

A Review of the Accuracy of Sentinel Lymph Node Biopsy by Comparing Frozen Section with Final Paraffin Block H&E Staining, and Correlation with the Final Axillary Lymph Node Dissection Results

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Objectives: 1) To determine the accuracy rate of sentinel lymph node biopsy (SLNB) by comparing frozen section (FS) with permanent formalin processed specimen by hematoxylin and eosin (H&E) staining; 2) To correlate with axillary lymph node dissection (ALND) histopathology results for those with positive SLNB.

Methods: This was a retrospective study on all breast cancer patients who underwent SLNB using methylene blue dye. The number of SLN harvested, number of lymph nodes confirmed on frozen section, frozen section result, number of SLN confirmed on paraffin block H&E, result of H&E. If frozen section of SLN was positive for metastasis, further data on the axillary lymph nodes harvested and its final H&E result were noted. The accuracy rate was determined. Whenever a discordance of FS and H&E findings occurred, the interpreting pathologists were interviewed for the cause

Results: A total of 34 SLNB procedures were performed. There were 24 SLNBs that turned out negative on frozen section while 10 were positive for metastasis and underwent further ALND. Three of the 24 negative SLNBs turned out to be positive (false negative rate of 12.5%). Out of the 10 positive SLNBs, 1 turned out to be negative (false positive rate of 10%). Only 6 of the 10 patients with (+) SLNB had more positive nodes harvested upon further ALND. The accuracy rate of SLNB on frozen section was 88%, with sensitivity and specificity of 90% and 87.5% respectively. There were 5 pathologists involved in the interpretations of the SLNB, and in 76% the pathologist interpreting the FS was different from the one interpreting the permanent specimen H&E. The reasons for discordance between frozen section and final H&E reading were inter-reader variability and tissue sampling limitation during frozen section.

Conclusion: The accuracy of frozen section in SLNB procedure is 88%.

Key words: Sentinel lymph node biopsy, axillary lymph node dissection, breast cancer

Axillary lymph node metastasis is considered to be the most important independent prognostic factor in breast cancer. To determine the axillary lymph node status, axillary lymph node dissection (ALND) has been the standard of care in patients with breast cancer in order to provide correct staging and obtain good local control. Complications of ALND include pain, paresthesia, seroma, infection, limitation of shoulder motion and lymphedema.¹ In recent years however, sentinel lymph node biopsy (SLNB), a minimally invasive method of assessing the axilla, has emerged as an alternative to ALND and has become the standard of care for clinically node negative breast cancer patients because it has significantly reduced the morbidity of breast surgery.^{2,3} The most common agents used are isosulfan blue dye and filtered technetium sulfur colloid. An increased sentinel lymph node (SN) identification rate with the use of the combination of both blue dye and radioisotope is well documented. However, Morrow, et al. showed that there was no difference between using blue dye alone or the blue dye and radioisotope combination.⁴ In resource-limited medical setting, the use of methylene blue dye has become popular. This technique involves the identification of first node(s) draining the tumor area by the introduction of 1% methylene blue dye in the subareolar space. Sentinel lymph nodes (SN) are examined by frozen section and if they are positive

for malignancy, axillary lymph node dissection is carried out in the same setting. On the other hand if they are negative for malignancy, the probability of tumor metastasis in non-sentinel lymph nodes is very low, so the ALND procedure is omitted.⁵

At Vicente Sotto Memorial Medical Center (VSMMC), sentinel lymph node biopsy (SLNB) using methylene blue dye has become the standard of care for clinically node negative breast cancer patients after the conclusion of a local validation study in 2010 involving 20 patients. The SLN identification rate was 100%. The accuracy rate was 95.0% with a false negative rate of 6.7%.⁶

Continuous monitoring of the accuracy of SLNB results is necessary to maintain the effectiveness of this procedure. This particular review was undertaken to determine the accuracy rate of sentinel lymph node biopsy (SLNB) by comparing frozen section (FS) with permanent specimen H&E staining results. Furthermore, when the SLNB was positive and further ALND was performed, the number of positive nodes further harvested was determined.

Methods

A chart review was done on all breast cancer patients who underwent SLNB using 1% methylene blue dye from November 2010 to December 2013 at VSMMC. The following variables were noted: number of SN harvested, number of SN confirmed on frozen section, frozen section results, number of SN confirmed on permanent specimen H&E and their results. If frozen section of SN was positive for metastasis, further data on the axillary lymph nodes harvested and their final H&E results were noted. Tissue specimens in frozen section were cut in 4-5 microns thin, compared to the permanent section at 2-3 microns. The pathologists performing the frozen section and permanent specimen H&E procedures were noted. When there was a discrepancy or discordance of findings between frozen section and final H&E, the interpreting pathologists were requested to review the slides and interviewed for the possible causes. All data were tabulated and analysed. The accuracy rate was computed.

