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Clinical study

Factors affecting failed localisation and false-negative rates of sentinel node biopsy in breast cancer – results of the ALMANAC validation phase

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Summary

Background. Despite the widespread application of sentinel lymph node biopsy (SLNB) for early stage breast cancer, there is a wide variation in reported test performance characteristics. A major aim of this prospective multicentre validation study was to quantify detection and false-negative rates of SLNB and evaluate factors influencing them.

Methods. Eight-hundred and fourty-two patients with clinically node-negative breast cancer underwent SLNB according to a standardised protocol that used a combination of radiopharmaceutical 99mTc-albumin colloid and Patent Blue V dye. SLNB was followed by standard axillary treatment at the same operation in all patients.

Results. Sentinel lymph nodes (SLNs) were identified in 803 (96.1%) of 836 evaluable cases. The median number of SLNs removed per patient was 2 (range 1–9). There were 19 false negatives, resulting in a sensitivity of 263/282 (93.3%) and accuracy 782/803 (97.6%). SLNs were successfully identified by blue dye in 698 (85.6%), by isotope in 698 (85.6%), and by the combination of blue dye and isotope in 782 (96.0%) of 815 patients. Among 276 node positive patients, one or more positive SLNs were identified by blue dye in 251 (90.9%), by isotope in 246 (89.1%) and by the combination of blue dye and gamma probe in 258 (93.5%). Obesity, tumor location other than upper outer quadrant and non-visualisation of SLNs on the pre-operative lymphoscintiscan were significantly associated with failed localisation ($p < 0.001$, $p = 0.008$, $p < 0.001$, respectively). The false-negative rate in patients with grade 3 tumors was 9.6%, compared with 4.7% in those with grade 2 tumors ($p = 0.022$). The false-negative rate in patients who had one SLN harvested was 10.1%, compared with 1.1% in those who had multiple SLNs (three or more) removed ($p = 0.010$).

Conclusion. SLNB can accurately determine whether axillary metastases are present in patients with early stage breast cancer with clinically negative axillary nodes. Both success and accuracy of SLNB are optimised by the combined use of blue dye and isotope. SLNB success decreases with increasing body mass, tumor location other than the upper outer quadrant and non-visualisation of hot nodes on the pre-operative lymphoscintiscan. This study demonstrates reduction in the predictive value of a negative SLNB in grade 3 tumors.

Introduction

The feasibility and accuracy of sentinel lymph node biopsy (SLNB) for patients with breast carcinoma has been investigated primarily in small, single institution studies in which a few surgeons with extensive experience performed most of the procedures. Most validation series excluded patients with T2/T3 tumors and those undergoing mastectomy. An important issue is lack of a standard technique of identifying the sentinel lymph node (SLN). In most series, participating surgeons did not attend a formal SLNB training program before recruiting patients. Results are therefore difficult to compare and cannot be generalised to other hospital environments.

Ongoing randomised trials will provide information on the long-term morbidity, local-regional control, disease-free survival and overall survival following SLNB compared to axillary lymph node dissection (ALND). However, the false-negative rate and factors influencing it can only be determined from validation series in which SLNB is followed by ALND.

Here we report results from the validation phase of the multicentre ALMANAC trial [1]. The same type and dose of blue dye and radiocolloid were used in all participating hospitals, with a standard protocol for SLN identification. In the interests of generalisability the study was designed to include patients of both sexes with clinically node-negative invasive breast cancer, irrespective of tumor size. We analysed factors

influencing the success and accuracy of sentinel lymph node biopsy.

Methods

Study design

The ALMANAC multicentre randomised trial compared sentinel lymph node biopsy with standard axillary treatment in the management of patients with early clinically node-negative breast cancer. The randomised phase of this trial is reported elsewhere [2] and shows that axillary staging using SLNB confers a marked benefit in several measures of arm and shoulder morbidity and quality of life.

The randomised phase was preceded by a validation phase, during which each participating surgeon was to perform at least 40 sentinel lymph node biopsies which were followed by either axillary lymph node dissection or four-node axillary sampling to stage the axilla, whichever was the current standard staging procedure in that centre. Surgeons who achieved a localisation rate of at least 90% and a maximum of two false-negatives for 40 consecutive cases were eligible to proceed to the randomised phase of the trial [1]. Results of the validation phase are presented in this paper.

