Innovating image-guided surgery: Introducing multimodal approaches for sentinel node detection

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Lymphoscintigraphy and SPECT/CT in multicentric and multifocal breast cancer: does each tumor have a separate drainage pattern? Results of a Dutch multicenter study (MULTISENT)

**Purpose:** To investigate whether lymphoscintigraphy and SPECT/CT after intralesional radiocolloid injection in each tumor separately in patients with multiple malignancies within one breast yields additional sentinel nodes compared to intralesional injection in the largest tumor only.

**Methods:** Patients were included prospectively in 4 centres in the Netherlands. Lymphatic flow was studied using planar lymphoscintigraphy and SPECT/CT until four hours after administration of 99mTc-Technetium-nanocolloid in the largest tumor. Subsequently, intratumoral injection of the smaller tumor(s) was performed followed by the same imaging sequence. Sentinel nodes (SNs) were intraoperatively localized using a gamma ray detection probe and vital blue dye.

**Results:** 50 patients were studied. Additional lymphatic drainage was depicted after the second and/or third injection in 32 patients (64%). Comparison of planar images and SPECT/CT after consecutive injections enabled visualization of the number and location of additional SNs (32 axillary, 11 internal mammary chain, 2 intramammary and one interpectoral. A SN contained metastases in 17 patients (34%). In 5 patients with a tumor-positive node in the axilla that was visualized after the first injection, an additional axillary involved node was found after the second injection. In 2 patients, isolated tumor cells were found in SNs that were only visualised after the second injection, whilst the SNs identified after the first injection were tumor-negative.

**Conclusion:** Lymphoscintigraphy and SPECT/CT after consecutive intratumoral tracer injections enable lymphatic mapping of each tumor separately in patients with multiple malignancies within one breast. The high incidence of additional SNs draining from tumors other than the largest one suggests that separate tumor related tracer injections may result in a more accurate approach for mapping and sampling of SNs in patients with multicentric or multifocal breast cancer.
INTRODUCTION

Sentinel node (SN) biopsy has become the standard staging procedure for patients with unifocal breast cancer. In case of multiple malignancies in one breast, axillary lymph node dissection is still commonly performed. More recently, the SN procedure has been evaluated for this patient category; however, both lymphatic mapping and SN identification have been related to the largest tumor only. Lymphatic mapping of patients with more than one tumor in the breast using a single injection of the radiotracer has led to identification rates ranging from 86% to 100%, yet false negative rates vary from 4-14%. If only one tracer injection is administered in or around the tumor with the largest diameter, lymphatic drainage related to solely that tumor will be detected, instead of the drainage pattern of each tumor separately. This theory is supported by former studies showing that drainage patterns may differ amongst tumors located in different quadrants. If the tracer is injected superficially, lymphatic drainage related to the subareolar plexus will be depicted. This will lead to the identification of a SN in the majority of the patients, but it may not represent lymphatic drainage related to the tumors themselves. Although superficial tracer administration is frequently applied for staging the axilla in patients with multicentric/multifocal breast cancer, a substantial number of hospitals and cancer centres also sample SNs in levels II/III or outside the axilla. For this purpose, peri- or intratumoral injections are routinely used. In this context, it is important to establish if the pattern of lymphatic distribution using two (or more) injections is different compared to the use of a single injection.

Therefore, the aim of the current study was to investigate whether lymphoscintigraphy and SPECT/CT after intralesional injection of the radiopharmaceutical in each tumor separately yields additional SNs compared to intralesional injection in the largest tumor only in patients with multiple malignancies within one breast.

METHODS

From June 2009 to April 2011 patients were asked to participate in a prospective multicenter study aimed to establish the feasibility of the SN procedure in multicentric and multifocal breast cancer (the MULTISENT study). The study was conducted in 4 participant centres in The Netherlands after approval of the protocol by their medical ethical committees.

Patients

Patients with multiple invasive tumors in one or more quadrants of the breast referred to one of the participating centres were included after obtaining written informed consent. Eligible patients should have at least 2 invasive tumors detectable by ultrasound. Both patients with multifocal (multiple tumors in one breast quadrant) and patients with multicentric (multiple tumors in more than one quadrant) disease were included, as long as the distance between the 2 tumors was at least...
2 centimeters. Before inclusion, histopathological confirmation of multicentric or multifocal breast cancer was obtained by fine-needle aspiration cytology or core biopsy of each breast lesion. Ultrasonography of the axilla with fine-needle aspiration cytology in case of a suspicious node, was routinely performed before lymphatic mapping. Patients were excluded if one of the tumors had a diameter of more than five centimetres, if the patient had more than three invasive foci in one breast, or if there was clinical evidence of disseminated breast cancer related disease.

