Effect of neoadjuvant chemotherapy on lymphatic pathways leading to sentinel lymph nodes in breast cancer

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Neoadjuvant chemotherapy (NAC) has been the standard therapy for breast cancer; however, whether sentinel lymph node biopsy (SLNB) should be performed after NAC in clinically node-positive patients is controversial. The indocyanine green (ICG)-fluorescence SLNB method (ICG-SLNB) provides a high identification rate as well as the ability to visualize lymphatic pathways, and previous studies have investigated the lymphatic pathways to sentinel lymph nodes (sentinel lymphatic pathways) using ICG-fluorescence method. In this study, we used ICG-SLNB to compare the sentinel lymphatic pathways before and after NAC to investigate whether they were affected by chemotherapy. Although the locations of the sentinel lymph nodes were unchanged, NAC did alter 42.8% of the sentinel lymphatic pathway routes. Our data indicate that SLNB after NAC can still detect the sentinel lymph nodes regardless of the presence or absence of metastasis. Since the false negative rates of SLNB after NAC for node-positive patients is higher than that for node-negative patients according to meta-analyses, further investigation is required to determine whether to perform SLNB after NAC for node-positive patients.

Keywords: neoadjuvant chemotherapy; indocyanine green-fluorescence method; sentinel lymph node biopsy; lymphatic pathways

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Introduction

Sentinel lymph node (SLN) biopsy provides reliable regional nodal staging and less complication compared with axillary lymph node dissection for the patients with clinically lymph node-negative breast cancer. Among the known methods of SLN biopsy (SLNB), the indocyanine green (ICG)-fluorescence method of SLNB (ICG-SLNB) offers a high identification rate as well as the ability to visualize lymphatic pathways. Neoadjuvant chemotherapy (NAC) has been the standard therapy for breast cancer. The pathological complete response by NAC is reported to be associated with long-term outcome in breast cancer patients with aggressive subtypes. However, the influences of NAC on lymphatic pathways leading to SLNs and on the location of the SLNs themselves are unclear. Furthermore, the optimal timing for SLNB is undetermined for patients who have breast cancer treated with NAC, particularly clinically node-positive patients.

Indocyanine green (ICG)-fluorescence SLNB method

ICG is excited by infrared light (760 nm), and emits at a fluorescent (830 nm) wavelength; hence, the fluorescent property of the ICG reagent makes it a suitable substitute for a dye. This method requires a photodynamic eye (PDE) camera but does not require employing stringent safety controls as in the case of radioisotopes. When performed using the axillary compression technique, ICG-SLNB can accurately identify SLNs as round-shaped fluorescent signals
that are visible through the skin.[3]

The ICG-fluorescence method is useful for SLNB because it offers a high SLN identification rate as well as subcutaneous visualization of lymphatic routes. Sugie et al. and Hirano et al. reported that the ICG-fluorescence method had significantly higher detection rate of SLN than the blue dye method in patients with early breast cancer.[4, 5] Kitai et al. reported that the detection rate of SLNs using ICG-fluorescence imaging was 94%, and that the mean number of detected SLNs was 2.8[2]; Abe et al. reported corresponding values of 100% and 3.1, while Tagaya et al. reported corresponding values of 100% and 5.5 [6, 7]. Our study has also demonstrated a high identification rate (97.4%) and a median of 3 SLNs detected for all cases[8]. Yi et al. and Gill et al. reported that the removal of a maximum of 5 SLNs at surgery was enough to recover more than 99% of positive SLNs in breast cancer patients [9, 10]. The ICG-fluorescence SLNB method thus appears to be the most suitable means of decreasing the false negative rate (FNR) because this method has a high SLN identification rate and can be used for more than 3 SLNs.

Some previous studies have investigated the sentinel lymphatic pathways using the ICG-fluorescence method. Specifically, Hojo et al. and Abe et al. reported the presence of variations in sentinel lymphatic pathways using this method [6, 11]. Hojo et al. reported two types of lymphatic route based on the drainage pattern: Type C converged to a single lymphatic route and Type S drained to separate routes. They showed that Type S and Type C lymphatic pathway were seen in 20.4% and 79.6% of cases, respectively. Abe et al. reported patterns in lymphatic routes consisting of 1-3 channels. They reported that 60% of patients had one lymphatic channel from the nipple to the axilla, 24% had two channels, and 16% had three channels.