Results

There were 33 patients who underwent SLNB, one of which had bilateral SLNB making it a total of 34 SLNB procedures done. The number of sentinel nodes harvested ranged from 1 to 6, with a mean of 2 nodes. Out of 34 SLNB procedures done, 24 turned out to be negative for metastasis on frozen section while 10 were positive for metastasis and underwent further ALND. Out of the 24 negative SLNBs in frozen section, 3 (12.5%) turned out to be positive for metastasis on final H&E (discordant), while the remaining 21 (87.5%) were negative for metastasis on final H&E (concordant). Out of the 10 SLNBs that were positive for metastasis on frozen section, 1 (10%) turned out to be negative for metastasis on final H&E (discordant), while the remaining 9 (90%) were truly positive for metastasis on final H&E. (Figure 1) Sentinel lymph node biopsy on frozen section had an accuracy of 88%, with sensitivity and specificity of 90% and 87.5% respectively. The false negative rate in this study was 12.5%. (Table 1)

Among 10 patients with positive SLNB by frozen section, a total number of 30 sentinel nodes were harvested, 14 was positive on frozen section and 16 was negative. When subjected to the final H&E staining, there was 1 false positive node and 1 false negative node respectively (Figure 2). Among 24 patients with negative SLNB by frozen section a total number of 50 sentinel nodes were harvested, when subjected to the final H&E staining the number of SLN detected increased to 55, with detection of 3 positive nodes and 52 negative nodes. (Figure 2)

Table 1. Accuracy of frozen section versus permanent specimen H&E in SLNB.

		H&E		Total
		Positive	Negative	
F.S	Positive	9	1	10
	Negative	3	21	24
	Total	12	22	34
Accuracy rate: 88.2%		Positive predictive value: 75%		
Sensitivity rate: 90%		Negative predictive value: 95.4%		
Specificity rate: 87.5%		False positive: 10%		
		False negative: 12.5%		

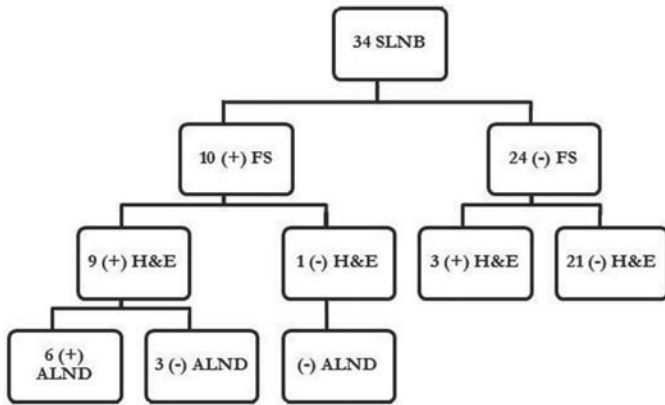


Figure 1. Algorithm of SLNB procedures based on outcome.

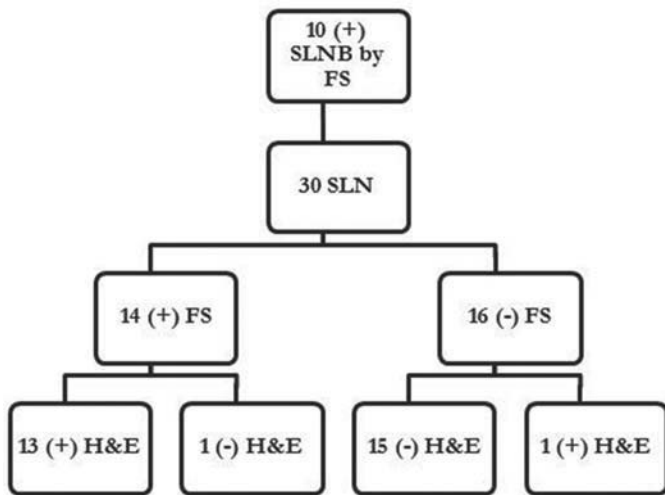


Figure 2. Algorithm of patients with positive SLNB based on number and outcome of nodes.

