

Does the Method of Biopsy Affect the Incidence of Sentinel Lymph Node Metastases?

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■ **Abstract:** More detailed examination of the sentinel lymph node (SLN) in breast cancer has raised concerns about the clinical significance of micrometastases, specifically isolated tumor cells detected only through immunohistochemical (IHC) staining. It has been suggested that these cells do not carry the same biologic implications as true metastatic foci and may represent artifact. A retrospective institutional review board-approved review was conducted on clinically node-negative breast cancer patients who underwent SLN biopsy (SLNB) between 1997 and 2003. Retrospective analysis of tumor characteristics and the method of the initial diagnostic biopsy were correlated with the presence and nature of metastatic disease in the SLN. Of 537 SLNBs, 123 (23%) were hematoxylin-eosin (H&E) positive. SLN positivity strongly correlated with tumor size ($p < 0.001$) and tumor grade ($p = 0.025$), but not with the method of biopsy (needle versus excisional biopsy). Prior to July 2002, we routinely evaluated H&E-negative SLNs with IHC ($n = 381$). Of the 291 H&E-negative patients, 26 had IHC-only detected micrometastases (9%). The likelihood of detecting IHC-only metastases did not correlate with tumor size or grade, but was significantly higher in patients undergoing excisional biopsy than core needle biopsy. While the method of biopsy has no demonstrable effect on the likelihood of finding metastases in the SLN by routine serial sectioning and H&E staining, it may significantly impact the likelihood of finding micrometastases by IHC. IHC should not be used routinely in the evaluation of the SLN and caution should be used when basing treatment decisions (completion axillary lymph node dissection or adjuvant therapy) on IHC-only detected micrometastases. ■

Key Words: breast cancer, immunohistochemistry, micrometastases, needle biopsy, sentinel lymph node

Intraoperative lymphatic mapping and sentinel lymph node biopsy (SLNB) has now become the standard of care in the axillary examination of patients with breast cancer. More accurate and less invasive than axillary dissection, it rapidly changed the management of breast cancer and has spared countless women the lymphedema and other morbidities associated with a complete node dissection (1,2). However, as with all new technologies, SLNB is not without its questions.

Sentinel lymph node biopsy is more sensitive than axillary node dissection in that it allows for detailed examination of the sentinel node by serial sectioning (3,4). While this improves accuracy, it also raises concerns about the clinical significance of micrometastases, specifically in patients with isolated tumor cells detected through immunohistochemical (IHC) staining. It is

possible that we may be detecting tumor cells in the sentinel lymph nodes (SLNs) that don't carry the same biologic implications as true metastatic foci. Why would these cells be present in the SLN if not for having gained the ability to metastasize?

One possibility is that tumors routinely shed cells into the lymphatic vasculature that will never develop into metastatic lesions. Another possibility is that instrumentation of the tumor, as is done with a needle or excisional biopsy, might displace tumor cells into the lymph node. Several authors have demonstrated the possibility that manipulation of the tumor could physically disperse tumor cells that might travel via lymphatic pathways to the regional lymph nodes (5–8). If this is the case, it might be true that the presence of micrometastases in the sentinel node may have more to do with the method of biopsy than with the biologic properties of the cancer.

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MATERIALS AND METHODS

A retrospective institutional review board-approved chart review was conducted on clinically node-negative

breast cancer patients who underwent SLNB from October 1997 to December 2003. Retrospective analysis was conducted of patient demographics, tumor characteristics, and the method of the initial diagnostic biopsy (fine-needle aspiration biopsy [FNAB], core needle biopsy [CNB], or excisional biopsy). It was also recorded whether the margins around both the invasive cancer and any in situ component were involved on initial biopsy. Lymphatic mapping was performed using a variety of injection techniques using 3–4 mCi technetium-99m and isosulfan blue dye (lymphazurin, US Surgical, Norwalk, CT). Lymph nodes with evidence of blue dye uptake or radioactivity as detected by an intraoperative gamma probe (Navigator, US Surgical) were labeled as sentinel nodes, as were lymph nodes that appeared suspicious on exploration.

For all patients, the sentinel node was measured and cut entirely along its longitudinal axis into sections 1.5 to 2 mm thick. The sections were submitted in formalin for paraffin section histology. Each paraffin block was sectioned at three levels and stained with hematoxylin-eosin (H&E). Prior to July 2002, the sentinel nodes also underwent evaluation with IHC for cytokeratin if the H&E stains did not reveal metastases. Cytokeratin protein expression was studied by immunohistochemistry using a keratin cocktail consisting of a mixture of Cam 5.2 (Becton Dickinson, Franklin Lakes, NJ, U.S.A.) and AE1/3 (Boehringer Mannheim, Mannheim, Germany). For the purposes of this study, a sentinel node was designated as positive by IHC only if the metastasis was a group of tumor cells identified only with the IHC stains.

