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# Intraoperative sentinel lymph node examination by frozen section, immunohistochemistry and imprint cytology during breast surgery – A prospective study

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## ABSTRACT

The aim of this study was to compare the sensitivity of intraoperative frozen section with hematoxylin-eosin (H&E) staining, immunohistochemistry (IHC) or imprint cytology (IC) in the analysis of sentinel node (SN) in breast cancer. Towards this end, a prospective study of 102 patients undergoing mastectomy or sector resection with SN biopsy was conducted. Frozen sections of SN with H&E, IHC staining and IC had sensitivities of 73.5%, 75.5% and 51.0%, respectively. The combination of H&E and IHC raised the overall sensitivity to 83.7%. Macrometastases (>2 mm) were detected in 100% of the cases with H&E, 92.6% with IHC and 81.5% with IC; and micrometastases (≤2 mm) in 35.0%, 45.0% and 5.0%, respectively. The combination of H&E and IHC staining raised the sensitivity to 55.0%. Frozen-section analysis with H&E staining showed high sensitivity in detecting macrometastases but not micrometastases. The sensitivity for detection of micrometastases was not substantially increased by the use of intraoperative IHC. Imprint cytology did not provide any additional information.

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## 1. Introduction

Axillary lymph node dissection with removal of about ten lymph nodes is a standard operative procedure, providing acceptable staging and local control in the treatment of breast cancer.<sup>1,2</sup> However, the axillary dissection is associated with a significant long-term morbidity, including upper arm pain, shoulder dysfunction, numbness and lymphoedema of the arm.<sup>2,3</sup> There is also a correlation between the number of nodes excised and the severity of the postoperative discomfort.<sup>3,4</sup> It is only patients with truly involved nodes who may benefit from complete axillary dissection.

Sentinel node (SN) biopsy has been developed as an alternative to full axillary lymph node dissection for staging node-negative breast carcinoma.<sup>5–7</sup> It combines the potential advantage of decreasing the morbidity associated with axillary lymph node dissection with high accuracy in the prediction of axillary lymph node status.<sup>8</sup> A key issue is how to study SN intraoperatively, both quickly and as accurately as possible, to be able to proceed to an axillary clearance in one procedure. Studies of sensitivity and specificity of frozen sections, intraoperative immunohistochemistry (IHC) and imprint cytology (IC) have revealed varying results, usually with high sensitivity for detection of macrometastases, but

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insufficient sensitivity for detection of micrometastases.<sup>9</sup> Few studies have tested combinations of intraoperative IHC of frozen embedded sections and routine hematoxylin-eosin (H&E) staining and IC. The aim of this report was to compare the sensitivity of different intraoperative histopathological procedures used separately and in combination in the analysis of sentinel nodes in breast cancer within a prospective study of consecutive patients.

## 2. Patients and methods

### 2.1. Surgical treatment and sentinel node biopsy

One hundred and two women with breast cancer and clinically negative axillary nodes underwent sentinel lymph node biopsy at the unit for breast cancer surgery at Huddinge University Hospital. Patients with multifocal tumours, clinically detected axillary metastases, earlier history of breast surgery, tumours larger than 3 cm, a need for neoadjuvant treatment, and pregnant women were excluded from the study. Sentinel node biopsy was performed using a dye and radioisotope technique as described previously.<sup>10</sup>

Between August 1999 and September 2000 all sentinel node biopsies were followed by axillary clearance, as part of a validation study of the sentinel node technique, irrespective of the outcome of the biopsy. After September 2000 patients were enrolled in a prospective study, where axillary clearance was performed only if sentinel node contained metastases larger than 0.2 mm in diameter. Only node-positive patients were included. Patient inclusion was stopped December 2001.

### 2.2. Definition of micrometastases

In the AICC New Staging System, an isolated tumour cell cluster (ITC) smaller than 0.2 mm, only found with immunohistochemistry, is classified as node-negative (N0); a finding between 0.2 and 2 mm is classified as node-positive (N+) and a macrometastasis is defined as a metastasis in a sentinel node larger than 2 mm.<sup>11,12</sup>

In this study micrometastases were defined as a unifocal group of cancer cells smaller than 2 mm, multifocal groups of cancer cells, each smaller than 2 mm. We also included three patients with single cancer cells in an area smaller than 0.2 mm (ITC), since the above mentioned definition was not in use when the patients were recruited.

