Original Study

Intraoperative Sentinel Node Fine-Needle Aspiration Biopsy as a Substitute for Whole Sentinel Node Excisional Biopsy in Breast Cancer Patients. Initial Report

Israel Barco,[1] Antonio García-Fdez,[1] Elena Vallejo,[1] Xavier Tarroch,[2] Montse Ysamat,[3] Cinthya J. Báez,[2] Manel Fraile[3]

Abstract

We aim at exploring intraoperative fine-needle aspiration biopsy (SN-FNAB) of the sentinel node (SN) as a replacement for whole SN excision. We included 80 patients with breast cancer (BC) undergoing SN biopsy. With Z0011 criterion for axillary dissection, the Negative Predictive Value rise to 100%. Intraoperative SN-FNAB is highly accurate for swiftly depicting both low and high axillary tumor burden.

Introduction: Sentinel Node Biopsy (SNB) is the choice procedure for axillary staging in Breast Cancer. Following the ACOSOG Z11 trial, axillary dissection is advised only in patients with more than 2 positive SNs. We aimed at exploring palpation-guided, intraoperative fine-needle aspiration biopsy of the SN as a replacement for whole SN excision in node-negative BC patients to minimize side-effects. **Patients and Methods:** We included 80 patients with BC undergoing SNB between December 2020 and May 2022. After identification of the SN, the breast surgeon performed SN-FNAB. Results were compared with definitive pathological assessment. ResultsDiagnostic yield was 80%, including a "learning curve." 58 of 64 patients with suitable samples tested negative. In this group, the Negative Predictive Value was 77.6% (IC 64.7%-87.5 %). If micro metastasis is disregarded, the NPV would increase to 86.2% (IC 74.6%-93.9%). If we accept the Z11 criterion for axillary dissection, the NPV would rise to 100%. Six patients had a positive SN-FNAB. They were all confirmed as having macro metastatic-positive SNs at the final pathological assessment, and 3 of them also displayed extra nodal extension (ENE). **Conclusion:** We believe that intraoperative SN-FNAB is highly accurate for swiftly depicting both low axillary tumor burden/negative cases, in whom axillary dissection is to be omitted, as well as high axillary tumor burden cases.

Clinical Breast Cancer, Vol. 000, No.xxx, 1–4
Keywords: Sentinel node biopsy, Axillary lymph-node dissection, Fine-needle aspiration biopsy, Axillary tumor burden, De-escalation axillary surgery

Background

During the last 2 decades, Sentinel Node Biopsy (SNB) has become the choice procedure for lymph-node axillary staging in most Breast Cancer (BC) patients, including those in the post-neoadjuvant setting.[1-3] Furthermore, systematic axillary lymph-node dissection (ALND) after a positive SN has gone into question in the wake of the ACOSOG Z11 trial.[4] Because of the proven non-inferiority of skipping ALND in selected SN positive patients, full axillary clearance is now generally acceptable only for cases with more than 2 positive nodes.[5]

Although SNB is a minimally invasive procedure, it nonetheless carries a definite risk of side effects.[6] In part, such untoward effects could be overcome if the SN could be sampled but not removed, provided that the relevant information it contains on its pathologic status can be safeguarded. Such rationale would be in keeping with the present trend towards de-escalation of axillary surgery for BC patients.[7]

We aimed at exploring the potential of palpation-guided, intraoperative fine-needle aspiration biopsy (FNAB) of the SN as a replacement for whole SN excision in node-negative BC patients.

[1]Breast Unit, Department of Gynecology. Hospital Universitari Mútua Terrassa. Universitat de Barcelona. Terrassa. Spain
[2]Breast Unit, Department of Pathology. Hospital Universitari Mútua Terrassa. Universitat de Barcelona. Terrassa. Spain
[3]Breast Unit, Department of Nuclear Medicine. Hospital Universitari Mútua Terrassa. Universitat de Barcelona. Terrassa. Spain

Submitted: Jun 13, 2022; Revised: Aug 3, 2022; Accepted: Aug 19, 2022; Epub: xxx

Address for correspondence: Israel Barco, MD, Breast Unit, Department of Gynecology. Hospital Universitari Mútua Terrassa. Universitat de Barcelona, Plaça Dr Robert 5, 08221 Terrassa, Spain.
E-mail contact: ibarco@mutuaterrassa.es

1526-8209/$ - see front matter
https://doi.org/10.1016/j.clbc.2022.08.008

Please cite this article as: Israel Barco et al, Intraoperative Sentinel Node Fine-Needle Aspiration Biopsy as a Substitute for Whole Sentinel Node Excisional Biopsy in Breast Cancer Patients. Initial Report, Clinical Breast Cancer, https://doi.org/10.1016/j.clbc.2022.08.008

Table 1 Clinico-Pathological Features of Included Patients.