**Lymphatic mapping**

Tracer injection of non-palpable tumors was guided by ultrasound. The largest tumor was injected first, followed by image acquisition. The same afternoon or in the morning of the second day (after 4-26 hours), the second tumor was injected, and the same scintigraphic imaging sequence was repeated using reference markers for patient positioning and image acquisition. In case of 3 tumors, the second and third tumor were injected at the same time. If the second/third tumor(s) were injected on the second day, a 5-minute static anterior image was performed before injection, in order to exclude other delayed draining lymph node stations (which were not seen on the first day) and to estimate remaining SN radioactivity.

An average dosage of 123 MBq (range 95-141 MBq) $^{99m}$Tc-nanocolloid (GE- Healthcare, Eindhoven, The Netherlands) was injected intratumorally for the first tumor and approximately 120 MBq (range 110-133 MBq) was injected in the second and/or third tumor(s). Lymphoscintigraphy was based on anterior and lateral planar images performed ten minutes, two and four hours after injection of the radiopharmaceutical. A cobalt-57 flood source was placed behind the patient to outline the body contour. SPECT/CT (Symbia T, Siemens, Erlangen, Germany) images were acquired immediately after the 4-hour conventional images. SPECT acquisition (matrix 128x128, 60 frames at 25 seconds per view) was performed using 6° angular steps steps. After correction for attenuation and scatter, SPECT and CT axial five millimetre slices were generated and fused. Both orthogonal multiplanar reconstruction and volume rendering were generated to visualize SPECT/CT. This imaging protocol was exactly repeated after the second or third injection.

The number and location of the SNs was determined for each tumor by an experienced nuclear medicine physician. Using lymphoscintigraphy and SPECT/CT, draining lymph nodes were localized in relation to the anatomical structures to enable accurate comparison with studies obtained after subsequent injection(s). Lymph nodes with an own afferent lymphatic vessel draining directly from the injection site were considered as SNs. In case of multiple nodes appearing with no afferent vessels on the lymphoscintigram, the first node appearing in the basin was considered as a SN. Following our experience, lymphatic distribution is usually completed 3-4 hours after tracer injection. Therefore, for the second scintigraphic study (4-26 hours after the first injection), lymph nodes appearing in other basins or besides the original SNs in the same basin were considered as additional SNs. The
location of each SN was marked on the skin with indelible ink before surgery.

**SN biopsy and histopathology**

Immediately before the operation, 1 mL patent blue dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was injected into or around each tumor (intra- or peritumoral injection). Intraoperatively, the dye and a gamma ray detection probe (Neoprobe, Johnson & Johnson Medical, Amersfoort, The Netherlands) were used to localize the SN(s). Frozen section histology was routinely performed, except in patients who received breast conserving therapy. All harvested nodes were postoperatively fixed in formalin, bisected, embedded in paraffin, and cut at a minimum of six levels at 50 to 150 μm intervals. Deferred pathological evaluation included haematoxylin-eosin and immunohistochemical staining (CAM 5.2; Becton Dickinson, San Jose, CA, USA). Axillary clearance was only performed in case of a tumor-positive SN.

**Study endpoints and analysis**

All data from each hospital were prospectively collected in one database at The Netherlands Cancer Institute. Analyses included: number and location of SNs after the first injection, additional SNs and other draining basins after second injection, as well as the differences in lymphatic drainage between patients with multifocal and multicentric tumors.

**RESULTS**

50 patients with a mean age of 56 years (range 31-84) were included. Tumors were located in one quadrant of the breast (multifocal disease) in 29 patients, and in 2-3 quadrants of the breast (multicentric disease) in 21 patients. Patient characteristics and SN results are outlined in Table 1.

In all 50 patients, lymphatic drainage was visualized after the first tracer injection of the largest tumor with a total of 76 SNs (mean 1.5 SN per patient, median 1, range 1-4). 2 of these nodes were only seen on SPECT/CT and not visualized on planar images. In 32 patients (64%), additional lymphatic drainage was depicted after the second and/or third injection with a total of 46 additional SNs. Visualization of additional drainage was seen in 18/29 (62%) patients with multifocal lesions (Figure 1) and in 14/21 (67%) patients with multicentric lesions (Figures 2 and 3). The mean number of SNs per patients after injection of all tumours was 2.4 (median 2, range 1-5). The extra SNs after second/third injection were localized as follows: 32 in the axilla (70%), eleven in the internal mammary chain (24%), two intramammary (4%) and one interpectoral SN (2%). In 18/32 (56%) patients with additional lymphatic drainage, SNs were localized in a different nodal basin than SNs after the first injection. All additionally identified SNs could be surgically removed, except for 2 internal mammary nodes (total excised SN range 1-5).