### 2.3. Histopathological examinations

After dissection of the sentinel node, the specimen was sent fresh to pathology for frozen-section examination with hematoxylin and eosin staining (H&E), immunohistochemistry with cytokeratin antibodies (IHC), and imprint cytology (IC). The lymph node was bisected. Imprint cytology was performed by pressing each cut surface of the node against a glass slide, which was then air-dried and stained with MGg (May-Grynewald giemsa). Three consecutive sections were taken from both cut surfaces and stained with H&E and one with cytokeratins, using a rapid staining method (EPOS anti-cytokeratins/HRP Dako) with the MNF 116 monoclonal antibody.<sup>13</sup>

The entire sentinel node was submitted for histological processing and was examined for evidence of metastasis. Routine processing of each half of the previously bivalved sentinel node specimen consisted of formalin fixation, paraffin imbedding and serial step sectioning at three levels, staining with H&E, and one last section for cytokeratin immunohistochemistry (MNF 116, Dako with protease pretreatment using a Tech-mate 500 plus). All the initial routine slides were later reviewed. The touch preparations were evaluated postoperatively without knowledge of the status of the sentinel node. Sensitivity was defined as the number of patients with a positive test, divided by the total number of patients with a positive axilla, as found by any of the methods. Accuracy is the number of patients in whom the test gave a correct result, positive or negative, divided by all patients.

## 3. Results

The mean age of all the 102 patients was 57 years (range 31–83). The median size of all measured primary tumours was 19.5 mm (range 5–62 mm). The mean number of SN was 1.8/patient; 44 patients had 1 SN, 37 had 2 SN and 21 patients had 3 or more SN. Of the 102 breast cancer patients, 48 (47.1%) had metastases in the axillary lymph nodes. Of the 48 with positive nodes, 20 (41.6%) were found to have micrometastases. A ductal carcinoma was found in 90 patients, lobular carcinoma in 9, two patients had a combined ductal and lobular, and one had mucinous cancer.

### 3.1. Overall sensitivity

The overall sensitivity of intraoperative frozen section with H&E staining was 73.5% (36/49), with IHC 75.5% (34/49) and with IC 51.0% (25/49). The accuracy of the three methods was 87.2%, 88.2% and 76.4%, respectively. The combination of intraoperative frozen section with H&E staining and IHC raised the sensitivity to 83.7% (41/49), whereas addition of intraoperative IC examination did not affect the results (Table 1).

### 3.2. Sensitivity in detecting macro- and micrometastases

Macrometastases were found in 27 patients, all of which were identified using intraoperative H&E staining, whereas IHC only detected 25 of them (sensitivity 92.3%). Imprint cytology detected 22 out of the 27 cases with macrometastases (sensitivity 81.5%).

Micrometastases ( $\leq 2$  mm) were detected with the intraoperative frozen-section examination and HE staining in 7 out of 20 patients (sensitivity 35%) and with intraoperative IHC staining on frozen section in 9 of 20 patients (sensitivity 45.0%). The combination of these two methods raised the detection rate to 11 out of 20 patients (sensitivity 55.0%). Six out of 20 micrometastases were found only with IHC and two only with H&E. Imprint cytology detected one of the cases of micrometastases, which was also found with H&E (sensitivity 5%), and thus added nothing to overall detection rate.

**Table 1 – Percentage and absolute sensitivity and accuracy of three different intraoperative analyses of sentinel lymph nodes in 102 breast cancer patients**

Intraoperative examination of SN	All metastases sensitivity (absolute number)	All metastases accuracy (absolute number)	Macrometastases >2 mm sensitivity (absolute number)	Micrometastases <2 mm sensitivity (absolute number)
Frozen section	73.5%	87.2%	100%	35.0%
Hematoxylin-eosin	(36/49)	(89/102)	(27/27)	(7/20)
Frozen section	75.5%	88.2%	92.6%	45.0%
Immunohistochemistry	(37/49)	(90/102)	(25/27)	(9/20)
Imprint cytology	51.0%	76.4%	81.5%	5.0%
	(25/49)	(78/102)	(22/27)	(1/20)
H&E and IHC combined	83.7%	92.2%	100%	55.0%
	(41/49)	(94/102)	(27/27)	(11/20)

#### 4. Discussion

Intraoperative frozen sectioning with H&E staining in sentinel node, used as a single procedure, had high sensitivity in detecting macrometastases but was not optimal in detecting micrometastases. Frozen section with IHC was somewhat superior to H&E in detecting micrometastases, but did not add anything to H&E concerning macrometastases. The combination of these two methods raised the sensitivity but still, only little more than half of the cases with micrometastases were found on intraoperative assessment. Imprint cytology detected 4 out of 5 macrometastases but did not find micrometastases and its value in our hands was not high.

