Methodology and implications of lymphatic mapping and sentinel lymphadenectomy
Tanis, P.J.

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CHAPTER NINE

Frozen Section Investigation of the Sentinel Node in Malignant Melanoma and Breast Cancer

Departments of Surgery and Pathology, The Netherlands Cancer Institute, Amsterdam, the Netherlands. Department of Surgery, Amstelveen Hospital, Amstelveen, the Netherlands. Department of Surgery and Pathology, Groningen University Hospital, Groningen, the Netherlands

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The concept of orderly progression of solid tumours through the lymphatic system implies that the decision to perform regional node dissection can be based on the tumour-status of the sentinel lymph node. Two techniques can be applied to assess this tumour-status intraoperatively: touch imprint cytology (contact cytology) and frozen section analysis. In this article, our results of frozen section investigation of sentinel lymph nodes in melanoma and breast cancer patients are described and a review of the literature on this technique is given.

**Patients and methods**

Between December 1993 and March 1995, 99 consecutive patients with clinically localised cutaneous melanoma underwent a sentinel node procedure with intraoperative frozen section investigation. Forty-five patients were operated at The Netherlands Cancer Institute and 54 at the Groningen University Hospital following the same protocol. The primary melanoma was located in the head and neck region in 9 patients, on the trunk in 25, on the upper limb in 18 and on the lower limb in 47 patients. The Breslow thickness was between 1.1 and 11.0 mm with a mean of 2.9 mm and a median thickness of 2.2 mm. Ulceration was present in 33 patients (33%).

A total of 262 consecutive T1-2N0 breast carcinoma patients underwent 265 sentinel node procedures with intraoperative frozen section investigation between January 1999 and June 2000 (three bilateral): 230 operations were performed at The Netherlands Cancer Institute and 35 at the Amstelveen Hospital. The mean size of the tumours was 1.9 cm (range 0.2 to 8.0 cm) with a pathological stage T1 in 178 cases (67%), T2 in 86 (32%) and T3 in 1 woman. The histology of the tumour was invasive ductal carcinoma in 214 tumours (81%), invasive lobular carcinoma in 34 (13%) and various other types in the remaining 17 patients (6%). Specimens from the two hospitals were assessed in the same pathology laboratory.

One day before operation, lymphoscintigraphy was performed after injection of technetium-99m labelled nanocolloid (Nanocoll, Amersham Cygne, Eindhoven, the Netherlands).

Surgery was performed guided by patent blue dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) and a hand-held gamma ray detection probe (Neoprobe and Europrobe). The technique has been described in detail elsewhere.

The excised lymph nodes were submitted fresh for frozen section investigation. While we awaited the result, wide local excision of the tumour site or mastectomy was performed. Immediate regional node dissection was performed when frozen section analysis revealed metastastic disease in the sentinel node. Some breast cancer patients received radiotherapy of the axilla when the sentinel node was tumour-positive depending on tumour stage and menopausal status.

All lymph nodes were bisected and one level frozen section of both cut surfaces was made. Care was taken to obtain complete cross sections of the maximum diameter, preferably including the hilum and marginal sinus. Each frozen section slide was stained with haematoxylin and eosin (H&E). The remaining tissue was formalin fixated, paraffin embedded and cut at three levels 50-100 μm apart. All these sections were evaluated with H&E staining. The first level was also examined with
immunohistochemical staining. The anti-cytokeratin reagent CAM5.2 (Becton Dickinson, San Jose, CA, USA) was used at a dilution of 1:250 in sentinel nodes of breast cancer patients. Both S-100 (Dako, Glostrup, Denmark) at a dilution of 1:40.000 and HMB45 (Dako) at a dilution of 1:200 was used in melanoma patients. The Fisher exact test was used to compare false-negative rates between subgroups with different tumour stages.

Results

In the 99 melanoma patients, 177 sentinel nodes were excised (mean 1.8 per patient, range 1 to 4). Frozen section investigation revealed metastatic involvement of the sentinel node in eight patients. In another nine patients, metastases were only found in step sections (eight patients) or after immunohistochemical staining (one patient). The sensitivity of frozen section investigation was 47%, the specificity 100%. The negative predictive value was 90% (82 of 91). The sentinel node was the only positive node in thirteen of seventeen patients (76%). The relation between the Breslow thickness and the sensitivity of the frozen section examination is displayed in table 1.

Table 1. Relation between Breslow thickness and sensitivity of frozen section investigation of the sentinel node in melanoma patients.

