Sentinel node biopsy. Evolving from melanoma to breast cancer

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CHAPTER 9

UPTAKE OF RADIOCOLLOID IN SENTINEL LYMPH NODES

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SUBMITTED
ABSTRACT

Introduction: Sentinel node biopsy is difficult when a sentinel node contains little radioactivity. This study was undertaken to identify factors that influence the level of radioactivity in sentinel nodes.

Methods: Lymphoscintigraphy was performed after injection of 64 MBq of $^{99m}$Tc-nanocolloid in 0.25 ml in 60 consecutive patients with a palpable malignant breast tumour and in 22 patients with melanoma. Sentinel nodes were retrieved the next day with the blue dye technique and a gamma detection probe (Neoprobe® 1500). Eight patients with breast cancer were excluded because scintigraphy did not reveal a sentinel node, one because the sentinel node was false-negative. After the operation, the level of radioactivity in 148 sentinel nodes of 73 patients was measured with an uncollimated 19 mm probe in a standardised fashion. Linear regression with backward stepwise elimination was performed to estimate the influence of the following parameters on uptake: tumour type, age, sex, nodal status, dose, and time between injection and start of operation.

Results: Median uptake was 6.5 kBq (0.16% of the injected dose, 95% range 0.03-102 kBq) in sentinel nodes in patients with breast cancer versus 33.3 kBq (0.82% of the injected dose, 95% range 2.6-147 kBq) in sentinel nodes in patients with melanoma ($p<0.001$). The level of radioactivity significantly decreased with increasing time between injection of the tracer and operation ($p=0.04$). Age, nodal status, sex and dose were not shown to be related to uptake.

Conclusions: Median uptake of $^{99m}$Tc-nanocolloid in sentinel nodes in patients with breast cancer was more than five times lower than in patients with melanoma. The level of radioactivity significantly decreased with increasing time between injection of the tracer and operation which
may be due to disintegration of the tracer. The operation should for this reason not be delayed unnecessarily although a two-day protocol is feasible.

**INTRODUCTION**

Sentinel nodes in patients with breast cancer seem to accumulate less of the injected radioactive tracer than sentinel nodes in patients with melanoma. Lymphoscintigraphy in patients with breast cancer may fail to visualise a sentinel node or may not depict a sentinel node until hours after administration of the tracer. The sentinel node cannot be identified in up to 20% of patients with breast cancer. In patients with melanoma, sentinel nodes are usually visible within 20 minutes and are harvested in almost all patients. It is important to understand which factors influence the uptake of radioactivity, because a higher uptake facilitates intraoperative identification of sentinel nodes.

Several technique related factors may be predictors of uptake: the tracer type, dose of radioactivity (Chapter 8), volume, concentration, site of injection, or the time that has elapsed since the injection. Several patient related factors may play a role such as age, previous excisional biopsy, ambient temperature, level of hydration, exercise or body site. Several authors have expressed concern that tumour-positive sentinel nodes would accumulate less of the tracer but this is probably only the case for grossly involved nodes.

We previously quantified the level of radioactivity in sentinel nodes in patients with melanoma. The aims of the present paper are to quantify the level of radioactivity in sentinel nodes in patients with breast cancer, to compare this with uptake in sentinel nodes in patients with melanoma and to estimate whether patient age, sex, lymph node status, injected dose or time between injection of the radioactive tracer and start of the operation influence the uptake in the sentinel node.
PATIENTS AND METHODS

Between December 1997 and March 1999, 60 patients with breast cancer underwent sentinel node biopsy. All patients had an operable palpable breast tumour that appeared malignant on clinical examination, on imaging (mammography, ultrasound or both) and with fine needle aspiration cytology. Patients were not enrolled when excisional biopsy had been performed or when there was clinical evidence of axillary lymph node metastases. Eight patients were excluded because no sentinel node was visualised with lymphoscintigraphy. The first 40 patients were part of a validation study and axillary dissection was performed regardless of sentinel node status. One patient was excluded because the sentinel node biopsy was false-negative and the hot lymph node that was seen on the lymphoscintigraphy had probably been left behind. In the same period, sentinel node biopsy was performed in 22 patients with clinically localised cutaneous melanoma of at least 1 mm thickness and no palpable regional lymph nodes. Diagnostic excision with narrow margins had been performed in all patients with melanoma before lymphatic mapping. The sentinel nodes could be identified on lymphoscintigraphy and during surgery in all patients with melanoma. The patient demographics are presented in Table 1.

