopsy allows the pathologist to perform a focused analysis of one or a few lymph nodes with multiple sectioning and, if required, immunohistochemical staining, and this may improve the accuracy of nodal staging. The objective of this prospective study is therefore to evaluate the feasibility of SLN mapping in NSCLC using Tc colloid and a handheld gamma detection probe, associated with blue dye technique.

**Materials and Methods**

The protocol was approved by the Ethics Committee of the Royal Brompton & Harefield NHS Trust/National Heart & Lung Institute, London, UK; and by the Consultative Committee for the Protection of Persons Assisting in Biomedical Research of the Rhône-Alpes-Loire region, France. Twenty-six patients were enrolled in this prospective study after informed and signed consent from October 1, 1999, through April 31, 2002. Inclusion criteria were as follows: >18 years of age, peripheral NSCLC (without bronchosopic evidence of invasion and more distal than the lobar bronchus), clinical stage I or II, and surgically operable and resectable. Exclusion criteria were as follows: legal minority, pregnancy, patient refusal, previous ipsilateral thoracic surgery, and multiple synchronous or metachronous carcinomas of the lung.

Routine preoperative staging was performed, including clinical examination, chest radiography, CT scans of the chest and the upper abdomen, and cervical mediastinoscopy in case of mediastinal enlarged node (>1 cm in the short axis). Two patients required mediastinoscopy for enlarged node in homolateral stations 2 and 4. At thoracotomy, if necessary, a frozen section of the tumor was undertaken to ensure diagnosis, and then a total of 2 mL Patent Blue Dye-V (Laboratoire Guerbet; Roissy, France), mixed with 1,600 µg. 99mTc-albumin (99mTc Nano-colloid; Mallincrodt; Biester, UK) or of 99mTc-sulfur colloid (Nanocs; CIS Bio International; Nancy, France; reduced heating time protocol and filter) was injected in four divided doses into each quadrant of lung tissue immediately surrounding the tumor in a collapsed lung. The first two patients had only patent blue dye injected. The systematic nodal dissection before lung resection as described by Graham et al was carried out immediately after injection. In a previous study in melanoma, preoperative dynamic lymphoscintigraphy correctly identified 90% of the SLNs in <15 min after a peritumoral injection. Dissection was carried out just after the injection because prolonged anesthesia remained a concern to us. SLN mapping by visual and handheld gamma probe guidance (Navigator intraoperative gamma probe; Tyco; Gosport, UK; or Neoprobe 2000; Ethicon Endo-surgery; Issy les Moulineaux, France) was performed every 5 min or 10 min or at each step of the lymphadenectomy. The first lymph nodes to stain blue or radioactive (three times the background) were considered to be the sentinel nodes. The exact station according the lymph node classification of Naruke et al was recorded. Each blue node, if any, was checked with the gamma probe, and the count per second was recorded after removal. We then verified the absence of residual radioactivity; a probe count <1% of the most radioactive lymph node was regarded as background radiation. The SLNs were sent separately for pathologic examination. In the absence of blue coloration, this was recorded and the probe alone was used to detect the SLN, and vice versa. The planned intervention then continued according to standard practice. On the specimen, all lymph nodes (stations 11, 12, 13, and 14) were dissected from the surrounding parenchyma and connective tissue and checked for blue staining with the gamma probe.

The nodes were processed in their entirety, and sections were stained with hematoxylin-eosin. If required, sections of SLN were stained immunohistochemically using epithelial markers: MAF116 (1/80 dilution; Dako; Cambridge, UK) and Ber-EP4 (1/30 dilution; Dako) to confirm or refute involvement by tumor. Tissues obtained during radioguided SLN biopsy contained low residual radioactivity, and pathologic examination was delayed by 48 h. A number of patients in whom one or more SLNs were found was used to calculate the detection rate of the SLN biopsy. The unit of analysis was the patients, not the number of SLNs removed. A false-negative SLN was defined as a negative SLN with other nodes (non-SLN) positive for NSCLC. Sensitivity was calculated by the number of patients with a positive SLN divided by the number of patients with positive SLN plus the number of patients with a negative SLN but positive nodes in the node dissection specimen (false-negative SLN). Specificity was calculated by the number of patients with a positive SLN and negative nodes at resection divided by the number of patients with a negative SLN but negative nodes at resection plus the number of patients with a positive SLN but negative nodes at resection (false-positive SLN).

**Results**

Twenty-six patients with resectable suspected NSCLC were included in this study. Two patients had benign lesions; therefore, 24 patients were eligible for evaluation. Median age was 62.5 years (range, 35 to 80 years). Four patients were women. The tumors were located in the left upper lobe (LUL) [n = 7], left lower lobe (LLL) [n = 8], right upper lobe (RUL) [n = 5], and right lower lobe (RLL) [n = 4]. Preoperative staging of the disease revealed 18 stage I tumors and 6 stage II tumors. A lobectomy was performed in 22 patients, and a
pneumonectomy was performed in 2 patients. The histologic types were squamous cell carcinoma in 11 cases and adenocarcinoma in 13 cases.

