Availability of tissue rinse liquid-based cytology for the rapid diagnosis of sentinel lymph node metastasis and improved bilateral detection by photodynamic eye camera

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Abstract

Objective: On sentinel lymph node navigation surgery for early invasive cervical cancers, to gain high sensitivity and specificity, the sentinel nodes should be detected bilaterally and pathological diagnosis should be sensitive to detect micrometastasis. To improve these problems, we tried tissue rinse liquid-based cytology and the photodynamic eye.

Methods: From 2005 to 2013, 102 patients with Stage Ib1 uterine cervical cancer were subjected to sentinel lymph node navigation surgery with Technetium-99 m colloid and blue dye. For the recent 11 patients with whom bilateral sentinel node detection was not available, the photodynamic eye was selectively examined. The detected sentinel node was cut along the minor axis into 2 mm slices, soaked in 10 ml CytoRich red and then subjected to tissue rinse liquid-based cytology at the time of surgery.

Results: With the accumulation of 102 Ib1 patients subjected to sentinel lymph node navigation surgery, the bilateral sentinel node detection rate was 67.7%. The photodynamic eye was examined for the recent 11 patients who did not have bilateral signals. Out of the 11, 10 patients obtained bilateral signals successfully. During the period of examining the photodynamic eye, a total of 34 patients were subjected to sentinel lymph node navigation surgery. Thus, the overall bilateral detection rate increased to 97% in this subset. Two hundred and five lymph nodes were available as sentinel nodes. The sensitivity of tissue rinse liquid-based cytology was 91.7%, and the specificity was 100%. False positivity was 0% and false negativity was 8.3%. Detection failure was observed only with one micrometastasis and one case of isolated tumor cells.

Conclusion: Combination of photodynamic eye detection and tissue rinse liquid-based cytology pathology can be a promising method for more rewarding sentinel node detection.

Key words: sentinel node, cervical cancer, TRLBC, ICG, photodynamic eye
Introduction

Sentinel lymph node navigation surgery (SNNS) for early invasive cervical cancers has been widely studied and its feasibility is now validated as a powerful tool for the intraoperative analysis of the nodal status. According to the systemic review of the literature, the combined technique using Technetium-99m colloid (99mTc) and blue dye achieved a sensitivity of 92% and a detection rate of 97% (1). On the other hand, to gain high sensitivity and specificity, the sentinel nodes (SNs) should be detected bilaterally and ultrastaging should be performed for the detection of micrometastasis (2).

Bilateral detection, which means at least one of both right and left sides of pelvic lymph node was recognized as SN, is not always feasible, and unless the bilateral detection, systemic lymph adenectomy on the non-detection side should be performed, which confer no beneficial advantage to the patient; in fact, the detection rate of SNs shown to be 97%, but the report includes the unilateral detection and the actual bilateral detection rate remains as low as around 70% (3–5). The omission of systemic pelvic lymphadenectomy demands the presence of a skilled pathologist, and establishment of accurate and speedy pathological diagnosis by intraoperative frozen section. Some laboratories have selected cyto-diagnosis with imprint preparation of cut surfaces of the lymph nodes. However, the results reported have varied widely due to the difficulties of the imprinting procedure and to the heterogeneity of the cytological features (6). A molecular biological approach that can detect the messenger ribonucleic acid for cytokeratin in SNs has been developed and accepted as one of high sensitive resolution (7). But it requires specialized instrument and involves high costs. In addition, this method cannot show the morphological characteristics of the metastatic lesion, which limits the differentiation of benign glandular inclusions with relatively high false positivity, and determination of the N category of the TNM classification especially in the case of breast cancer. Ultrastaging with serial sectioning and immunohistochemistry (IHC) showed that one-quarter of patients with SN metastasis exhibited micrometastasis (8). Although the prognostic relevance of micrometastasis and isolated tumor cells (ITCs) remains undetermined in the case of uterine cervical cancers, the increase of the relative risk of recurrence in the presence of true micrometastases has been pointed out (9). However, under the term of serial sectioning in the works in the literatures, various conditions are involved. One of the recommended ultrastaging protocols for a negative SN consisted of three consecutive sections (μm thick), each obtained at five levels (40 μm intervals) with hematoxylin and eosin (HE) staining and IHC (10). Even if this procedure can be performed, it will be time-consuming and has some difficulties as a routine clinical work.