Sentinel node biopsy was performed as has been described elsewhere. Lymphoscintigraphy was performed after injection of a mean dose of 64 MBq (1.7 mCi) of 99mTc-Technetium-labelled human albumin colloid (99mTc-nanocolloid) (Nanocoll®, Amersham Cygne, Eindhoven, the Netherlands). A mean volume of 0.25 ml was injected into the tumour in the patients with breast cancer and around the biopsy site in the patients with melanoma. Images were obtained during 30 minutes after injection and after two hours (melanoma) or four hours (breast cancer). About 24 hours later, 1 ml of patent blue dye (Bleu Patente V, Laboratoire Guerbet, Aulnay-sous-Bois, France) was injected into and around the tumour. Sentinel nodes were sought guided by the blue dye and a gamma ray detection probe (Neoprobe® 1500, Neoprobe Corporation, Dublin, Ohio, USA).

UPTAKE OF RADIOCOLLOID
Table 1 Patient demographics

<table>
<thead>
<tr>
<th>Patients with breast cancer $\quad n = 51$</th>
<th>Patients with melanoma $\quad n = 22$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>51 (36-74)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
</tr>
<tr>
<td>Tumour stage</td>
<td></td>
</tr>
<tr>
<td>T1N0</td>
<td>28 (55)</td>
</tr>
<tr>
<td>T2N0</td>
<td>23 (45)</td>
</tr>
<tr>
<td>T3N0</td>
<td>-</td>
</tr>
<tr>
<td>T4N0</td>
<td>-</td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
</tr>
<tr>
<td>UOQ</td>
<td>22 (43)</td>
</tr>
<tr>
<td>LOQ</td>
<td>9 (18)</td>
</tr>
<tr>
<td>UIQ</td>
<td>13 (26)</td>
</tr>
<tr>
<td>LIQ</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Central</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Nodal stage</td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>26 (51)</td>
</tr>
<tr>
<td>pN1</td>
<td>25 (49)</td>
</tr>
</tbody>
</table>

*Values are mean (range). All other values in parenthesis are percentages. UOQ, upper outer quadrant; LOQ, lower outer quadrant; UIQ, upper inner quadrant; LIQ, lower inner quadrant.

Variability of technique related factors is listed in Table 2.

The level of radioactivity in each sentinel node was measured immediately after removal at a site with low background radioactivity. Measurements were performed during ten seconds with an uncollimated 19 mm gamma probe (Neoprobe® 1500) in direct contact with the node. Sensitivity of the probe was about 150 counts per second per kBq $^{99m}\text{Tc}$ in this geometric configuration. This method has previously been shown to provide a reliable estimate of the total amount of radioactivity present in the node\(^7\).
Table 2 Technique related factors

<table>
<thead>
<tr>
<th></th>
<th>Patients with breast cancer (n = 51)</th>
<th>Patients with melanoma (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected dose (MBq)*</td>
<td>64.0 (8.6)</td>
<td>60.0 (15)</td>
</tr>
<tr>
<td>Injected volume (ml)*</td>
<td>0.22 (0.05)</td>
<td>0.30 (0.07)</td>
</tr>
<tr>
<td>Time between injection and start surgery (hours)</td>
<td>23.8 (1.8)</td>
<td>23.5 (2.1)</td>
</tr>
</tbody>
</table>

*Values are mean (standard deviation).

Statistical analysis was performed with Statistical Package for the Social Sciences software (SPSS, Chicago, Illinois, USA). Linear regression with backward stepwise elimination of non-significant variables was performed to estimate the influence of the following parameters on the level of radioactivity present in each node at the time of surgery: tumour type, age, sex, nodal status, dose and time between injection and operation. If more than one sentinel node was removed in one patient, the logarithmic mean of the nodes was used as an estimate of uptake.

