Thoracoscopic Segmentectomy with Intraoperative Evaluation of Sentinel Nodes for Stage I Non-Small Cell Lung Cancer

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Objectives: Segmentectomy is the treatment of choice for small-sized non-small cell lung cancer (NSCLC); however, it is difficult to decide the surgical procedure because accurate evaluation of hilar lymph node metastasis remains unclear. We here report the outcome of video-assisted thoracic surgery (VATS) segmentectomy with and without the assessment of sentinel nodes.

Materials and Methods: Eighty-three patients with stage IA NSCLC underwent VATS segmentectomy between January 2003 and December 2010. Twenty patients underwent indocyanine green fluorescence imaging for sentinel node biopsy (SNB) and 63 did not. Intraoperative real-time quantitative RT-PCR to determine the expression of CK-19 was used for evaluation of metastasis. Perioperative outcome, local recurrence rates and survival were compared in both groups.

Results: Sentinel lymph nodes were identified in 16 of 20 patients (80%) with segmentectomy in the SNB group. The false negative rate was 0%. By RT-PCR for CK-19 expression, only one of these patients showed positive sentinel nodes, which indicated isolated tumor cells; however, segmentectomy was not converted to lobectomy. Seven of 63 patients with VATS segmentectomy without SNB and none of the SNB group relapsed. In the relapsed patients without SNB, 4 (6.3%) were local recurrences and 3 (4.7%) were distant metastases. Recurrence-free survival rates in both groups were not significantly different because of the short follow-up period of the SNB group.

Conclusions: Our study demonstrated that VATS segmentectomy with SNB was useful for deciding intraoperatively to perform segmentectomy with an accurate lymph node status.

Keywords: lung cancer, segmentectomy, VATS, sentinel node, micrometastasis

Introduction

A randomized trial that compared lobectomy and sublobar resection of the lung was reported in 1995 by the Lung Cancer Study Group (LCSG) and showed that sublobar resection had the potential for local recurrence; subsequently, the standard treatment for stage I non-small cell lung cancer has been lobectomy. However, the LCSG study might have been misleading because a number of wedge resections were included and large tumors over than 3cm were allocated in the sublobar resection group.
As recently reported, sublobar resection showed an equivalent outcome to lobectomy regarding the oncologic effects in small NSCLC, if patient selection for sublobar resection was adequate. Video-assisted thoracic surgery (VATS) has been accepted in a variety of thoracic operations, including small-sized lung cancer. VATS is less invasive and provides benefits due to rapid postoperative recovery and shortening the hospital stay; VATS sublobar resection as definitive management for early stage lung cancer remains controversial and might be associated with increased local recurrence rates. Most reports related to VATS segmentectomy for early stage NSCLC showed no significant difference in the oncologic outcome compared to VATS lobectomy or open segmentectomy; however, intraoperative surgical decision making about the procedure is still uncertain and depends on the evaluation of lymph node metastases in frozen sections. Furthermore, it is difficult to know which lymph nodes should be examined.

The sentinel lymph node concept is that the first lymph node within the lymphatic basin from the tumor would allow the assessment of sentinel nodes that represent the state of the remaining regional nodes. Nomori et al. reported the usefulness of sentinel node navigation surgery for open segmentectomy using intraoperative frozen sections. Sentinel navigation surgery may have the advantage of a more focused lymph node status evaluation. We also reported that the new method of thoracoscopic indocyanine green fluorescence imaging was useful for the identification of sentinel nodes. The purpose of this study was to evaluate the outcome between VATS segmentectomy with and without intraoperative evaluation of sentinel nodes in stage I NSCLC.

Materials and Methods

Patients

In this retrospective study, 83 consecutive stage IA patients underwent surgery at Oita University Hospital (Oita, Japan) between September 2003 and December 2010. VATS segmentectomy with sentinel node biopsy (SNB) was performed in 20 patients and without sentinel node biopsy in 63 patients. Open segmentectomy and conversion from segmentectomy to lobectomy were excluded from this retrospective analysis. VATS segmentectomy with sentinel node biopsy was started from January 2009, and patients with complete informed consents were selected. Patients with severe preoperative comorbidity were selected for wedge resection. Three patients with obstructive lung disease were included in SNB group and twelve in without SNB group. Both procedures were accompanied by systemic radical lymphadenectomy. Principally, all patients underwent surgery with curative intent by VATS. We performed the standard preoperative evaluation and followed the algorithm of operative assessments and procedures, described in a previous report. Clinically, T1 N0 M0 for segmentectomy was eligible in this retrospective analysis, and bi- or trisegmentectomy (multi-segmentectomy) was followed when the segmental margin was less than 1 cm. The assessment of free surgical margin was confirmed by intraoperative measurement of the distance from the tumor to the segmental margin.