In this study, to improve the above problems in the detection of positive SNs in early-stage cervical cancers, we tried tissue rinse liquid-based cytology (TRLBC) (11) for rapid and sensitive intraoperative pathological diagnosis, and used the photodynamic eye (PDE) (12) for the more sensitive dye-positive node detection, and their advantages were discussed.

Patients and methods

Sentinel node mapping

From 2005 to 2013, 102 patients with Stage Ib1 uterine cervical cancer have been subjected to lymphscintigraphy to detect sentinel lymph nodes (SNs), following the approval of the ethical committee in Hokkaido Cancer Center. Thirty-six patients had adeno- or adenosquamous carcinoma, 63 had squamous cell carcinoma and 3 had undifferentiated carcinoma. At 20 h before the radical hysterectomy, each 0.2 ml of 99mTc-Phytate (148MBq) was injected into the sub-epithelial area of uterine cervix at the four quadrants: 0, 3, 6, 9 o’clock sites. Four hours later, lymphscintigraphy was performed to confirm the SN detection. If bilateral clear imaging was not available, blue dye (patent blue) was injected into the cervix during the surgery. Intraoperatively, SNs were scanned with a gamma probe (Navigator GPS, Furuno electric Co. Ltd, Nishinomiya, Japan) and nodes with more than 10-fold counts above the background were identified.

Tissue rinse liquid-based cytology

The detected SN was cut on the minor axis into 2 mm slices, soaked in a small bottle filled with 10 ml CytoRich red (Becton Dickinson, NJ, USA) and rinsed thoroughly with tweezers. The bottle was set on a vibrator for 10 s, and cells on the cut surface were shaken off. The tissue slice was recovered from the bottle and subjected to HE staining, and IHC with anti-keratin antibody (AE1/AE3 Nichirei, Tokyo, Japan). CytoRich Red in the bottle was centrifuged at 900 g for 2 min. The pellet was divided and fixed onto the SurePath PreCoat slides (Becton Dickinson, NJ, USA), one of which was stained by the Papanicolaou method and other was immunostained by anti-keratin antibody with a Sentinel Lymph Node Rapid IHC kit (Invitrogen, CA, USA) (11).

Even when rapid TRLBC diagnosis showed no metastasis, we performed backup lymphadenectomy in the earlier 50 cases, which included obturator, internal iliac, external iliac, internal supra-inguinal and common iliac lymph nodes, to evaluate the accuracy of our sentinel navigation surgery. In recent cases, the systemic backup was omitted.

Indocyanine green fluorescence detection by photodynamic eye

For the recent 11 patients with whom bilateral SN detection was not available, PDE was selectively examined. Indocyanine green (ICG) was diluted by 100 times and each of 1 ml was injected in the directions of 0, 3, 6, 12 o’clock after the opening of the retroperitoneal cavity. During operation, SNs were detected with PDE-neo (Hamamatsu Photonics, Hamamatsu, Japan) (Fig. 1).

Results

SN detection

By the accumulation of 102 Ib1 patients subjected to SNNS, the average number of detected SNs per case was 1.93. Detection rate of SNs on the right side was 81.3% (83/102); and on the left side was 79.4% (81/102). The detection rate (at least unilateral) was 89.2% (91/102). Bilaterally positive detection was available in 67.7% (69/102) and bilaterally negative detection was in 10.8% (11/102) by an ordinary Tm-phytate isotope method plus blue dye method (Table 1).

We did not experience any prominent adverse event by the procedures of SN detection. But, in two cases with patent blue, slight skin staining in the lower abdominal wall and pubic area by the dye was observed, which disappeared in several days.