Our colleague Yamaguchi also found that, in 226 patients with clinically node-negative early-stage breast cancer, 43.4% had multiple lymphatic pathways to SLNs. We further investigated the relationship between the routes of lymphatic pathways and tumor location from the surgical anatomy viewpoint. The number of sentinel lymphatic pathways ranged from one to four (one route, 56.6%; two routes, 38.5%; three routes, 4.4%; and four routes, 0.44%). In 69.1% of the patients, the sentinel lymphatic pathways only passed through the upper outer quadrant (UOQ) area. Interestingly, these pathways passed from the areola to the UOQ via a non-UOQ area, such as the lower inner/outer quadrant and upper inner quadrant area, in 30.9% of the patients. When each of the multi-sentinel lymphatic pathways of a single patient was counted separately, 81.1% of the sentinel lymphatic pathways passed only through the UOQ area, while 18.9% passed through the non-UOQ areas (Figure 1). Takeuchi et al. compared the variation of lymphatic pathways using the ICG-fluorescence method as well as computed tomographic (CT) lymphography, reporting that the ICG-fluorescence method was superior to CT lymphography for the detection of multiple routes of subcutaneous lymphatic pathways[12].

These results demonstrated that using the ICG-fluorescence method is useful to confirm the route of lymphatic pathways to sentinel nodes.

**Sentinel lymph node biopsy before or after neoadjuvant chemotherapy**

Neoadjuvant chemotherapy has been increasingly used to downstage breast cancer patients. Pathologic complete response rates vary in accordance with the histological subtype of breast cancer[13, 14]. Cortazar et al. reported that the pathological complete response was strongly associated with the long-term outcome in patients with aggressive breast cancer subtypes (Triple negative type, Luminal B type, and HER2-enrich type)[11]. The use of neoadjuvant chemotherapy has expanded beyond classically unresectable, locally advanced breast cancer and is now being indicated more frequently for some smaller tumors, especially aggressive breast cancer subtypes.

![Figure 1. The variations in the passing area of sentinel lymphatic pathways from the nipple to the axilla (n = 226). 81.1% of the sentinel lymphatic pathways passed only through the upper outer quadrant (UOQ) area, while 18.9% passed through the non-UOQ areas.](http://www.smartsctech.com/index.php/ccm)
Table 1. The change of Number of sentinel lymphatic pathways by NAC (Reprinted with permission[8])

<table>
<thead>
<tr>
<th></th>
<th>Number of sentinel lymphatic pathways</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-NAC</td>
<td>Post-NAC</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>2</td>
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</tr>
</tbody>
</table>

c N0: clinical node-negative cases, c N+: clinical node-positive cases

Table 2. The metastasis of SLN and non-SLN in clinical node-positive cases

<table>
<thead>
<tr>
<th>Case #</th>
<th>Pathological response Grade</th>
<th>Positive SLN (n)</th>
<th>Removed SLN (n)</th>
<th>Positive LN in back-up Ax</th>
<th>Subtype</th>
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<tbody>
<tr>
<td>7</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
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</tr>
<tr>
<td>9</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
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</tr>
<tr>
<td>10</td>
<td>2b</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>Triple negative</td>
</tr>
<tr>
<td>15</td>
<td>2a</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>Triple negative</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>Triple negative</td>
</tr>
<tr>
<td>29</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>HER2+</td>
</tr>
<tr>
<td>35</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>Triple negative</td>
</tr>
<tr>
<td>37</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>Triple negative</td>
</tr>
<tr>
<td>38</td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>HER2+</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>Triple negative</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>ER+HER2+</td>
</tr>
<tr>
<td>31</td>
<td>2a</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>ER+HER2+</td>
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<tr>
<td>14</td>
<td>3</td>
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<td>4</td>
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<tr>
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<td>1</td>
<td>3</td>
<td>2</td>
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<tr>
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<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>Triple negative</td>
</tr>
</tbody>
</table>

Pathological response was evaluated according the Classification of response criteria established by the Japanese Breast Cancer Society[20]. Grade 0: No response, 1a: Mild response, 2a: Marked response with High grade changes, 2b: Marked response with Extremely high grade changes, 3: Complete response.

