

Sentinel lymph nodes and breast carcinoma: analysis of 70 cases by frozen section

Khalid I. Al-Shibli, MD; Hiba A. Mohammed MD; Kari S. Mikalsen

BACKGROUND: The sentinel node biopsy (SNB) is a reliable method for determining the status of the regional lymph nodes in patients with breast cancer. SNB technology is evolving rapidly, but no standardization has yet been accomplished. The aim of this study is to discuss the accuracy of this procedure and the optimal method for identifying micrometastases.

METHODS: We collected data from 70 women with primary invasive breast carcinoma who underwent SNB for breast cancer. We examined two frozen sections levels from each half of each lymph node, as well as a cytology imprint before arriving at the frozen section diagnosis. Immunohistochemistry with pancytokeratin (AE1/AE3) was done on the paraffin sections. For the association between the lymph node size and the possibility of metastases, Student's t test was used and a *P* value of less than 0.05 was regarded as significant.

RESULTS: The number of patients with metastases in SNB was 19, from which 15 cases were correctly diagnosed in frozen sections/imprints and four cases were false negative. The axillary toilet from all cases with SNB metastases smaller than 2 mm showed no additional positive nodes. Lymph node diameter showed a significant association with sentinel node status ($P < 0.0001$).

CONCLUSION: Frozen section examination of SNB from patients with breast carcinoma is both specific (100%) and sensitive (79%). Diagnosis of lobular carcinoma can be difficult, and may require immunohistochemistry with cytokeratin for diagnosis. Small metastases in a non-optimal frozen section may be difficult to discern. Cytology imprints add nothing to the diagnosis.

*From Pathology Department,
Nordlands Hospital
Bodo, Norway*

*Correspondence to:
Dr. Khalid I. Al-Shibli
Consultant Histopathologist
Pathology Department,
Nordlands Hospital
8092 Bodo, Norway*

*Accepted for publication:
November 2004*

Ann Saudi Med 2005;25(2):111-114

Recent studies have demonstrated that the sentinel node biopsy (SNB) is a reasonably reliable and minimally invasive method for determining the status of the regional lymph nodes in patients with clinically node-negative invasive breast cancer.¹ It may be as accurate as the standard axillary lymph-node dissection for the evaluation of axillary lymph-node status in breast cancer patients.² SNB technique is also used for ductal carcinoma in situ grade III. However, its role is less clear than in invasive tumours.¹ The technique is of great value in terms of avoiding unnecessary axillary toilet with its known side effects. Morbidity following complete axillary dissection is high, with pain (81%), lymphoedema (10%), general discomfort (39%) and loss of work (27%).^{3,4} SNB technology is evolving rapidly, but variation in technique is widespread, with no standardization yet accomplished. The question whether the sentinel lymph node predicts the axillary status in all cases is difficult to answer, and there is no agreement on how many lymph nodes must be submitted to represent accurately the axillary status. Kennedy et al thought that removal of two sentinel nodes accurately stages the axilla in breast cancer,⁴ while Cody said that SNB reliably predicts axillary status in 98% of all patients, and in 95% of those who are node positive.⁵

There is also no agreement on the optimal pathological protocol. The College of American Pathologists recommends that sentinel lymph nodes be sectioned as close to 2 mm as possible and entirely submitted for histological evaluation, regardless for node size, although this may take one or more cassettes per lymph node. A single microscopic section from each block is then regarded as sufficient and would identify virtually all patients with metastases more than 2 mm.⁶

The aim of this study is to assess the accuracy of this procedure and the optimal method for identifying micrometastases as well as finding any association between the lymph node size and the possibility of metastasis.

Materials and Methods:

Data were collected and analysed retrospectively from 70 women with primary invasive breast carcinoma less than 3 cm in diameter and with no axillary lymphadenopathy who underwent SNB for breast cancer, between October 2002 and June 2004, at Norlands Hospital, Bodo, Norway. Four cases of SNB were done in patients with grade III ductal carcinoma in situ, but these cases were excluded from the study.

According to the protocol used in our hospital, all nodes that were hot and/or blue were removed and analysed. We used the combined technique (blue dye-gamma detection) to localize the SNB, which is reportedly associated with higher sensitivity and reproducibility.^{7,8} Each node was sliced in two halves through the hilus, with imprints taken from each half (for the first 40 cases). A minimum of two haematoxylin and eosin (H&E)-stained frozen sections from each half of each lymph node were examined. After paraffin embedding, two additional H&E-stained and two cytokeratin-stained levels (one from each half) from each SLN were examined. This procedure is supposed to correctly identify the status of the node in 97.9% of patients.⁹ The cytokeratin used for immunohistochemistry was a pancytokeratin (AE1/AE3), which is thought to be both a sensitive epithelial marker and more specific than CAM5.2 in recognizing epithelial cells in SNB.¹⁰

For the association between the lymph node size and the possibility of metastases, the Student's *t* test was used and a *P* value of less than 0.05 was regarded as significant.