<table>
<thead>
<tr>
<th>Breslow thickness</th>
<th>Metastases intraoperatively / total no. patients</th>
<th>Metastases in permanent sections / total no. patients</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.5 mm</td>
<td>0 / 15 (0%)</td>
<td>0 / 15 (0%)</td>
<td>-</td>
</tr>
<tr>
<td>1.5 - 4.0 mm</td>
<td>5 / 65 (8%)</td>
<td>12 / 65 (18%)</td>
<td>42</td>
</tr>
<tr>
<td>&gt; 4.0 mm</td>
<td>3 / 19 (16%)</td>
<td>5 / 19 (26%)</td>
<td>60</td>
</tr>
</tbody>
</table>

In breast cancer patients, lymph node metastases were found by frozen section investigation in 71 of 265 procedures (27%). Twenty-five procedures were false negative (sensitivity 74%). In ten patients, the tumour-positive lymph node was not send for frozen section investigation because of its small size or localisation in the internal mammary chain or in the breast parenchyma. One frozen section result was false-positive (specificity 99%), because no tumour cells were seen after serial sectioning and immunohistochemical staining. The negative predictive value was 87% (169 of 194). In the 265 sentinel node procedures in breast cancer patients, 444 lymph nodes (406 sentinel nodes, 38 second-tier nodes) were evaluated with H&E frozen sections (mean 1.7, range 1 to 6). Definitive evaluation showed tumour cells in 122 of 444 assessed lymph nodes (27%), corresponding with a sensitivity of 73% (89 of 122) per node. Twenty-nine of 33 lymph nodes which were negative in the frozen sections contained metastases smaller than 2 mm. Eighteen of these 29 micrometastases were found only by immunohistochemical staining with CAM5.2. Six of 24 lymph node metastases of a lobular carcinoma (25%) were not detected by frozen section investigation and 26 of 95 lymph node metastases (27%) in ductal carcinoma. The false-negative rates of the frozen sections for tumour stage T1a,b, T1c
and T2 or greater were 40% (2 of 5), 25% (13 of 51) and 25% (10 of 40) respectively (table 2). Review of the frozen sections showed tumour cells in the marginal sinus originally interpreted as histiocytes in one patient. The sentinel node was the only positive node in 53 of 82 cases (65%) with complete axillary lymph node dissection.

**Table 2.** Relation between tumour stage and sensitivity of intraoperative frozen section investigation of the sentinel node in breast cancer.

<table>
<thead>
<tr>
<th>Tumour stage</th>
<th>Metastases intraoperatively / total no. patients</th>
<th>Metastases in permanent sections / total no. patients</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1a,b</td>
<td>3 / 41 (7%)</td>
<td>5 / 41 (12%)</td>
<td>60</td>
</tr>
<tr>
<td>T1c</td>
<td>38 / 137 (28%)</td>
<td>51 / 137 (37%)</td>
<td>75</td>
</tr>
<tr>
<td>T2 / T3</td>
<td>30 / 87 (34%)</td>
<td>40 / 87 (46%)</td>
<td>75</td>
</tr>
</tbody>
</table>

**Discussion**

Intraoperative pathological assessment of the sentinel node allows for a regional lymph node dissection during the same session. Sensitivity and specificity are important factors in deciding whether a technique should be used. When the sensitivity is too low, the efforts and costs will exceed the benefits and an unreliable method gives undue distress to the patients. The specificity of frozen section analysis is 100% in almost all studies (tables 3 and 4) and, therefore, overtreatment will rarely occur.

This study reveals a low sensitivity (47%) of frozen section analysis in melanoma patients and is in agreement with two other studies (table 3). Reliability of frozen section analysis in breast cancer patients appears to be more favourable. The sensitivity of frozen section at one level in this study was 74%. Three other studies, which also included more than 75 patients with lymph node metastases, showed a similar sensitivity of 68%, 77% and 87% (table 4). However, a study with 890 patients revealed a much lower sensitivity of 58%.

**Table 3.** Results of intraoperative pathological investigation of lymph nodes in melanoma patients in literature.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Proportion of patients with Breslow thickness (%)</th>
<th>Intraoperative technique</th>
<th>Definitive pathological examination</th>
<th>Metastases in intraoperatively / total no. patients</th>
<th>Metastases in permanent sections / total no. patients</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibbs</td>
<td>1999</td>
<td>51 / 43 / 6</td>
<td>FS; 2L</td>
<td>PS; ≥ 4L; H&amp;E and IHC (S-100, HMB 45)</td>
<td>6 / 64 (9%)</td>
<td>14 / 64 (22%)</td>
<td>43</td>
<td>100</td>
</tr>
<tr>
<td>Clary</td>
<td>1999</td>
<td>30 / 54 / 16</td>
<td>FS</td>
<td>Step-sectioning and IHC</td>
<td>31 / 312 (10%)</td>
<td>55 / 312 (18%)</td>
<td>56</td>
<td>100</td>
</tr>
<tr>
<td>Present study</td>
<td>2000</td>
<td>15 / 66 / 19</td>
<td>FS; 1L</td>
<td>PS; 3L; H&amp;E and IHC (S-100, HMB 45)</td>
<td>8 / 99 (8%)</td>
<td>17 / 99 (17%)</td>
<td>47</td>
<td>100</td>
</tr>
</tbody>
</table>