There were 5 pathologists involved in the FS/H&E interpretations of the SLNB. Among the 33 cases performed, the FS and the H&E were interpreted or read by the same pathologist in 8 occasions (24%); while in 25 occasions, the pathologist interpreting the FS was different from the one reading the H&E (76%). According to the reviewing pathologists, the reason for the false positive result was the difference in interpretation of the frozen section and final H&E. The frozen section was interpreted or read by a pathologist different from the one reading the final H&E. The reason for the false

negative result was tissue sampling limitation on frozen section. Deeper cuts in permanent section showed that there was a very small focus of metastasis not seen or missed on frozen section. In the case of documenting additional 5 nodes among the 24 SLNB negative procedures, the reason forwarded by the pathologists was also tissue sampling limitation during frozen section. More meticulous dissection and study done on the permanent specimen by the pathologist who was not under time pressure showed that there were more nodes not seen or missed out on frozen section. Therefore, the main reasons for the discordance between FS and permanent specimen H&E were inter-reader variability and tissue sampling limitation on frozen section.

Discussion

Of the 10 patients in this study who had positive SLNB, 6 (60%) had further nodal metastases identified in their axillary lymph nodes on H&E thus benefited from further ALND, on the other hand, 4 (40%) did not have any further nodal metastases identified in their axillary lymph nodes on H&E and thus did not benefit from further ALND. In the study by Ali et al involving 23 patients who had positive SLNB, 16 (70%) had further nodal metastases identified in their axillary lymph nodes thus benefited from further ALND, while 7 (30%) did not have any further nodal metastases.⁷ This is in contrast to studies by Hashmi et al and Cedolini et al wherein greater percentage of SLNB positive patients did not benefit from further ALND at 61% and 55% respectively.^{8,9} (Table 2)

Table 2. Frequency of ALN metastasis after a positive SLNB.

Reference	Year	No. of SLNB positive patients	ALN metastasis after positive SLNB (%)
This review	2014	10	67%
Ali, et al.	2008	23	70%
Cedolini, et al.	2014	84	45%
Hashmi, et al.	2013	62	39%

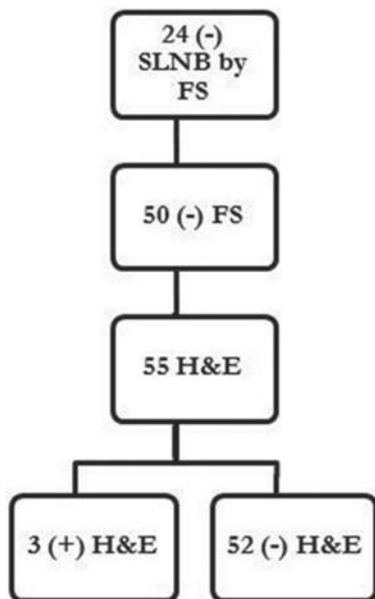


Figure 3. Algorithm of patients with negative SLNB based on number and outcome of nodes.

The final H&E examination of sentinel lymph nodes identified metastasis in 3 out of 24 patients whose initial frozen section were negative for metastases (FNR of 12.5%) and should have undergone ALND at a second operation but was not done. In the study by Ali, et al. the final H&E examination of sentinel lymph nodes identified metastasis in 7 out of 71 patients whom the initial frozen section was negative for metastases.⁷ All 7 patients underwent ALND at a second operation and in 2 out of 7 patients (28.6%), the sentinel nodes were the only involved nodes while the other 5 patients (71.4%) had additional metastases in the axillary lymph nodes. This is in contrast to the study done by Wada, et al. wherein 13 of false-negative patients underwent ALND and in 11 out of 13 patients (84.6%) the sentinel nodes were the only involved nodes.¹⁰ In the study by Geertsema, et al. of 112 false-negative patients who underwent further ALND, 95 out of 112 patients (85%) the sentinel nodes were also the only involved nodes.¹¹

According to studies by Carlson, et al. and Giuliano, et al. complete ALND may not be necessary in selected patients with a positive SN in less than 3 nodes because the need for systemic therapy is established and the risk

of an axillary recurrence appears to be low.^{12,13} Trends to omit ALND in SN-positive patients have been documented over the last 15 years, especially among patients with low tumor burden in the SN. Bilimoria and colleagues identified 97,314 clinically node-negative patients found to have a positive SN from the National Cancer Database from 1998-2005. Approximately 20% of SN-positive patients did not have completion ALND and the proportion of patients with micro-metastatic SNs who had SLNB alone increased from 25% to 45%. With a median follow-up of 63 months, the authors did not find a difference in regional recurrence or survival between those who had SLNB with completion ALND and those who had SLNB alone.¹⁴ A meta-analysis of 48 studies that included 14,959 SN-negative patients followed for a median of 34 months demonstrated an axillary failure in 67 patients (0.3%).¹⁵