Postoperative pathological findings were made by two pathologists, and the final staging and histology were evaluated according to the UICC TNM classification. Postoperative follow-up was practiced every 3 months by blood tests, chest x-ray, and CT scan at 6-month intervals. If we were to find any relapse, patients would undergo subsequent radiotherapy and/or chemotherapy. Re-operation was one of the options to treat local recurrence. All patients with stage IB were treated by UFT (tegafur and Uracil) for two years.

Surgical technique

Our surgical technique is similar to a previous report; if necessary, a utility access port of 3-4 cm in length was added to the 4 ports. All procedures were performed by visualization through a monitor, so-called complete VATS or total thoracoscopy. The intrathoracic procedure regarding hilar and intralobar vessel treatment was the same in segmentectomy with or without sentinel node biopsy; however, the difference was segmental plane management by electrocautery or a stapler. Theoretically, the segmental artery is divided and ligated or stapled first, following pulmonary vein ligation, stapling or bronchus stapling. If the intraoperative examination of hilar or intralobar lymph nodes using frozen sections showed metastases, segmentectomy was converted to lobectomy. Both procedures were followed by systemic mediastinal lymph node dissection.

Sentinel node biopsy

Our technique for the identification of sentinel nodes was previously reported, and an indocyanine green (ICG) fluorescence imaging system (Olympus, Tokyo, Japan) was applied. Two milliliters (5 mg/ml) of ICG was injected around the tumor using 25 gauge needles and
sentinel nodes were detected immediately and approximately 10 minutes after injection. The most critical point was avoidance of ICG leakage from the injection site or intrathoracic spreading because these effects make true sentinel nodes invisible; therefore, we immediately closed the injection site using an EndoClip™ (Covidien, Mansfield, MA) or Endoloop™ (Medcompare, South San Francisco, CA) after injection. Cases which were not diagnosed as lung cancer preoperatively were diagnosed intraoperatively by core needle biopsy or wedge resection. Sentinel node sampling preceded lobal or segmental resection of the lung. Green fluorescent nodes were dissected as sentinel nodes. This procedure is done only once because the background of dye leads to difficulties in the identification of the sentinel node. All systemic lymph node dissections, which mean full hilar and mediastinal node dissection, were followed by lung cancer resection.

Pathologic examination
After sentinel node dissection, sentinel nodes were examined by standard histology using H & E staining. Half of the sentinel nodes were fixed in formalin and embedded in paraffin for histological examination.

Quantitative PCR analysis
The other half of the sentinel nodes was evaluated using real-time RT-PCR to detect cytokeratin 19 (CK-19) expressions for micrometastases. Taqman PCR methods (TaqMan® Gene Expression Assays; Applied Biosystems, Tokyo, Japan) were used for quantitative evaluation of the RNA expression by PCR, as previously reported. The CK-19 gene was amplified by the following primer set: forward: GTC ATG GCC GAG CAG AAC, reverse: CCC GGT TCA ATT CTT CAG TC. The CK-19 gene internal probe was the universal probe library probe #89 (Roche, Mannheim, Germany). The PCR amplification condition was one cycle at 60°C for 25 seconds, and 95°C for 10 min followed by 45 cycles at 95°C for 10 seconds and 40°C for 30 seconds. The measured value was calculated by comparative Ct methods, and GAPDH gene amplification was used as a control. All reactions were duplicated. CK-19 mRNA was expressed as n-fold GAPDH mRNA, and the levels were compared relative to normal lymph node samples.

Statistical analysis
All statistical analysis was performed using SPSS 14.0 (SPSS Japan Inc., Tokyo, Japan). The different variables were analyzed with the chi-square test or Fisher’s exact test. Recurrence-free and overall survivals were analyzed using the Kaplan-Meier method and evaluated by the log-rank test. Statistically significant differences were accepted at p < 0.05.

Results
Patients and tumor characteristics are summarized in Table 1. There were no significant differences in age, gender, histological type, pathological T factor, and tumor size between VATS segmentectomy with SNB and without SNB. Although all patients were clinically and pathologically Stage IA, T1a was 17 of 20, 85% in SNB group and 53 of 63, 84% in without SNB group. Sentinel nodes were identified in 16 of 20 SNB group patients (80%), and only one patient showed faint amplification by real-time RT-PCR; however, the surgical procedure was not converted from segmentectomy to lobectomy. The false negative rate was 0%.