Participants

In the validation phase of the trial, 842 patients (including 5 men) aged 18–80 years with clinically node-negative, invasive breast cancer irrespective of the size of the tumor, were enrolled by 31 surgeons, in 18 centres in the United Kingdom between February 1998 and December 2001. Fifteen surgeons completed at least 40 cases in the validation phase. Patients underwent wide local excision or simple mastectomy. For all patients axillary procedure was SLNB followed by standard axillary treatment. Signed informed consent was required from every participant according to a protocol approved by local ethics committees and in accordance with the Declaration of Helsinki. Exclusion criteria were pregnancy, known multicentric cancer, prior ipsilateral axillary surgery or breast surgery except previous benign biopsy, previous irradiation of the ipsilateral axilla or breast, pre-existing limb disease causing swelling and known allergy to human albumin or Patent Blue V.

Sentinel node biopsy

All surgeons together with their team of nuclear physicians and pathologists attended a training course on SLNB. SLNB followed a standardised protocol using a combination of radiopharmaceutical compound and blue dye. Briefly, 2 ml of the radiopharmaceutical 99mTc-albumin colloid (Nanocoll®, GE Healthcare, USA) was injected at four sites peritumorally on the day before surgery (dose = 40 MBq) or on the day of surgery (dose = 20 MBq) and the injected area was massaged gently for approximately 5 min to facilitate lymphatic drainage. Static scintigraphic images, in anterior and oblique projections, were obtained approximately 3 h after injection of the radiocolloid tracer. The locations of axillary and any non-axillary sentinel lymph nodes were marked on the patient's skin. The patient was given general anaesthesia in the operating room, and 3–5 min before the first incision was made, 2 ml of Patent Blue V dye (Laboratoire Guerbet, Aulnay-sous-Bois, France) diluted to a total volume of 5 ml with saline was injected peritumorally. Intra-operative identification of SLN(s) was based both on blue dye mapping and gamma probe detection, preferably before breast surgery. A SLN was defined as any blue-stained node or any node with radioactive count more than 10 times the background count which was measured on the arm. Extra-axillary sites were not routinely removed as this is not the standard of care.

Pathological examination

All lymph nodes were examined by standard hematoxylin-eosin staining. Lymph nodes smaller than 5 mm were bisected and stained; those 5 mm or larger were sectioned at 3 mm intervals and single sections were stained with hematoxylin-eosin. Intraoperative histologic examination or immunohistochemical staining techniques were not used to examine the lymph nodes.

Statistical analysis

For comparison of sentinel node identification and false-negative rates, Mann–Whitney and chi-square tests were performed according to distributional form, as indicated. All p-values are two-sided.

Results

Adequate data on SLN identification and outcome was available for 836 (99.3%) of 842 patients. Their clinicopathological characteristics are summarised in Table 1. The axillary basin was tumor positive in 282 (33.5%) patients.

Table 2 shows the results of sentinel node biopsy. Sentinel lymph node localisation was successful in 803 (96.1%) of 836 patients. Two-hundred and eighty-two patients were node positive, of whom 19 were false negatives, resulting in a sensitivity of 263/282 (93.3%) and accuracy 782/803 (97.6%). The false negative rate is defined in the normal manner as the complement of the sensitivity, 19/282 (6.7%). But the impact of false negatives on the whole series is better represented by the fact that they amounted to only 2.4% of all the patients with successful localisation.

The median number of sentinel nodes removed per patient was 2 (range 1–9). The numbers of patients

Table 1. Characteristics of 836 patients evaluable for SLNB status[a]

Characteristic	$n = 836$
Age (years) median (range)	57 (26–89)
BMI (kg/m^2) median (range)	25.4 (16.5–46.9)
Sex	
Male	5
Female	831
Initial presentation	
Palpable mass	531 (82.8%)
Non-palpable abnomality	110 (17.2%)
Nodal metastases	282
Type of breast surgery	
Wide local excision	574 (73.1%)
Mastectomy	211 (26.9%)
Tumor size	
Up to 20 mm	458 (54.4%)
20–50 mm	235 (27.9%)
> 50 mm	12 (1.4%)
Tumor grade	
I	137 (19.8%)
II	318 (46.0%)
III	237 (34.2%)
Pathological findings	
Invasive ductal	525 (74.4%)
Invasive lobular	77 (10.9%)
Other	104 (14.7%)

[a]There are missing values for some variables.