Statistical significance for binary or categorical covariates was calculated using the chi-squared test, with a *p*-value less than 0.05 considered statistically significant.

RESULTS

Between October 1997 and December 2003, 537 SLNB procedures were performed for invasive breast cancer in 531 clinically node-negative patients (528 women and 3 men). The average age was 54.4 years (range 22–85 years). The majority of tumors were invasive ductal carcinoma (84%), including patients with ductal carcinoma in situ (DCIS) and microinvasion. Lobular carcinoma accounted for 10%, while the remaining 6% were other histologies (tubular, medullary, mucinous, and papillary). The mean tumor size was 14 mm, ranging from microinvasive to 7 cm.

More than half of the cancers were diagnosed by core needle biopsy (52%). This includes core biopsies of palpable masses performed in the office and image-guided

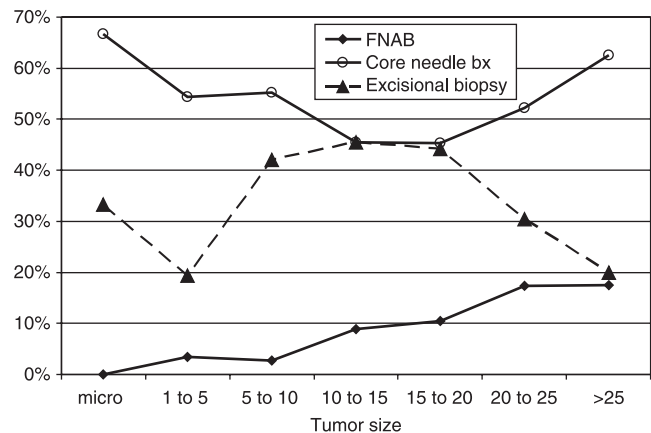


Figure 1. Method of biopsy by size of primary tumor. Core needle biopsy was used more commonly for small lesions (stereotactic core biopsy) or large lesions (percutaneous core biopsy) while both core biopsy and excisional biopsy were utilized equally for lesions between 1 and 2 centimeters.

core biopsies of mammographically detected lesions. Forty-one patients underwent FNAB (8%) and the remaining 40% were diagnosed by excisional biopsy, including both excisions of palpable lesions and wire localized excisional biopsies of nonpalpable lesions. Of the 215 excisional biopsies performed, 136 (63%) had either invasive cancer or in situ cancer extending to one margin, while 79 (37%) had negative margins obtained on diagnostic biopsy. The method of biopsy varied with the size of the lesion (Fig. 1).

All 537 procedures had sentinel nodes evaluated by serial sectioning and H&E staining. A total of 123 patients were positive by these criteria (23%). SLN positivity strongly correlated with the size of the tumor (Fig. 2). In addition to tumor size, tumor grade was significantly associated with the likelihood of finding a positive node by H&E (Table 1). The presence of estrogen receptors (ERs), progesterone receptors (PRs), and expression of HER-2/*neu* did not correlate with node positivity.

When we examined the presence of lymph node metastases detected by H&E as compared to the method of biopsy, there was no correlation found. Patients who underwent needle biopsy, by either FNA or core needle biopsy, had the same likelihood of having metastases in the sentinel node as patients who had an excisional biopsy. When we broke down this group to patients who had positive margins and those who had negative margins after excision, we also found no statistically significant difference in finding metastases.

A different result was obtained when we looked at IHC-only micrometastases. Of the 537 procedures, 381

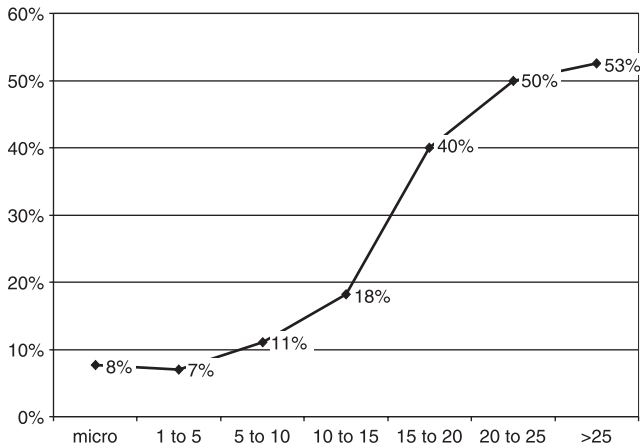


Figure 2. Incidence of a positive sentinel lymph node based on the size of the primary tumor.

