Sentinel lymph node mapping in early breast cancer – Our experience

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Abstract

Background The axillary lymph node status is the most important determinant of prognosis in patients with breast cancer. Sentinel lymph node (SLN) biopsy is a safe alternative for axillary clearance with an equal efficacy limiting the morbidity caused by axillary clearance.

Patient and methods From May 1996 till September 2009, 523 clinically node negative, early breast cancer patients attending our clinic at All India Institute of Medical Sciences were included in the study. They underwent sentinel lymph node biopsy by either combined technique or blue dye alone. All patients irrespective of the axillary status underwent axillary lymph node dissection (ALND).

Results Of 523 patients, 267 underwent combined technique of sentinel node mapping and 256 underwent blue dye technique alone. The identification rate of sentinel lymph node was 94.3% (253/267) for combined technique and 87.8% (225/256) for blue dye alone. Of 523 patients SLN was identified in 478 patients. The identification rate was 91.3%. The sensitivity = 91.5% (141/154), false negative = 8.4% (13/154), negative predictive value = 96.14% (324/337), and accuracy being 97.2% (465/478).

Conclusion Sentinel node mapping is a simple and safe technique of identifying the axillary node involvement. Sentinel lymph node biopsy is associated with less arm oedema and shoulder morbidity compared to ALND. However, the results of long term effects of sentinel node approach on tumor recurrence or patient survival are awaited.

Keywords Sentinel lymph node biopsy · Axillary lymph node dissection · Axillary node sampling · Lymphatic mapping · Early breast cancer · Immunohistochemistry.

Introduction

The axillary lymph node status is a strong determinant of the long and short-term outcomes in patients with breast cancer with reference to loco-regional recurrence, five year survival and for deciding adjuvant therapy. Since time of William Stuart Halsted, the evaluation of axillary

lymph node status has traditionally been performed by complete removal of all the lymph nodes and fibro-fatty tissue contained in the axilla from its apex to lowest level (Level one, level 2 and level 3 lymph nodes) called axillary lymph node dissection (ALND). The presence of axillary metastases decreases survival by 28–40%.^{1,2} A meta-analysis of six trials by Orr et al, in patients with

breast cancer and clinically negative axillae who underwent standard treatment (modified radical mastectomy or breast conservation surgery and ALND or breast irradiation) to standard treatment without ALND had shown average survival benefit of 5.4% (Range 4–16%).³ However, the limitation of the study was that these trials were conducted in an era when adjuvant therapy was not used. Another limitation of Orr's meta-analysis was that the 5 year data of Institute Curie trial was included which showed a benefit of ALND. The same trial at 15 years of follow up revealed no survival benefit. Today most patients with breast cancer with more than 1 cm tumor receive adjuvant therapy which is known to reduce the risk of both locoregional as well as systemic recurrences and improve disease free survival.

In a more recent meta-analysis, Sanghani, Balk and Cady reported that in women with early breast cancer and a clinically negative axilla, there is no survival benefit with axillary node dissection when compared with observation alone. They reported overall survival (odds ratio [OR] 1.55; 95% confidence interval [CI], 0.74–3.24), metastases (OR 0.91; 95% CI, 0.65–1.29), or ipsilateral breast recurrence (OR 1.11; 95% CI, 0.68–1.83) associated with axillary treatment compared to no axillary therapy.⁴

The failure of locoregional clearance may increase if the number of nodes involved are more requiring post-operative radiotherapy. The lymph node metastases is found in 14–60% of patients with early breast cancer.⁵ In T1 tumors of less than 1 cm in size, with grade 1 histological differentiation the risk of axillary metastases is 14%. With grade 3 and T2 tumors, the risk of axillary metastases may increase to 60%. If we subject all women with breast cancer to axillary dissection, we would be performing this procedure unnecessarily to about 40 to 60 % subjects. ALND is associated with significant morbidity like lymphoedema, seroma, anaesthesia or parasthesia in arm, restriction of shoulder movement and a diminished quality of life. Hence, there is a need for a less invasive procedure with lesser morbidity and good prognostic value comparable to axillary lymph node dissection.

