Abstract: Background. The sentinel node concept has become one of the most interesting topics in the treatment of head and neck cancer. The aim of this article is to report the results of our feasibility study and clinical application of sentinel lymph node (SLN) radiolocalization and biopsy in patients with clinically negative neck oral cancer.

Methods. Individuals with previously untreated N0 oral cancer participated in the study. The radioactive tracer used was 99m Tc phytate. Lymphoscintigrams were taken in the feasibility study, and fusion images of SPECT and CT were obtained in the clinical SLN biopsy (SLNB) group. In the feasibility study, metastases to SLNs and other nodes were analyzed in permanent specimens. In the clinical application group, we investigated the comparative effectiveness of multi-slice frozen section analysis and imprint cytology for the intraoperative diagnosis of SLNB.

Results. Fifteen individuals participated in the feasibility study. Six SLNs in five patients were cancer-positive, and two thirds of the SLNs were micrometastases. The SLN concept was established, and SLNs with the highest to the third highest radioactivity reflected the patients’ neck status accurately. Twelve patients participated in the clinical application group of SLNB. Intraoperative diagnosis of the three hottest SLNs correctly predicted the neck status of 10 patients. Three patients underwent modified radical neck dissection on the basis of the intraoperative diagnosis of cancer metastasis to SLNs, whereas neck dissections were spared in patients with no evidence of such metastases. There were two false-negative cases. One involved a failure of the intraoperative diagnosis of SLNB, and the other had cancer-negative SLNs and cancer-positive non-SLNs. Considering intraoperative diagnosis, multi-slice frozen section analysis was found to be superior to imprint cytology in its sensitivity, specificity, and overall accuracy on a lymph node basis. No differences were found in any of these indices of intraoperative SLNB on a patient basis. The fusion images of SPECT and CT proved very useful during intraoperative SLNB.

Conclusions. The sentinel node concept was established in the head and neck region. Analyzing the three hottest SLNs suffices to predict a patient’s neck status. Multi-slice frozen section analysis was shown to be superior to imprint cytology for detecting micrometastasis to SLN. Intraoperative SLNB based on fusion images of SPECT and CT proved to be an easy, accurate, and reliable method.

Keywords: sentinel lymph node; radiolocalization; fusion images of SPECT and CT
Gould et al\(^1\) reported the indication of neck dissection for parotid cancer using the word “sentinel lymph node” in 1960. They were surprisingly prescient to have foreseen the sentinel node concept of head and neck cancer more than 40 years ago. In 1977, Cabanas\(^2\) described the sentinel node concept of penile cancer. Morton et al\(^3\) reported the clinical application of this concept to cutaneous melanoma in 1992. Recently, many investigators in the head and neck field have been studying and reporting sentinel lymph node (SLN) localization and biopsy.\(^4\)–\(^11\)

In November 2000, we started our feasibility study of SLN radiolocalization in N0 neck oral cancer. We investigated the SLN radiolocalization with backup neck dissection and concluded that the sentinel node concept could be applied to the head and neck region. We recognized that the SLN reflected the patient’s neck status accurately and that the SLN radioactivity count had important implications for selecting SLNs that should be referred for intraoperative pathologic analysis in each individual. On the basis of our results, we commenced the clinical application of intraoperative SLN biopsy (SLNB) in August 2003. In this context, indications of neck dissection were determined on the basis of the patient’s neck status. If the intraoperative diagnosis by SLNB was positive, the patient underwent neck dissection, but the surgical option was excluded if it was not. Furthermore, for a more precise intraoperative SLNB, we obtained fusion images of SPECT and CT for visualizing SLNs. This method proved very useful in detecting SLNs before surgery of the primary site.

In this article, we describe the results of our study of SLN radiolocalization in patients with clinically N0 neck oral cancer.

