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Sentinel Lymph Node Biopsy in Breast Cancer: An Analysis of the Maximum Number of Nodes Requiring Excision

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■ **Abstract:** Sentinel lymph node biopsy (SNB) is now the standard of care in assessment of patients with clinically staged T1-2, N0 breast cancers. This study investigates whether there is a maximum number of sentinel lymph nodes (SLN) that need to be excised without compromising the false-negative (FN) rate of this procedure. Data were prospectively collected for 319 patients undergoing SNB between February 2001 and December 2006 at our institution. This data were analysed, both in terms of the order of SLN retrieval and relative isotope counts of the SLNs, in order to determine the maximum number of SLNs that need to be retrieved without increasing the FN rate. Furthermore, we investigated the relationship between SLN blue dye concentration and the presence of SLN metastases. The SLN identification rate was 97% with no false-negative cases amongst patients undergoing simultaneous axillary clearance historically during technique validation. In patients with SLN metastases, excision of the first 4 SLNs encountered results in the identification of a metastatic SLN in all cases. Although the majority (86%) of SNB metastases are in the hottest node, the SLN containing the metastasis is in the first 4 hottest nodes in 99% of patients with nodal metastases. The remaining 1% of SLN metastases were identified by blue dye. There was no statistically significant association between the SLN blue dye concentration and the presence of SLN metastases. A policy to remove a maximum of four blue and/or hot SLNs along with any palpably abnormal lymph nodes does not result in an increased false-negative rate of detection of SLN metastases. ■

Key Words: breast neoplasms, isotope count, patent blue dye, sentinel lymph node biopsy, technetium

Following 10 years of procedure validation worldwide (1–4), sentinel lymph node biopsy (SNB) is now considered the operative procedure of choice for axillary node staging in patients with clinically staged T1-2, N0 breast cancer. Various methods of SNB detection were initially reported, utilizing radioisotope alone (5), blue dye alone (6), and both in combination (7). Despite good results from single centers using blue dye or radioisotope alone, SNB mapping using a combined radioisotope and blue dye technique emerged in 2001 as the method with the greatest sentinel lymph node (SLN) identification rate (93%) and the lowest false-negative rate (5%) (8). Furthermore, the ALMANAC trial (9) has now shown both an improved quality of life and decreased arm/shoulder morbidity with SNB as compared with axillary node clearance (ANC).

The number of SLNs that should be excised has been previously examined. In 2001, two multicenter studies, using the combined blue dye/radioisotope mapping technique, found that the SNB false-negative rate was significantly decreased when more than one SLN was excised (3,10). Two further studies (11,12) examining this issue concluded that all nodes containing isotope and/or blue dye should be excised in order to reduce the SNB false-negative rate. The relationship between the intensity of radioactivity in the SLNs and node positivity, based on a single histologic node section, has been studied (13) and concluded that while the hottest SLN was the most likely to contain metastases, a significant number of less hot SLNs contained metastases, necessitating the removal of all blue and/or hot nodes.

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It is not uncommon to encounter an axilla in which a number of lymph nodes are radioactive. In order to keep the morbidity of SNB to a minimum, as few nodes as possible should be excised while maintaining a satisfactory false-negative rate for this technique.

The current study was undertaken with the primary aim of examining whether there is an upper limit to the number of SLNs that need to be excised while preserving a low false-negative rate. It aimed to identify this number on the basis of the degree of SLN isotope positivity and the order in which the SLN was retrieved in any given SNB procedure. A secondary aim of the study was to investigate whether there is an overall correlation between the intensity of SLN blue dye staining and the likelihood of SLN metastases.

PATIENTS AND METHODS

Between February 2001 and December 2006, 319 patients with biopsy proven clinical stage T1-2, N0 breast cancer underwent SNB at the Royal Berkshire Hospital. All patients had SNB mapping using the combined Patent blue dye (Guerbet laboratories, Paris, France) and technetium (Tc^{99})-labeled albumin nanocolloid (GE Healthcare, Amersham, Buckinghamshire, UK) technique.

