Clinical relevance and refinement of the sentinel node procedure in breast cancer and melanoma
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EIGHT FALSE NEGATIVE SENTINEL NODE PROCEDURES IN BREAST CANCER: WHAT WENT WRONG?

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CHAPTER 9

INTRODUCTION

In addition to the identification of the sentinel node, the success of the procedure is determined by a low false negative rate. Few authors, however, discuss the following two questions: what went wrong in the false negative cases and what can be done to avoid these failures in the future. In this study, we share our experience with false negative sentinel node biopsies in palpable and non-palpable breast cancer patients and discuss what we have learned from these cases.

MATERIALS AND METHODS

Between January 1997 and November 2001, 599 cN0 breast cancer patients underwent sentinel node biopsy at The Netherlands Cancer Institute. Seven patients had a bilateral tumor and 112 patients a non-palpable tumor. Pathologic proof of breast cancer was routinely obtained by core biopsy or fine needle aspiration and the primary tumor was still present in all patients. Routine axillary clearance was performed until January 1999 in 81 patients as part of the learning phase. In the subsequent patients, axillary clearance was omitted in the case of a tumor-negative axillary sentinel node. Since November 1999, all patients had preoperative axillary ultrasonography to ensure the cN0 status. The median follow-up duration of patients studied after the learning phase was sixteen months (range 1-34).

A two-day protocol was used. On the day before surgery, $^{99m}$Tc-nanocolloid (Nanocoll®, Amersham Cygne, Eindhoven, the Netherlands) was injected into the lesion in a mean volume of 0.2 ml and a mean radioactivity dose of 105.4 MBq (2.8 mCi). In case of non-palpable breast cancer, the intratumoral injection was guided by ultrasound or stereotaxis. Immediate dynamic imaging was performed followed by static imaging at 30 minutes and four hours post-injection. A dual-head gamma camera (ADAC Vertex®, Milpitas, California, U.S.A.) was used. Since July 1999, additional views were made after two hours (455 cases) and in August 2000 dynamic imaging was abandoned (236 cases). Both anterior and lateral images were obtained. The location of the node was marked on the skin with indelible ink. After the last lymphoscintigraphy, a localization wire with a catheter was positioned in the non-palpable tumors. The next day, patent blue dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) in a mean volume of 1.0 ml was administered into the palpable lesion or through the catheter. Intra-operative detection of radioactivity was measured with a gamma ray detection probe (Neoprobe®, Johnson & Johnson Medical, Hamburg, Germany). Both tracers were used to identify the sentinel node. All procedures were performed by one of four experienced surgeons or under their supervision by a resident or fellow. A hot spot on the lymphoscintigram was considered to be a sentinel node if an afferent lymphatic channel was visualized, the hot spot was the first one seen in a sequential pattern, or the hot spot was the only one depicted. An afferent blue lymphatic vessel coming directly from the tumor also defined a node as the sentinel node. Our policy is to perform a complete axillary lymph node dissection if no sentinel node can be identified during surgery. These cases are not defined as false negative, because no sentinel node has been removed.

All sentinel nodes were formalin-fixated, bisected, paraffin-embedded and cut at a minimum of six levels at 50 to 150 µm intervals. On most sentinel nodes a frozen section was performed. Pathological evaluation included hematoxylin-eosin and immuno-histochemical staining (CAM 5.2, Becton Dickinson, San Jose, California, U.S.A.). Immuno-histochemical staining was also performed on the lymph nodes in the remainder of the axilla during the learning phase. A sentinel node procedure was considered false negative if the initial pathology evaluation revealed no tumor cells in the sentinel node while one or more non-sentinel nodes were tumor-
positive. These cases can either be detected right away by the incidental or deliberate removal of other nodes or during follow up as a clinical recurrence. The standard definition of sensitivity was used: it was calculated over the total number of patients with an involved axilla.

**RESULTS**

Axillary sentinel nodes were identified intraoperatively in 565 out of 606 procedures (93.2%). The sentinel node revealed metastasis in 204 out of 565 of these cases (36.1%) and was false negative in eight. Patient characteristics are found in Table 1. The false negative rate was 2 out of 32 (6.3%) during the learning phase.

