Sentinel node biopsy. Evolving from melanoma to breast cancer
Jansen, L.

Citation for published version (APA):
CHAPTER 8

EVALUATION OF MAMMARY LYMPHOSCINTIGRAPHY BY SINGLE INTRATUMORAL INJECTION FOR SENTINEL NODE IDENTIFICATION

R.A. Valdés Olmos¹, L. Jansen², C.A. Hoefnagel¹, O.E. Nieweg², S.H. Muller¹, E.J.Th. Rutgers², B.B.R. Kroon²

Departments of ¹Nuclear Medicine and ²Surgery, The Netherlands Cancer Institute / Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands

J NUCL MED 2000; in press
CHAPTER 8

ABSTRACT

Introduction: The aim of the present study was to evaluate the findings of mammary lymphoscintigraphy performed by single intratumoural injection in 150 patients with breast carcinoma: 100 patients (group A) investigated in the validation phase of the study, and 50 (group B) studied after the tracer dose was optimised.

Methods: Immediately after injection of $^{99m}$Tc-nanocolloid using a 25 G needle and 0.2 ml volume, simultaneous anterior and lateral images were acquired with a dual head gamma camera during 20 min followed by sequential static anterior and prone lateral breast images after 30 min, 2 and 4 hours. $^{57}$Co-assisted skin marking defined the sentinel node location for subsequent gamma probe/blue dye guided sentinel node biopsy.

Results: In group A (mean dose 61.6 MBq, range 42-88 MBq) scintigraphy visualised lymph nodes in 83 patients (83%) with an increase in the rate of visualisation from 72% for the first 40 patients to 90% for the last 60; patient age (p=0.01) as well as administered tracer dose (p=0.04) were found significant factors for visualisation with optimal results obtained with tracer doses higher than 65 MBq. Lymph nodes were visible in 34 patients (41%) during the first 30 min after injection whereas in 49 patients appearance occurred at 2-4 hours. A total of 97 lymphatic basins were visualised (80 in the axilla, 3 in the clavicular region, 14 in the internal mammary chain). In group B (mean dose 90.8 MBq, range 68-124 MBq), visualisation rate was 94% with early lymph node appearance in 27 patients (57%) and a total of 53 basins (45 axillary, 8 internal mammary regions). In combination with intraoperative blue dye mapping and gamma probe the identification rate increased to 90% in group A and 98% in group B. Prone lateral images contributed to identification of
intramammary lymph nodes in a total of 14 patients and axillary nodes close to the injection site, not seen on anterior images, in 8 other patients.

Conclusions: Mammary lymphoscintigraphy by single intratumoural injection is a valid method for lymphatic mapping and identification of both axillary and non-axillary sentinel nodes. Lymph node visualisation appears to be improved with higher tracer doses. The compactness of the injection site enables high quality additional lateral images which can depict intramammary or axillary lymph nodes located adjacent to the injection site.

INTRODUCTION

Since the first description of the technique and its introduction for melanoma by Morton et al., the procedure of sentinel node biopsy has been adopted rapidly for application in breast cancer. Although some groups do not perform lymphoscintigraphy as a part of the sentinel node procedure, there is an increasing interest in this modality, since its enables the preoperative lymphatic mapping and indicates the site of the sentinel node(s) which can subsequently be found by use of the gamma probe in the operating room.

Mammary lymphoscintigraphy after multiple peritumoural injections has been shown to be effective for preoperative lymphatic mapping and sentinel node localisation. However, the bloom of activity from multiple injection depots may obscure intramammary and axillary lymph nodes located close to the injection site. This may lead to incorrect identification of the sentinel node. Furthermore, there is a large variation in lymphatic drainage pattern from the four quadrants of the breast. The use of multiple injections around the tumour may increase variability of results. Finally, lymphatic vessels, which are the most important criterion to recognise the sentinel node, are less frequently visualised after peritumoural injection.
Mammary lymphoscintigraphy by single subdermal injection has also been found to be effective for detection of axillary sentinel nodes but the method appears to be less sensitive to depict lymphatic drainage to the internal mammary chain. Administration of the tracer further away from the tumour also increases the chance that a lymphatic watershed is crossed and that the node that is visualised is not the node that drains the tumour.