Sentinel Node Technique

The Tc^{99}-labeled albumin was injected subdermally in the periareolar region of the breast quadrant in which the cancer was situated. This was injected either on the day before surgery (40 mBq) or on the day of surgery (20 mBq). Preoperative lymphoscintigraphy was carried out at the operating surgeons' discretion. At the time of surgery, 4 mL of Patent blue dye in normal saline (50:50) was injected subdermally at the same site as the radioisotope injection. Intraoperatively, all blue and/or radioactive lymph nodes were identified, either visually or with a gamma probe (Europrobe; Euromedical, Le Chesney, Paris, France), and excised from the ipsilateral axilla. These SLNs were considered to be a radioisotope success if the 10 second ex vivo SNB count was greater than 10 times the background count. The count over the injection site, a background count and ex-vivo counts for all SLNs were recorded at the time of surgery. The color of excised lymph nodes was assessed at the time of operation and graded on a scale from 0 to 3, where 0 was indicative of no blue-dye infiltration and 3 representing avid blue-dye uptake within the lymph node.

For the purpose of this study when more than one lymph node was excised en bloc (on histopathology), and considered to be a single SNB at the time of surgery, all nodes in that specimen were counted as one node with one radioisotope count value. Palpably abnormal lymph nodes (LN) were excised at surgery but were considered to be an abnormal node sample rather than a SNB if they contained no isotope or blue dye. Operative and pathologic data were collected prospectively as for the New Start Programme (Royal College of Surgeons, England).

The first 50 patients underwent a concurrent ANC as part of the validation phase of one consultant surgeon entering patients into the ALMANAC trial (9). A further 49 patients underwent a concurrent axillary node sample (ANS) as part of the New Start training programme undertaken by the other two consultant surgeons. All procedures were carried out by the three consultant surgeons or their trainees under supervision.

Patients with multifocal breast cancer, and those who had undergone previous breast surgery, were included in the study but usually underwent a concurrent ANS as a backup measure. Exclusion criteria included: (a) patients with clinically involved lymph nodes and latterly if a fine-needle aspiration (FNA) of an ultrasound (USS)-detected abnormal LN confirmed metastases, (b) pregnancy, and (c) previous axillary surgery.

Histologic Lymph Node Analysis

All SNBs underwent the same histologic analysis. Each SNB was fixed in 10% formalin for 24 hours. The node was then sliced into 2 mm thick slices along its longitudinal axis. All of the slices were processed using standard histologic tissue processing methods (Tissue-Tek VIP processor; Sakura, Thatcham, Berkshire, UK) and embedded in paraffin wax blocks. Three-micrometer-thick sections were cut at three levels through each block, stained using Harris's hematoxylin and eosin (H + E) method and examined under the microscope. Immunohistochemical staining (e.g., Pancytoytokeratin and S-100 staining) was carried out if the reporting pathologist deemed it necessary. All nodes excised in an ANC underwent a standard histologic analysis with H + E staining of one section of the bisected node.

Statistical Analysis

All data were analysed using Statistical Package for Social Sciences (SPSS; SPSS, IBM UK, Feltham,

Middlesex, UK). To identify whether the intensity of blue-dye staining was predictive of nodal metastases, a binary logistic regression analysis was performed.

RESULTS

General Characteristics

Between February 2001 and December 2006, 319 patients underwent SNB at the Royal Berkshire Hospital. Two patients had bilateral breast cancer, taking the number of SNB procedures to 321. Table 1 shows patient demographics. These are similar to the breast cancer population overall. Stand-alone SNB took place in 2% of patients prior to primary medical therapy and immediate breast reconstruction. There was a failure of SNB localization by both blue dye and isotope in nine procedures. These patients consequently underwent ANS. All subsequent analyses have taken place on the 312 successful SNB procedures.

