Sentinel lymphnode biopsy in early breast cancer using methylene blue dye and radioactive sulphur colloid – a single institution Indian experience

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Abstract

Background Axillary lymph node dissection is an established procedure in breast cancer staging. However, it is associated with unpleasant side effects. A promising alternative to assess axillary lymph node status in early breast cancer patients is Sentinel Lymph Node Biopsy (SLNB). Isosulfan blue has traditionally been the dye used to identify the Sentinel Lymph Node (SLN). This article is a validation study of SLNB using methylene blue dye and radioactive sulphur colloid in early breast cancer Indian patients.

Materials & Methods With written informed consent, 100 patients with cytology or biopsy proven carcinoma breast, clinical stage T1/ T2 N0 M0, underwent SLNB using combination of methylene blue dye & radioactive technetium 99m sulphur colloid as a part of validation study from June 2003 to February 2006. After validation study, from March 2006 to February 2007, 35 patients have undergone SLNB followed by complete axillary clearance in only those patients with SLNB being positive for metastases.

Results In all 100 patients of the validation study SLN was identified. Total number of cases with positive axillary nodes was 27, out of which SLN was only positive node for metastases in 69% of cases. The overall sensitivity, specificity, positive predictive value and negative predictive value of SLNB 96.2%, 100%, 100% and 98.6% respectively with false negative rate of 3.7%. In subsequent 35 patients who underwent SLNB followed by complete axillary clearance, SLNs was identified in all the cases.

Conclusions SLNB is effective in early breast cancer patients of Indian population. SLNB using combination of methylene blue dye and radio-active Tc99m sulphur colloid can stage the axilla with high accuracy & low risk of false negativity in early breast cancer patients.

Keywords Sentinel lymph node biopsy · Methylene blue dye · Tc99m radioactive sulfur colloid · Early breast cancer · Indian patients

Introduction

Modern screening methods and increased self awareness about breast cancer has made it possible to diagnose breast cancer at an early stage. Axillary lymph node status is one of the most important prognostic indicators in breast cancer. Routine axillary dissection does not benefit the 70–80% of early breast cancer patients who are clinically node negative [1]. Also axillary lymphadenectomy results in significant morbidity like chronic lymph edema of ipsilateral extremity (3–12%), [2, 3] frozen shoulder syndrome and long term sensory abnormalities [4, 5].

A promising alternative to assess axillary lymph node status in early breast cancer patients is Sentinel Lymph Node Biopsy (SLNB). The Sentinel Lymph Node (SLN) concept represents a biological model that assumes the

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presence of a specific afferent lymphatic drainage pathway from a primary tumor to a principal, “Sentinel”, lymph node in the regional lymphatic basin [6–8]. The definition of SLN in breast carcinoma is the node most likely to harbor metastases if they are present.[9] The assumption is that if SLN is negative, all other axillary lymph nodes will be negative [10, 11]. Thus the technique of SLNB was developed to provide surgeons with information that allows axillary dissection to be avoided if SLN is negative, which is less invasive and with least morbidity. Most of the literature related to SLN in breast cancer patients are mainly from western centers and traditionally isosulfan blue has been the dye used to identify SLN. We have performed SLNB study using combination of methylene blue dye and radioactive sulphur colloid in early breast cancer patients. We have used methylene blue dye in our study as it is more readily available, safe, less expensive and equally effective [14–19]. In the following article we want to share our experience of validity of SLNB in early breast cancer using combination technique in Indian patients.

Materials and methods

With written informed consent, all patients with pathologically proven carcinoma breast, clinical stage T1/ T2 N0 M0, were included in our study (inclusion criteria). Prior clearance from ethical board of our Hospital was taken for the study. Fine Needle Aspiration Cytology (FNAC) was done in most of the cases for the diagnosis of carcinoma breast. If patient already had a trucut biopsy or incisional biopsy or excision biopsy done, then review of the slides of the same was done at our hospital for confirmation. Total number of patients were 135, 100 patients as a part of validation study (all these patients underwent SLNB followed by axillary lymphadenectomy) from June 2003 till February 2006 and 35 patients from March 2006 to February 2007 who underwent SLNB followed by axillary lymphadenectomy only, if SLNB was positive for metastases. Patients of carcinoma breast with clinical stage T3/T4, clinically palpable lymph nodes, presence of systemic metastases, pregnant women, lactating women, patients with multicentric and multifocal tumors and with h/o previous radiotherapy and/ chemotherapy were excluded. 0.2ml (0.2mCi/7.4MBq) of radioactive technetium 99m (Tc99m) sulphur colloid was injected peri-tumorally in all four quadrants and intradermally in skin anterior to lump (total of 1ml and 1mCi/ 37MBq) two hours prior to surgery. 1ml methylene Blue dye (10%) was injected peritumorally in all four quadrants (total of 4ml), 20 min prior to surgery. In patients with prior lumpectomy Tc99m-radioactive sulphur colloid was injected into perillumpectomy cavity area in all 4 quadrants and subareolar region and methylene blue was injected into the perillumpectomy cavity area in all 4 quadrants. Sterile packed hand held gamma ray detecting probe (Europrobe – Boulevard vivier Mier, Lyon cedex, France, Fig. 1) was