	N-80
Patient Age - mean (range)	56.5 y (31-91)
Neoadjuvant Therapy	Eight patients - 3 with complete pathologic response
Clinical T Stage	1 - T1a
	11 - T1b
	36 - T1c
	31 - T2
	1 - T3
Breast Surgical Procedure	9 - Mastectomy
	71 - Conservative
Tumour Surgical Pathology	61 - Ductal Ca
	11 - Lobular Ca
	8 - Other
Phenotype	31 - Luminal A
	36 - Luminal B
	10 - Her2 +
	3 - Triple Negative
Axillary Surgical Procedure	62 - Negative SN
	10 - Positive SN + Axillary dissection 8 - Positive SN - Z11[a] (No Ax dissect)

[a] ACOSOG Z0011 trial.

Patients and Methods

We included 80 patients with infiltrating breast tumors submitted to our Breast Unit for surgical treatment from December 2020 to May 2022. All of them had an indication for SNB after a US or US-FNAB negative axillary assessment. Patients with clinically or US-positive nodes before neoadjuvant therapy were not included. Mean age was 56.50 years (range 31-91). Eight (node-negative patients at onset) had received neoadjuvant chemotherapy. Pathologic mean tumor size for invasive lesions was 16 mm (range 5-50). Table 1 summarizes the clinical features of included patients.

All patients received the standard SNB procedure. Our technique for SNB has been described elsewhere.[8] It is based on intra-peritumoral injection of 99m-technetium nano colloids, preoperative lymphoscintigraphy, and intraoperative use of a gamma probe. In the event of finding more than one "hot spot" in the axilla, the SN was identified as the lymph node/s with the highest gamma probe signal. Number and location of SNs shown in the preoperative lymphoscintigraphy images were always considered during surgery. After identification of the actual "sentinel" lymph nodal structure and just before excision, the breast surgeon performed a palpation-guided SN-FNAB by inserting a 25G needle under continuous negative-pressure drive using a 10 mL syringe. Several passes were applied to the SN, taking care to reach the cortex from the hilar side. The procedure was considered completed once some aspirate was apparent in the adaptor. Thereafter, without delay to avoid drying artefacts, the assembled needle and syringe were sent to the pathology department where the sample was processed as follows. Cytological smears were obtained from the sample which were immediately double stained (Diff-Quick and Papanicolaou). In some cases, cell blocks were obtained, and these were stained with H&E and even supplemented by immunohistochemistry if necessary. A rapid smear evaluation was discarded in our study as its results would not be considered intraoperatively, especially considering the blinded layout.

The final histopathological assessment of sentinel nodes was done after buffered ethanol fixation. Thereafter, serial slicing at 4 microns and H&E staining was performed. At least 2 independent pathologists assessed the SNs. In negative SN cases, further slicing and H&E was repeated, also supplemented by Cytokeratin immunostains (CK AE1-3). This was also done in the case of isolated tumor cells or micro metastasis present in the SN, to reassess metastatic size.

Intraoperative results from the SN-FNAB were completely blinded to the surgical team. A decision to perform immediate or delayed ALND was taken based on our current SNB protocol, which follows international guidelines in accordance with the ACOSOG Z11 trial, namely, to proceed to ALND only if there are more than 2 positive SNs. ALND was also considered if there was a positive SN with extra nodal extension (ENE) greater than 2 mm, no matter the number of positive SNs.

All patients gave their written consent to participate in the study, which was formally accepted by the Review Board of our center.

Data analysis was based on estimating the Negative Predictive Value (NPV), of US-FNAB results by comparison with the final histopathological assessment, along with correspondent 95% Confidence Intervals (CI), assuming the normal approximation.

Results

Feasibility

According to the report by an independent pathologist unaware of other findings from each patient, on arrival, the SN-FNAB sample was deemed suitable for cytologic diagnosis in 64 of the 80 patients, amounting to 80% of the series. The overall 20% failure rate points to a rather demanding technique. Failure rates were seen to improve overtime, from 20% during the first semester down to 12.5% during the last semester, suggesting a "learning curve."