FS = frozen section; L = level; PS = paraffin section; H&E = hematoxylin and eosin; IHC = immunohistochemistry
Several factors can influence the reliability of intraoperative pathological examination of lymph nodes. The first is the tumour load of the sentinel node. In melanoma patients and patients with lobular breast cancer, often small foci or single metastatic cells are found, detectable only with step sections or immunohistochemistry. However, the percentage of false negative frozen sections in lobular carcinoma compared with ductal carcinoma was not different in this study, 25% and 27% respectively. The second factor is the size of the lymph node. It may be impossible to capture one entire cross section of a large lymph node in one frozen section. The third factor is the number of assessed levels per node. We made a single frozen section of each lymph node in both malignancies resulting in a relatively small chance of encountering the metastasis (sampling error). Gibbs and co-authors improved the sensitivity by adding a second frozen section level. In breast cancer patients, Viale and co-workers made 15 pairs of frozen sections with H&E and rapid cytokeratin staining of each of two halves (60 sections). They found 64% of the sentinel lymph node metastases in the first pair of sections, but did observe metastases as far as the 15th cutting level. A disadvantage of these large numbers of frozen sections is that no tissue remains for more sensitive pathological examination. Also, this approach is time consuming and requires additional personnel. Some authors suggested that the sensitivity of frozen section analysis is lower in small tumours. An explanation for
this phenomenon might be the smaller sampling error in large tumours, because of the relatively higher incidence of macrometastases compared to smaller tumours. A difference between the sensitivity of frozen section investigation in melanomas with a Breslow thickness of more than 4.0 mm and from 1.5 to 4.0 mm was seen in this study (table 1), but this difference was not significant (P = 0.5). The difference between the sensitivity of frozen section examination in T1a,b and T1c breast cancer (table 2) was also not significant (P = 0.4). Finally, the quality of the frozen section and the experience of the pathologist are important factors.

The calculated sensitivity of an intraoperatively used method depends on the type and the quality of the definitive pathological examination (gold standard). The use of H&E stained step sections in breast cancer was reviewed by van Diest et al., who found that 9% of initially negative lymph nodes were converted to tumour-positive with this approach. The increase in sensitivity by immunohistochemical staining in melanoma was 45% in one study. Three studies addressed the additional value of immunohistochemical staining in breast cancer and found 14%, 31% and 36% of all positive sentinel nodes only with this technique. These metastases would have been missed with frozen section examination. This results in substantial overestimation of the frozen section sensitivity in studies without immunohistochemistry as the gold standard. Molecular analysis with the reverse-transcriptase polymerase chain reaction (RT-PCR) may increase the number of ultimately detected (micro)metastases even further, although this remains to be established.

Explanations for the difference in sensitivity of frozen section examination in melanoma and breast cancer may be the higher incidence of micrometastases with a single tumour cell distribution in melanoma and the more difficult recognition of that disease.

The a priori chance of detecting a lymph node metastasis of melanoma is approximately 20%. The sensitivity of approximately 50% means that occult metastases are identified with frozen section microscopy in only 10% of melanoma patients. In the other 90% of cases, operation time is reserved for a regional lymph node dissection that is not performed. Even in thick melanomas (more than 4.0 mm), reservation of operation time for a standard lymphadenectomy will be needless in 85% of the patients. Therefore, we have abandoned frozen section analysis in this disease.

In breast cancer, the higher a priori chance of lymph node metastases (40%) together with the higher sensitivity of frozen section examination (75%) results in unused operation time in 70% of cases. Thirty percent of the patients are spared a second admission and operation. This is acceptable in our setting and we continue to perform frozen section investigation of sentinel nodes in breast cancer patients. A disadvantage of this approach is that patients experience great distress when the frozen section is false negative, despite the fact that this risk has of course been discussed ahead of time. An alternative approach would be to exclude stage T1a,b breast cancer patients because of the low incidence of lymph node metastases and a possibly lower sensitivity of intraoperative frozen section analysis in this subgroup (table 2).

In our series, the only false-positive frozen section of a sentinel node in breast cancer was encountered. We actually performed an unnecessary axillary lymph node
dissection in this patient, but fortunately there was another indication for axillary clearance because of the multicentricity of the tumour. The risk of a false-positive frozen section report can be limited by awaiting the definitive pathological examination in case of doubt.

In conclusion, this study shows a lower sensitivity of a single H&E stained frozen section of the sentinel lymph node in melanoma (47%) as compared with breast cancer (74%). Frozen section examination allows immediate axillary lymph node dissection in the majority of node-positive breast cancer patients. We do not recommend frozen section examination in melanoma.

References

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