With this view in mind, surgeons developed various techniques to identify the spread of cancer in the axillary nodes before the nodes became palpable. Some of the methods of nodal evaluation are axillary sampling, sentinel lymph node biopsy (SLNB), ultrasound assessment of axillary nodes followed by aspiration cytology of suspicious lymph node and PET scan.

Axillary sampling

Axillary nodal sampling (ANS) also called four node sampling was introduced in United Kingdom 30 years

ago by Forrest and colleagues in 1982 in which four palpable nodes were excised and sent for histopathological examination. In patients with positive nodes on ANS, radiotherapy was administered to axilla. In this technique at least four palpable lymph nodes were removed from axillary tail or lower axillary fat. The technique is reported to have an accuracy of 95% in staging of axilla. In two randomized trials from Edinburgh breast clinic, comparing ALND with axillary sampling, there was similar locoregional recurrence and survival in both the groups. The morbidity was least in patients with axillary node sampling who had not received radiotherapy.^{6,7} After a period of 11 years of follow up, 62.6 percent are alive after clearance and 65.0 percent after sampling. There was only a minor difference in axillary relapse, favouring axillary clearance (3.0 vs 5.4%). Though it is a simple, non expensive procedure with no special training required it has limitations. It is a blind procedure. It has no anatomical or oncological basis. We do not know even the optimal number of nodes to be removed in axillary sample specimen that is representative of axilla.

A larger study from the Danish Breast Cancer Group of 13,851 patients found that those with more than ten negative nodes retrieved from the axilla, had significantly better axillary recurrence-free survival, overall recurrence free survival and overall survival than if fewer than ten nodes were removed. These conflicting findings serve to highlight the risk of false-negative staging of the axilla following sampling as opposed to clearance because less than 10 nodes are sampled.⁸

Sentinel node mapping

The sentinel lymph node biopsy (SLNB) is based on the hypothesis proposed by Cabanas in penile cancer. The *sentinel* node is the *first* node to be involved during the spread of metastases from a primary tumour.⁹ The word sentinel means '*sentry*'/*Guard* who stands on the gate of a building to keep a watch (Fig. 1). In medicine it is used to describe a thing that acts as indicator of presence of disease e.g.: HIV *sentinel surveillance*. Sentinel node stands like a guard on the gate of the axilla and keeps an eye on cancer cells that pass from the primary on their passage to axilla. Just as the guard knows who enters the gate, the sentinel node has the information whether the cancer cells have entered the axilla or not. The results of SLNB vary in various studies and has a learning curve in acquiring the skills required for performing a successful SLNB. Giuliano performed first SLNB using blue dye alone with identification rate of 66%,¹⁰ Krag, using 99 m Tc Sulfur Colloid achieved identification rate of 82%¹¹ and Albertini using combined technique accomplished an identification rates of 92%.¹² With improved experience Guiliano later achieved an identification rate of 94%¹³.

Fig. 1 Guard standing in front of Palace alike to sentinel node at entry of axilla

NSABP-32 and ALMANAC trials were randomized clinical trials in which patients with clinically node negative disease in early breast cancer were randomized for SLNB versus standard axillary treatment (ALND or axillary sampling depending on the choice of the operating surgeon).^{14,15} In AL-MANAC trial, 1031 patients were randomized either to undergo sentinel node biopsy (515 patients) or standard axillary treatment (516 cases). The standard axillary treatment comprised axillary node sampling (123 cases) or full level I, II and III dissection (373 cases). Sentinel node was mapped by combined technique using preoperative lymphoscintigraphy using 99 m Tc albumin colloid injection and intraoperative Patent blue V dye injection. ALMANAC trial had shown that the relative risk for lymphoedema of arm was 0.37 (absolute rates: 5 vs 13%) and for sensory loss 0.37 (absolute rates: 11 vs 31%) for the sentinel lymph node biopsy group compared to standard axillary dissection at 12 months. Drain usage, length of hospital stay and time to resumption of normal day-to-day activities after surgery were statistically significantly lower in the sentinel lymph node biopsy group (all $P < 0.001$). Overall 'patient recorded quality of life' and arm functioning scores were statistically significantly better in the sentinel lymph node biopsy group throughout (all $P = 0.003$). These benefits were seen with no increase in anxiety levels in sentinel lymph node biopsy group ($P > 0.05$).