SNB Characteristics

Table 1 shows the details of the SNB procedures. A preoperative scintigram was carried out initially on all patients during the individual surgeons' learning curve, and subsequently took place variably depending on surgeon preference. A mean number of 1.9 sentinel nodes were excised and the SNB metastasis rate was 27% overall. The majority (87%) of sentinel nodes contained both isotope and blue dye. Forty-eight patients had a total of 96 palpably abnormal LNs excised. Three of these patients were found to have metastases (two were micrometastases) in the abnormal LNs and no metastases in the SLNs. We have not considered these to be false-negative cases, in this study, as the SNB procedure included the removal of all palpably abnormal LNs. In all three cases no further LNs containing metastases were found on subsequent ANC. The false-negative rate (0%) in this series was calculated from patients who had lymph node metastases in a simultaneous ANC.

A breakdown of the number of patients having different numbers of sentinel nodes excised is shown in Table 2. The majority of patients (84%) had up to two SLNs excised and only 1% of patients had more than four nodes excised. Within these groups the number of patients with SNB metastases are also shown in Table 2.

SLN Metastases

In the 85 patients with SLN metastases, the position of the first SLN containing a metastasis with respect to the order in which it was removed is shown in Table 3. In the majority of cases (98%), a SLN containing metastases was identified in the first 2 SLNs removed. One hundred percent of the first positive SLNs were found in the first 4 SLNs removed.

Only two patients with SLN metastases had no isotope in any of the nodes excised. Both had two equally blue nodes excised, with one of the two sentinel nodes containing metastases. The remaining 83 patients all

Table 1. Clinicopathologic Characteristics of Patients Undergoing Sentinel Lymph Node Biopsy

Patient characteristics	Number
Number of patients	319
Number of SNB procedures	321 (2 bilateral)
Number of successful SNB procedures	312
Age median (years) (range)	61 (22–90)
Tumor size median (mm) (range)	15 (1–48)
Tumor histology	
Invasive ductal	192
Invasive lobular	41
Mixed invasive adenocarcinoma	40
DCIS	21
Other	18
Surgery	
Mastectomy	60
Wide local excision	245
Pre-operative scintigram	120
Mean number of SLNs excised (range)	1.87 (1–7)
SNB metastases positive	85/312
SNB found by	
Blue dye only	11
Isotope only	30
Isotope and blue dye	271
Simultaneous ANS	49
Simultaneous ANC	50
False-negative SNB rate (patients undergoing simultaneous ANC)	0/50

ANC, axillary node clearance; ANS, axillary node sample; DCIS, ductal carcinoma in situ; SNB, sentinel lymph node biopsy; SLN, sentinel lymph node.

Table 2. The Number of Patients Overall and With Sentinel Lymph Node Biopsy Metastasis Per Sentinel Lymph Node Site Sampled

No. sentinel nodes excised	No. patients overall (n = 312)	No. patients with SNB metastasis (n = 85)
1	170	43
2	93	22
3	33	10
4	12	8
5	3	1
7	1	1

SNB, sentinel lymph node biopsy.

Table 3. Location of the First Sentinel Lymph Node Containing a Metastasis in the Order Removed

First SLN containing metastases	No. patients (n = 85)
1st	71
2nd	12
3rd	1
4th	1

SLN, sentinel lymph node.

had isotope in one or more of the sentinel nodes. These 83 patients were analysed in order to determine which node (from highest count to lowest count) contained the first metastasis. This data, inclusive of the 43 patients with only one SNB excised, are shown in Table 4. Eighty-six percent of SNB metastases are in the hottest node. The sentinel node containing the metastasis is in, the first 3 hottest nodes in 98% and, the first 4 hottest nodes in 99% of patients with nodal metastases.

The SNB contained isotope at greater than 10 times the background level in all of the 43 patients with metastases who had only one node excised. The other 40 patients, who had two or more SNBs excised with at least one containing metastases, had a total of 114 nodes excised. Eighteen of these nodes did not contain isotope at a level of 10 times the background count and were detected by blue dye.