Diagnostic Accuracy

Cytologic SN-FNAB findings were compared with the independent final histopathological assessment of the same SNs which was regarded as the "Golden Standard" (Table 2). Other significant findings from the surgical pathology work-up were also accounted for. Axillary Low Tumor Burden (LTB) was considered if there were up to 2 positive axillary nodes with macro metastasis. High Tumor axillary Burden (HTB) was considered if more than 2 axillary nodes showed macro metastasis.

Of the 64 patients with successful SN-FNAB, 58 patients had a negative result (Table 2). Out of these, 45 had a truly negative SN-FNAB. Five patients presented with micro metastatic involvement only of the SN (thereby ALND being omitted), while 8 patients had macro metastatic involvement of the SN, 3 of whom also displayed ENE. In 1 of these 3 latter cases ALND was omitted because ENE itself was just under 2 mm in size. None of the negative SN-FNAB patients had greater than 2 involved axillary nodes. Therefore, even with the caveat of such a small sample, we may say that the Negative Predictive Value was 77.6% (IC 64.7%-87.5 %). If a SN with only micro metastasis is considered the same as a negative SN for the

Please cite this article as: Israel Barco et al, Intraoperative Sentinel Node Fine-Needle Aspiration Biopsy as a Substitute for Whole Sentinel Node Excisional Biopsy in Breast Cancer Patients. Initial Report, Clinical Breast Cancer, https://doi.org/10.1016/j.clbc.2022.08.008

Table 2 Intraoperative SN-FNAB Results.

	N - 80
Negative SN- FNAB (vs. histopathology)	58
	Forty-five True Negative
	Five SN micro metastasis
	Eight SN Macro metastasis
	(3 with ECE[a]) (4 ALND[b])
	One non-SN metastasis
	0 > 2 axillary positive nodes
Positive SN- FNAB (vs. histopathology)	6
	Six True Positive
	Six macro metastases
	(Three with ECE + ALND)
	Three non-SN metastasis
	2 >2 axillary positive nodes
Unsuccessful SN- FNAB sample	16
	Eight SN negative
	One SN micro metastasis
	Four SN Macro metastasis
	(Two with ECE) (3 ALND)
	Two non-SN metastasis
	1 > 2 axillary positive nodes

[a] ENE = extra nodal extension.
[b] ALND = Axillary lymph-node dissection.

purpose of performing subsequent ALND, then the NPV would increase to 86.2% (IC 74.6%-93.9%). If we accept the Z11 criterion for ALND, eg, metastasis to more than 2 axillary nodes, then the NPV would rise to 100%. However, if we accept ENE > 2 mm as an additional criterion for ALND, then the NPV would ebb back to 96.6% (IC 88.0%-99.6%).

There were 13 false negatives (Table 2), none of them had more than 2 positive axillary nodes. The axillary surgical procedure would have gone unchanged, vg skipping axillary dissection according to the ACOSOG Z11 assay as already mentioned. However, the question remains whether such false negatives could have some influence on any ensuing adjuvant therapy. Postoperative chemotherapy would have been given irrespective of the SN result in 2 patients (1 HER2-positive and 1 patient with only partial response to neoadjuvant therapy). In 1 additional patient chemotherapy was not even considered because of severe comorbidity. The ten remaining patients were all luminal tumor cases. As per routine, the decision to give adjuvant chemotherapy in them was based on a combination of clinical judgement (including age, histological grade, Ki67 expression, and lymphovascular invasion), and the result of genetic testing (Oncotype associated with a Recurrence Risk over 16). Irrespective of the SN result, in 7 cases chemotherapy was thus discarded. In the 3 remaining patients, adjuvant chemotherapy was instituted based on the same principles, also regardless of the SN result. The occurrence of some low axillary tumor burden false negatives SNs did not have a great influence on postoperative chemotherapy in our study.

Six patients presented with a positive SN-FNAB. They were indeed all confirmed as having macro metastatic-positive SNs at the final pathological assessment, while 3 of them also displayed ENE > 2 mm. ALND was performed in these 3 cases, showing non-SN metastasis in all of them, and greater than 2 positive SNs in 2. ALND was skipped in 3 cases with only 1 positive SN and no ENE.

Discussion

SNB certainly holds a pivotal role for surgical decision-making in BC patients and has been evolving along the lines of the "de-escalation" policy. To avoid unnecessary side-effects, ALND is no longer indicated in patients with SN micro metastasis or in patients with up to 2 positive SNs with macro metastasis.[5,9] Although SNB is much less harmful than ALND, it is per se not free from some untoward surgical effects, including subclinical lymphoedema, shoulder dysfunction, and arm numbness.[6] Therefore, there seems to be enough room for further development aimed at decreasing SNB surgical morbidity.