used to assess radioactivity of the injected primary tumor area and survey of the nodal basins for focal sites of increased counts. While surveying the nodal area, a small lead shield to prevent shine through effect from primary site was used. The area of axillary skin (hot spot) with audible tone (acoustic signal) and high counts as shown by the probe (at least one tenth of the count at the injected primary area and/ or counts of 10:1 ratio of SLN to background) was marked over axillary skin just prior to the surgery.

Intraoperatively, SLN was searched through a short dedicated incision over the marked axillary skin area along skin crease in cases of breast conservation surgery, (so that incision can easily be extended to complete the axillary dissection) or after raising upper flap (in cases of mastectomy). Axillary tissue is gently dissected until a blue lymphatic is identified which is traced proximally and distally to identify all SLNs. Blue stained nodes (Fig. 2) and/or nodes with high count and audible tone (hottest node) as identified by hand held gamma probe were excised and sent for final HPE in a separately labeled bottle. Verification was done by ex-vivo SLN counts compared with residual in- vivo background counts using the probe. Additional radioactive SLNs were removed, if present until background was less than one tenth the value of the hottest node. All patients underwent complete axillary dissection subsequently. Sensitivity, specificity, positive predictive value and negative predictive value of the sentinel lymph node biopsy was analyzed after final histopathology report was available in comparison with rest of the axillary lymph nodal status (in first hundred patients of validation study). Adjuvant therapy was advised as per final pathological stage.

Results

Between June 2003 and February 2006, 100 consecutive patients underwent SLNB followed by axillary lymphad-

Fig. 1 Europrobe: Hand held gamma ray detecting probe and counter

Fig. 2 Methylene blue stained SLN in axilla (blue nodes)

enectomy using methylene blue dye and radioactive Tc 99m Sulphur Colloid as a part of validation study. Subsequently 35 patients (between March 2006 and February 2007) underwent SLNB followed by axillary lymphadenectomy only if SLNB was positive for metastases. The results of analysis of first 100 patients are included here. The

average age of the patients was 52 yrs (range 24–82 years). 38 patients were pre menopausal and 62 were postmenopausal. Table 1 shows various tumor characteristics.

Table 2 shows the salient features of SLN. Sentinel Lymph Node was identified in all cases (100%). SLN identified in internal mammary region (one case) was biopsied and it showed tuberculosis and no metastases. The mean number of sentinel lymph nodes detected was 2 and mean number of axillary nodes dissected was 16. The mean size of SLN was 0.7cm. Total number of cases with positive axillary nodes was 27, out of which SLN was the only positive node for metastases in 18 cases (69%), both SLN and rest of the axilla also being positive for metastases in 8 cases and SLN was negative for metastases in 1 case (false negative rate of 3.7%). Total number of cases with negative axillary nodes was 73. In all these 73 cases, SLN was also negative for metastases. With above mentioned results, the overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of SLNB in predicting axillary node status was 96.2%, 100%, 100% and 98.6% respectively (Table 3). The overall accuracy was 99%.

In 35 cases who underwent SLNB followed by axillary lymphadenectomy only if SLNB was positive for metastases after 100 cases of validation study, SLNs were identified in all patients. All the patients are on regular follow up. Till to date, none of these 35 patients have axillary or systemic recurrence.