The hottest node contained metastases in 28 of 40 patients with SNB metastases that had more than one SNB excised. Of the remaining 12 patients, six had metastases in the second hottest node of which one was detected by blue dye only (count present but not at 10 times background level). Four patients had the first nodal metastasis in the third hottest node of which one was detected by blue dye only. Both patients with the fourth and fifth hottest node being the first positive node had isotope in those nodes but the ex vivo count was <10 times background level. They were both identified using blue dye.

Sixteen patients had more than three SLNs excised; six of whom had no SNB metastases. Of the remaining 10 patients, nine had the SLN containing metastasis in the first 3 SLNs retrieved and one in the 4th SLN retrieved. In eight of these 10 cases, the SLN containing metastasis was one of the three hottest nodes excised. In the other two cases, it was the fourth and fifth hottest node; both of which were blue and contained isotope counts below 10 times the background count.

Of the four patients that had more than four SLNs excised; two had no SNB metastases. The SLN containing metastasis was the second SLN retrieved in both cases. In one of these two cases this SLN was the hottest node; in the other it was the fifth hottest node which had an isotope count below the threshold level but contained blue dye identifying it as a SLN.

Table 4. Position of Positive Sentinel Lymph Node in Relation to the Degree of Isotope Count Positivity

Positivity of SLN with metastasis (hottest SLN positive)	No. nodes (n = 83)
1st	71
2nd	6
3rd	4
4th	1
5th	1

SLN, sentinel lymph node.

SLN Blue Dye Intensity

When the intensity of SLN blue-dye staining seen intraoperatively was compared to no uptake of blue-dye with regards to the presence of metastatic deposits, no significant differences were seen (Table 5). When blue-dye was absent from a lymph node there was a 24% likelihood of metastatic deposit. This fell to 13% for those nodes with the weakest uptake of blue-dye. Regression analysis failed to identify a significant relationship between the intensity of blue-dye staining and the probability of the node containing metastases.

DISCUSSION

This longitudinal cohort study examining the relationship between SLN metastases and isotope counts has shown that the false-negative rate of this technique is not increased by removing a maximum of four SLNs. A SNB identification rate of 97%, together with a 0%

Table 5. Regression Analysis to Assess Whether Depth of Blue-dye Staining is Predictive of Nodal Metastases

Intensity of blue stain	No. nodes	Number with mets	Odds ratio*	p-value
0	76	18	1.000	0.250
1	106	14	0.490	0.070
2	305	66	0.890	0.701
3	51	10	0.786	0.587

*Odds ratio relative to no blue-dye staining.

false-negative rate, based on 50 cases in which a simultaneous ANC was performed, has been demonstrated. Furthermore, to date, no patient has had an axillary recurrence. Both the patient characteristics and SNB characteristics (Table 1), including the 27% SNB metastasis rate and the mean number of SLNs excised, are comparable to other studies (10,12).

Number of SLNs Requiring Excision

In this study, 99% of SNBs undertaken retrieved four or less SLNs. In the remaining 1% of SNBs, if the four SLN sites containing the highest isotope counts only are excised, one SNB with metastases would be missed. This SLN with metastasis contained blue dye and did not have an isotope count above the threshold level required to be an isotope success. As the second SLN retrieved in the procedure it would have been removed with a policy to excise up to the first 4 SLNs encountered, resulting in no increase in the false-negative rate.

On a practical basis, this study has shown that by excising up to the first 4 SLNs encountered, no SLN containing metastases would be missed. A number of studies have examined which SLN contains metastasis in relation to the order in which it was removed from the axilla. In a large multi-institutional study, Martin et al. (11) found that the SLN containing the metastasis was one of the first 4 nodes removed in all patients who did not have a false-negative SNB based on a simultaneous ANC. Two further studies by McCarter et al. (14) and Woznick et al. (15) found that the SNB false-negative rate would increase by 0.7% and 1.2% respectively if only the first 4 SLN sites excised were examined. This small difference may be due to both studies injecting a different blue dye and radioisotope in a different site, than that used in the present study. Furthermore, the number of SLNs excised in the study by Woznick et al. (15) was greater than most published series, with a mean of 4.4 (range 1–21) and with 7% of SNB procedures retrieving 10 or more SLNs. This difference may account for the small false-negative rate increase that would have been recorded if a maximum of four SLNs were excised in their study. All three studies concluded that all blue and/or hot SLNs should be excised in order to decrease the false-negative rate.