**Table 1. Patient characteristics of all eight false negative sentinel node procedures.**

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Gender</th>
<th>Age</th>
<th>Size of primary (cm)</th>
<th>Grade of primary</th>
<th>Location</th>
<th>Presumptive cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>44</td>
<td>1.7</td>
<td>2</td>
<td>UOQ</td>
<td>Surgical delay</td>
</tr>
<tr>
<td>2a</td>
<td>F</td>
<td>44</td>
<td>1.8</td>
<td>1</td>
<td>LOQ</td>
<td>Pathological sampling error</td>
</tr>
<tr>
<td>2b</td>
<td>F</td>
<td>45</td>
<td>1.8 / 1.5</td>
<td>2</td>
<td>UOQ</td>
<td>Pathological sampling error</td>
</tr>
<tr>
<td>2c</td>
<td>M</td>
<td>54</td>
<td>2.2</td>
<td>2</td>
<td>C</td>
<td>Pathological sampling error</td>
</tr>
<tr>
<td>3a</td>
<td>F</td>
<td>46</td>
<td>2.1</td>
<td>1</td>
<td>UOQ</td>
<td>Tumor blocking</td>
</tr>
<tr>
<td>3b</td>
<td>F</td>
<td>66</td>
<td>2.0</td>
<td>1</td>
<td>UOQ</td>
<td>Tumor blocking</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>84</td>
<td>2.1</td>
<td>1</td>
<td>UOQ</td>
<td>?</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>43</td>
<td>3.0</td>
<td>2</td>
<td>UOQ</td>
<td>?</td>
</tr>
</tbody>
</table>

UOQ: upper outer quadrant, LOQ: lower outer quadrant, C: central

**CASE 1**

A 44 year-old woman presented with a 1.7 cm left breast cancer. On the day before surgery at 8:58 AM, a total of 88.0 MBq (2.4 mCi) $^{99m}$Tc-nanocolloid was injected into the lesion. The last lymphoscintigraphy image (four-hour scan) was reported as showing one axillary sentinel node and no second-echelon nodes. Because of a conflicting schedule, the surgeons were forced to postpone the operation until the afternoon. At 2:30 PM a volume of 1 ml patent blue dye was administered into the lesion. A 4-cm incision was made along the lower hairline of the left axilla. After tracing a blue lymphatic vessel, a blue node was found with a disappointing amount of radioactivity (seventeen counts per second and a background of eight counts per second). The existence of a second sentinel node was considered and exploration was continued. One additional blue lymphatic vessel could be identified, leading to an unstained node with again a low count rate. After harvesting two additional non-blue, low-radioactive nodes, the axilla still showed some radioactivity. At this point the surgeons decided to perform the wide local excision of the primary tumor first. In this way they hoped to lower the background radiation. Nevertheless, no other clear hot or blue nodes could be identified. At that time, intra-operative palpation of the axilla was not routinely performed. A total of three sentinel nodes and one non-sentinel node were submitted for pathological evaluation. Because this patient was operated during the learning phase, a completing axillary lymph node dissection was performed. All three sentinel nodes and the non-sentinel node were free from metastases. The axillary lymph node dissection specimen contained seventeen additional nodes. Five of these nodes
showed metastatic foci, with a size of 2 mm to 1.2 cm. The primary tumor was a 1.7 cm infiltrating ductal carcinoma grade III.

The left breast, axilla and parasternal region were treated with radiotherapy. Adjuvant systemic chemotherapy was instituted, followed by Tamoxifen. Forty months later, the patient was doing well without evidence of disease.

Remarks: A 30 hour delay and a physical half-life of six hours leave us with a mere 3.1% of the injected radioactivity dose at the time of surgery. Beside that, most of the tracer stays at the injection site. After 24 hours, the median uptake in a sentinel node is just 0.16% of the injected dose. This is sufficient for the injected amount of radioactivity. With these facts in mind, one must conclude that the extra delay in this patient is accountable for creating a situation in which the intraoperative assistance of the radioactive tracer is diminished. If surgery takes place the next day, it should be done as the first case in the morning. In our 599 patients the mean time interval was 23.5 hours (range 18.5 - 31), thus within the acceptable 24 hours range.

CASE 2A

A 44 year-old woman presented with a 1.8 cm left breast cancer. Lymphoscintigraphy showed one sentinel node in the axilla. No second-echelon nodes were seen. The next morning, a volume of 1.0 ml patent blue dye was injected into the lesion. A blue lymphatic vessel was identified and followed to a threesome of nodes. One node was only blue, the second was only radioactive and the third one was neither. The first two nodes were considered to be the sentinel nodes and were removed. The primary tumor was excised.

Neither one of the two sentinel nodes showed evidence of tumor at pathological evaluation. The primary tumor was a 1.8 cm infiltrating ductal carcinoma grade II.