Although tracer administration into the tumour has been described for sentinel node identification in gastrointestinal, thyroid, and head and neck tumours, its use for sentinel node visualisation in breast cancer has not yet been validated. The migration of radiolabelled colloid particles from the tumour site to the lymph nodes after intratumoural administration has been documented by mammary lymphoscintigraphy used for axillary staging in breast cancer. The satisfactory imaging results found in these previous reports as well as our own experience with intratumoural administration of blue dye for sentinel node identification in breast cancer led us to evaluate the technique of mammary lymphoscintigraphy by single intratumoural injection for sentinel node detection. In addition, this study aimed at evaluating tracer dose-dependent aspects of lymph node visualisation and at improving the depiction of intramammary or axillary lymph nodes located close to the injection site by performing sequential anterior and prone lateral (hanging breast) images.

MATERIAL AND METHODS

Lymphoscintigraphy data were evaluated of the first 100 consecutive patients with breast cancer who underwent sentinel node biopsy in the trial institution (group A) and of 50 consecutive patients who were included after increasing the standard tracer dose (group B). Patients were enrolled with an operable palpable breast tumour, shown to be malignant with clinical examination, imaging (mammography, ultrasound or both) and fine needle aspiration cytology. Exclusion criteria were clinical
evidence of axillary lymph node metastases, previous excisional biopsy and pregnancy. The study protocol was approved by the Ethical Committee of the Netherlands Cancer Institute and informed consent was obtained from all patients. Patient characteristics are summarised in Table 1.

Lymphoscintigraphy was performed the day before surgery after administration of $^{99m}$Tc-labelled nanocolloid which has a particle size of less than 80 nanometer (Amersham Cygne, Eindhoven, the Netherlands). The tracer was administered into the tumour with a single slow injection (0.2 ml) using a fine needle (25G). Syringes were measured after injection in order to calculate net administered doses. The mean injected dose was 61.6 MBq in group A (range 42-88) and 90.8 MBq (range 68-124) in group B. Immediately after injection, simultaneous anterior en supine lateral dynamic lymphoscintigraphy of the affected region was performed acquiring 20 sec images over a period of 20 min, using a dual-head gamma camera with low-energy high-resolution collimators. Subsequently, 5-min anterior, supine and prone lateral (hanging breast) planar images were obtained after 30 min, 2 and 4 hours with simultaneous transmission scanning using a $^{57}$Co flood source. The location of the sentinel node was defined using $^{57}$Co markers and marked on the skin with ink. Criteria to identify the sentinel (first-echelon) node were the visualisation of an afferent lymphatic vessel leading from the injection site to this node or, if no afferent vessels were seen, the first lymph node appearing in each basin.

Shortly before to surgery, 1.0 ml patent blue dye (Bleu Patenté V, Laboratoire Guerbet, Aulnay-sous-Bois, France) was injected into the tumour. Subsequently, measurements were made over the skin marks with a gamma-ray detection probe (Neoprobe7 1000/1500, Neoprobe Corporation, Dublin, Ohio, USA) to confirm the location of the sentinel node as seen on scintigraphy and to indicate the site for the incision. If no sentinel nodes were seen on scintigraphy, the lower axilla was explored. Sites other than the lower axilla were explored only when scintigraphy revealed sentinel nodes in these basins. Following a small skin incision, the sentinel node was identified and removed after careful dissection of the
afferent blue vessel and after confirmation with the probe that the blue node was radioactive. The sentinel nodes were submitted for microscopic evaluation separately from non-sentinel nodes or axillary lymph node dissection specimen. Microscopic evaluation included step-sectioning, haematoxylin and eosin staining and immunohistochemistry staining (CAM 5.2).

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 100)</th>
<th>Group B (n = 50)</th>
<th>Total (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)*</td>
<td>53.2 (29-83)</td>
<td>54.1 (34-83)</td>
<td>53.5 (29-83)</td>
</tr>
<tr>
<td>Affected breast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>52</td>
<td>28</td>
<td>80 (53)</td>
</tr>
<tr>
<td>Right</td>
<td>48</td>
<td>22</td>
<td>70 (47)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1N0M0</td>
<td>57</td>
<td>34</td>
<td>91 (61)</td>
</tr>
<tr>
<td>T2N0M0</td>
<td>42</td>
<td>16</td>
<td>58 (39)</td>
</tr>
<tr>
<td>T3N0M0</td>
<td>1</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Breast quadrant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper outer</td>
<td>45</td>
<td>25</td>
<td>70 (47)</td>
</tr>
<tr>
<td>Upper inner</td>
<td>27</td>
<td>10</td>
<td>37 (25)</td>
</tr>
<tr>
<td>Lower outer</td>
<td>9</td>
<td>8</td>
<td>17 (11)</td>
</tr>
<tr>
<td>Lower inner</td>
<td>10</td>
<td>4</td>
<td>14 (9)</td>
</tr>
<tr>
<td>Central</td>
<td>9</td>
<td>3</td>
<td>12 (8)</td>
</tr>
</tbody>
</table>

*Values in parenthesis are ranges. All other values in parenthesis are percentages.
RESULTS

In group A, lymph nodes were visualised by scintigraphy in 83 of the 100 patients (83%). The rate of visualisation was 65% for the first 20 patients, 80% for the second 20, and increased to an average of 90% for the last three sub-groups of 20 patients (Fig. 1A). Using multiple linear logistic regression analysis, age (p = 0.01) and tracer dose (p = 0.04), but not patient order number, were found to be significant factors for lymph node visualisation. Visualisation was nearly 100% with doses above 65 MBq (Fig. 1B).