Our results are in accordance with previously published results. In a review, Van Diest<sup>14</sup> pointed out that under optimal conditions, frozen section with H&E could have a sensitivity of 80–85% at best, due to the limited number of sections available at intraoperative workup. This agreed very well with their own experience.<sup>15</sup> More recently however, a larger follow-up study, with an increased number of lobular carcinomas and frozen sections done by less experienced pathologists, found a sensitivity of only 60%.<sup>16</sup> A study of frozen sections of lymph nodes retrieved during axillary sampling showed a sensitivity of 73%; the diagnosis was correct in 81 out of 88 node examinations but missed seven of the 26 cases with involved nodes.<sup>17</sup> Two later reports on sentinel node showed a sensitivity between 73% and 86%.<sup>18,19</sup> The most plausible reason for diverging results is the technique of sectioning of the sentinel node, both intraoperatively and postoperatively. In general, the sentinel node concept has led to a more careful examination of the first node and has highlighted the larger number of micrometastases that this new technique detects. In order to minimise the number of intraoperative ‘false negative’ analyses of the sentinel node, various histopathological methods have been considered. Multiple sections and H&E staining of the sentinel node increased the frequency of detected micrometastases by 3%, with a further increase of 13% when combined with IHC.<sup>20</sup> Using multiple sectioning of sentinel node intraoperatively as the only histopathological examination, Viale achieved a general agreement of 96.7% between sentinel node and axillary lymph node status.<sup>21</sup> However, multisectioning of sentinel node is time-consuming, besides involving excessive tissue loss. The shortage of breast pathologists in Sweden is

a reality and while cutting the SN into 60 slices intraoperatively as described by Viale, does not achieve optimal sensitivity, is time-consuming and expensive. It is also important to remember that even with serial sections and IHC, only about 1–2% of the node will be examined and that some SN tissue is bound to be lost, with the theoretical risk that some micrometastases will be missed.

The addition of IHC when using standard sectioning of the sentinel node, could not be expected to increase the sensitivity more than marginally, which is in accordance to both our results, and others.<sup>21,22</sup> Nähnig and colleagues, in small material, found an increase in sensitivity from 82% for H&E to 88% for IHC. However, the only case detected intraoperatively with IHC that did not show on H&E was a case with isolated tumour cells. In our hands the addition of IHC prolonged the pathological procedure with about 15 min, and the cost of the cytokeratine antibodies was 80 Euro per examination. The cost and time for IHC as addition to H&E therefore does not seem warranted.

Intraoperative use of frozen sections has been shown in several studies to be highly accurate for the detection of macrometastases, but suboptimal for the detection of micrometastases.<sup>9,18,19,23,24</sup> This is confirmed in our study, with 100% sensitivity for macrometastases, but only a little over 50% for micrometastases, even when combining H&E and IHC. The clinical importance of micrometastases has been debated, and results from different studies differ.<sup>25,26</sup> The consensus today is to consider micrometastases between 0.2 and 2 mm in diameter as node-positive, whereas smaller deposits of tumour cells are regarded as node-negative.<sup>11,12</sup> A small number of patients will therefore be subject to a reoperation in the axilla after intraoperative frozen sections, due to findings of micrometastases in the final pathological workup.

Imprint cytology (IC) has been evaluated for detecting sentinel node metastases.<sup>27–31</sup> The sensitivity varies greatly between 34% and 96.4%. The reason for this great variability is hard to interpret, but is probably due both to differences in methodology and experience. Two formal comparisons of IC and frozen sections stained with H&E have shown comparable sensitivity within each study, but completely different level between the two studies, 57% vs. 89%.<sup>9,32</sup> As for frozen sections the sensitivity is much better for macroscopic metastases than for microscopic disease.<sup>9,29–31</sup> The

sensitivity of imprint cytology in our hands was not acceptable for detecting the total number of metastases but identified 4 out of 5 macrometastases. However, even if this is a cheap and quick method, the reliability seems inferior to frozen sections with H&E. This is a standard procedure that most pathology laboratories are familiar with, and is now recommended as intraoperative method in sentinel node biopsy for breast cancer in Sweden.

In conclusion, frozen section with H&E staining is capable of detecting all macrometastases in the sentinel node in breast cancer patients. Addition of IHC intraoperatively is not necessary since it only marginally increases overall accuracy at considerable expense. None of the methods was optimal for detection of micrometastases and a few patients with microscopic metastases will require second operation in the axilla.

### Conflict of interest statement

None declared.

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