Pathological examination of the mastectomy specimen showed more tumor foci
than the preoperatively detected (2-3) lesions in 4 patients. In 3 other patients, one of the preoperative detected foci showed ductal carcinoma in situ on pathologic examination, while preoperative cytology and imaging were suspect for invasive disease.

The SN biopsy was tumor-positive in 17 patients (34%) and isolated tumor cells were found in 5 other patients (10%). Five patients with additionally identified drain-age patterns after second injection showed an extra SN that was tumor-positive (10%). One of these nodes was palpable on careful palpation during the operation and would therefore have been excised anyway. Furthermore, in all 5 patients the first injection had already revealed an involved SN in the same nodal basin. In 1 patient, isolated tumor cells were found in both an axillary and an additional internal mammary SN. In 2 patients, isolated tumor cells were found in SNs that were only visualised after the second injection, whilst the SNs identified after the first injection were tumor-negative.

**DISCUSSION**

This study demonstrates that lymphoscintigraphy and SPECT/CT after separate tracer injections in each tumor in patients with multiple tumors within one breast may yield additional SNs compared to intralesional injection in the largest tumor only. More than half of the patients in the present study showed additional SNs after tracer injection into a second tumor at a different location. Interestingly, this was even true for separate tumors located in the same quadrant of the breast. These results emphasize once again that the breast should not necessarily be regarded as one single entity with regards to its lymphatic drainage.8-11

The percentage of patients with multiple carcinomas within one breast that have lymph node involvement may range from 41% to 71%.3-5,18-22 Coombs and Boyages reported that 52.1% of patients with multicentric/multifocal disease have lymph node involvement, compared to 37.5% in patients with unifocal lesions.23 In view of these numbers, accurate staging seems particularly relevant in case of multiple malignancies within one breast. Yet, the reported false negative rate of the SN biopsy is high.7, 24-25 Ozmen et al. found a significant association between multicentric/multifocal disease and a false-negative procedure and Fearmonti et al. particularly mention the risk of a false-negative procedure in case of large additive tumor burden.24-25 A recent prospective multi-institutional study by Giard et al. reported a 13.6% false negative rate of SN biopsy after a single tracer injection in the breast.7 Possibly, the fact that not all breast lesions are taken into account can cause (tumor-positive) SNs to be missed.

Whether harvesting additionally identified SNs after tracer injection in the second or third tumor is relevant, depends on the potential of these smaller tumors to spread before the larger tumor disseminates. Vlastos et al. found comparable outcomes for patients with unifocal versus multicentric disease and conclude that the diameter of the largest tumor only can be used to stage tumor size.26 Other
studies however, have shown that the risk of lymphatic dissemination in multicentric tumors is dependent on the tumor-load of all lesions.\textsuperscript{18,21} Andea et al. found that tumor specimens with multiple nodules had a higher frequency of lymph node involvement compared to unifocal tumors of a similar volume or area.\textsuperscript{20} This might imply that an aggressive small lesion could give rise to a positive SN, while the first draining node from the largest lesion can be negative. Additionally detected nodes (belonging to tumors other than the largest one) contained tumor in 5 cases in the present study. This finding will only lead to upstaging if no positive SNs belonging to the largest tumor are found in the same nodal basin, which has not been the case in our population. In 2 patients, the additional SN found after injection of the second lesion contained isolated tumor cells (one internal mammary node, one axillary node), whereas the SNs identified after injection of the first tumor were tumor-negative. Although the demonstration of isolated tumor cells in the SN did not change the clinical management in these patients, its occurrence does suggest that the additional SNs draining from tumors other than the largest one can potentially be a route for occult metastases. A larger prospective study is currently in preparation to establish the clinical relevance of this assumption.

Although sequential lymphoscintigraphy remains mandatory to identify the SNs, the rather laborious methodology used in the current study can be simplified in daily clinical practice by simultaneous administration of both injections followed by only one scintigraphic study.