This review documented the failure to identify a few lymph nodes during frozen section and small foci of metastasis in final H&E. Perhaps a more detailed SN histopathologic processing technique can improve the SLNB accuracy. The American College of Pathologists has established guidelines to process the SN with frozen sections or permanent formalin processed specimens. The SN is bivalved along the longitudinal axis, serially sectioned at 1.5 to 2mm thickness blocks and each block is sectioned at 3 levels. If metastases are identified in the SN, the size of the metastasis is reported as macrometastasis (>2.0 mm), micrometastasis (>0.2 to 2.0mm), or isolated tumor cells (≤ 0.2 mm). The method of detection of metastasis is by H&E, IHC, or reverse transcription polymerase chain reaction (RT-PCR).¹⁶

The limitations of this study are: 1) small sample size; 2) Frozen section SLNB-negative patients did not undergo confirmatory ALND.

Conclusion

The accuracy of frozen section in SLNB procedure is 88% with a false negative rate of 12.5%. The main reasons for the discordance between frozen section and permanent specimen H&E were inter-reader variability and tissue sampling limitation on frozen section.

References

1. Ashikaga T, Krag DN, Land SR, et al. Morbidity results from the NSABP B-32 trial comparing sentinel lymph node dissection versus axillary dissection. *J Surg Oncol* 2010;102:111-8.
2. Veronesi U, Paganelli G, Galimberti V, et al. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph nodes. *Lancet* 1997; 349: 1864-7.
3. Giuliano AE, Kirgan DM, Guenther JM, et al. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994; 220: 391-401.
4. Morrow M, Rademaker AW, Bethke KP, et al. Learning sentinel node biopsy: results of a prospective randomized trial of two techniques. *Surgery* 1999; 126: 714-20.
5. Veronesi U, Paganelli G, Viale G, et al. Sentinel lymph node biopsy and axillary dissection in breast cancer: results in a large series. *Asian Pacific J Cancer Prev* 1999;14(4): 2657-62.
6. Siguan SS, Castillo ET, Tagab HC, Baking STAG. Sentinel Lymph Node Biopsy - The Vicente Sotto Memorial Medical Center Experience. *Phil J Surg Spec* 2011; 66(2): 41-4.
7. Ali R, Hanly AM, Naughton P, et al. Intraoperative frozen section assessment of sentinel lymph nodes in the operative management of women with symptomatic breast cancer. *World J Surg Oncol* 2008; 6: 69.
8. Hashmi AA, Faridi N, Khurshid A, et al. Accuracy of frozen section analysis of sentinel lymph nodes for the detection of Asian breast cancer micrometastasis - experience from Pakistan. *Asian Pacific J Cancer Prev* 2013; 14(4): 2657-62.
9. Cedolini C, Bertozzi S, Seriau L, et al. Eight-year experience with the intraoperative frozen section examination of sentinel lymph node biopsy for breast cancer in a North-Italian university center. *Int J Clin Exp Pathol* 2014; 7(1): 364-71.
10. Wada N, Imoto S, Hasebe T, Ochiai A, Ebihara S, Moriyama N. Evaluation of intraoperative frozen section diagnosis of sentinel lymph nodes in breast cancer. *Japan J Clin Oncol* 2004; 34(3): 113-7.
11. Geertsema D, Gobardhan PD, Madsen EVE, et al. Discordance of intraoperative frozen section analysis with definitive histology of sentinel lymph nodes in breast cancer surgery: complementary axillary lymph node dissection is irrelevant for subsequent systemic therapy. *Ann Surg Oncol* 2010; 17: 2690-5.
12. Carlson GW, Wood WC. Management of axillary lymph node metastasis in breast cancer: making progress. *JAMA* 2011; 305: 606.
13. Giuliano AE, McCall L, Beitsch P, et al. Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: the American College of Surgeons Oncology Group Z0011 randomized trial. *Ann Surg* 2010; 252: 426.
14. Bilimoria KY, Bentrem DJ, Hansen NM, et al. Comparison of sentinel lymph node alone and completion axillary lymph node dissection for node-positive breast cancer. *J Clin Oncol* 2009; 27: 2946-53.
15. Van der Ploeg IM, Nieweg OE, van Rijk MC, et al. Axillary recurrence after a tumour-negative sentinel node biopsy in breast cancer patients: a systematic review and meta-analysis of the literature. *Eur J Surg Oncol* 2008; 34: 1277-84.
16. Fitzgibbons PL, LiVolsi VA. Recommendations for handling radioactive specimens obtained by sentinel lymphadenectomy. Surgical Pathology Committee of the College of American Pathologists, and the Association of Directors of Anatomic and Surgical Pathology. *Am J Surg Pathol* 2000; 24: 1549-51.