As we hypothesized that intraoperative sampling of the SN using FNAB could work as a substitute for whole SN excision, we first committed ourselves to validate the technique of Intraoperative SN-FNAB. That meant reaching a fair degree of cytologic sample yield, as well as a good diagnostic value by comparison with the "golden standard," which corresponds to fully fledged histopathologic assessment of the SN. SN-FNAB seems technically demanding and is associated with significant failure rates. We probably went through a "learning curve," and our diagnostic yield was seen to increase as more cases were being accrued. Considerable failure rates and learning curves for palpation- guided FNAB performed by surgeons have been reported in other clinical contexts. Jashan et al reported a rate of up to 30% non-diagnostic FNAB samples in patients with head and neck masses.[10] Fernandes et al reported a clear-cut increase in diagnostic yield of thyroid FNAB, from 70.8% to 94.3% at the end of the learning process.[11] Although it was not included in our initial research layout, we have to acknowledge that a rapid intraoperative smear evaluation of FNA samples would have been helpful in order to decrease the rate of cytologically unsuccessful results and to shorten the learning curve.

Preoperative axillary US-guided FNAB has been used for some time in BC patients carrying suspicious nodes at US scans. Iwamoto et al were able to achieve a 90.5% rate of successful cytologic samples, with 100% PPV.[12] Most patients with false negative results had LTB at the final histopathological workup, while most patients with a positive result had more than 2 metastatic nodes, which is very much in keeping with our present results. Layfield et al also found a clear-cut correlation between sensitivity of US-FNAB and axillary tumor burden.[13]

The clinical bearing of histologically proven ENE in sentinel nodes remains controversial. Although SN ENE is a predictive factor for increased rates of positive non-SN nodes, it is not, however, associated with significant detrimental effects on patient outcomes.[14] Therefore, using ECE as a criterion to perform completion ALND also comes into question. It has been recently suggested that the presence of microscopic ENE in the SN should not be considered an indication for ALND[15]

Please cite this article as: Israel Barco et al, Intraoperative Sentinel Node Fine-Needle Aspiration Biopsy as a Substitute for Whole Sentinel Node Excisional Biopsy in Breast Cancer Patients. Initial Report, Clinical Breast Cancer, https://doi.org/10.1016/j.clbc.2022.08.008

On the issue of oncological safety of SN FNAB without excision, we must acknowledge that implementation of this technique would result in some LTB nodal disease not being removed in a few patients. This is not a far cry from the residual macro metastatic nodal disease that is not being removed in up to 27% of patients when we routinely apply the Z11 trial corollary.[4,5] There is even some ongoing clinical investigation on the prospects of altogether omitting SNB in BC patients with clinically negative nodes.[16,17]

Although it might seem counterintuitive at first glance, the relative lack of sensitivity of SN-FNAB for low tumor burden cases is in fact a good opportunity. Most node-positive patients are now being managed by omitting ALND, and certainly such de-escalation policy has conveyed a dwindling effect on the role of intraoperative SN work-up.[18] However, systematic omission of Sentinel Node assessment and ALND will always lead to a second operative procedure in higher tumor burden cases.

To safely preserve the SN and to make a swift intraoperative management decision of skipping unnecessary ALNDs, we rely on the correct classification of a case as negative, even if the SN contains micro metastasis or there are less than 3 nodes with macro metastasis. We also rely on accurately classifying as positive those cases with a significantly increased chance of axillary HTB, so we can proceed with ALND right away. This is exactly the clinical scenario that our initial results attest to. Given that our patient selection scheme for SNB includes a negative axillary US scan, the expected prevalence of HTB cases is low. In such a context, and based on our present results, we believe that intraoperative SN-FNAB is highly accurate for depicting both LTB/negative and HTB/positive cases. Further work must be done to consolidate our initial experience and to investigate whether SN-FNAB is safe and leads to a decrease of surgical morbidity.

Clinical Practice Points

- Although SN biopsy (SNB) is a minimally invasive procedure, it nonetheless carries a definite risk of side effects. In part, such untoward effects could be overcome if the SN could be sampled but not removed.
- Based on our results, we believe that intraoperative SN-Fine-needle aspiration biopsy is highly accurate for swiftly depicting both low axillary tumor burden/negative cases, in whom axillary dissection is to be omitted, as well as high axillary tumor burden cases.