Fig. 1 A) Lymph node visualisation analysis in the first 100 consecutive patients (group A) referred for mammary lymphoscintigraphy showing a stable rate of visualisation of 90% for the last 3 sub-groups of 20 patients. After the tracer dose was optimised (group B) visualisation rate increased to 94%.
Fig. 1 B) Patient age and administered tracer dose analysis of group A (mean dose 62 MBq) showing that non visualisation occurs more often in older patients and after administered doses of less than 65 MBq $^{99m}$Tc-nanocolloid.

Fig. 1 C) Patient age and administered tracer dose in 50 patients studied after the tracer dose was optimised (group B, mean dose 91 MBq).
Lymphatic drainage exclusively to the lower axilla was seen in 67 of the 83 patients (81%) with lymph node visualisation. Drainage outside the lower axilla was found in 16 patients (19%). In 12 patients, lymphatic drainage to both the axilla and the internal mammary chain was seen. In one patient lymph nodes both in the axillary and clavicular regions were visualised and in another patient both infraclavicular and internal mammary nodes were depicted (Fig. 2). Exclusive internal mammary flow was seen in one patient and only an infraclavicular sentinel node in another one.

In 32 patients (39%) only 1 node, and in 51 patients (61%) 2 to 8 nodes were visualised. Lymphatic flow was seen in 34 patients (41%) during the first 30 minutes after injection: in 24 of these during the dynamic acquisition (0-20 min) and in 10 others on early static images. Late appearance (2-4 hours) was observed in 49 patients (59%).

Fig. 2 Anterior images obtained at 30 min (A) and 4 hours (B) showing lymphatic flow to the internal mammary chain and to the infraclavicular region (arrow) after administration of $^{99m}$Tc-nanocolloid at the tumour site (T) in the lower inner quadrant of the left breast.
Drainage to a total of 97 basins was demonstrated: 80 axillae, 14 internal mammary chains, 3 clavicular regions. Considering the first appearing lymph node and the visualisation of an afferent lymphatic vessel as the two most important criteria to identify the sentinel node (Fig. 3 and 4), scintigraphy was conclusive for 73 basins (75%). For 24 basins (23 axillae) in which multiple nodes appeared simultaneously without lymph vessel visualisation, scintigraphy was considered not conclusive as to which of these nodes was the sentinel node (Fig. 5).

Blue dye mapping, which was always performed, was able to identify axillary sentinel nodes in 7 patients with non-visualisation on scintigraphy, and in all but 4 basins with non-conclusive lymphoscintigraphy (i.e. total identification rate of 90%).

Fig. 3 Sequential lymphoscintigraphy of a patient with a T1 carcinoma in the lower outer quadrant of the right breast. Direct drainage to the right axilla (solid arrow) and to an intramammary node (dotted arrow) is observed on anterior (A) and prone right lateral (C) views performed 30 min after administration of $^{99m}$Tc-nanocolloid at the tumour site (T). After two hours (B and D), a substantial number of secondary (non-sentinel) nodes is observed in the axilla, the breast and along the internal mammary chain (arrows).
Fig. 4 Anterior (A) and prone right lateral (B) images showing a single lymph node in the right axilla at 4 hours after administration of $^{99m}$Tc-nanocolloid into the tumour (T) in the upper inner quadrant of the right breast.

Fig. 5 Anterior (A) and left lateral (B) images showing three lymph nodes in the left axilla 4 hours after intratumoural administration of $^{99m}$Tc-nanocolloid (T) in the upper inner quadrant of the left breast. Additional intraoperative blue dye administration allowed identification of two sentinel nodes with separate afferent lymphatic vessels and one secondary lymph node in the area indicated by lymphoscintigraphy.
A total of 152 sentinel nodes (mean 1.5 per patient, range: 1-5) was excised. In the axilla an average of 1.3 sentinel nodes was removed. One or more axillary sentinel nodes contained tumour in 35 patients, 3 of whom also had a tumour-positive sentinel node outside the axilla; one additional patient had a non-axillary tumour-positive sentinel node only (in other words the incidence of pathological non-axillary nodes was 4/16 = 25%). Two patients had metastases in second-echelon lymph nodes despite tumour-negative sentinel nodes (false negative).