The left breast was treated with radiotherapy. No adjuvant systemic chemotherapy was instituted. After 21 months, the patient presented with a palpable left axillary recurrence for which she underwent an axillary lymph node dissection. Pathological evaluation of the axillary lymph node dissection specimen showed massive tumor invasion and lymph nodes were hardly recognizable. Adjuvant radiotherapy to the axilla, supraclavicular and parasternal regions was given as well as chemotherapy and Tamoxifen. The patient was doing well with no evidence of disease three years after the first operation. The pathologist was asked to review the original sentinel nodes. Additional serial sectioning of these nodes revealed two clusters of approximately ten tumor cells in the marginal sinus of one sentinel node.

Remarks: This false negative sentinel node procedure was due to a pathological sampling error. Two other patients in our series were initially reported to have tumor-negative sentinel nodes and tumor-positive second-echelon nodes (no. 2b and 2c in the table). In case 2b a tumor-positive non-sentinel node was found in the lateral part of a simple mastectomy specimen. After palpation of the biopsy wound in case 2c, a suspicious non-sentinel node was harvested and found to be tumor-positive. Additional serial sectioning of the sentinel nodes of both patients showed micrometastases after all.

CASE 3A

A 46 year-old woman presented with a 2.1 cm right breast cancer. Lymphoscintigraphy demonstrated one axillary sentinel node with two second-echelon nodes. During surgical exploration, a blue lymphatic vessel was followed to a blue-stained, radioactive node. The sentinel node was harvested. Palpation of the biopsy wound revealed two firm lymph nodes of less than 1 cm, which were neither radioactive nor blue. Both nodes were removed because they
felt suspicious. On frozen section examination, the sentinel node was found to be free of disease, but one of the two non-sentinel nodes was tumor-positive. A wide local excision of the primary tumor was performed followed by a complete axillary lymph node dissection. Serial sectioning of the sentinel node at fourteen levels confirmed the tumor-negative result of the frozen section investigation. The involved non-sentinel node was 0.4 cm and showed massive tumor invasion. One of the other eighteen axillary lymph nodes contained metastasis as well. The primary tumor was a 2.1 cm infiltrating ductal carcinoma grade II. The right breast, axilla and parasternal region were treated with radiotherapy. Adjuvant systemic chemotherapy was instituted, followed by Tamoxifen. The patient was doing well without evidence of disease 26 months later.

Remarks: One additional patient (no. 3b in the Table) was similar to Case 3a. A tumor-positive non-sentinel node was found in the axilla by means of intra-operative palpation. A complete axillary lymph node dissection was performed. She was free of tumor and doing well eight months later.

Lymph flow obstruction by tumor within the node is the presumptive cause in these cases. Uptake of the radioactive tracer and the patent blue dye will not occur if a sentinel node is completely invaded by tumor. The lymph flow will be rerouted to a neo-sentinel node and so will be the tracers. How can we recognize this feature? Sometimes it is possible to identify a blue lymphatic vessel approaching a node and stopping right in front of it, without actually staining the node. In these cases blocking of the dye by tumor inside the node must be considered and such a node should be named the sentinel node. We have experienced that palpation of the biopsy wound may be helpful. In this way solid lymph nodes might be detected which could not be felt through the skin at physical examination. Preoperative axillary ultrasonography also provides information about the lymph node status. Both preoperative ultrasonography and intraoperative palpation have now become standard at our institution.

CASE 4

An 84 year-old woman presented with a 2.1 cm left breast cancer. Lymphoscintigraphy showed one sentinel node and one second-echelon node in the axilla. Before the incision, at 8:30 AM, a volume of 1.0 ml patent blue dye was injected into the lesion. A blue lymphatic vessel was followed to a radioactive and blue node. Another blue and radioactive node was found very close to the first and both were harvested. No other suspicious lymph nodes were found after palpation of the biopsy wound. The primary tumor was excised by means of a simple mastectomy. Both nodes were free of metastatic tumor. In the lateral part of the mastectomy specimen, ten additional lymph nodes were found. Two of them contained metastases: 0.5 mm and 4-mm. Additional serial sectioning of the sentinel nodes still revealed no tumor cells. The primary tumor was a 2.1 cm infiltrating ductal carcinoma grade I. Adjuvant hormone therapy was instituted (Tamoxifen). No radiotherapy was given because of cardiac problems and advanced age. The patient was doing well without evidence of disease four months later.

Remarks: No cause could be found to explain this false negative procedure.