Results

The total number of patients included in the study was 70, which included 52 with ductal, and 18 with lobular, invasive breast carcinoma. For each patient we received between 1 and 6 lymph nodes for frozen section (Table 1). The total number of lymph nodes was 139, and sizes ranged from 4 to 26 mm with a mean of 11.8 mm. The number of patients with metastases in SNB was 19, from which 15 cases were correctly diagnosed in frozen sections/imprints and 4 cases were false negative ($4/19=21\%$) (Table 2).

Table 1. Number of lymph nodes received per case for 70 sentinel lymph node cases examined for breast carcinoma.

Number of cases	Number of lymph nodes per case
30	1
20	2
15	3
2	4
2	5
1	6

There were no false positive results. The total number of the lymph nodes with metastases was 33.

Metastases were located in the subcapsular sinus in 5 cases, the sinuses and parenchyma in 9 and were massive (sinuses, parenchyma, capsule and extranodal fat) in 5 cases. Three of the false negative cases were ductal carcinoma in which the metastases were small and located only in the subcapsular sinus. In two cases the metastases were seen only in paraffin sections, while in the third case a small focus of about 1 mm was missed in a frozen section with a lot of cracking artefact. The fourth false negative case was a lobular carcinoma with large metastases in the sinuses and parenchyma, but the cells were misinterpreted as histiocytes in frozen section.

The cytology imprints show malignant cells only when they were easily seen in histology. There was a significant association between the size of the lymph node and the possibility of metastases, the cut point was a lymph node size more than 13 mm (*P* value =0.0001). The size of the metastases was less than 0.2 mm in 2, between 0.2 and 2 mm in 6 and more than 2 mm in 11 (Table 3).

The most common reactive changes in the lymph nodes were sinus histiocytoses, followed by follicular hyperplasia. Two of the lymph nodes with large metastases showed also non-caseating granulomas.

Discussion

Axillary dissection in patients with breast carcinoma has a proven higher survival (about 5%) and better regional control (close to 100%) than in women with breast carcinoma treated with no initial axillary dissection, as seen in six randomised trials.⁶ There is no phase III randomised study that has tried to compare clinically node negative patients managed by SNB technique alone with those undergoing axillary dissection. The NSABP-32 study involves 73 institutions in North America, and its primary aims

Table 2. Diagnosis and type of breast carcinoma in the 70 cases.

	Number of cases
SNB frozen section diagnosis	
Negative for carcinoma	51
Positive for carcinoma	19
Type of breast carcinoma	
Ductal carcinoma	52
Lobular carcinoma	18

Table 3. Size of the metastases in the sentinel lymph node biopsies and the state of axillary toilet.

Size of metastasis	The state of non-sentinel (axillary toilet) lymph nodes with metastatic carcinoma			Total
	Negative	Not done	Positive	
<0.2 mm	0	2	0	2
0.2 to 2 mm	6	0	0	6
>2 mm	2	0	9	11
Total	8	2	9	19

are to determine if removal of only sentinel lymph nodes provides survival and regional control equivalent to those of axillary dissection while associated with much less morbidity¹¹

Frozen section examination of a SNB from patients with breast carcinoma had a high specificity (100%) and sensitivity (79%), in our study. Interpretation can be difficult especially in cases of lobular carcinoma in which the cells can be small and diffusely dispersed and can be mistaken for lymphoid or histiocytic cells. This was the cause of one false negative result in our series. Although there were large collections of lobular carcinoma cells, they were mistaken for histiocytes in the frozen section. Immunohistochemistry, on the frozen sections, with cytokeratin can eliminate this problem.

