

Sentinel node investigation in breast cancer: detailed analysis of the yield from step sectioning and immunohistochemistry

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Abstract

Aims—To evaluate in detail the extent to which step sectioning and immunohistochemical examination of sentinel lymph nodes (SNs) in patients with breast cancer reveal additional node positive patients, to arrive at a sensitive yet workable protocol for histopathological SN examination.

Methods—This study comprised 86 women with one or more positive SN after a successful SN procedure for clinical stage T1–T2 invasive breast cancer. SNs were lamellated into pieces of approximately 0.5 cm in size. One initial haematoxylin and eosin (H&E) stained central cross section was made for each block. When negative, four step ribbons were cut at intervals of 250 µm. One section from each ribbon was stained with H&E, and one was used for immunohistochemistry (IHC).

Results—When taking the cumulative total of detected metastases at level 5 as 100%, the percentage of SN positive patients increased from 80%, 83%, 85%, 87% to 88% in the H&E sections through levels 1 to 5, and with IHC these values were 86%, 90%, 94%, 98%, and 100%. Three of nine patients in whom metastases were detected at levels 3–5 only had metastases in the subsequent axillary lymph node dissection.

Conclusions—Multiple level sectioning of SNs (five levels at 250 µm intervals) and the use of IHC detects additional metastases up to the last level. Although more levels of sectioning might increase the yield even further, this protocol ensures a reasonable workload for the pathologist with an acceptable sensitivity when compared with the published literature.

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Sentinel node (SN) biopsy is rapidly gaining acceptance as a staging procedure in breast cancer.^{1–3} When the SN is identified successfully, the absence of tumour in this node predicts with a high degree of accuracy the absence of metastases in the remaining axillary lymph nodes.^{4–9}

The reliability of the SN procedure as an accurate staging procedure is dependent on the ability to identify the true SN and the extent of histopathological examination of the SN. Both

are instrumental in limiting the false negative rate of the SN procedure.

Multiple level sectioning has been shown to increase the detection rate of SN metastases in several studies.^{10–13} However, complete serial sectioning would result in an unacceptable workload for the pathologist. Therefore, a compromise must be found between workload and sensitivity by limiting the degree of step sectioning and immunohistochemistry (IHC) on multiple levels. Up to now, no consensus exists on the most (cost-)effective protocol.^{9 14–17} Furthermore, no studies have yet reported on the precise yield of each additional level of step sectioning.

Our study was performed to evaluate in detail the yield of multiple levels and the use of IHC for detecting metastases in SNs, to arrive at a protocol for optimal SN investigation.

Methods

From a total of 250 consecutive women with clinical stage T1–T2 N0M0 invasive breast cancer who underwent a successful SN procedure from October 1994 to October 1999, we identified 86 patients in whom one or more SNs proved to be positive. The mean age of the patients was 56 years (range, 33–84), and pathological tumour size was on average 2 cm (range, 0.6–6).

We evaluated the extent of SN processing required before these patients were found to be positive. All patients who were initially negative by haematoxylin and eosin (H&E) on the first SN section subsequently underwent standard multiple sectioning at 250 µm intervals and staining with both H&E and IHC. We assessed at which additional level patients who were initially found to be negative were subsequently converted to SN positive.

SENTINEL LYMPH NODE BIOPSY

The day before surgery, 40 MBq of ^{99m}Tc colloidal albumin was injected in two to four depots peritumorally. Lymphoscintigraphy was done to detect the presence, location, and number of focal accumulations.

Just before surgery, 0.5 ml of 2.5% patent blue solution (Guerbet, Aulnay-sous Bois, France) was injected intracutaneously just around the areola. During surgery, axillary focal tracer accumulations were localised using a handheld γ probe (c-track; Carewise, Morgan Hill, California, USA). All hot and blue nodes were removed as an SN. Furthermore, all radioactive nodes were biopsied until less than 10% of residual radioactivity, compared with the activity of the hottest SN, remained in the axilla.

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Table 1 Cumulative number of patients with breast cancer sentinel node metastases found with each additional level (250 µm intervals) in a total of 86 patients

	H&E	%	IHC	%
Level 1	69	80	74	86
Level 2	71	83	77	90
Level 3	73	85	81	94
Level 4	75	87	84	98
Level 5	76	88	86	100

H&E, haematoxylin and eosin staining; IHC, CAM5.2 immunohistochemistry.

TISSUE PROCESSING

SNs smaller than 0.5 cm were processed intact, those between 0.5 and 1 cm were halved, and SNs larger than 1 cm were lamellated into pieces of approximately 0.5 cm in size. In most cases, intraoperative fresh frozen section analysis of all individual pieces was performed.¹⁹ After frozen section analysis, the SN was fixed in neutral buffered formaldehyde and embedded completely. One initial 4 µm thick H&E stained section was made for each block. When negative, an additional section was done at the first level for IHC and four step ribbons were cut at an interval of 250 µm. From these ribbons one section was stained with H&E, and one was used for IHC with the CAM5.2 antibody (Becton Dickinson, San Jose, California, USA). All slides were examined by the same pathologist (PJvD), taking the frozen section and its control paraffin wax embedded section as level 1.

All SNs containing any cell consistent with an epithelial morphology (and immunophenotype) were considered metastase positive.

Results

Of the 86 patients eventually found to have positive sentinel nodes, 69 patients were found to be positive on the first SN section stained with H&E. Additional step sectioning and IHC were required in the remaining 20% of patients.