In group B, lymph node visualisation rate was 94% (Fig. 1). Non-visualisation occurred in 3 patients aged 73, 83 and 58; in two of these patients sentinel nodes could be identified by blue dye (i.e. total identification rate of 98%). In this group a total of 53 basins (45 axillae, 8 internal mammary chains) were depicted. Lymphoscintigraphy was considered conclusive for 42 basins (79%); additional blue dye identified sentinel nodes in all basins with non-conclusive scintigraphy. Early visualisation occurred in 27 patients (57%) and in the remaining 20

| Table 2 Results of Group A (validation phase) and Group B (optimised tracer dose) |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | Group A (n = 100) | Group B (n = 50) | Total (n = 150) |
| Mean dose (MBq)*                | 61.6 (42-88)     | 90.8 (68-124)   | 130 (87)        |
| Scintigraphic visualisation rate| 83 (83)          | 47 (94)         | 125 (96)        |
| SN axilla                       | 80 (96)          | 45 (96)         | 24 (18)         |
| SN outside axilla               | 16 (19)          | 8 (17)          | 49 (98)         |
| Intraoperative identification rate| 90 (90)          | 49 (98)         | 139 (93)        |
| Sentinel node tumour-positive   |                  |                 |                 |
| Axilla                          | 35 (40)          | 15 (32)         | 50 (37)         |
| Outside axilla                  | 4 (25)           | 2 (25)          | 6 (25)          |

*Values in parenthesis are ranges. SN= sentinel node. All other values in parenthesis are percentages.
patients lymph nodes were seen after 2-4 hours. A total of 94 sentinel nodes (mean 1.9 per patient, range 1-4) was excised. In the axilla an average of 1.8 nodes were removed. Sentinel node metastases were found in 16 patients: in 14 in the axilla, in one in both axilla and internal mammary region, and in one exclusively in the internal mammary chain. The results in group A versus group B are summarised in Table 2.

Prone lateral images with hanging breast contributed to identify intramammary lymph nodes (Fig. 6) in 14 patients (7 in group A and 7 in group B). Axillary nodes close to the injection site were seen on the lateral and not on the anterior images in 8 other patients (6 in group A and 2 in group B).

Fig. 6 A) Anterior lymphoscintigraphy showing three lymph nodes of the left internal mammary chain (arrows) at 4 hours after intratumoural administration of $^{99m}$Tc-nanocolloid at the tumour site (T) in the lower inner quadrant of the left breast. Note the difference between supine (B) and prone (C) lateral views which leads to visualisation of an intramammary lymph node just behind the injection site on C (arrow).
DISCUSSION

The findings of the present study indicate that preoperative lymphoscintigraphy by single intratumoural tracer injection using a small volume is a valid method to depict the sentinel node in the axilla and other non-axillary drainage routes. Lymphoscintigraphy is an important guide for intraoperative sentinel node localisation. During the first 100 procedures, the lymph node visualisation rate increased from 65% to 90% and it remained stable for the last 60 investigated patients. This effect was caused by variations in net injected tracer dose and patient age. This leads to the conclusion that for optimal results, using a fixed volume, it is important that the specific activity of the tracer solution is sufficiently high so that at least 65 MBq can be injected. Lymph node visualisation increased to 94% after the tracer dose was adjusted.

The visualisation rate found by us appears to be similar to that of lymphoscintigraphy by peritumoural administration (75%-98%) reported by other groups\(^7\)\(^9\)\(^{17-21}\). An advantage of using a single small-volume injection is that the injection site is compact. This, in combination with the acquisition of prone lateral images with hanging breast, allowed a better visualisation of the space between the injection site and the thoracic wall. Axillary lymph nodes located close to the injection site or intramammary nodes could be seen in 16% of the patients with lymph node visualisation. Furthermore, early visualisation of lymph nodes was possible in 47% of patients which is helpful for identification of the sentinel node through the depiction of afferent lymph vessels or the sequence of appearance.