In two of our false negative cases the metastases were between 2 to 4 mm and only in deeper paraffin sections. Using the recommendation of the College Of American Pathologists in sectioning the SNB as close to 2 mm as possible and submitting them entirely for histological evaluation (see above),⁶ may help to avoid such false negative results. Moreover, a non-optimal frozen section may make small metastases very difficult to be recognized. This was the cause of false negative report in one of our cases. These foci were revealed easily by AE1/AE3 cytokeratin immunohistochemistry done on paraffin

block sections. A rapid immunostaining will be of help in disclosing these cases in frozen sections.¹²⁻¹⁴ Moreover, the preliminary data of the randomized phase III NSABP-32 study indicates that between 10% and 20% of the cases that are node-negative by H&E will be positive by cytokeratin immunohistochemistry. The secondary aim of the above study is to determine whether the survival of patients who have occult metastases is worse than that of patients who are negative by both H&E and immunohistochemistry.¹¹

Localizing the area within the lymph node most likely to harbour metastases could be useful in determining the optimal method for evaluation, and may help the pathologist to concentrate on it in the limited frozen section time. Many studies have shown that a metastatic tumour has a higher probability of being present in the region of the inflow junction of the afferent lymphatic vessels.^{15,16} This area cannot be identified by histology alone, but it corresponds to the site of entry of the blue dye to the lymph node or the maximum radioactive count in SNB mapping. The surgeon can mark the area, but such technique is uncommon and is not used in our hospital. In our cases the metastases was mostly localized in the convex part of the lymph node (13 out of 19 cases), in the coronal plane, which represents mostly the region of the inflow junction of the afferent lymphatic vessel.⁶

Cytology imprints for the fresh SNB tissue were done for the first 40 cases in this series. We found the imprints to be time and effort consuming and think that they have added nothing to the diagnoses, in our cases. They revealed a good number of malignant cells only when the metastases were big and easily seen on frozen histological sections. Therefore we have stopped doing cytology imprints as a routine procedure.

New staging guidelines have established a low limit for micrometastases and defined metastases no larger than 0.2 mm. In our hospital, axillary clearance is done for patients with a metastatic focus more than 0.2 mm. However, in all cases in which the metastases were smaller than 2 mm (6 cases) the rest of the nodes in the axilla were negative. The number of cases is too small for any conclusion. Viale et al. concluded that when sentinel nodes contain metastases no larger than 1.0 mm, the likelihood of nonsentinel node metastases is 16%; however, when sentinel node metastases are between 1 and 2 mm, the likelihood of nonsentinel node metastases is 36%.¹⁷ The precise size

stratification for predicting clinical significance of micrometastases in SNB is difficult to predict, and larger studies are needed on this issue. In this study, axillary toilet from all our six SNB cases with metastases less than 2 mm showed no metastatic carcinoma (Table 3). This may be consistent with Weaver's argument that "preliminary data suggest that the historic definition of 2.0 mm for clinically significant metastases may not be far from the mark prognostically".¹⁸ A study on a series of 696 patients from the John Wayne Cancer Centre found that the only group with decreased 5-year disease-free and overall survival were those with nodal metastases more than 2 mm.¹⁹ Even with metastases larger than 2 mm in SNB, the nonsentinel lymph nodes were negative in 2 of 11 cases in this series. This is consistent with numerous studies that have shown that SNB may be the only positive lymph node in 40% to 70% of cases.²⁰ Stitzenberg et al showed that only extranodal extension, and not the size or the tumor type, of the SNB metastasis was significantly associated with tumor involvement of the non-sentinel nodes on multivariate analysis.²¹ Extranodal extension was seen in 8 of our 19 cases, all showed metastases in the non-sentinel axillary nodes.

Lymph node diameter showed a significant association with sentinel node status. The mean size

for the negative lymph nodes was 10.5 mm and for the positive 15.6 mm (Figure 1). Only 3 out of 33 positive SNB were smaller than 13mm in maximum diameter, all others were equal or larger than 13mm ($P<0.0001$) (Figure 2). Finally, in all our cases in which there were many nodes sent for assessment, the metastases were seen in the biggest node.

In conclusion, the definite usefulness of SNB technique over conventional axillary dissection is yet to be proved by randomised studies. One such study is now in an advanced stage.¹¹ Frozen section examination of a SNB from patients with breast carcinoma is both specific (100%) and sensitive (79%). Lobular carcinoma or a non-optimal frozen section with small metastases can represent a difficulty, which can be eliminated by cytokeratin immunohistochemistry. Cytology imprints add nothing to the diagnoses. The precise size stratification for predicting clinical significance of micrometastases in SNB is difficult to predict, and larger studies are needed on this issue. Lymph node diameter showed a significant association with sentinel node status in our study ($P<0.0001$).

This paper was presented at the 16th meeting of the International Academy Of Pathology, Arab division, which took place in Tunisia, 20-22 September, 2004.