**MATERIALS AND METHODS**

**Feasibility Study of SLN Radiolocalization.**

Patients. Patients with previously untreated N0 neck oral cancer whose negative necks were diagnosed by physical examination and imaging evaluation (CT, MRI) were candidates for this study. From November 2000, 15 individuals were registered. The primary sites included 10 cases of the tongue, three cases of the oral floor, and one case each of the lower gingiva and buccal mucosa. Pathologic diagnoses of all cases showed squamous cell carcinoma except for one case diagnosed as mucoepidermoid carcinoma in the buccal mucosa. Informed consent was obtained from all participating patients. The institutional ethical committee of our center approved these protocols.

Injection of Radioactive Tracer and Imaging Study. The radioactive tracer used was technetium 99m (99m-Tc) phytate, which was injected in the nuclear medicine room 24 hours before surgery. The injected dose was 37 MBq for the first 10 patients and 18.5 MBq for the other five. The tracer was injected submucosally around the primary tumor at six points hexagonally for the first group of patients and four points in a quadrant for the second group. A static lymphoscintigram, anterior and bilateral oblique view, was performed.

SLN Radiolocalization. In the feasibility study, we identified SLNs using a hand-held gamma probe and performed backup selective neck dissection. Using a gamma probe on the operating table, we identified and extracted the radiolabeled SLN before starting selective neck dissection. The level of SLN and its gamma probe count were recorded. We then continued with the planned surgery. After finishing the operation, we once again identified and extracted residual radiolabeled SLN in the dissected specimen.

We performed a unilateral supraomohyoid neck dissection on the affected side for the backup neck dissection. When an SLN was visualized on the healthy side by the lymphoscintigram, if the laterality of a patient’s tumor was distinct, the SLN on the healthy side was simply extracted without neck dissection. If a bulky cancer was located on the midline, a bilateral supraomohyoid neck dissection was indicated.

Histopathologic Evaluation of SLN. The SLNs and all other dissected lymph nodes were examined for disease. The attending pathologist examined multiple SLN sections of approximately 2-mm thickness with hematoxylin-eosin stain. The same pathologist examined the remaining neck lymph nodes in a single representative cross section. Cancer metastases to the SLN and the remaining neck lymph nodes were then compared.

Clinical Application of Intraoperative SLN Biopsy.

Patients. In August 2003, we commenced clinical application of intraoperative SLNB. The indication of neck dissection was decided on the basis of
the diagnosis of intraoperative SLNB. Twelve individuals had participated in the study by the end of February 2004, including 10 cases of the tongue and one case each of the soft palate and upper gingiva. Except for a case of tongue muco-epidermoid carcinoma, all were cases of squamous cell carcinoma. Informed consent of all participating patients and approval of our institutional ethical committee were obtained.

Injection of Radioactive Tracer and Imaging Study. The radioactive tracer was the same as the one used in the feasibility study, and the dosage was 18.5 MBq. In these patients, SPECT and CT images were taken after the usual lymphoscintigraphy. The attending radiology department technician produced the fusion images of SPECT and CT by which SLNs were visualized beforehand. Figure 1 shows the level I SLN in case 27. These images revealed all SLNs and their relation to anatomic structures.

**FIGURE 1.** Fusion images of SPECT and CT showing a level I sentinel lymph node (SLN) in case 27. The right column is CT; the center and left are SPECT and fusion images, respectively. The fusion image visualizes an SLN and its relation to nearby anatomic structures. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

**RESULTS**

Feasibility Study of SLN Radiolocalization. Table 1 shows the results of the first 15 cases. All cases and their SLNs are displayed in the order of application and identification, respectively. One or more sentinel nodes were identified in every case with an average 3.3 SLNs per person detected. A maximum of nine SLNs were found in case 6. SLNs with high radioactivity counts were localized before selective neck dissection. Many of the SLNs in the specimens usually showed lower radioactivity than others. However, a level I SLN with the highest radioactivity was found in the specimen of case 8. This SLN localizing failure might have been due to overlapping radioactivity from the primary site.

Six SLNs were involved in five cases. The SLNs with the highest radioactivity (hottest) counts were positive for cancer in four cases, and one case showed a positive SLN with the second highest radioactivity count. Cancer metastases were found in the SLNs with the hottest and third hottest counts in case 3. All cancer-positive SLNs were the only nodes affected without extracapsular spread. The three hottest SLNs accurately reflected the patients’ neck status. Consequently, investigating the
three hottest SLNs was enough to predict a patient’s neck status when more than three were identified.