Uptake was corrected for physical decay with the following equation to t = 24 hours after injection:

\[
A(t) = A(0) * e^{-\ln2 * (t / t_{1/2})}
\]

A(t) is the amount of radioactivity that remains at a certain time and is related to the physical decay rate of the nuclide. Age, injected dose and the time between injection and operation were treated as continuous variables. All statistical tests were two-sided; \( P \leq 0.05 \) was considered to be statistically significant.
RESULTS

A total of 103 sentinel nodes was removed from 51 patients with breast cancer (median 2 nodes per patient, range 1-4). Sixty-four of these nodes (62%) were also blue. Median uptake per sentinel node was 6.5 kBq \( (1.7 \cdot 10^4 \text{ mCi}, 0.16\% \text{ of the injected dose}) \) at 24 hours after injection of 64 MBq in the patients with breast cancer. The 95\% range was 0.03-102 kBq \( (0.001\% - 2.5\% \text{ of the injected dose}) \). The 50\% range was 2.5-16.3 kBq.

Forty-five sentinel nodes were removed from 22 patients with melanoma. Thirty-six of these nodes (80\%) were blue. Median uptake per sentinel node in the patients with melanoma was 33.3 kBq \( (0.82\% \text{ of the injected dose}) \), which is 5.1 times the median uptake in patients with breast cancer. The 95\% range was 2.6-147 kBq \( (0.06\% - 3.6\% \text{ of the injected dose}) \). The 50\% range was 14-91 kBq.

The results of the linear regression model are shown in Table 3. Uptake in sentinel nodes was significantly higher in patients with melanoma than in patients with breast cancer. Age, sex, nodal status, dose and time between injection of the tracer and operation were not related to uptake. Because a previous study did show a relation between dose and visualisation of sentinel nodes on lymphoscintigraphy (Chapter 8), a correction for dose to 64MBq was introduced in the model. A linear relation between dose and uptake was assumed.

The results of the linear regression model after correction for dose are shown in Table 4. Uptake in sentinel nodes was in this model also related to the tumour type. Uptake in sentinel nodes in patients with melanoma was 190\% higher than in patients with breast cancer \( (95\% \text{ confidence interval 125-255\%}) \). A longer time between injection of the tracer and operation was associated with a lower level of radioactivity in the nodes. The amount of radioactivity decreased with about 17\% per hour \( (95\% \text{ confidence interval 0.5-33\%}) \). This is shown in Figure 1. Age, sex and nodal status were again not shown to be related to uptake after correction for injected dose.
Table 3 Linear regression model estimating effect on uptake. Backward stepwise elimination method. Uptake was corrected for physical decay to 24 hours.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( P )</th>
<th>( P )</th>
<th>( P )</th>
<th>( P )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour type: breast cancer or melanoma</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.37</td>
<td>0.30</td>
<td>0.22</td>
<td>0.22</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>0.60</td>
<td>0.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nodal status</td>
<td>0.55</td>
<td>0.58</td>
<td>0.62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Time between injection and start of surgery (hours)</td>
<td>0.17</td>
<td>0.19</td>
<td>0.18</td>
<td>0.17</td>
<td>0.12</td>
</tr>
<tr>
<td>Dose</td>
<td>0.66</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\( P > 0.15 \) was set as the cut-off for variable elimination

Table 4 Linear regression model estimating effect on uptake. Backward stepwise elimination method. Uptake was corrected for physical decay to 24 hours and for dose to 64 MBq.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( P )</th>
<th>( P )</th>
<th>( P )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour type: breast cancer or melanoma</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.48</td>
<td>0.36</td>
<td>0.36</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>0.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nodal status</td>
<td>0.47</td>
<td>0.51</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Time between injection and start of surgery (hours)</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\( P > 0.15 \) was set as the cut-off for variable elimination
DISCUSSION

The tumour type was the strongest predictor of uptake in sentinel nodes ($p<0.001$). Median uptake per sentinel node was 5.1 times higher in patients with melanoma than in patients with breast cancer. This confirms that the lymphatic drainage from the breast parenchyma is less efficient than from the skin\textsuperscript{1,6,22,26}.

The level of radioactivity per sentinel node decreased with time from injection. This factor was included in the model because uptake is a dynamic process. Studies in animal models\textsuperscript{17,18,27} and healthy volunteers\textsuperscript{28} with a variety of tracers showed that accumulation in lymph nodes continues for several hours after injection. The present data show that one
day later the amount of tracer in the nodes decreases. This may be explained by disintegration of the tracer. Besides this effect, physical decay accounts for decrease of the level of radioactivity at a rate of 11% per hour. Although a two-day protocol is feasible, the operation should not be delayed unnecessarily because of these two factors.