The localization of the tumor was 42 in the right lung and 41 in the left lung. In our protocol, a variety of segmentectomies were performed. Multi-segmentectomy combined with an adjacent segment, to keep the margin at more than 1 cm, was performed for 38 patients, and non-adjacent segment due to multiple cancers was performed for 4 cases.

The perioperative outcome is shown in Table 2. Mean numbers of dissected lymph nodes and the mean operating time in SNB group (15.9 and 303 minutes) were significantly higher in the without SNB group (10.9 and 241 minutes); however, blood loss, duration of chest tube drainage and the length of postoperative hospital stay
were similar in both groups. Postoperative morbidity and mortality are summarized in Table 3. There were no significant differences in overall morbidity between SNB group, 15% and without SNB group, 22%. The most frequent morbidity was air leakage, however, the rates of air leakage were not significant different between two groups (p = 0.28). Furthermore, the frequency of chronic obstructive pulmonary disease (COPD) in both groups were not significantly different (SNB group, 3 (15%); without SNB group, 12 (19%), p = 0.68). No mortality occurred in either group, in this study. The most common complication was prolonged air leakage longer than 7 days in 11 cases.

Local recurrence, distant metastases and cancer-related deaths are shown in Table 4. Although 7 of 63 without SNB group relapsed, no without SNB group patients relapsed during the follow-up period (median 18.1 months). In the relapsed patients without SNB, 4 (6.3%) were local recurrences and 3 (4.7%) were distant metastases. Two patients showed recurrence in one year (visceral pleura of residual lobe and resected margin). The other two patients recurred at intrapulmonary in two years or pleura, in four years, respectively. Three of four patients underwent re-operation for completion lobectomy; one is alive with disease and the other two patients were dead. All four patients received systemic chemotherapy. On the other hand, two patients with distant disease died due to cancer in the SNB without group. Significant differences between SNB group and without SNB group were not found in these events (Fisher’s exact test).

Furthermore, there was no significant difference in recurrence-free (Fig. 1A) and overall survival (Fig. 1B) between SNB group and without SNB group. The 2-year recurrence-free and overall survival rates were 100%, 80% in SNB group and 84%, 90% in without SNB groups, respectively.

**Discussion**

The finding of the Lung Cancer Oncology Group study showed a three-fold increase of local recurrence in patients who received sublobar resection1); however, recent reports revealed that anatomical segmentectomy provided feasible results in the recurrence rates and overall survival if adequate selection (stage IA and IB) was performed for NSCLC.10,11) Current reports support segmentectomy as one of the operative options to cure small NSCLC.

Many factors influencing the outcome of survival and local recurrence in the segmentectomy have been proposed, such as lymph node dissection or sampling, and surgical margins from the tumor.12, 13) One of the most important points for deciding to perform a segmentectomy was intraoperative accurate assessment of intrasegmental, hilar and mediastinal lymph nodes. Sentinel node navigation surgery was a useful tool to evaluate the metastases of lymph nodes and molecular diagnosis to detect micrometastasis.14) On the basis of this protocol, we compared the results of the perioperative outcome and interim result for survival between segmentectomy with

### Table 2  Perioperative outcome

<table>
<thead>
<tr>
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<th>SNB mean ± SD</th>
<th>without SNB mean ± SD</th>
<th>P</th>
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<tbody>
<tr>
<td>Number of dissected</td>
<td>15.9 ± 10</td>
<td>10.9 ± 8.8</td>
<td>0.03</td>
</tr>
<tr>
<td>LN</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blood loss (ml)</td>
<td>182 ± 291</td>
<td>118 ± 127</td>
<td>0.37</td>
</tr>
<tr>
<td>Operating time (min)</td>
<td>303 ± 103</td>
<td>241 ± 82</td>
<td>0.01</td>
</tr>
<tr>
<td>Duration of chest tube drainage (days)</td>
<td>4.6 ± 3.4</td>
<td>5.1 ± 3.8</td>
<td>0.75</td>
</tr>
<tr>
<td>Length of postoperative hospital stay (days)</td>
<td>10.4 ± 3.5</td>
<td>12.8 ± 9.1</td>
<td>0.34</td>
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SNB: sentinel node biopsy; LN: lymph node

### Table 3  Morbidity and mortality

<table>
<thead>
<tr>
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<th>SNB (n = 20)</th>
<th>without SNB (n = 63)</th>
<th>P</th>
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<tbody>
<tr>
<td>Air leakage’</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Prolonged drainage of effusion’</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>Empyema</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>Chylothorax</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>Hoarseness</td>
<td>1</td>
<td>0</td>
<td></td>
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<tr>
<td>Arrythmia</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3 (15%)</td>
<td>14 (22%)</td>
<td>p = 0.48</td>
</tr>
<tr>
<td>Mortality</td>
<td>0</td>
<td>0</td>
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</table>

SNB: sentinel node biopsy; IP: interstitial pneumonia ‘air leakage and prolonged drainage of effusion were defined as drainage needed for longer than 7 days.