According to the classification of Hermanek et al,12 which defined micrometastasis as an infiltration of lymph node parenchyma by tumor cells less than 2 mm in diameter, four of six cancer-positive SLNs in this study were diagnosed as micrometastases and the others as metastases.

In summary, we concluded that the SLN concept in the head and neck region was established by our feasibility study of SLN radiolocalization and that a histopathologic examination of the three hottest SLNs would probably suffice for predicting the neck status of a patient. When considering an intraoperative diagnosis of SLNB, a reliable method of intraoperative diagnosis that can detect micrometastasis in SLNs is necessary.

**Clinical Application of Intraoperative SLNB.** For 12 patients, we applied intraoperative SLNB for deciding whether to perform neck dissection; Table 2 shows the results. Frozen section analysis and imprint cytology successfully predicted the patients’ neck status in 10 cases. Imprint cytology yielded one false-positive (case 21, level II 18) result. There were no false-positive results in frozen section analysis. Intraoperative SLNB revealed metastases to five SLNs in three patients, all of whom underwent modified radical neck dissections, whereas neck dissections were not performed on seven patients with no evidence of such metastasis. These metastases found in the three hottest SLNs matched the result of our feasibility study. There were two false-negative cases (16 and 25). In case 16, frozen section analysis was performed on only a single representative slice, and permanent analysis revealed metastasis in another slice of the SLN. Imprint cytology was unable to detect these cancer cells. Case 25 had cancer-negative SLNs on the healthy side of the neck and cancer-positive non-SLNs on the affected side, even though a preoperative

<table>
<thead>
<tr>
<th>Case/site/T classification</th>
<th>Localized sentinel lymph node, Level counts (metastasis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/tongue/T3</td>
<td>II 129(–) II 58(–) I ns(–)*</td>
</tr>
<tr>
<td>2/lower gum/T3</td>
<td>II 20(–) I 15(+)</td>
</tr>
<tr>
<td>3/buccal mucosa/T4</td>
<td>I 77(+) II 32(–) I 18(+)m</td>
</tr>
<tr>
<td></td>
<td>I ns(–) II ns(–)*</td>
</tr>
<tr>
<td>4/tongue/T2</td>
<td>II 200(–) II 88(–) III 37(–)*</td>
</tr>
<tr>
<td></td>
<td>III 19(–)*</td>
</tr>
<tr>
<td>5/oral floor/T2</td>
<td>II 59(–) I 51(+)</td>
</tr>
<tr>
<td>6/tongue/T2</td>
<td>II 221(–) II 151(–) II 135(–), I 161(–) II 63(–) II 49(–) II 207(–) II 77(–) II 35(–)*</td>
</tr>
<tr>
<td>7/tongue/T2</td>
<td>I 94(–) III 47(–)</td>
</tr>
<tr>
<td>8/tongue/T3</td>
<td>II 25(–) II 14(–) I 87(–)*</td>
</tr>
<tr>
<td>9/oral floor/T2</td>
<td>I 61(–) II 58(–) I 49(–)</td>
</tr>
<tr>
<td>10/tongue/T1</td>
<td>II ns(–)</td>
</tr>
<tr>
<td>11/tongue/T4</td>
<td>I 27(–) III 25(–) II 34(+)m</td>
</tr>
<tr>
<td>12/tongue/T2</td>
<td>II 116(+)m II 37(–) I 13(–) I</td>
</tr>
<tr>
<td></td>
<td>I 16(–) I 23(–)* I 12(–)* II 7(–)*</td>
</tr>
<tr>
<td>13/tongue/T2</td>
<td>III 89(+)m</td>
</tr>
<tr>
<td>14/tongue/T2</td>
<td>III 22(–)</td>
</tr>
<tr>
<td>15/oral floor/T1</td>
<td>II 109(–) II 11(–) II 26(–) I 3(–)</td>
</tr>
</tbody>
</table>

Abbreviations: m, micrometastasis; ns, not stated.
*Sentinel node in specimen.
†Sentinel node on healthy side of the neck.