References

1. 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;349:1864–1867 Jun 28.
2. Fraile M, Rull M, Julian FJ, et al. Sentinel node biopsy as a practical alternative to axillary lymph node dissection in breast cancer patients: an approach to its validity. *Annals of Oncology*. 2000;11:701–705 Jun.
3. Lyman GH, Somerfield MR, Bosserman LD, et al. Sentinel lymph node biopsy for patients with early-stage breast cancer: American society of clinical oncology clinical practice guideline update. *J Clin Oncol*. 2016;35:561–564.
4. Giuliano AE, Hunt K, Ballman K, et al. Axillary dissection vs. no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA*. 2011;305:569–575 Feb 9.
5. Giuliano AE, Vallman KV, McCall L, et al. Effect of axillary dissection vs. no axillary dissection on 10-year overall survival among women with invasive breast cancer and sentinel node metastasis: The ACOSOG Z0011 (Alliance) randomized clinical trial. *JAMA*. 2017;318:918–926.
6. 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;102:111–118.
7. Jatoi I, Benson JR, Toi M. De-escalation of axillary surgery in early breast cancer. *Lancet Oncol*. 2016;17:e430–e441.
8. Barco I, Chabrera C, Fraile M, et al. The appropriate axillary procedure after a positive sentinel node in breast cancer patients: the "Hôpital Tenon" score revisited. A two-institution study. *Clin Transl Oncol*. 2016;18:1098–1105.
9. Galimberti V, Cole B, Viale J, et al. Axillary dissection versus no axillary dissection in patients with breast cancer and sentinel-node micro metastases (IBCSG 23-01): 10-year follow-up of a randomized, controlled phase 3 trial. *Lancet Oncol*. 2018;19:1385–1393.
10. Jahshan F, Doweck L, Ronen O. Learning curve of fine-needle aspiration cytology of head and neck masses. *IMAJ*. 2016;18:350–353.
11. Fernandes VT, De Santis RJ, Enepekides DJ, et al. Surgeon-performed ultrasound guided fine-needle aspirate biopsy with report of learning curve; a consecutive case-series study. *J Otolaryngol Head Neck Surg*. 2015;44:42–49.
12. Iwamoto N, Aruga T, Horiguchi S, et al. Ultrasound-guided fine-needle aspiration of axillary lymph nodes in breast cancer: Diagnostic accuracy and role in surgical management. *Diagnostic Cytopathology*. 2019;47:788–792.
13. Layfield LJ, Zhang T, Esebua M, et al. Axillary lymph node metastasis: Fine-needle aspiration biopsy sensitivity as a function of node size, percent replacement of lymph node by tumor and tumor deposit size. *Diagnostic Cytopathology*. 2021;49:181–186.
14. Nowikiewicz T, Kurylcio A, Głowacka–Mrotek I, et al. Clinical relevance of a degree of extracapsular extension in a sentinel lymph node in breast cancer patients: a single-centre study. *Sci Rep*. 2021;11:8982 Apr 26.
15. Barrio AV, Downs-Canner S, Edelweiss M, et al. Microscopic extracapsular extension in sentinel lymph nodes does not mandate axillary dissection in Z0011-eligible patients. *ANN Surg Oncol*. 2020;27:1617–1624.
16. van Roozendaal LM, Vane NL, van Dalen T, et al. Clinically node negative breast cancer patients undergoing breast conserving therapy, sentinel lymph node procedure versus follow-up: a Dutch randomized controlled multicentre trial (BOOG 2013-08). *BM Cancer*. 2017;17:459.
17. Araujo D, Mendes G, Menezes R, et al. Sentinel lymph node biopsy vs. no axillary surgery in early breast cancer clinically and ultrasonographically node negative: A prospective randomized controlled trial-VENUS trial. *Breast J*. 2020;26:2087–2089.
18. van der Noorda ME, Peters MT, Rutgers EJ. The intraoperative assessment of sentinel nodes - Standards and controversies. *Breast*. 2017;34(S1):S64–S69.

Please cite this article as: Israel Barco et al, Intraoperative Sentinel Node Fine-Needle Aspiration Biopsy as a Substitute for Whole Sentinel Node Excisional Biopsy in Breast Cancer Patients. Initial Report, Clinical Breast Cancer, https://doi.org/10.1016/j.clbc.2022.08.008