The effect of dose and age did not reach statistical significance. The influences may have been obscured by the large individual variation in uptake and the small variation in age and dose. Valdés Olmos et al. found that older age and lower dose were indeed associated with a lower rate of visualisation of sentinel nodes on lymphoscintigraphy (Chapter 8). Krag et al. found a decreased sentinel node identification rate in patients of 50 years or older and suggested that fatty degeneration of lymph nodes may decrease the capacity for uptake of the tracers. A larger sample size than in the present study is needed to show the influence of dose and age on uptake.

Maximal 3% (123 kBq) of the injected dose was found in a sentinel node after injection in the breast parenchyma. Krag et al. have also estimated uptake in sentinel lymph nodes in patients with breast cancer. They found uptake values up to 9% of the injected dose per node. Most of the tracer stays behind at the injection site. This is an important disadvantage for lymphatic mapping of the breast because it may interfere with probe measurements in the axilla. Injecting a small volume in or close to the tumour has the advantage that the tracer is administered in a relatively small area that can easily be excised with the tumour. This reduces the background radioactivity considerably and facilitates gamma probe guided detection of sentinel nodes with little uptake. The tracer $^{99m}$Tc-albumin colloid is frequently used in Europe, $^{99m}$Tc-sulfur colloid is a common tracer in the United States. Larger particles migrate less well from the injection site. Smaller particles may flow more easily to the sentinel nodes but are less well retained there. The ideal tracer would easily be taken up into the lymphatic system to leave a minimal residue at the injection site and would be taken up in large amounts in the sentinel node(s) without “overflow” to second-echelon nodes.
Median uptake was 6.5 kBq in the sentinel nodes in patients with breast cancer. With the four gamma probes tested by Tiourina et al., this would give count rates as low as 4.4 to 39 cps at 1 cm distance in water. The two least sensitive probes tested would not register more than 5 counts per second for 52% and 32% of the sentinel nodes respectively at 1 cm distance. These sentinel nodes have to be found with the blue dye technique. This problem would not occur that often in the patients with melanoma: 11% and 4% of the nodes would then be below the detection limit of the two least sensitive probes. The recently developed smaller probes are easier to use for the surgeon but are probably even less sensitive because the surface of the measurement unit is smaller. Increased uptake will facilitate sentinel node identification in patients with breast cancer, shorten the operation time, increase the identification rate and may prevent false-negative biopsies.

One may argue that the values for uptake found were low because non-sentinel nodes may have been included in the measurements. However, it is unlikely that this was the cause for the low values of uptake because 77% of nodes with an uptake below the 25th percentile (2.5 kBq) and 100% of nodes below the 10th percentile (0.6 kBq) were identified with the blue dye technique. Afferent blue lymphatic ducts were seen from the tumour-site and showed that these lymph nodes were indeed first-echelon (sentinel) nodes.

How could uptake in sentinel nodes in patients with breast cancer be improved? Increasing the dose will probably lead to higher uptake in the sentinel node, but the increased amount of radioactivity in the breast may interfere with the probe measurements. The optimum tracer volume is still unclear. Krag et al. have stated that uptake is increased with injection of 8 ml instead of 4 ml. In contrast, De Cicco et al. showed that uptake was higher when 0.5 ml was injected than with 1.5 ml. They also described that the advantage of a small volume is that the “blur” at the injection site is smaller. Uptake may be concentration dependent, which would explain De Cicco’s findings. The large volume used by Krag et al. may cause higher uptake despite the low concentration by increasing the lymphatic flow. Bass et al. investigated the effect of massaging the
injection site. This was shown to improve the percentage of blue nodes, but it did not seem to contribute to the uptake of the radioactive tracer. Comparative studies are needed to learn what combination of factors leads to optimal uptake in sentinel nodes in patients with breast cancer. Future research should also be directed at development of a more selective tracer: modifications of surface characteristics may increase uptake in macrophages in the sentinel nodes.

In conclusion, uptake of $^{99m}$Tc-nanocolloid in sentinel nodes in patients with breast cancer was significantly lower than in patients with melanoma. The level of radioactivity decreased with increasing time between injection of the tracer and operation which may be due to disintegration of the tracer. Besides this, physical decay decreases the amount of radioactivity in the lymph nodes at a similar rate. The operation should for this reason not be delayed unnecessarily although a two-day protocol is feasible.
REFERENCES