**Table 2.** Diagnosis by frozen section and imprint cytology and final histopathologic diagnosis of sentinel lymph nodes.

<table>
<thead>
<tr>
<th>Case/site/T classification</th>
<th>Cancer-positive sentinel node by frozen section/imprint cytology</th>
<th>Final histopathologic diagnosis level counts (metastasis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16/soft palate/T2</td>
<td>None/None</td>
<td>II 122(–) II 52(+) II 10(–)*</td>
</tr>
<tr>
<td>17/tongue/T2</td>
<td>None/None</td>
<td>II 134(–) II 127(–)</td>
</tr>
<tr>
<td>18/tongue/T2</td>
<td>None/None</td>
<td>II 159(–) IV 31(–) IV 23(–) I 17(–)</td>
</tr>
<tr>
<td>19/tongue/T2</td>
<td>None/None</td>
<td>II 71(–) III 44(–) II 22(–)</td>
</tr>
<tr>
<td>20/tongue/T3</td>
<td>None/None</td>
<td>III 53(–) II 18(–) III 12(–) II 3(–)</td>
</tr>
<tr>
<td>21/tongue/T2</td>
<td>IV 68 II 20/II 20 II 18</td>
<td>IV 68(+)m II 20(+)m II 18(–), micrometastasis to level I node</td>
</tr>
<tr>
<td>22/tongue/T1</td>
<td>None/None</td>
<td>I 80(–) I 69(–) II 64(–)</td>
</tr>
<tr>
<td>23/upper gingiva/T2</td>
<td>None/None</td>
<td>II 46(–) II 18(–)</td>
</tr>
<tr>
<td>24/tongue/T1</td>
<td>None/None</td>
<td>II 37(–) II 156(–) III 128(–)</td>
</tr>
<tr>
<td>25/tongue/T2</td>
<td>None/None</td>
<td>II 61(–)m III 31(–) II 20(–)*, metastases to level I and level III nodes with extracapsular spread on the affected side</td>
</tr>
<tr>
<td>26/tongue/T2</td>
<td>I 71 I 71</td>
<td>I 71(+)m II 65(–)</td>
</tr>
<tr>
<td>27/tongue/T2</td>
<td>I 134 III 33 I 134</td>
<td>I 134(+) II 62(–) III 33(+)m</td>
</tr>
</tbody>
</table>

Abbreviation: m, micrometastasis.
*Sentinel node on healthy side of the neck.
imaging evaluation could find no metastases. Because a subtotal glossectomy with free anterolateral thigh flap reconstruction was necessary in this case because of a large T3 tumor over the midline, we performed a bilateral supraomohyoid neck dissection; positive non-SLNs were confirmed by this procedure.

A final histopathologic analysis revealed that six lymph nodes of intraoperative SLNB were positive for cancer and four were micrometastases. Frozen section analysis revealed all micrometastases, whereas imprint cytology was able to detect only two, indicating that our intraoperative multi-slice frozen section analysis was more sensitive in detecting micrometastases. Figure 2 shows a micrometastasis to a level IV SLN in an intraoperative frozen section specimen.

When evaluating the results of frozen section analysis and imprint cytology of SLNB on a patient basis, the sensitivity, specificity, and overall accuracy of both were the same, ie, 60%, 100%, and 83.3%, respectively. When evaluating the intraoperative diagnosis of frozen section analysis and imprint cytology on an SLN basis (except for case 25, which had cancer-negative SLNs and cancer-positive non-SLNs), the sensitivity, specificity, and overall accuracy were all superior in frozen section analysis, ie, 83.3%, 100% and 96.9%, respectively, against 50%, 96.2%, and 87.5%, respectively in imprint cytology.