We performed backup lymphadenectomy in the earlier 50 cases, which included obturator, internal iliac, external iliac, internal supra-inguinal and common iliac lymph nodes. We have found no metastasis in these resected backup nodes. Because the accuracy and availability of our sentinel navigation surgery was shown by this result, backup lymphadenectomy was omitted in recent cases. Even so, on the side where SN detection was not available, we had to add systemic lymphadenectomy. Improvement of successful bilateral
detection was desired to keep the significance of SNNS as a morbidity-reducing operation. Then, the PDE was examined for 11 patients who did not have bilateral signals by isotopes or the ordinary blue dye method. Of the 11, 10 patients obtained bilateral signals successfully (Table 2). During the period of examining the PDE from case 1 to 11, a total 34 patients were subjected to SNNS. Thus, the overall bilateral detection rate was increased to 97% in this subset.

Feasibility of TRLBC

We have applied TRLBC method to the 102 patients for the intraoperative, rapid detection of SNs. Two hundred and five lymph nodes were available as SNs. Sensitivity of TRLBC to permanent slides was 91.7%, and specificity was 100%. False positivity was 0% and false negativity was 8.3% (Table 3). Detection failure was observed only with one micrometastasis and one case of ITCs. All the general metastasis more than micrometastasis have not been overlooked. Nevertheless, other three ITCs could be detected by TRLBC (Fig. 2 and Table 4).

**Table 1.** The detection of SNs by an ordinary Tm-phytate isotope plus blue dye method

<table>
<thead>
<tr>
<th>Detection 91 (89.2%)</th>
<th>Bilaterally</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right (81.3%)</td>
<td>Left (79.4%)</td>
</tr>
<tr>
<td>83 (81.3%)</td>
<td>81 (79.4%)</td>
</tr>
<tr>
<td>69 (67.7%)</td>
<td>11 (10.8%)</td>
</tr>
</tbody>
</table>

| SNs, sentinel nodes. |

**Table 2.** Improvement of bilateral sentinel node detection by photodynamic eye with ICG

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>RI</th>
<th>ICG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rt</td>
<td>Lt</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>Ob</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>Ob</td>
</tr>
<tr>
<td>3</td>
<td>Ob</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>Ob</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>Ob/ii</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>Ob</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>Ob</td>
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<tr>
<td>8</td>
<td>Ob</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>Ob</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>li</td>
<td>ND</td>
</tr>
</tbody>
</table>

RI, radio isotope; ICG, indocyanine green; ND, not detected; Ob, obturator node; li, internal iliac node; Ci, common iliac node; Ei, external iliac node.

**Discussion**

By the successful bilateral detection of SNs, we can omit the systemic lymphadenectomy and pursue a morbidity-reducing operation; in addition, a multicenter cohort group also showed that patients with optimal bilateral SN detection were significantly more likely to have metastasis detected (2). However, identification of bilateral SNs has been reported to be 64–72% (2,3,5,13,14), which is not satisfactory for clinical application. Also in our study, with the combination of

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**Figure 1.** (A) Photodynamic eye (PDE) camera system. Photodetector is connected to controller and video recorder. (B) Sentinel node with diluted indocyanine green (ICG) was intraoperatively searched by the PDE camera. (C) Fluorescence of ICG is obtained as an image on the monitor screen. With fluorescence mode, anatomical images cannot be seen. After marking the spot, mode was changed to the normal view camera and the node was recognized precisely.
radio isotope and dye, the bilateral detection rate was as low as 67.7%.

As shown by the Arbeitsgemeinschaft Gynaekologische Onkologie (Germany) study group, patients with tumor diameter ≤20 mm may profit from this concept, with bilateral detection (87.2%) which was much higher than the previous reports (15). Similarly, in our study, patients with tumor diameter ≤20 mm showed a bilateral detection rate of 82.8% (data not shown). If sentinel navigation can be applied only to these cases, the rate will be much improved. Still, the rate is not enough to achieve reliable feasibility.