The postoperative pathologic staging ranged from stage IA to stage IV. Eleven patients (45.8%) had pathologic stage I disease: T1N0M0 in 4 patients (16.6%) and T2N0M0 in 7 patients (29.1%). Seven patients (29.1%) had stage II disease: T1N1M0 or T2N1M0 in six patients (25%) and T3N0M0 in one patient (4.1%). Five patients (20.8%) had stage IIIA disease (T1N2M0 or T2N2M0). One patient (4.1%) had stage IV disease (T2N0M1). Median tumor size in the larger diameter was 38 mm (range, 11 to 60 mm).

The first two patients had only blue dye injected, while patient 15 had only radiotracer injected. No complications were related to the SLN biopsy; notably, no adverse effects of the tracers (patent blue dye or 99mTc) occurred.

Median time from injection to identification of the 17 SLNs successfully identified in 13 patients was 18.5 min (range, 5 to 30 min). In nine cases, we found the SLNs during the operation, but in four cases it was only identified on assessment of the resected specimen within the operating theater. The locations of the SLNs picked up on the resected specimens were station 12 (n = 3) and station 10 (n = 1). The intrapulmonary location, the background of the tumor, or the airway diffusion of the 99mTc made the intraoperative detection impossible in these cases.

At the least, one SLN was found in 13 patients; the detection rate of the method was 54.2% (Fig 1). We did not find an SLN in 11 cases (45.8%) [Table 1]. For two patients, no lymph node uptake of either dye or 99mTc was seen, but a translobar passage from segment 6 to segment 2 of the blue dye was noticed, and for one patient directly to a metastatic nodule (<1 cm in diameter). For two patients, a blue lymphatic channel was evident in the direction of stations 7 and 2, but the anthracotic nodes hampered a colorimetric identification. In two negative cases, dissection of intense adhesions may have disrupted the lymphatic channels. In four patients, we identified no migration of both tracers, but we did notice blue sputum on bronchial aspiration and a high background of the bronchus. This suggested an airway injection of the tracers.

Of the 17 SLNs identified, 9 were blue and radioactive, 6 were only blue, and 2 were only radioactive (Table 2). In 10 patients (13 SLNs), both tracers were utilized and successful radionuclide migration occurred once without any dye, six times with both, and three times with dye only. Two of 7 patients injected with the 99mTc-nanocolloid (at the beginning of the study) had an SLN identified (28.5%), while 6 of 15 patients injected with the 99mTc-sulfur colloid had an SLN identified (40%).

The false-negative rate of the SLN biopsy was assessed by the presence of a metastatic lymph node below an uninvolved SLN. Histologic examination revealed seven patients with negative SLNs. In one case (patient 8), a blue SLN in station 12 was found negative for metastatic disease, while on the routine lymphadenectomy a single node in station 10 proved to be metastatic. The sensitivity of the method is 75%, and the false-negative SLN rate is 14%. The pathologic results detected six patients with at least one SLN involved (46.1%). In one case, the SLN was the sole metastatic site. Also, for case 15, the SLN was the sole distant metastatic node. The specificity of the method is 67%.

Among the 17 SLNs, 4 were noted to be mediastinal lymph nodes (23.5%). Two were in stations 5 and 6 for two LUL tumors, and two were in station 7 for the same LLL.

Finally, a translobar passage of the blue was noticed in three patients (patients 6, 23, and 24). In one case, the blue lymphatic channel led to a metastatic nodule.

**Discussion**

The SLN concept is based on the presence of orderly and predictable lymphatic drainage pathways. Tumor cell progression within the lymphatic system generally follows a sequential pattern. Lymph nodes are able to retain and to fight tumor cells efficiently. The SLN is defined as the first node draining the tumor and should be the first site affected in case of lymphatic dissemination. Morton and colleagues developed the concept in malignant melanoma through a blue dye injection technique associated with a systematic lymphadenectomy. They
proved that “skip metastases” beyond an uninvolved sentinel node was a rare event (<2%). In a previous study without performing systematic lymphadenectomy in case of negative SLNs in melanoma, we showed that the rate of nodal recurrence developed in the same drainage area for negative SLN patients was 2.7%. If the SLN concept is applicable in NSCLC, a negative SLN biopsy result could eliminate the need for radical lymphadenectomy.