Table 1 Tumor characteristics

Breast carcinoma	No of cases
Side	
Right sided tumors	38
Left sided tumors	62
Location	
Outer quadrant tumors	74
Inner quadrant tumors	13
Central quadrant tumors	13
T Stage	
T1 tumors	17
T2 tumors	83
Histology	
Invasive ductal carcinoma	87
Invasive lobular carcinoma (all unilateral)	6
Mucinous carcinoma	2
Medullary carcinoma	2
Tubular carcinoma	1
High grade DCIS with micro invasion (who underwent mastectomy)	2

Table 2 SLN Characteristics

	No of cases
Sentinel node identified	100
Levels of SLN identified	
Only Level 1	86
Only Level 2	6
Both Level 1 and 2	6
Intramammary node	1
Internal mammary node only	1
SLN identification	
Both by gama probe and blue dye	87
By gama probe only	9
By blue dye only	4
Distribution of metastases in SLN and non SLN	
a)Total no of cases with positive axillary nodes	27
a) SLN being the only positive node	18
b) Both SLN and other axillary node also being positive	8
c)Negative-SLN–false,negative	1
b)Total no of cases with negative other nodes and negative SLN	73

Fig. 3 Hand held Gamma Probe to identify SLNB- Both Hot and Blue node (a) Deep Blue SLNB (blue nodes) (b) *In vitro* reconfirmation by hand gamma probe for same activity of hot node

Discussion

The main premise of SLNB is that patients with negative nodes can be identified as accurately as with an axillary dissection while surgical morbidity can be decreased. Taking above into consideration, we conducted this study of SLNB study in early breast cancer patients to validate the process in our setup.

Between June 2003 and February 2006, 100 patients underwent SLN biopsy with methylene blue dye and radioactive Tc99m sulphur colloid as a part of validation study. All these patients underwent routine standard axillary lymph node dissection as part of the procedure. The results of this validation study, have been compared with other studies on the sentinel node biopsy in early breast carcinoma.

Chemical agents used for lymphatic mapping fall into two categories. In the first category there are various vital dyes that are used in SLNB, viz lymphazurin (isosulfan) blue, patent blue, methylene blue, and indigo carmine. The absorption of dyes by the lymphatic system appears to be the function of their protein binding capacity, which depends on their molecular structure [12]. Despite differences in protein binding, no distinct clinical superiority in sentinel node identification or false negative rates have been demonstrated with any particular dye in indirect, or direct comparisons [13–15]. Methylene blue dye demonstrated poor lymphatic uptake in initial studies but recent work shows that it can be substituted for isosulfan blue [14, 15]. We are using methylene blue dye to identify SLNs and also it is less expensive and more readily available. In our study we found that it readily reaches SLN and stains the node deeply blue. Also, its superiority in SLN identification is reflected by our good results (Table 3).

In the second category, there are radiolabelled large molecules, the most common of which are various colloids, albumin, and dextran. Here also, there appears to be no distinct clinical superiority with one agent over other by indirect comparisons [20–22]. Debate continues to exist, however, regarding optimal particle size and whether filtration of the particle is necessary. In some studies filtration did not change SLN identification rates [23, 24], but in other studies, unfiltered colloid resulted in superior rates of SLN identification [25, 26]. Unfiltered particles with an average size of 305–340 nm are retained better by the SLN [27, 28]. Larger doses filtered colloids if used, are required to counterbalance the penetration of the lymphatics [27]. A dose of less than 0.5 mCi/ 18.5MBq has been used by some [29, 30], 1mCi/ 37MBq [31,32] or more than 1mCi/ 37MBq by others[33] with good results. Injection volumes have varied from 0.5 ml to larger volumes (8 ml) [28, 34]. We are using filtered radioactive Tc99m sulfur colloid (total of 1 ml and 1 mCi/ 37MBq, particle size 150–200 nm) as we can achieve good results and also it is easily available and economical.

We have not used preoperative lymphatic mapping by lymphoscintigraphy in our study as it adds on to the expenditure of the procedure and more importantly adds little if anything to intraoperative lymphatic mapping [35, 36]. In a large multicenter trial (Louisville Multicenter Trial), lymphoscintigraphy did not improve the ability to identify an axillary SLN or decrease the false negative rate [37].

Various sites of injection of the mapping agent are peritumoral, intratumoral, intradermal over the tumor site and subareolar. Intradermal or subareolar injection appear to increase the identification rate when compared to peritumoral