In our patients with SNB metastasis, 86% of metastases were found in the hottest node, 93% in the first 2 hottest nodes, 98% in the first 3 hottest nodes and 99% in the four hottest nodes. These figures correlate reasonably with the two other studies (13,16) assessing the relationship between isotope levels and nodal metastases. The study by Camp et al. (16), in which 249 patients underwent SNB using isotope only, showed that all SLNs containing metastases were included in the first 4 hottest nodes excised. The hottest node contained metastases in 76% of their patients with lymph node metastases as compared with 86% of patients in this study. Wada et al. (13) examined 503 patients undergoing SNB. They looked at which SLN contained metastases in relation to positivity of the isotope count and found that of the 123 node positive cases, 90% had metastases that were detected within the first 3 hottest SLNs, as compared with 98% in the current study. Of the remaining 12 cases not detected in the three hottest nodes, four had metastasis in SLNs containing blue dye only and the other eight were false-negative cases in which none of the SLNs contained metastasis but non-SLNs did.

In both the current study and that by Camp et al. (16), the SNBs underwent a more extensive histologic workup than in the study by Wada et al. (13), in which the SNBs underwent analysis of a single node section with H + E staining only. This may explain the discrepancies between the current study and that by Wada et al. (13), in terms of their lower percentage of cases with metastases identified in the first 3 SLNs and higher false-negative rate. Both studies (13,16) concluded that all nodes containing blue dye and/or radioisotope should be excised.

We have demonstrated that all patients with SLNs containing metastasis would have been identified by excising only the three hottest or blue LNs in this study. However, a policy to retrieve a maximum of three SLNs would have resulted in one patient having a false-negative SNB as the SLN containing metastasis was the fourth node retrieved.

SLN Isotope Success Definition

There have been several proposed definitions of a SNB isotope count that is considered to be an isotope success. These include absolute counts above a certain value (1), any count above background (13), SLN-to-background ratio (17) and SLN-to-hottest SLN ratio (2). In this study an isotope success has been defined as a SLN containing at least 10 times the background count, however, all SLNs with an isotope count above background were excised in order to ascertain whether this arbitrary cut-off value is valid. The presented results have shown that, when

combined with a policy to remove all SNBs containing blue dye up to a maximum of four SLNs, this definition of a SNB success does not increase the false-negative rate.

Morbidity Associated with SNB

Two large randomized trials (9,18) have shown a reduced arm morbidity and better quality of life following SNB when compared with standard axillary dissection. To date, there have been no studies comparing the morbidity of removing different numbers of SLNs. It would be reasonable, however, given the data comparing the morbidity of SNB with axillary dissection, to assume that removal of an increasing number of SLNs increases arm morbidity and decreases quality of life.

To determine the absolute false-negative rate in this study, it would need to have been undertaken by removing up to the four hottest and/or blue SLNs and followed by a concurrent ANC in all cases. However, SNB is a fully validated technique and this would now be an unethical and unacceptable trial to participate in given the increased morbidity of ANC.

SLN Blue Dye Intensity

This study has shown no statistically significant association between the intensity of staining and the probability of the node containing metastases. The degree of blue due staining is a subjective intraoperative assessment which may account for the lack of association.

CONCLUSION

The present study has determined that, by using the combined blue dye and radioisotope identification technique and examining the SLNs in multiple sections, retrieval of a maximum of the four hottest and/or blue SLNs along with any palpably abnormal LNs does not increase the false-negative rate in SNB procedures. On the basis of these results it would appear that no more than four SLNs require excision, unless it becomes subsequently obvious that the SLN containing the highest radioisotope count has not been excised.

CONFLICTS OF INTEREST

The authors have no conflict of interest.

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