Now internationally SLNB has become an accepted procedure in patients with early breast cancer. The advantages of SLNB are – it is a less invasive procedure giving few lymph nodes for detailed pathological examination. It is associated with less postoperative morbidity with good efficacy. We in our institute have evaluated SLNB using combined and blue dye techniques in patients with early breast cancer along with completion axillary dissection.

In 1492, Queen Isabella of Spain sent Christopher Columbus to search and have trade with India. Columbus took a voyage in uncharted seas without the help of any Global Positioning System with Satellite navigation (GPS) and landed in the Americas. Today we do not want to go astray and wish to hit the target with a very high accuracy. The blue dye and gamma probe are the GPS in the hands of breast surgeon. Since the lymphatic pathways in axilla vary from individual to individual and there is no constant route that cancer cells will take on their sojourn to axilla, it will only be prudent for the breast surgeon not to commit the mistake that Columbus had committed. Hence a blue dye guided and radioguided mapping of the regional nodal basin with precise localization of *node(s)* at highest risk i.e. *sentinel node* should be adopted in preference to a *blind* axillary nodal sampling.

Limitations of sentinel lymph node biopsy are that it is expensive and a Nuclear medicine department and Gamma probe may not be available in smaller institutions. Delayed assessment by haematoxylin and eosin staining of sentinel nodes results in 25–30% of patients with SLN metastases needing further surgery.¹⁶ Frozen section and imprint cytology offer an advantage that decision for axillary dissection can be taken in one sitting if SLN turns to be positive. However, frozen section facility is not available in many hospitals and its sensitivity is low especially in hands of inexperienced pathologists. This led to development of the newer techniques like Gene search (a real time qualitative polymerase chain reaction technology)¹⁶ and one step nucleic acid amplification (OSNA).¹⁷ With the help of these newer molecular biology techniques, the presence of cancer cells in the sentinel nodes can be ascertained within 20 minutes in the operation theatre and full axillary dissection performed if SLN is found positive. We require large scale multicentric clinical studies to validate these new procedures.

Our Experience of Sentinel Node mapping at All India Institute of Medical Sciences New Delhi.

Patient and methods

Five hundred and twenty three (523) patients with Tx, T1, T2, N0 disease with histologically confirmed breast carcinoma on core needle biopsy without palpable axillary node (N0) were included in the study after informed consent from May 1999 to September 2008. Exclusion criteria considered in the study were patients with irradiation to breast, breast tuberculosis, patients with T3/T4 disease and patients who refused consent.

Sentinel lymph node mapping

The patients scheduled for surgery for primary breast cancer in the study were injected with radioactive isotope

(Tc 99 m sulphur colloid) of 200–300 microcurie in 0.1–0.2 ml normal saline in subareolar region 1–3 h before surgery for patients scheduled for operation as second or third case in the day. Radiotracer was injected in the afternoon a day before surgery, if the patient was scheduled as first case next day morning. Lymphoscintigraphy was performed using a large field view gamma camera with a high-resolution collimator. Static images were obtained using both anterior and lateral projections for 1–2 h following radiotracer injection (Fig. 2). All the draining lymph nodes identified on lymphoscintigraphy were marked on patient's skin with indelible ink.

Intra-operative identification of SLN

A gamma-detecting probe was used intra-operatively to identify radioactivity positive node(s). Prior to the performance of procedure the background count (gamma count taken on outer aspect of arm away from axillary lymphatic basin for ten seconds) was noted. Initially hot node was identified with the gamma probe and incision was made close to it on skin crease. The lymph node was considered as hot node if the gamma probe count was 10 times more than background count in 10-seconds duration.