Table 2. Results of sentinel lymph node biopsy[a]

	n (%)
Total number of evaluable cases	836
Failed mapping procedures	33/836 (3.9%)
Sentinel node pathological findings	
True positive	263
True negative	521
False negative	19
False negative rate – normal definition	19/282 (6.7%)
Impact of false negatives on whole series	19/803 (2.4%)
Sensitivity	263/282 (93.3%)
Test accuracy	782/803 (97.6%)
Success rate in identifying the sentinel node related to mapping technique	
Dye success	698/815 (85.6%)
Isotope success	698/815 (85.6%)
Combined success	782/815 (96.0%)
Success rate in identifying the POSITIVE sentinel node related to mapping technique	
Dye success	251/276 (90.9%)
Isotope success	246/276 (89.1%)
Combined success	258/276 (93.5%)
Number of sentinel nodes removed median (range)	2 (1–9)
Pre-operative lymphoscintigraphy	
Axillary drainage	490/707 (69.3%)
IM drainage	62/707 (8.8%)
Axillary + IM	48 (6.8%)
IM only	10 (1.4%)
Axillary drainage unknown	4 (0.6%)

[a]There are missing values for some variables.
IM: internal mammary drainage.

shown in the following analyses vary as patients with unknown values for particular variables were excluded.

Lymphoscintigraphy visualised axillary hot spots in 490 (69.3%) of 707 patients. In 62 patients (8.8%), hot spots were noted in the internal mammary chain and drainage exclusively to the internal mammary nodes was seen in 10 patients.

Relative value of blue dye and radioisotope in localising the sentinel node

Using our combination of blue dye and gamma probe, one or more sentinel node was detected intra-operatively in 782 (96.0%) of 815 patients with adequate data. Had blue dye been used alone, localisation would have been successful in only 698 (85.6%) of them. Likewise, had the hand-held gamma probe been used alone, localisation would have been successful in only 698 (85.6%). Thus, we estimate the failed localisation rate would increase by a factor of 3.6 (14.4 versus 4.0%) when only a single localisation method is used.

Two-hundred and seventy-six of 815 patients had at least one positive SLN. One or more positive sentinel node was identified intra-operatively using the blue dye alone in 251 of them (90.9%), by hand-held gamma probe alone in 246 (89.1%) and by the combination of blue dye and gamma probe in 258 (93.5%). The false negative rate would increase by 2.5% (7 additional false negatives) if blue dye alone was used, or by 4.3% (14 additional cases) if isotope alone was used (Table 2).

Unsuccessful mapping procedures

Table 3 shows the factors associated with unsuccessful mapping procedures. High body mass index, tumor location other than upper outer quadrant and non-visualisation of SLN on pre-operative lymphoscintiscan were significantly associated with failed localisation ($p < 0.001$, $p = 0.008$, $p < 0.001$, respectively). Age, tumor size, tumor histology, tumor grade and presence of multifocality on final histology did not significantly affect sentinel node identification intra-operatively.

False negatives

Factors associated with false negatives are summarised in Table 4. The false-negative rate in patients with grade 3 tumors was 9.6%, compared with 4.7% in those with grade 2 tumors ($p = 0.022$). The false-negative rate in patients who had one SLN harvested was 10.1%, compared with 1.1% in those who had multiple SLNs (three or more) removed ($p = 0.010$). Age, BMI, sentinel node

Table 3. Factors associated with unsuccessful mapping procedures[a]