CASE 5

A 43 year-old woman presented with a 3 cm left breast cancer. At 9:12 AM on the day before surgery, a total of 63.1 MBq (1.7 mCi) $^{99m}$Tc-nanocolloid was injected into the lesion. One sentinel node and three second-echelon nodes were seen in the axilla. Before incision, at 11:17
A volume of 1.0 ml patent blue dye was injected around the lesion in the left breast. Intratumoral injection failed because the tumor was too solid. An incision was made along the lower hairline in the left axilla. A big blue lymphatic vessel was followed to a blue-stained, radioactive node. A second blue vessel led to another blue-stained, radioactive node. After harvesting both level I axillary nodes, scanning of the wound still showed some radioactivity in level II of the axilla. Exploration of this area revealed one more radioactive node with a small blue spot. Since this patient was part of the learning phase, a completing axillary lymph node dissection was performed in addition to a wide local excision of the primary tumor. All three sentinel nodes were free of tumor. The axillary lymph node dissection specimen contained twelve additional nodes, one of which contained a micrometastasis in the marginal sinus. The primary tumor was a 3 cm infiltrating ductal carcinoma grade II, with a slight in situ component.

The patient underwent a bilateral oophorectomy two months after breast surgery. Additional radiotherapy was given to the left breast and parasternal region. She was doing well without evidence of disease 34 months later.

Remarks: The unusual aspects of this procedure were the small amount of radioactivity, the longer interval between lymphoscintigraphy and operation and the injection of the blue dye around the tumor instead of into the actual lesion. However, no definite cause for the failure was identified.

Discussion

A total of eight patients out of a group of 212 with a tumor-positive axilla had a false negative sentinel node biopsy. Only one of these eight patients developed an actual recurrence in the axilla. Establishing the true incidence of axillary recurrence requires a longer period of follow-up, but several lessons can already be learned from the false negative sentinel node procedures described above. First of all, it makes sense to perform the sentinel lymphadenectomy as early as possible because more counts facilitate identification of the correct node. In a two-day protocol, the sentinel node biopsy should be the first case in the morning. Intraoperative palpation of the biopsy wound should be done during every procedure to diminish the chances of leaving behind involved nodes in the presence of a false negative sentinel node. The use of both a radioactive tracer and blue dye has been shown to be preferable to the use of a single agent. Wong et al. supported the fact that injection of both tracers improved the ability to detect multiple sentinel nodes. Case 2a demonstrates again that a sentinel node can be only blue or only radioactive. For pathological evaluation a minimum amount of three slices at a maximum of 250 μm intervals is recommended in the Netherlands. At our institute, almost all sentinel nodes are first cut in half. At least three slices of 50 to 150 μm are then made of both parts, for a total of seven slides including the frozen section. Although in some cases this was not enough, we feel that our protocol strikes a balance between sensitivity and workload for the pathologist. A total of 1200 sentinel nodes were pathologically examined in the present series of patients. The pathological false negative rate is three out of 221 tumor-positive sentinel nodes so far (1.3%). The chance that an initially reported tumor-negative node becomes positive after additional serial sectioning is three out of 982 (0.3%), but we must emphasize that not all of these 982 sentinel nodes have been re-examined. If that were to be done, one might expect more false negative cases, although this does not mean that all these patients will develop axillary recurrence.
Further reduction of the incidence of false negative sentinel node biopsies may be accomplished by meticulous preoperative evaluation of the axilla including ultrasonography and fine needle aspiration cytology of suspicious nodes.\(^7\)\(^,\)\(^8\) A 1999 review of learning phase studies showed a wide variety of false negative rates: 0-40\%.\(^9\)

Subsequently, three large observational studies showed just a single false negative case in patients who did not undergo confirmatory axillary lymph node dissection after harvesting tumor-free sentinel nodes. Veronesi et al. described 379 procedures with 285 tumor-negative sentinel nodes cases.\(^10\) No axillary recurrences were observed but the follow up duration was still short with 343 person-years of risk. The authors expect that up to seven patients may recur in the axilla in the future. A total of 133 patients with a median follow-up of 39 months (range: 24-51 months) and no axillary recurrences were described by Giuliano et al.\(^11\) In a series of 100 patients with a median follow-up of 24 months, Roumen and coworkers encountered one patient with axillary recurrence after a tumor-free sentinel node had been removed.\(^12\) A review of the literature revealed just one other false negative case after abandoning routine axillary node dissection. That case was seen at our institution and is included in the present series.\(^13\)

**CONCLUSIONS**

In conclusion, a meticulous technique is necessary in sentinel node procedures in breast cancer patients. Intraoperative palpation of the biopsy wound can identify suspicious lymph nodes. To further reduce the false negative rate, we recommend preoperative ultrasonography of the axilla. The combination of both a radioactive tracer and blue dye is preferred and in a two-day protocol surgery must be performed first thing in the morning. Seven slices of 50 to 150 \(\mu m\) strike an acceptable balance between sensitivity and work load for the pathologist.

**REFERENCES**

CHAPTER 9