Table 3 Statistics

Parameter	Calculations		Values
Sensitivity	$\frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}} \times 100$	$\frac{26}{26+1} \times 100$	96.2%
Specificity	$\frac{\text{True Negative}}{\text{False Positive} + \text{True Negative}} \times 100$	$\frac{73}{0+73} \times 100$	100%
False Negativity	$\frac{\text{False Negative}}{\text{True Positive} + \text{False Negative}} \times 100$	$\frac{1}{26+1} \times 100$	3.7%
Positive Predictive Value	$\frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}} \times 100$	$\frac{26}{26+0} \times 100$	100%
Negative Predictive Value	$\frac{\text{True Negative}}{\text{False Negative} + \text{True Negative}} \times 100$	$\frac{73}{1+73} \times 100$	98.6%
Accuracy	$\frac{\text{True Positive} + \text{True Negative}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}} \times 100$	$\frac{26+73}{26+73+0+1} \times 100$	99%

injection in most studies [38–40], although the two methods have been found equivalent in other studies [41, 42]. Compared to peritumoral injection, intradermal injection results in sentinel nodes that are more significantly more radioactive, which simplifies sentinel node localization and may shorten the learning curve [38]. There is data indicating presence of retromammary lymphatic pathways from deeper portion of breast tissue that drains to both axillary lymph nodes as well as internal mammary nodes [43]. This is distinct from superficial lymphatic pathways which primarily drains to axilla. Therefore lymphatic mapping after a combination of deep (peritumoral) and superficial injections of the radiocolloid will improve SLN mapping. In our series we had one case where SLN was an internal mammary node which turned out to be tuberculosis on histopathological examination and rest of the axilla was negative for metastases in the same case. The impact of identification and removal of sentinel internal mammary lymph nodes on patient management is still the subject of debate [44–46]. Intratumoral injection of the radioactive colloid as though another valid method of lymphatic mapping, requires higher tracer doses [47]. After taking into consideration the above mentioned facts, we inject radioactive colloid peritumorally and intradermally over the tumour site, in post lumpectomy cases to peritumpectomy cavity area and subareolar region and blue dye peritumorally for optimal sentinel node identification.

A unique, albeit, rare, complication of isosulfan blue dye is the manifestation of allergic reactions in the form of urticaria, blue hives, generalized rash, pruritus and rare anaphylactic shock to the blue dye used for the lymphatic mapping [48, 49]. 1.6% of patients experienced allergic reactions due to use of isosulphan blue in an large MSKCC study [49]. None of our patients had any of the above reactions proving safety value of the methylene blue dye [16]. The administration of isosulfan blue dye has been shown to artificially decrease the intraoperative pulse-oxymetric readings without any associated ill effects or changes in arterial oxygen tension [50, 51]. In our series none of the

patients had any interference during intraoperative monitoring. Skin lesions at the site of injection of methylene blue is seen in 20% of patients [52]. Therefore intradermal injection of methylene blue dye is not recommended, which is not done in our study. Also by avoiding intradermal methylene blue dye injection, there is no blue tattooing effect on skin. Fat necrosis at the injection site has also been described following use of methylene blue dye [53]. None of our patients had any fat necrosis (even in patients with breast conservation surgery).

Average size of the the SLN in our series was 0.7 cm. These subcentimeter lymph nodes are unlikely to be detected by preop axillary palpation. Axillary staging with sentinel lymphadenectomy has demonstrated the SLNB to be the only site of metastases in an average of 40–60% of nodal dissections from high volume breast centers [29–32, 54–56]. In our series, sentinel node was the only site of metastases in 69% of cases. SLN being the only positive axillary lymph node in these cases confirms the logical basis of the sentinel lymph node technique.

There is increased risk of false negativity if blue dye alone is used. One of the factors associated with higher rate of sentinel node identification is use of combination of radiocolloid and blue dye for lymphatic mapping [57, 58]. The optimal technique in a large study (The University of Louisville Breast Cancer Sentinel Lymph Node Study enrolling 99 surgeons) with lowest false negative rate was isotope plus dye technique (11.8% vs 5.8%, $p < 0.05$) [59]. Multiple single-institution series have evaluated SLNB followed by axillary dissection (Table 4). SLN identification was higher when combination was used (86–98%) [57, 60, 63–65, 67–70] as in our series (100%), rather than when dye alone was used (74–94%). [57,65,66] False negative rates varied between 0–19%, higher when radiocolloid was not used.

Multi-institution studies evaluating SLNB are summarized in Table 5. Identification rates ranged from 86–93% and false negative rate of 4–13%. These multi-institution

studies are important because they reflect the ability to successfully perform lymphatic mapping and SLNB in various clinical settings and by surgeons with different levels of experience in this procedure. In our series, blue dye could not identify SLN in 9 patients while gamma probe could not identify SLN in 4 cases. The identification of these sentinel lymphnodes would have been missed if both blue dye and radioactive sulphur colloid were not used in combination. Also, effectiveness of the combination technique to identify SLNB is reflected by low false negative rate (3.7%) in our study.