Changing the ordinary dye method which was recognized by naked eye to the PDE raised the rate up to 97% in our SNNS. Improvement of the SN detection rate by using the PDE has been confirmed in cases of breast cancer. The detection rate of axillary SN was 50% by green color alone, but increased to 94% by a fluorescence signal (16). The rate was 100% and the mean number of fluorescent SLNs was 5.5 (17). Smaller molecular weight, which makes the dye penetrate the cervical stromal tissue and lymphatic flow easier, and higher sensitivity, which makes the non-green nodes visible, could contribute to the increase of the detection rate. The PDE method is not perfect because mechanical obstruction due to tumor embolism in lymphatic channel or inflammation cannot allow the ICG fluorescence in SNs. In fact, in our case (case 9 in Table 2), we observed that the ICG signal stayed in the para-uterine artery area, and the signal did not move into the iliac vessel area. The case had inflammatory change and broad adhesion due to the endometriosis in left pelvic broad ligament. We supposed that had disrupted normal lymphatic drainage. In the cases of Stage IB1, we do not have to consider the lymphatic obstruction, caused by cancer cells, which is often occurred in more advanced, bulky cases. But still the disruption can be caused by other inflammatory changes even in those with smaller lesion volume (tumor diameter ≤20 mm). Nevertheless, ICG fluorescence must be a useful tool for the improvement of SN detection.

According to meta-analysis of 22 studies with 842 patients, 92% is the pooled sensitivity using technetium and dye (1). In our study using a combined detection technique and TRLBC, the sensitivity was 91.7% and specificity was 100%. False positivity was 0% and false negativity was 8.3%. These results are comparable to the average of meta-analysis. By performing ultrastaging, the sensitivity was 91% and the false negative rate was 2.8% (2). The chance of finding micrometastases can be increased with ultrastaging. A review of studies on micrometastases showed that the percentage of women with micrometastasis ranged from 0 to 47.4% with a mean value of 28.3% (8). Our study with TRLBC and the following HE staining and IHC showed that the percentage of ITC or micrometastasis was 31.3% (5 of 16 metastases). Thus, the detection rate by combination with TRLBC and conventional permanent pathology in this study was comparable to ultrastaging. In case of breast cancer, 47 of 83 positive nodes (56.6%) showed ITC or micrometastases with TRLBC (11). To finish the ultrastaging during the operation is a time-consuming and cost-incurring matter and not all institutes can implement it. TRLBC is easier and shorter (35 min to one node and additional 5 min per node) technique for intraoperative rapid diagnosis. The rate of finding ITC or micrometastasis during operation referring to permanent pathology was 68.1% in breast cancer (11) and 60% in our study. More sensitive detection for these small metastases may be obtained by increasing sliced sections, which will be our future work.

The significance of micrometastasis or ITC is not yet elucidated in uterine cervical cancer, but in a recent RTC report on breast cancer, it was shown that the extended lymphadenectomy is not necessary in

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**Table 3. Feasibility of TRLBC to detect SN metastasis**

<table>
<thead>
<tr>
<th>TRLBC</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permanent pathology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>2 (ITC ×1, Micrometa ×1)</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>181</td>
</tr>
</tbody>
</table>

Sensitivity was 91.7%, and specificity was 100%.

TRLBC, tissue rinse liquid-based cytology; ITC, isolated tumor cell.

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**Table 4. Detection of ITC and micrometastasis by TRLBC**

<table>
<thead>
<tr>
<th>Detection (−)</th>
<th>Detection (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITC</td>
<td>1*</td>
</tr>
<tr>
<td>Micrometastasis</td>
<td>1*</td>
</tr>
</tbody>
</table>

Tumor cell nest under the diameter of 200 µm.

Micrometastasis; tumor cell nest with a diameter from 200 µm to 2 mm.

*Taken by permanent pathology.

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**Figure 2.** (A) Isolated tumor cells detection by tissue rinse liquid-based cytology with Papanicolaou staining during the operation. One tumor cell cluster showing a 3-dimensional appearance (arrow) is deposited on the glass slide against the background of numerous lymphocytes. (B) Immunohistochemical staining as permanent pathology using anti-keratin antibody AE1/AE3. A tumor cell cluster ~60 µm in size (arrow) is seen in the lymph vessel of the sentinel node.
patients with micrometastasis (18). Depending on future study with uterine cervical cancer, the detection of micrometastasis or ITC would not be important if it is not related to prognosis.

In conclusion, combination of PDE detection and TRLBC pathology can be a promising method for more rewarding SNNS.

Conflict of interest statement
None declared.

References