	Number of failed localisations (%)	*p* value
All patients	33/836 (3.9%)	
Age (years)		
< 50	6/200 (3.0%)	0.14[b]
> 50	27/631 (4.3%)	
Body mass index (kg/m^2)		
< 30	8/425 (1.9%)	< 0.001[b]
> 30	14/113 (12.4%)	
Pre-op lymphoscintiscan		
Positive	9/593 (1.5%)	< 0.001[c]
Negative	23/230 (10.0%)	
Tumor location		
Upper outer quadrant	5/280 (1.8%)	0.008[c]
Lower outer quadrant	4/63 (6.3%)	
Upper inner quadrant	8/96 (8.3%)	
Lower inner quadrant	5/46 (10.9%)	
Central	0/13 (0.0%)	
Mixed	4/142 (2.8%)	
Tumor size		
Up to 20 mm	16/458 (3.5%)	0.79[b]
20–50 mm	8/235 (3.4%)	
> 50 mm	1/12 (8.3%)	
Tumor type		
Invasive ductal	19/525 (3.6%)	0.89[c]
Invasive lobular	2/77 (2.6%)	
Other	4/104 (3.8%)	
Focality		
Multifocal	4/75 (5.3%)	0.45[c]
Unifocal	20/559 (3.6%)	
Histologic grade		
I	3/137 (2.2%)	0.32[b]
II	11/318 (3.5%)	
III	10/237 (4.2%)	

[a]There are missing values for some variables.
[b]Mann–Whitney test.
[c]Chi-square test.

Table 4. Factors associated with false negative sentinel node biopsy[a]

	False negatives as a proportion of node positive patients (%)	*p* value
All patients	19/282 (6.7%)	
Age		
< 50	7/93 (7.5%)	0.79[b]
> 50	12/187 (6.4%)	
Body mass index (kg/m^2)		
< 30	9/146 (6.2%)	0.10[b]
> 30	5/36 (13.9%)	
Pre-op lymphoscintiscan		
Positive	13/192 (6.8%)	0.89[c]
Negative	6/83 (7.2%)	
No. of SLN removed		
1	10/99 (10.1%)	0.010[c,d]
2	8/94 (8.5%)	
≥3	1/89 (1.1%)	
Tumor location		
Upper outer quadrant	9/96 (9.4%)	0.95[c]
Lower outer quadrant	1/20 (5.0%)	
Upper inner quadrant	2/24 (8.3%)	
Lower inner quadrant	2/21 (9.5%)	
Central	0/6 (0.0%)	
Mixed	3/42 (7.1%)	
Tumor size		
Up to 20 mm	7/135 (5.2%)	0.51[b]
20–50 mm	10/130 (7.7%)	
> 50 mm	0/7 (0.0%)	
Tumor type		
Ductal	14/209 (6.7%)	0.74[c]
Lobular	2/30 (6.7%)	
Other	1/32 (3.1%)	
Focality		
Multifocal	3/34 (8.8%)	0.75[c]
Unifocal	13/180 (7.2%)	
Histologic grade		
I	0/38 (0.0%)	0.022[b]
II	6/128 (4.7%)	
III	10/104 (9.6%)	

[a]There are missing values for some variables.
[b]Mann–Whitney test.
[c]Chi-square test.
[d]One or two versus three or more nodes removed.

visualisation on pre-operative lymphoscintiscan, tumor location, tumor size, tumor histology and presence of multifocality on final histology did not influence the false-negative rate.

Discussion

Accurate assessment of axillary lymph node status in patients with early breast cancer is important for staging, prognosis and therapeutic decisions. Axillary lymph node dissection (ALND) is the most accurate method for assessing spread of disease to the lymph nodes. However, ALND may result in lymphedema, nerve injury, shoulder dysfunction, and other complications that may compromise function and quality of life. Sentinel lymph node biopsy (SLNB) is associated with reduced arm morbidity and better quality of life compared with ALND [2,3] and is being widely practiced in Europe, the United States and Australia. The present study confirms observations from previous validation studies suggesting that SLNB can be reliably performed in selected patients with early stage breast cancer [4–9].

The sentinel node identification rate in the present study was 96% and the false-negative rate was 6.7%, which compares favourably with the results of other validation studies [4–7,9]. Learning curve data for the 13

surgeons who completed at least 40 cases in the validation phase by July 3, 2001 was published recently [10]. There was little evidence of variation of sentinel node identification rate and false-negative rate between the 13 surgeons. Only the surgeon's first procedure within the series had a higher risk of failed localisation or false-negative result; apart from this no learning curve was identified. These findings suggest that the learning curve is very short if the surgeon completes a standardised training program including hands-on training.