With the introduction of a new generation of gamma cameras with the possibility to perform SPECT/CT, lymphatic mapping in breast cancer has become more accurate providing relevant information for the surgeon in cases concerning patients with single malignant breast lesions.\textsuperscript{27} In the present study, the combination of lymphoscintigraphy and SPECT/CT enabled determination of the separate drainage patterns of each lesion using a sequential protocol of image acquisition and well-defined criteria to identify SNs.\textsuperscript{29} This led to the depiction of 64% additional SNs in the same or in a different node basin.
CONCLUSION

Lymphoscintigraphy and SPECT/CT after consecutive intratumoral tracer injections enable lymphatic mapping of each tumor separately in patients with multiple malignancies within one breast. The high incidence of additional SNs draining from tumors other than the largest one suggests that separate tumor related tracer injections may result in a more accurate approach for mapping and sampling of SNs in patients with multicentric or multifocal breast cancer.

ACKNOWLEDGEMENTS

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Figure 1. Additional draining basins after second injection in a patient with 2 tumors in the lower outer quadrant of the right breast (multifocal breast cancer). A) Planar lymphoscintigram 4 hours after injection in the largest tumor (T1) showing drainage towards one SN in the axilla (SN1). B) Axial SPECT/CT slice depicting the axillary SN. C) Planar lymphoscintigram 4 hours after injection in the second tumor the next morning, showing a second axillary SN (*) which was not seen on the residual image acquired shortly before the second injection. The planar image also shows a clear (additional) SN in the internal mammary chain (SN2), and a (supraclavicular) second-echelon node. D) Axial SPECT/CT image showing the internal mammary chain SN in the third intercostal space.

Figure 2. An additional SN after a second injection of 99mTechnetium-nanocolloid in a patient with 2 tumors in different quadrants of the breast. A,B) After the first injection in the largest tumor (T1), drainage towards a SN (SN1) in the left axilla is visualized with planar lymphoscintigraphy and with volume rendered SPECT/CT 4 hours after injection. C) After intratumoral injection of the second tumor located centrally in the left breast (T2), drainage towards a second SN (SN2) in the axilla is depicted on the early planar lymphoscintigram 15 minutes after injection. D) 3D volume rendered SPECT/CT shows an overview of the 2 injection sites (T1, T2) and the SNs (SN1, SN2).

Figure 3. Additional SN after separate injections of 99mTc-nanocolloid in a patient with 2 tumors in different quadrants of the breast. A) 3D volume rendered SPECT/CT image four hours after intratumoral tracer injection in the largest tumor (T1), showing drainage towards an internal mammary chain SN (SN1). B) After intratumoral injection of the second tumor (T2), drainage towards an additional SN in the axilla was observed, as depicted on the SPECT/CT image (SN2).
### Table 1: Patient characteristics and sentinel node results

<table>
<thead>
<tr>
<th></th>
<th>Multifocal</th>
<th>Multicentric</th>
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<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td><strong>Median age</strong></td>
<td>53 years (range 31-84)</td>
<td>53 years (range 39-81)</td>
</tr>
<tr>
<td><strong>Mean tumor diameter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Largest lesion</td>
<td>2.0 cm (range 1.0-3.8)</td>
<td>2.3 cm (range 0.7-4.5)</td>
</tr>
<tr>
<td>- Smaller lesion(s)</td>
<td>1.0 cm (range 0.6-1.6)</td>
<td>1.4 cm (range 0.6-3.4)</td>
</tr>
<tr>
<td><strong>Number of tumors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Two</td>
<td>26 patients</td>
<td>18 patients</td>
</tr>
<tr>
<td>- Three</td>
<td>3 patients</td>
<td>3 patients</td>
</tr>
<tr>
<td><strong>Additional drainage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Extra SN</td>
<td>18 patients (62%)</td>
<td>14 patients (67%)</td>
</tr>
<tr>
<td>- In other basin</td>
<td>9 patients</td>
<td>9 patients</td>
</tr>
<tr>
<td><strong>SN status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Negative</td>
<td>15 patients (52%)</td>
<td>13 patients (62%)</td>
</tr>
<tr>
<td>- ITC*</td>
<td>4 patients (14%)</td>
<td>1 patients (5%)</td>
</tr>
<tr>
<td>- Positive</td>
<td>10 patients (34%)</td>
<td>7 patients (33%)</td>
</tr>
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</table>

* Isolated tumor cells
^ extra SN seen in another draining basin (basin which the largest tumor does not drain to)
REFERENCES