were done prior to July 2002, when we abandoned the routine use of IHC. Of these 381 patients, 90 (24%) had H&E-positive sentinel nodes, leaving 291 patients who were H&E negative. Of these 291 patients, 26 had micrometastases detected by IHC only (9%). In this case, the likelihood of detecting IHC-only micrometastases did not correlate with tumor size or grade (Table 2). As with H&E-detected metastases, ER, PR, and HER-2/*neu* status

Table 1. Factors Associated with a Positive SLNB by Routine H&E Staining (n = 532)

	SLN negative	SLN positive	p
Size			
0–5	89 (93%)	7 (7%)	<0.001
5–10	128 (89%)	16 (11%)	
10–15	99 (82%)	22 (18%)	
15–20	51 (60%)	34 (40%)	
20–25	23 (50%)	23 (50%)	
>25	20 (47%)	21 (53%)	
Grade			
I	98 (85%)	17 (15%)	<0.025
II	162 (75%)	54 (25%)	
III	55 (69%)	25 (31%)	
ER			
Positive	273 (77%)	84 (23%)	NS
Negative	79 (73%)	29 (27%)	
PR			
Positive	226 (77%)	70 (23%)	NS
Negative	125 (74%)	43 (26%)	
HER-2/<i>neu</i>			
Positive	68 (72%)	27 (28%)	NS
Negative	238 (77%)	71 (23%)	
Biopsy method			
Needle	239 (75%)	79 (25%)	NS
Excision	170 (79%)	44 (21%)	
Margins after excisional biopsy			
Positive	104 (78%)	31 (22%)	NS
Negative	66 (84%)	13 (16%)	

Table 2. Factors Associated with IHC Positivity in H&E-Negative Lymph Nodes (n = 381)

	SLN IHC negative	SLN IHC positive	p
Size			
0–5	67 (96%)	4 (6%)	0.10
5–10	78 (91%)	8 (9%)	
10–15	65 (94%)	4 (6%)	
15–20	26 (79%)	7 (21%)	
>20	27 (90%)	3 (10%)	
Grade			
I	65 (98%)	1 (2%)	NS
II	92 (88%)	13 (12%)	
III	35 (95%)	2 (5%)	
ER			
Positive	170 (90%)	18 (10%)	NS
Negative	45 (92%)	4 (8%)	
PR			
Positive	149 (90%)	16 (10%)	NS
Negative	65 (92%)	6 (8%)	
HER-2/<i>neu</i>			
Positive	37 (86%)	6 (14%)	0.20
Negative	143 (93%)	11 (7%)	
Biopsy method			
Needle	146 (95%)	8 (5%)	<0.025
Excision	117 (87%)	18 (13%)	
Margins after excisional biopsy			
Positive	70 (84%)	13 (16%)	NS
Negative	47 (90%)	5 (10%)	

also did not correlate with finding micrometastases. However, a statistically significant increase in IHC-only micrometastases was seen in patients having excisional biopsy (13%) compared with patients having a needle biopsy (5%). This was the only factor that correlated with IHC micrometastases. There was no statistically significant difference between positive margin and negative margin excisions, although these numbers are quite small.

DISCUSSION

The results of this retrospective study suggest that while the method of biopsy has no demonstrable effect on the likelihood of finding metastases in the SLN by routine serial sectioning and H&E staining, it may significantly impact the likelihood of finding micrometastases by IHC. Two previous studies, one from Memorial Sloan-Kettering Cancer Center (MSKCC) (9) and one from the John Wayne Cancer Institute (JWCI) (10), also found a significant correlation between the method of biopsy and the finding of metastases in the SLN. However, the two articles had somewhat different findings.

Moore et al. (9), from MSKCC, examined more than 4000 SLN procedures and found that when examining the sentinel nodes by serial sectioning and H&E, the presence of metastases correlated with tumor size and the presence

of lymphovascular invasion (LVI), but not with the method of biopsy. As they had a significant portion of patients who underwent surgery based on clinical and radiographic findings, without tissue biopsy, they had a control group who had no manipulation of the tumor prior to the sentinel node procedure (save mammography or physical examination). When they looked at sentinel nodes positive only by IHC, they found that size and LVI were not significant, but the method of biopsy was, with excisional biopsies having a higher risk than needle biopsies, which had a higher risk than no preoperative biopsy. Our findings at the University of Michigan, though a smaller sample size, are almost the same as the findings from MSKCC. As we routinely do not bring patients to the operating room without some type of tissue diagnosis, we have no similar control group to compare to.