### Table 4  Frequency of recurrence

<table>
<thead>
<tr>
<th></th>
<th>SNB (n = 20)</th>
<th>without SNB (n = 63)</th>
<th>P</th>
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<tbody>
<tr>
<td>Locoregional</td>
<td>0</td>
<td>4 (6.3)</td>
<td>0.25</td>
</tr>
<tr>
<td>Distant</td>
<td>0</td>
<td>3 (4.7)</td>
<td>0.32</td>
</tr>
<tr>
<td>Cancer-related death</td>
<td>0</td>
<td>4 (6.3)</td>
<td>0.25</td>
</tr>
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SNB: sentinel node biopsy
Thoracoscopic Segmentectomy with Sentinel Nodes Biopsy in NSCLC

The perioperative outcome showed no inferiority of the SNB group except for the operating time due to the procedure of dye injection and detection of sentinel nodes. Furthermore, as segmentectomy was preceded by SNB, it may take longer to dissect the target nodes. It was not clear why the mean numbers of dissected lymph nodes in SNB groups were significantly higher in the without SNB group; however, a performance bias to dissect more nodes may have been an influence. Also the selection bias that the patient with well-lobulated and non-adhesive lung was a candidate for SNB may lead to the difference of harvested nodes number. However, systemic hilar and mediastinal lymphadenectomy may decrease the possibility of stage migration in both groups, even if the number of harvested nodes were different. The lack of difference in morbidity and mortality between segmentectomy with SNB and without SNB suggested that this procedure was feasible and safe.

Although one patient showed faint amplification, i.e. suspicious of isolated tumor cells in hilar lymph nodes, the procedure was not converted from segmentectomy to lobectomy because the prognostic significance of micrometastasis or isolated tumor cells by molecular evaluation has not been elucidated. This patient has shown no evidence of recurrence two years after the operation. We found no significant difference in survival and local recurrence between segmentectomy with and without SNB; however, VATS segmentectomy without SNB might lead to a higher recurrence rates. Taken together, the segmentectomy without SNB group may include nodes that are occult positive even if node negative by histological examination, and the SNB group may show the true node negative according to the molecular evaluation of nodal status.

Several reports have revealed that the distance from the tumor to the resected margin was a good predictor of local recurrence. One-centimeter margins were proposed or the margin distance might have been greater than the maximum diameter of the tumor. According to these reports, our policy for segmentectomy was successful in maintaining a 1 cm distance from the tumor to the resected margins and, if impossible, multisegmentectomy (i.e., bi- or trisegmentectomy) or lobectomy was applied during the operation. When the tumor was located in the intersegmental portion, multisegmentectomy was performed. On the basis of this indication, we performed 38 multisegmentectomy in both groups, and local recurrence did not occur. Taken together, multisegmentectomy was a good option for segmentectomy at the above locations.

VATS segmentectomy is more difficult than segmentectomy by open thoracotomy, as reported in few papers, especially the comparison between open and VATS segmentectomy. The Cancer and Leukemia Group B (CALGB) 140503, phase III trial is ongoing to compare the recurrence-free survival of patients with a small tumor less than 2 cm in NSCLC undergoing lobectomy versus sublobar resection. Also, the aim of this study is to compare the results of lobectomy by open thoracotomy to VATS or the results of sublobar resection by open thoracotomy to...
VATS. The results of this study will provide more precise and reliable information about the advantages or disadvantages of VATS segmentectomy.

The limitations of this study include the retrospective analysis and single institutional study. Since this was not a blind, randomized study, selection bias by surgeons was possible. We plan to perform a randomized trial comparing VATS segmentectomy with or without SNB. Another limitation is the relatively short period of follow-up to observe differences in local recurrence and overall survival between the two methods. Longer follow-up and increasing the number of patients in both groups may lead to further confirmation of the results.

In conclusion, although the sample size is small, VATS segmentectomy with sentinel node navigation surgery may be an appropriate procedure with accurate staging, for stage I NSCLC.

Acknowledgments

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References