Most of the literature on SLNB is from western centers. Two series on SLNB from India has so far been published. In an Indian series published earlier Parmar et al [73] using blue dye (isosulphan blue) alone have reported an identification rate of 77%, false negativity rate of 16.6% and a negative predictive value of 90.3%. They have concluded that axillary sampling is a feasible alternative to SLNB. The other series is by Deo et al [74] using blue dye alone. The reported sensitivity, specificity and accuracy of SLNB in predicting the axillary node status being 84.2%, 100% and 91.3% respectively. The superior result of our study (Table 3) using the combination of methylene blue dye and radioactive sulfur colloid for lymphatic mapping on Indian patient population proves that SLNB using the above mentioned combination technique is safe and effective.

A very good coordination between members of the multidisciplinary team including surgeons, nuclear physicians and pathologists is required for a successful SLNB as in our series. Our data indicate that SLNB can reliably predict the state of the axilla, so that when this node is negative for metastases, axillary dissection can be safely avoided. Several surgeons offer SLNB alone without completion axillary dissection to patients with negative sentinel lymph node(s) based on data of single institution experience and

multicentre studies. Participants of the consensus conference in Philadelphia in April 2001 concluded that SLNB is a staging technique that can replace axillary lymph node dissection in experienced hands [9]. A recent survey on SLNB distributed by American Society of Breast Diseases Rapid Response Panel demonstrates that SLNB is considered to be the standard of care by 85% of the members who responded [75]. It has been suggested that surgeons should demonstrate an SLN identification rate of $\geq 90\%$ and a false negative rate of $\leq 5\%$ before they offer SLNB without completion axillary dissection [76]. It is only after our validation study, we started doing SLNB therapeutically. The American Society of Breast Surgeons recommends a minimum of 20 SLNB procedures in conjunction with complete axillary lymph nodal dissection or with mentoring to minimize risk of false negative results, which is endorsed by American Society of Clinical Oncology (ASCO) also [77]. However, before SLNB becomes the undisputed standard of care, randomized trials will have to show no difference in axillary recurrence and over all survival (OAS) between SLNB alone and SLNB followed by axillary dissection in patients with negative sentinel node(s). Complete final results of two large randomized trials (NSABP B-32, ALMANAC) are awaited in this regard [78, 79]. Outside clinical trials, patients who are found to have a positive sentinel lymph node by H and E should undergo a completion axillary dissection, since a number of studies have shown that nonsentinel nodes can also be involved in 30–50% of cases [59, 71, 80].

Large scale implementation of SLNB technique will reduce the cost of treatment as a result of shorter hospitalization time and will also reduce indirect costs because motor compromise and lymphedema as a result of axillary dissection will become rare. Two decades ago, treatment of breast cancer was transformed by demonstration that breast

Table 4 Single institution studies evaluating SLNB followed by axillary dissection

Authors	Mapping method	Identification rate (%)	False negative (%)
Cox et al [60]	BD+COL	94	1
Paganelli et al [61]	COL	98	6
Veronesi et al [62]	COL	99	7
Zervos et al [63]	BD+COL	89	4
Villa et al [64]	BD+COL	98	14
Noguchi et al [65]	BD+ COL	94	10
BD	74		
Motomura et al [57]	D	84	19
D + COL	95	0	
Yu et al [66]	BD	86	9
Nano et al [67]	BD+COL	87	8
Yong et al [68]	BD+COL	86	17
Fortunato et al [69]	COL+/- BD	97	6
Bobin et al [70]	BD+ /- COL	93	2

BD: blue dye. Col: radiocolloid. D: dye (indigo carmine)

Table 5 Multicenter studies evaluating SNB followed by axillary dissection.

Authors	Mapping method	Identification rate (%)	False-negative rate (%)
Krag et al [37]	COL	93	11
McMasters et al [59]	BD or COL	86	12
	BD + COL	90	6
Tafra et al [71]	BD + COL	87	13
Shivers et al [72]	BD + COL	86	4

BD: blue dye. COL: radiocolloid.

conservative therapy is as effective as mastectomy. Today we seem to be on the threshold of a development that may allow routine axillary dissection to be foregone in a large percentage of patients with early breast cancer.

Conclusions

Sentinel lymph node biopsy using combination of methylene blue dye and radioactive Tc99m sulphur colloid can stage the axilla with high accuracy and low risk of false negativity in early breast cancer patients. Methylene blue dye is an effective and safe blue dye for SLNB. SLNB is effective in Indian set of patient population. A very good coordination between members of multidisciplinary team including surgeons, nuclear physicians and pathologists is required for a successful SLNB.

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