When we began this study, we sought to address the following concerns: (1) Is the SLN technique applicable to a deep malignancy such as the lung cancer? (2) Could the adjunction of a radiotracer increase the sensitivity? (3) Is the SLN concept applicable in NSCLC? One of the goals of this study was to see if SLN techniques were practicable in the clinical setting. We therefore made the decision to start the lymphadenectomy as soon as the tracers had been injected in order to avoid prolonged anesthesia. Our data show that the average migration time of 18 min (range, 5 to 30 min) was compatible with the course of the dissection adding only few minutes to the operation. Liptay et al reported an average migration time of 63 min (range, 23 to 170 min), while Sugi et al reported an average migration time of 135 min (range, 78 to 300 min). Melfi et al waited a mean of 1 h before performing any procedures after the intraoperative injection of the isotope. This technical difference probably contributes to the relatively low detection rate (54.2%) found in our study, compared to the 82% found by Liptay et al, the 64.3% found by Sugi et al, or the 96% found by Melfi et al using only radioisotope SLN mapping.

However, others factors could explain our low detection rate. We did not find any SLNs in 11 patients, and in 4 patients (36%) we noticed an airway diffusion of the two tracers. The site of injection was peritumoral on the edge of the tumor or in the bed of the tumor if a wedge resection had been required for frozen section to confirm diagnosis. This could have facilitated airway injection. Also,

<table>
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<tr>
<th>Case No.</th>
<th>Tracers</th>
<th>Pathologic TNM</th>
<th>Tumor Location</th>
<th>SLN Location</th>
<th>SLNs, No.</th>
<th>Characteristics of SLNs</th>
<th>Pathologic Status of SLNs</th>
<th>Other Stations Involved</th>
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<tbody>
<tr>
<td>6</td>
<td>BD/RI</td>
<td>T2N1</td>
<td>RLL</td>
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<td>2</td>
<td>B/B</td>
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<td>4, 6</td>
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<td>BD/RI</td>
<td>T2N0M1</td>
<td>RLL</td>
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<td>7</td>
<td>BD/RI</td>
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<tr>
<td>16</td>
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<td>T2N2</td>
<td>RUL</td>
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<tr>
<td>20</td>
<td>BD/RI</td>
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<td>23</td>
<td>BD/RI</td>
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*B = blue node; R = radioactive node; BR = blue and radioactive node. See Table 1 for expansion of other abbreviations.
the radioactive background in the bronchus limited the probe detection. Others modalities of injection such as preoperative injection under CT scan control, intraoperative subpleural injection, or preoperative bronchoscopic injection have to be explored in future studies. Sugi and colleagues gained access to the tumor with a preoperative injection under imaging guidance in 14 patients. They reported several pneumothoraces, although they did not require chest drainage. The potential risk of complications such as pneumothorax, bleeding, and pleural tumor seeding does, however, remain a concern.

At the onset of this study, Little et al had already reported a detection rate of 47% using isosulfan blue dye mapping in lung cancer patients. The direct injection of both dye and Tc improves the detection rate. We therefore recommend, as did Schmidt et al, to use both tracers. In our experience, three patients in whom both tracers were injected had only blue SLNs, while only one patient had a non-blue but a radioactive SLN. Moreover, visual inspection found a blue translobar pathway on three occasions, in one case leading to a small metastatic nodule. This finding has not been described before.

We found direct mediastinal lymphatic drainage in four cases (23%), which is consistent with others. The intraoperative sentinel node mapping is an accurate method to identify “skip metastasis,” although in our study routine systematic nodal dissection had removed the stations where the N2 SLNs were located.

Finally, our study provides supportive data for the SLN concept being applicable to lung cancer with a sensitivity in this study of 75% and a false-negative SLN rate of 14%. The sentinel node status is a reliable predictor of the mediastinal nodes histologic status. Sugi et al detected one false-negative SLN in nine patients using the dye technique. The false-negative SLN rate for Little et al and Schmidt et al was 0%, while it was 5% for Liptay et al and 3.8% for Melfi et al.

If the future goal of this method is to avoid a futile mediastinal lymphadenectomy after a negative SLN on frozen section, one major limitation of the technique is its inability to easily detect SLNs located in stations 12 or 13. This required a dissection of the nodes on the specimen because the background of the tumor hampered the handheld gamma probe detection, and unfortunately the blue dye crosses nodes rapidly with the risk of picking up a satellite node rather than sentinel nodes.

In conclusion, intraoperative detection of the SLN with radioisotopic and blue dye techniques in peripheral NSCLC is feasible and safe. Further larger prospective studies are desirable to answer remaining questions about the site of injection, the timing of injection, and the detection methods in order to refine the technique. If SLN technology is to be useful to the surgeon, it should, ideally, allow the tracer to be injected prior to surgery. This would remove any conflict between the time required for migration and the need to avoid prolonged anesthesia. This would also allow the realization of a preoperative lymphoscintigraphy; in the 25% of patients in whom the SLN is identified in a mediastinal station, selective biopsy by mediastinoscopy prior to thoracotomy might allow the use of induction therapy with a possible improvement in prognosis.

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