Many studies have sought to determine the optimal technique for SLNB. Using a combination of isotope and blue dye for sentinel node localisation drastically reduces the rates of failed and false-negative procedures. In the current study, in most patients both dye and isotope found the sentinel nodes, however, in approximately 10% of patients only one of the agents located the sentinel node. More importantly, in approximately 4% of patients the positive SLN was found by dye alone and in 3% by isotope alone; these would have been missed by relying on a single technique of localisation. This is in line with other studies which show that the combination of radiolabeled colloid, lymphoscintigraphy, and blue dye offers the highest success rate with the fewest false negatives [7–9]. A small prospective randomised study in which the use of blue dye alone was compared with a combination of blue dye and radiolabeled colloid showed that the combined technique significantly improved the intra-operative SLN identification rate (100 versus 86%; $p = 0.002$) [11].

We have shown previously that replacement of the SLN by tumor significantly reduces the radioisotope uptake and may adversely affect intra-operative SLN identification [12]. However, SLN identification using blue dye is not compromised by increased SLN tumor burden. The afferent lymphatic leading to the blocked node may be patent. The surgeon can identify the tumor-replaced node by following the blue lymphatic leading to the node [12]. This result further suggests that a combination of blue dye and radioisotope should be used to optimise the localisation rate.

We found that patient age, BMI, sentinel node visualisation on pre-operative lymphoscintiscan, tumor location, tumor size, tumor histology and presence of multifocality did not alter the false-negative rate.

We have shown that high BMI adversely influences the successful mapping of SLNs. Patient age did not alter SLN localisation, though it has been reported in several other studies which have shown that accurate identification of the SLN decreases with increasing age as well as weight [13,14]. The specific causes for mapping failure in overweight patients are unclear. Sentinel node identification may be difficult in obese women because of the higher content of subcutaneous and axillary adipose tissue. Furthermore, the increased fatty tissue may impede the flow of the tracer through the lymphatics in the breasts of these patients. Or the lymph nodes in obese patients may have undergone fatty degeneration reducing their capacity to concentrate the tracer. These findings, however, do not contraindicate SLNB in obese individuals as the rate of successful localisation remains high and unsuccessful mapping does not adversely affect their prognosis or treatment.

Arguments have been made in favour of pre-operative lymphoscintigraphy as a ‚road map' for surgeons. Our results indicate that SLN visualisation on pre-operative lymphoscintigraphy significantly improves the intra-operative SLN identification rate ($p < 0.001$). If an SLN takes up enough radiocolloid to image with a camera, it should be easily detected with the intra-operative probe. Similar findings were reported by Birdwell et al. [15].

We found that tumors located in the upper outer quadrant had a higher SLN identification rate compared to other tumor locations. The simplest explanation relates to the transit distance for the blue dye or radioisotope from the peritumoral injection site to the axilla.

High false-negative rates may have a direct adverse impact on patient care including accurate staging, treatment decision making and long-term outcomes including survival. Clearly, the potential for both local as well as systemic under-treatment of patients increases as the false-negative rate increases. This study demonstrates a reduction in the predictive value of a negative SLNB in grade 3 tumors. Grade 3 tumors have a higher incidence of nodal metastases, thereby have an increased risk of lymphatic obstruction and re-routing of tracer leading to a false-negative result. Therefore, caution is required when applying the SLNB procedure in patients at considerably increased risk for lymph node positive disease.

The data from this study suggest that surgeons should not stop after finding just one SLN but should search thoroughly to be certain there are not more. This is important as the false-negative rate in patients who had multiple sentinel nodes (3 or more) removed was 1.1% compared with 10.1% in those with 1 sentinel node removed.

In conclusion, sentinel node biopsy is a safe and accurate alternative to routine axillary dissection for patients with early stage breast cancer with clinically negative axillary nodes. The success and accuracy of sentinel node mapping in breast cancer is optimised by the combined use of blue dye and isotope. Body mass index > 30, tumor location other than upper outer quadrant and non-visualisation of SLN on pre-operative lymphoscintiscan adversely affect the SLN identification rate. SLNB may not be appropriate for patients who have grade 3 tumors, and the decision to perform the procedure should be determined on the basis of the clinical judgement of the treating surgeon.

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