References

1. SinghRanger G, Mokbel K. The evolving role of sentinel lymph node biopsy for breast cancer. *Eur J Surg Oncol*. 2003 Jun; 29(5):423-5. Review.
2. Schrenk P, Shamiyeh A, Wayand W. Sentinel lymph-node biopsy compared to axillary lymph-node dissection for axillary staging in breast cancer patients. *Eur J Surg Oncol*. 2001 Jun; 27(4): 378-82.
3. Ivens D, Hoe A L, Podd T J, Hamilton C R, et al. Assessment of morbidity from complete axillary dissection. *Br J Sur* 1992;66:136-8
4. Ozaslan C, Kuru B. Lymphedema after treatment of breast cancer. *Am J Surg*. 2004 Jan; 187(1):69-72.
5. Kennedy RJ, Kollias J, Gill PG, Bochner M, Coventry BJ, Farshid G. Removal of two sentinel nodes accurately stages the axilla in breast cancer. *Br J Surg*. 2003 Nov; 90(11):1349-53.
6. Cody HS. Sentinel lymph node mapping in Breast cancer. *Breast Cancer* 1999 Jan 25;6(1): 13-22.
7. Hoyek T, Benoit L, Fraisse. The sentinel node: the Dijon experience. *J Gynecol Obstet Biol Reprod (Paris)*. 2002 Oct; 31(6):565-71.
8. Howard-Alpea G. Sentinel node localization and biopsy in breast cancer. *Clin Oncol (R Coll Radiol)*. 1999; 11(2):111-7.
9. Yared MA, Middleton LP, Smith TL, Kim HW, Ross MI, Hunt KK, et al. Recommendations for sentinel lymph node processing in breast cancer. *Am J Surg Pathol*. 2002 Mar; 26(3):377-82.
10. Xu X, Roberts SA, Pasha TL, Zhang PJ. Undesirable cytokeratin immunoreactivity of native nonepithelial cells in sentinel lymph nodes from patients with breast carcinoma. *Arch Pathol Lab Med*. 2000 Sep; 124(9):1310-3.
11. David N Krag, Thomas B. Julian, Seth P Harlow, Donald L weaver et al: NSABP-32: Phase III, randomised trial comparing axillary resection with sentinel lymph node dissection: A description of the trial. *Annals of Surgical Oncology* 11(3): 208S-210S.
12. Pargaonger AS, Beissner RS, Snyder S. Evaluation of immunohistochemistry and multiple-level sectioning in sentinel lymph nodes from patients with breast cancer. *Arch Pathol Lab Med*. 2003 Jun; 127(6):701-5.
13. Ozmen V, Muslimanoglu M, Cabioghr N, Tuzlali S et al. Increased false negative rates in sentinel lymph node biopsies in patients with multi-focal breast cancer. *Breast Cancer Res Treat*. 2002 Dec; 76(3):237-44.
14. Estourgie SH, Nieweg OE, Valdes Olmos RA, Rutgers EJ, Peterse JL, Kroon BB. Eight false negative sentinel node procedures in breast cancer: what went wrong? *Eur J Surg Oncol*. 2003 May; 29(4):336-40.
15. Leslia K. Diaz, Kelly Hunt, Fredrick Ames, Funda Meric, et al: Histological Localization of sentinel lymph node metastase in breast cancer. *Am J Surg Pathol*. 2003; 27(3):385-9.
16. Ceserni G. Mapping metastase in sentinel lymph nodes of breast cancer. *Am J Clin Pathol*. 2000; 113:351-4.
17. Hansen Nm, Grube BJ, Te w, et al. Clinical significance of axillary micrometastases in breast cancer: how small is too small? *Proc ASCO* 2001; 20:249.
18. Donal L. Weaver. Sentinel lymph nodes and breast carcinomam, which micrometastases are clinically significant? Special article *Am J Surg Path*. 2003; 27(6):842-5.
19. Viale G, Maiorano E, Mazarrol G. Histological detection and clinical implication of micrometastases in axillary sentinel lymph nodes for patients with breast carcinoma. *Cancer* 2001; 92:1378-84.
20. Changsri C, Prakash S, Sandweiss L, Bose S. Prediction of additional axillary metastasis of breast cancer following sentinel lymph node surgery. *Breast J*. 2004 Sep-Oct; 10(5):392-7.
21. Stitzenberg KB, Meyer AA, Stern SL, et al. Extracapsular extension of the sentinel lymph node metastasis: a predictor of nonsentinel node tumor burden. *Ann Surg*. 2003 May; 237(5):607-12; discussion 612-3