Where the tracer should be administered is the subject of much discussion. We chose our intrallesional injection site after careful consideration of accuracy and safety. Lymphatic drainage from the skin and the subareolar plexus is richer than drainage from the tumour and administration of a tracer at these sites is supposed to enhance the identification rate of a sentinel node. However, injection of the tracer further away from the tumour increases the risk that a lymphatic
watershed is crossed and that the node that is visualised is not the node that drains the tumour. Depositing the tracer around the tumour is in theory virtually as accurate as injecting the tracer into the lesion but has several drawbacks. For instance, it is more difficult to distribute the tracer all around the tumour. A theoretical problem is that the needle tip may wind up underneath the fascia when the tumour is lying deep since the needle is inserted blindly. The danger of needle tract metastasis is real since the needle may pass through tumour protrusions. Peritumoural injection spreads the tracer over a fairly wide region which may hamper visualisation of a sentinel node close to the primary tumour. Another practical drawback is that excision of the tumour will not remove all of the tracer from the injection site, which is often desired when probe detection is difficult with the tumour in situ. Advantages of intratumoural tracer administration are that the resistance of the tumour is felt after insertion of the needle, the tracer can be deposited accurately and the area with a high concentration of tracer is small.

The safety of inserting a needle into a neoplasm has been questioned in the light of fine needle aspiration cytology but there is no evidence in the literature that suggests that this approach increases recurrence rates. The average breast cancer is present for many years before it is detected. Tumours are known to shed malignant cells into the blood stream continuously. Based on the knowledge of the biology of the disease it is difficult to perceive that administration of the tracer into the tumour would increase the number of cells shed into the blood. It is unlikely that a single injection for scintigraphy significantly adds a risk in a patient group exposed to clinical examination, mammography, ultrasound examination, fine needle aspiration cytology, core biopsy, excisional biopsy, wide local excision and mastectomy.

It has been suggested that any attempt to administer a tracer directly into a tumour will only lead to a leakage of the tracer from the tumour into the peritumoural tissue at the injection site. The visualisation of intramammary lymph nodes appears to be, at least partially, in contradiction with this assumption. The lymphatic system of the mammary gland is postulated to contain cutaneous and parenchymal
lymphatics. A multi-directional model has been described for the lymphatic drainage of the breast including pathways to the axillary, the internal mammary and -rarely- to the posterior intercostal lymph nodes. Our findings suggest that migration of colloid particles from the tumour site after intratumoural injection may follow a multidirectional pattern; both to the periphery of the skin and to the parenchymal compartment which can drain to the internal mammary chain or to the axillary lymph groups. This observation is in agreement with a previous experience in which we have observed homogeneous distribution within the tumour after blue dye administration. A 16% to 35% visualisation of internal mammary lymph nodes reported for peritumoural administration and the high rate in the present study are in contrast to the low incidence of internal mammary chain visualisation (2%) described after subdermal injection. That technique appears to visualise the axillary lymph nodes only, following the superficial lymphatics.

In spite of the high lymph node visualisation rate found in the present study, in about a quarter of the cases with visualised lymph nodes it is not possible to identify with certainty which node is the sentinel node by means of lymphoscintigraphy. In these cases, additional intraoperative blue dye lymphatic mapping is recommended. By this combined approach we were able to identify the sentinel node conclusively not only in the majority of the patients with non-conclusive lymphoscintigraphy but also in a significant number of patients with non-visualisation on lymphoscintigraphy.

The application of interpretation criteria for lymphoscintigraphy strictly in accordance with the sentinel node concept is of vital importance since its determines the strategies to be followed in intraoperative sentinel node localisation. In cases with conclusive lymphoscintigraphy the use of the gamma probe may be sufficient provided that the sentinel node has been correctly marked on the skin during the scintigraphic procedure. Nevertheless, it is our experience that the blue dye may occasionally reveal an additional sentinel node. In cases with non-conclusive scintigraphy, the use of the gamma probe may support intraoperative detection of radioactive lymph nodes but not identification of the sentinel node.
node because the probe cannot visualise the lymphatic channels that determine the order in which nodes receive drainage. Blue dye mapping in these cases is indispensable to visualise afferent lymphatic vessels enabling adequate identification.

CONCLUSION

Mammary lymphoscintigraphy by single intratumoural injection is a valid method for lymphatic mapping and visualisation of both axillary and non-axillary sentinel nodes. Lymphoscintigraphy in combination with intraoperative use of blue dye and a gamma probe enables sentinel node identification in virtually all cases. Visualisation appears to depend on patient age and tracer dose with improved depiction at doses higher than 65 MBq of $^{99m}$Tc-nanocolloid. The compactness of the injection site and additional prone lateral breast images allow depiction of intramammary or axillary lymph nodes located close to the injection site.

ACKNOWLEDGEMENTS

We thank the technical staff of the Department of Nuclear Medicine and the Audio-visual Department of The Netherlands Cancer Institute for their support.
REFERENCES