The optimal timing of SLNB is uncertain for patients who have breast cancer treated with NAC. The SENTINA (SENtinel NeoAdjuvant) study determined that SLNB performed after NAC is associated with a lower detection rate and a higher FNR compared to SLNB performed before NAC[15]. Some investigators have suggested that the FNRs of SLNB after NAC may be due to (i) possible changes to intramammary lymphatic pathway, (ii) potential multiple sources that the lymphatic pathway become obscure and undetected for large tumors, and (iii) possible non-uniform cytotoxic responses of axillary lymph node metastases[16, 17]. However, there has been no clear evidence of NAC-induced changes in the lymphatic routes to the SLNs.

NAC affects the route of lymphatic pathways to SLNs

To investigate whether chemotherapy affects lymphatic pathways, we compared sentinel lymphatic pathways before and after NAC while employing the ICG-fluorescence method of SLNB[8].

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ICG-SLNB was performed before NAC for 22 clinically node-negative patients. The locations of the sentinel lymphatic pathways were recorded before NAC and then reexamined after NAC using the same method. In 16 node-positive patients, the sentinel lymphatic pathways and SLN locations were tracked using ICG-fluorescence method before NAC. After NAC, ICG-SLNB with axillary dissection was performed while recording the sentinel lymphatic pathway locations.

Our study revealed that, regardless of the presence or absence of lymph node metastasis, the sentinel lymphatic pathways were detectable irrespective of the influence of NAC in all cases. Although the locations of the SLNs were not affected by NAC, the sentinel lymphatic pathways in 42.8% of the affected breasts (16/38) were altered by NAC (Table 1). The number of sentinel lymphatic pathways increased in 6 cases (15.8%) and decreased in 10 cases (26.3%) after the completion of NAC (Figure 2). In those patients whose sentinel lymphatic pathways were altered by NAC, each had at least 1 sentinel lymphatic pathway whose route had not been changed despite several other pathways that had. This may explain why the locations of the SLNs were not affected by NAC.

These results indicated that SLNB after NAC can detect the true SLN regardless of the presence or absence of lymph node metastasis, and suggested that SLNB can be safely performed after NAC for clinically node-negative patients.

**SLNB after NAC for clinical node-positive breast cancer**

There is still debate over allowing SLNB to be performed after NAC in clinically node-positive patients. In a recent systematic review of 15 published studies of patients with clinically node-positive breast cancer undergoing NAC, the identification rate ranged from 78% to 98% (overall, 89%), whereas the FNR ranged from 5% to 25% (overall, 14%) [18]. Tan et al. reported that the FNR of clinically node-negative patients after NAC was 7% in their meta-analysis. It was similar to that in patients without NAC [19]. Based on these results, the FNR of SLNB after NAC for node-positive patients was high in relative to that for node-negative patients. Our study also evaluated the metastatic rate of non-SLNs in back-up dissections after NAC for clinically node-positive patients. The identification rate was 93.4% (15/16), and we further found that 25% of SLN-negative cases had non-SLN metastases in back-up dissection. Interestingly, among the eight patients who had pathological complete response (therapeutic response Grade 3: Necrosis and/or disappearance of all tumor cells) [20] and SLN negativity, one case (12.5%) had no SLN metastasis, despite the fact that the number of removed SLNs was 2-9 (Table 2). The FNR was 50%, probably due to the small number of patients in this study. The SENTINA-trial reported FNRs of 18.5% in patients with node-positive breast cancer who converted to node-negative after NAC with two or more SLNs removed [15]. The Update Committee of ASCO Clinical Practice Guideline considers that the FNR of SLNB after NAC in clinically node-positive patients (10% to 30%) is unacceptable. That suggests that the removed SLN may not be the same lymph node which was diagnosed with metastasis by the previous biopsy [21].

Boughey et al. reported that false-negative SLNs were significantly decreased when SLNB was performed using the combination of blue dye and radiolabeled colloid in clinical node-positive disease when examining at least three SLNs [22]. According to this report, utilizing both the ICG-fluorescence method and radiolabeled colloid methods may help reduce the FNR in clinical node-positive patients.

Most of patients who receive NAC have aggressive subtype tumors; e.g. triple negative and HER2-positive types. Some investigators reported that the response of axillary lymph nodes to the NAC was a good prognostic indicator.
compared with that of the primary tumor [23, 24, 25]. Based on these results, even if true SLNs could be detected and contained no metastasis, we should nevertheless consider carefully whether to perform SLNB after NAC for clinically node-positive patients. Further examinations will be required to evaluate performing SLNB after NAC for clinically node-positive patients.

Conflicting interests

The authors declare that they have no conflicting interests.

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