Blue dye injection

4 ml of 1% isosulphane blue dye was injected in the subareolar region using plastic syringe with 26 G needle and 1 ml injected in the breast parenchyma in the peritumoral region towards the axillary side of the lump. After 5–10 min of blue dye administration, a small incision 2–3 cm long was made along the skin crease between

axilla and breast centered at anterior axillary fold (Fig. 3). After raising the skin flaps blue stained lymphatic tracts were meticulously searched and traced towards axilla. The blue stained lymph node to which a blue stained lymphatic tract leads was considered as sentinel lymph node and resected along with perinodal fat.

A hot, blue or palpable node was considered to be sentinel node and sent for detailed pathological examination by paraffin fixation processing. A complete axillary lymph node clearance was done and the specimen was sent for histopathological examination. The information of full axillary clearance was used for validating the results of sentinel lymph node biopsy.

The sentinel lymph nodes were formalin fixed and paraffin embedded. All SLNs were evaluated by Haematoxylin and Eosin (H&E) staining and those with negative histology for metastasis, immunohistochemical staining with monoclonal antibody against cytokeratin.

Results

We at All India Institute of Medical Sciences, from 1996 to 2003 included 523 patients with early breast cancer with clinically node negative disease to validate sentinel lymph node biopsy at our centre. Of these 523 patients, 259 were premenopausal and 264 were postmenopausal. Two hundred and sixty seven (267) of them underwent combined technique and 256 underwent blue dye technique alone. Among these 523 patients, 108 had tumor less than 2 cm, 369 had 2–5 cm and 46 were Tx tumors. The identification rate of SLNB was 94.7% (253/267) for combined technique and 87.8% (225/256) for blue dye alone. Of 523 patients, SLN was identified in 478 patients, so the identification rate overall was 91.3%. The sensitivity = 91.5% (141/154), false negative = 8.4%

Fig. 2 Preoperative Lymphoscintigraphy for localization of axillary sentinel lymph node (SLN)

Fig. 3 Incision for SLNB centered over anterior axillary fold on the skin crease between axilla and breast

(13/154), negative predictive value = 96.14% (324/337), and accuracy being 97.2% (465/478). Axillary metastases were present in 154 patients. In 13 cases of 154, sentinel lymph node was negative but higher node in axilla turned out to be positive. Immunohistochemistry was performed in 241 patients in whom SLN was identified with micro-metastases in 8 patients.

Discussion

With developing technology and demand for better health system there is constant pressure on surgeon to develop better techniques which are less invasive, less morbid but with an efficacy equal to the older ones. In this constant struggle many inventions are being made through constant research. Initially radical mastectomy was considered to be the standard procedure which later changed to modified radical mastectomy decreasing the morbidity of previous procedure and now to breast conservation surgery with equal survival rate as with modified radical mastectomy. Axillary dissection used to be the standard procedure for locoregional control for many decades till DL Morton introduced sentinel lymph node biopsy in melanoma patients which later was applied to breast cancer by Guiliano bringing a change in axillary management with equal efficacy.^{18,10} SLNB is an attractive alternative to ALND because of less risk of lymphoedema and sensory loss. Many studies have shown that SLNB is accurate in predicting the absence of axillary metastases, i.e. it has a good negative predictive value. In 38–67% of cases with metastatic axillary lymph nodes, the sentinel lymph node is the only node involved.¹⁹

The identification rate with combined technique is 90–100%, and with blue dye alone is 65–95% in different studies.²⁰ We could infer from our study as well as from literature, that combination technique of preoperative lymphoscintigraphy, intraoperative gamma probe and isosulphane blue dye would be more sensitive in successful SLN identification, compared to blue dye alone or gamma probe alone. The accuracy of SLN in detecting axillary metastases was 97.2% in our study which was comparable with world literature of 91–100%.²⁰

The reported false negative rate of sentinel examination in the literature was 0–29.4%.¹⁹ In our study 13 patients of 478 patients in whom SLN was identified had skip metastases and the false negative rate was 8.4% (13/154). In 13 patients of skip metastases six patients had involvement of whole node with metastases blocking the lymphatic channel. The cause of skip metastases can be explained by complete blockade of the lymphatics or by alternative drainage of lymphatics as described by Ludwig. Ludwig described 5 different arrangements of afferent and efferent lymphatics in relation to the draining lymph nodes, in which he described that few

lymphatics may bypass the filter function of the node, i.e. the lymphatics traverse through a lymph node without actually opening in to it. In this type of lymphatic anatomy the node traversed by the lymphatics will seem to be a sentinel node as a blue lymphatics is seen to enter it but the node will not show any cancer cells. This anatomical variation explains some cases of a false negative node.²¹ The negative predictive value is 96.14% implying the capability of the test in ruling out metastases in majority of the cases.