On the other hand, Hansen et al. (10), from JWCI, examined 663 patients who underwent SLNB. As with us, they do not have a group that did not undergo some type of preoperative biopsy. They found that on multivariate analysis, the type of biopsy did correlate with finding metastases in the sentinel node. However, this was any type of metastases, including those found on H&E, and they found that there was a decreased chance of finding disease in the SLN with excisional biopsy as compared to either FNAB or core needle biopsy. When they examined the size of the SLN metastases, FNAB actually favored macrometastases rather than IHC-detected metastases. Core needle biopsy showed no difference when compared to excisional biopsy.

Although there is no clear explanation why the JWCI findings differ, these results confirm the findings from MSKCC. Preoperative needle biopsy does not significantly increase the likelihood of finding metastases in the sentinel node when the node is examined by serial sectioning and H&E, as is the recommendation. In addition, excisional biopsies with positive margins (suggesting the tumor has been cut across) do not lead to increased detection of SLN metastases. These results also imply that IHC-only detected micrometastases may have more to do with artifact than with tumor biology. This raises questions regarding the clinical significance of IHC-only detected micrometastases.

The addition of IHC to routine H&E examination will dramatically increase the detection rate of cancer cells within the SLN (3,11–22). The clinical significance of these micrometastases remains in question, as retrospective studies have suggested no prognostic significance (17,20,22,23), a correlation with disease-free survival but not overall survival (18,19,21), or a correlation with over-

all survival (12,15,16). Few of these studies used multivariate analysis, so it is difficult to answer the question from the existing retrospective data. The fact that IHC-only micrometastases correlate more with the method of biopsy than with tumor factors known to increase the risk of true nodal spread further increases the suspicion that these findings are not clinically significant. Great care must therefore be taken when using IHC-only micrometastases for clinical decision making, as this seems likely to lead to overtreatment. Unless prospective, randomized trials prove otherwise, IHC should not be used in the routine evaluation of sentinel nodes, and if used, patients found to harbor IHC-only micrometastases (American Joint Committee on Cancer [AJCC] stage N0(i+)) should be treated as node negative.

The 2001 Consensus Conference on the Role of Sentinel Lymph Node Biopsy as well as the College of American Pathologists have both recommended that the decision for completion axillary lymph node dissection (ALND) should not be based on IHC-only micrometastases alone (24,25). Of the 26 IHC-only positive patients in this series, 18 had completion ALND, with only 1 patient having additional disease in the nonsentinel nodes. This is comparable to other institution's experiences with IHC-only positive SLNs. Chu et al. (26) reported none of 33 patients with IHC-only positive SLNs had additional disease at completion ALND, and concluded that there is no benefit for patients with T1 or T2 breast cancer and IHC-only micrometastases. Wong et al. (27) reported 6 of 58 patients with nodal metastases on completion ALND for IHC-only positive nodes. The authors concluded that although there may be a slight decrease in the false-negative rate with the addition of IHC, it is not significant enough to subject patients to the morbidity of ALND. Grey et al. (28) found additional disease in 2 of 28 patients with IHC-only micrometastases in the SLN. Both patients had advanced lobular tumors.

Not all reports have similar conclusions. Jakub et al. (29) described 9 of 62 patients with nodal metastases after completion ALND for IHC-only positive lymph nodes, 12% with T1 lesions, and concluded that IHC should be considered in all cases to minimize false-negative rates.

The status of the regional lymph nodes remains the most important prognostic indicator in breast cancer. Lymphatic mapping, SLNB, and completion ALND for sentinel node-positive patients have become the standard of care. Until pending data from prospective trials (American College of Surgeons Oncology Group [ACOSOG] Z0010 and Z0011) are available, it is still recommended that patients with positive SLNs as detected by routine

H&E staining methods undergo completion ALND. The clinical significance of IHC-only positive nodes remains in question.

Based on our results and the growing body of evidence, we recommend against the routine use of IHC in the pathologic evaluation of breast cancer SLNs. For patients who have IHC-only metastases, we recommend a multidisciplinary approach, with patient counseling on the unclear clinical significance, and do not recommend completion ALND for micrometastases detected by IHC staining alone.

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