Interestingly, all 15 patients with more than one positive sentinel node were found to have SN metastases on the first H&E stained section. The percentage of SN positive patients increased from 80%, 83%, 85%, 87% to 88% in the H&E sections through levels 1 to 5, and with IHC these figures were 86%, 90%, 94%, 98% and 100%. At the first level, IHC revealed five additional SN positive patients and the additional levels 2 to 5 revealed metastases in three, three, four, and two more patients, respectively (table 1). Of the nine patients with SN metastases only detected at levels 3–5, three had further metastases in the subsequent axillary lymph node dissection.

Looking at all harvested nodes in these 86 SN positive patients, a total of 103 tumour positive SNs were found. When taking the cumulative total of detected metastases at level 5 as 100%, the percentage of positive SNs increased from 81%, 83%, 85%, 86% to 87% in the H&E sections through levels 1 to 5. With IHC, the increase was from 86%, 90%, 94%, 98% to 100%. The first level failed to detect metastases in 14 SNs. Additional levels 2 to 5 yielded metastases in four, four, four, and two more positive SNs, respectively (table 2).

Table 2 Cumulative number of breast cancer sentinel node metastases found with each additional level (250 µm intervals) in 103 sentinel nodes

	H&E	%	IHC	%
Level 1	84	81	89	86
Level 2	86	83	93	90
Level 3	88	85	97	94
Level 4	89	86	101	98
Level 5	90	87	103	100

H&E, haematoxylin and eosin staining; IHC, CAM5.2 immunohistochemistry.

Discussion

Because validation studies have shown high accuracy rates for the SN procedure,^{4–9} it can now be used as a staging procedure without the need for complete axillary lymph node dissection in patients with a negative SN.¹

Provided that the surgeon has performed a reliable SN biopsy procedure, it is up to the pathologist to determine whether axillary metastases are present or not.

In the days of routine axillary dissection, it was shown that a large proportion of patients are converted from node negative to node positive with more elaborate histological examination of axillary nodes.^{16 20 21} This has important consequences for the clinical management of these patients because, in general, lymph node positive patients will receive adjuvant treatment. For these reasons, the histopathological examination of SNs tends to be even more elaborate, with the additional argument that it can be done so with an acceptable workload for the pathologist because only a few nodes need to be examined this way.

Our study clearly shows that more patients are converted to node positive with each additional step of sectioning (at 250 µm intervals) and that with each additional step IHC has a higher sensitivity than H&E. The clinical relevance of finding these metastases is underlined by the fact that several patients with SN metastases, only visible at level 3–5, had second echelon metastases.

However, even with this intensive protocol not all nodal metastases will be detected. Such a 100% sensitivity can theoretically only be reached with complete serial sectioning of the SN at 12 µm intervals.²²

However, the question arises whether one really needs to find all metastatic cells. Therefore, one needs to arrive at a method that has a high sensitivity with an acceptable workload for the pathologist.

In a few studies complete serial sectioning of the SN has been done. For instance, Cserni¹¹ serially sectioned the SN up to extinction with 3–5 µm thick slices and examined every 10th to 20th level. In the final analysis, 15 of the 21 patients with metastases limited to the SN were positive on the initial central cross section. Dowlathshahi *et al* performed complete serial sectioning at 250 µm intervals and found that only six of 30 SN positive patients were positive on the initial section examined by H&E and IHC.²³ This difference is remarkable, also in view of the fact that Cserni's intervals were smaller and therefore it would be expected that a greater number of additional metastases

would be found. The greater yield on the initial section in Cserni's study could be ascribed to the fact that they tried to cut the central cross section guided by the blue lymphatic vessel.²⁴ In addition, the average size of the metastases may have been greater in patients in Cserni's study (68% T2 tumours versus 25% in the study by Dowlatsahi *et al*).

Nevertheless, the fact remains that the use of IHC and additional step sectioning improves the detection rate of metastatic deposits. The question for practical purposes then remains: how many additional levels (and at what interval) need to be examined?

Turner *et al* examined 10 levels at 40 µm intervals in 42 SN positive patients in whom the initial H&E section was negative.¹⁰ In these patients, with the use of IHC the first two levels of SN examination found additional metastases in all but one patient. They therefore concluded that the additional eight levels of examination did not significantly contribute to the detection of additional metastases. However, with 10 levels at 40 µm intervals only 400 µm of the entire SN is examined, which might not be sufficient for the detection of all metastases. Rather than taking many sections at small intervals, it may be more efficient to take fewer sections at larger step intervals.

Our present study shows in detail the yield of step sectioning and IHC at five levels with an interval of 250 µm. Because we routinely perform frozen section analysis, which leads to some loss of material, this ensures sampling through the larger part of the SN. In practice, this has proved to be an acceptable workload. Clearly, the yield increased with additional levels. The first level failed to detect metastases in 14 SNs (14% of the total number of metastases found). Additional levels 2 to 5 yielded metastases in four, four, four, and two more SNs, respectively. Thus, additional levels clearly reveal more metastases and even the fourth and fifth level together reveal 6% additional metastases. Not surprisingly, the yield with IHC was higher than with H&E only. IHC facilitates the detection of single metastatic cells, and speeds up the screening of the sections dramatically. Therefore, some investigators omit H&E staining when IHC is performed. However, we prefer to make H&E control sections because they are helpful in the detection of artefacts and benign inclusions.

In conclusion, step sectioning of SNs with ICH is very useful for finding the smallest metastases in SNs of breast cancer that may be clinically relevant. We therefore propose step

sectioning and ICH at four additional levels, separated by 250 µm intervals, when the original H&E section is tumour negative. Despite the fact that our protocol might still miss some metastases we believe this protocol ensures a reasonable workload for the pathologist with an acceptable sensitivity.

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