We applied fusion images of SPECT and CT for localizing the SLN in this series. Figure 1 shows the level I SLN located in front of the submandibular gland and on the undersurface of the mandible in case 27. We were able to recognize the correct position of SLN preoperatively on anatomic structures. This method was convenient, especially in level I. Usually it is very difficult to identify level I SLN using only a gamma probe, because overlapping radioactivity from the primary site interferes with the operator trying to identify the radioactivity from the SLN. However, this method enables us to extract the SLN without the adverse effect of radioactivity from the primary site and to confirm the SLN radioactivity. Fusion images of SPECT and CT proved very useful in intraoperative SLNB.

**DISCUSSION**

**Feasibility Study of SLN Radiolocalization.** In our feasibility study, we extracted all radiolabeled lymph nodes as SLNs as long as they showed any radioactivity, whether high or low. Because we had no clear idea of the relation between the radioactivity of SLN and cancer metastasis, we were determined to clarify this point and to establish a procedure for intraoperative SLNB.

In our feasibility study, four of five cases with positive SLN had the hottest radioactivity, and the other one had the second hottest positive SLN. In one case with two positive SLNs, they were the hottest and third hottest. This finding indicates that SLNs that have the hottest to third hottest radioactivity are very important in investigating the histopathologic analysis and determining the patient's neck status. It is necessary to analyze these SLNs intensively to detect cancer metastasis, whereas meticulous investigation into SLNs with lower radioactivity is of less value when more than three SLNs have been identified. Porter et al reported an interesting relationship between the radioactivity of SLNs and the order of SLNs harvested from patients with melanoma. They concluded that the overall histopathologic status of a nodal basin was always established by the first or second SLN harvested and that 95% of the positive SLNs were hottest or stained blue during the intraoperative SLN mapping using a radioactive tracer and dye. Dünne et al reported metastasis in the hottest or the second hottest SLN. Their results are in agreement with ours. Analyzing the three or three hottest SLNs may be all that is needed to forecast a patient's neck status. In our third case, the three hottest SLNs revealed that the patient's neck status was N2b, rather than being merely positive or negative. Of course, many more cases must be examined to

**FIGURE 2.** Frozen section analysis of intraoperative sentinel lymph node biopsy revealed micrometastasis to level IV sentinel lymph node in case 21. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
conclude whether analyzing the three hottest SLNs is the better option. On the basis of our feasibility study results, we conclude that it is better to analyze SLNs with the highest to third highest radioactivity to predict a patient’s neck status from an intraoperative SLNB.

As for the intraoperative SLNB, overlapping radioactivity from the primary site adversely affected SLN identification only when a handheld gamma probe was used, especially in level I. In case 8, despite careful use of a gamma probe, we failed to identify the hottest level I SLN. Fortunately, this SLN was negative for cancer; if the same situation occurred in a clinical setting, the intraoperative SLNB would not have proven to be a safe method for predicting a patient’s neck status. Because a more reliable method of intraoperative SLN detection is necessary for accurate intraoperative SLNB, we applied fusion images of SPECT and CT together with planar lymphoscintigram images to patients in the subsequent intraoperative SLNB study group.

When considering the intraoperative diagnosis of SLN and determining an indication for neck dissection, there is a need for a more sensitive diagnostic method that is able to detect micrometastases, which account for two thirds of our cases. Frozen section analysis has usually been performed for an intraoperative diagnosis of lymph node metastasis or a marginal study of a surgical specimen. Some authors have reported a high overall accuracy of frozen section analysis in the intraoperative SLNB of breast cancer.\(^{14,15}\) Although such an analysis is a reliable method for intraoperative histopathologic diagnosis, its disadvantages include the difficulty in interpretation because of freezing artifacts, a loss of tissue for processing, high expenditures of labor and time, etc. The Augsburg consensus has discouraged the use of frozen section analysis in evaluating the SLNs in cutaneous melanoma.\(^{16}\) Recently, the superiority of imprint cytology of SLNs in detecting micrometastasis during breast surgery has been reported.\(^{17,18}\) Asthana et al\(^{11}\) also reported the usefulness of imprint cytology in SLNB of oral cancer. In the next section, we examine whether frozen section analysis or imprint cytology was more effective in the diagnosis of intraoperative SLNB on the basis of our study.

**Clinical Application of Intraoperative SLNB.** On the basis of the results of our feasibility study, we began the clinical application of intraoperative SLNB to determine the indication for neck dissection. As shown in Table 2, intraoperative SLNB successfully predicted patients' neck status in 10 cases. Among them, our intraoperative SLNB of the hottest three SLNs correctly predicted one case of N1 and two cases of N2b. Thus, our policy of analyzing the three hottest SLNs would seem to be a better method for predicting a patient’s neck status in detail. In practice, however, a positive SLN calls for an indication of modified radical neck dissection. Surgery thus depends exclusively on either a positive or negative result from intraoperative SLNB. When focusing only on the indications for surgery, investigating just the two hottest SLNs would probably suffice. However, it is necessary to investigate more cases to clearly determine whether analyzing the three hottest SLNs to predict the patient’s neck status in detail is in fact unnecessary for the adequate management of patients with clinically N0 neck oral cancer.

Two failures in predicting neck status were observed: one was the failure of an intraoperative diagnosis of SLN (case 16), and the other was misdiagnosis of clinical N0 because of the limitations of a conventional imaging study (case 25). The SLN is occasionally localized on the healthy side of the neck in a large tumor invading beyond the midline, as in cases 11 and 25. The SLN on the healthy side of the neck in case 11 showed micrometastasis; on the other hand, case 25 had showed cancer-negative SLNs and cancer-positive non-SLNs. In this latter case, cancer-occupied non-SLNs diverted the lymphatic flow to the healthy side of the neck, resulting in cancer-negative SLNs being visualized only on the healthy side. The difference between these two cases is that the former SLNs were visualized bilaterally, whereas the latter were visualized only on the healthy side of the neck. Thus, careful interpretation of the fusion images of SPECT and CT or a lymphoscintigram would be necessary whenever SLNs are visualized only on the healthy side.

In our intraoperative SLNB group, our multislice frozen section analysis detected all metastases and micrometastases except in case 16, whereas imprint cytology was only able to detect two micrometastases. On a lymph node basis, multi-slice frozen section analysis was superior to imprint cytology in sensitivity, specificity, and overall accuracy. This was an entirely unexpected finding, because some current studies of the SLNB of breast cancer have reported the superiority of imprint cytology, especially for detecting
micrometastases.\textsuperscript{17,18} Because there are far fewer patients with oral cancer than breast cancer, our intraoperative diagnoses of SLNB cases have numbered only 12 to date, requiring us to continue this study and collect much more data to clarify which is the more reliable method of intraoperative SLNB diagnosis. This question can only be resolved by ongoing investigations.

In this latter series, we applied fusion images of SPECT and CT together with planar scintigrams to facilitate intraoperative SLNB. This method was extremely useful, especially for localizing SLNs in level I, which are usually very difficult to localize with only a gamma probe because of overlapping radioactivity from the primary site. Fusion images of SPECT and CT have enabled us to preoperatively recognize all SLNs and their relation to nearby anatomic structures (eg, the mandible or the submandibular gland). We were able to extract SLNs based on the fusion images and to confirm its radioactivity with a gamma probe without the adverse effect of overlapping radioactivity from the primary site. Consequently, by use of fusion images of SPECT and CT, intraoperative SLNB proved to be easy, accurate, and reliable.

In conclusion, the SLN concept was established as an effective treatment option for the head and neck region. Analyzing the three hottest SLNs sufficed for a reliable prediction of a patient’s neck status. In our study, multi-slice frozen section analysis was superior to imprint cytology for an intraoperative histopathologic diagnosis by SLNB to detect micrometastases to SLNs. Further investigation is necessary to determine more accurately which method is more effective for the intraoperative diagnosis of SLNB. It is clear, however, that the fusion images of SPECT and CT have proven very useful in intraoperative SLNB.

Acknowledgments. We thank Dr. Hiroki Iwata (Department of Breast Surgery, Aichi Cancer Center Hospital) and Radiology Technician Hiroki Miyamura for their technical instruction on sentinel lymph node radiolocalization and for producing fusion images of SPECT and CT, respectively.

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