Various techniques of SLN mapping were described and differ according to the materials used: blue dye and or 99 mTc-labelled Sulfur colloid or 99mTc-labelled albumin, volume of agent, timing and site of injection. The major variation in results was found with injection techniques. Dye or the tracer has been injected into the peritumoral or peri/subareolar tissue, subdermally or intradermally. Some advocate intradermal injection because of ease of injection, rapid migration of the isotope, higher uptake by the sentinel node, and lower false negative rates. In a large retrospective analysis in patients who had undergone SLND and ALND, the sentinel node identification rate was 98% for intradermal injection and 89% for peritumoral injection.²² The dermis contains a richer plexus of lymphatics than the breast parenchyma, resulting in more rapid uptake by the lymphatics. Although false negative rates are statistically similar among the various techniques, it was 9.5% for peritumoral injection, 7.8% for subdermal and 6.5% for intradermal injection.²³ One concern with intradermal injection is that internal mammary nodes cannot be visualized, these are detected in 1.2% cases with intradermal injection and 9% of cases with peritumoral injection.²² The advantage of subareolar injection is that it can be performed in nonpalpable tumors, in tumors with increased distance from axilla for upper and lower inner quadrant lesions and in multicentric disease. Moreover, subareolar injection of technetium avoids the problem of overlap of the radioactive zone of diffusion of the injection site with the radioactive sentinel lymph node in upper outer quadrants causing ‘*shine through*’ effect.

In our study subareolar radioisotope injection was given, isosulphane blue dye was injected 4 ml subareolarly and 1 ml in peritumoral space. Traditional pathological evaluation of axillary lymph nodes in breast cancer has consisted of bi-valving all identified nodes with subsequent microscopic examination. Although this approach is efficient for the processing of large number of nodes, random sectioning of the lymph node reduces the chance of detecting micrometastatic disease which is defined as microscopic foci of less than 2 mm maximum diameter of metastases in the lymph nodes (0.2 to 2 mm). The advent of SLNB has dramatically changed the pathological evaluation of sentinel nodes. Serial sectioning of SLN at 2 mm intervals offers an opportunity of

more detailed pathological evaluation of the node. Few centres are routinely using Immunohistochemistry for evaluating SLN.

Usually there is a delay of several days between the time of SLNB and the final histopathological reporting. This 'lag time' in SLNB processing precludes the performance of a synchronous ALND. Therefore, completion ALND for patients with a positive SLN biopsy usually requires a separate hospitalization, a second operative procedure, with its attendant anaesthetic risk and cost, increased surgical risk in view of distorted anatomy because of prior surgery, additional time demands on personal or professional commitments, prolonged anxiety over the biopsy results and the possible need for additional surgery. These factors represent potentially increased emotional and financial costs for the patient. If ALND is to be performed for cases with positive SLN, then it would be ideal to identify the positive node in the initial surgery. Patients with an intraoperative positive SLN biopsy can then go for synchronous ALND.

The intraoperative pathological evaluation was initially done with frozen section, imprint cytology, and other techniques, but they have a high false negative rates of 6–35%.²⁴ Now a days immunohistochemistry is been used for SLN and with this method there is up-staging in around 2–20% patients.²⁵ Most metastatic foci detected by this technique are usually micrometastases or isolated tumor cells. There is a small possibility that cells other than metastatic carcinomas may be positive (false positive staining), such as small macrophages in few patients.

Significance of axillary micrometastases: Presence of small deposit of cancer cells of 0.2 mm to 2 mm in a lymph node is defined as micrometastasis. In a recent cohort study from Netherlands women having a sentinel node biopsy before 2006 were studied to assess the impact of isolated tumour cells and micrometastasis in the sentinel node. In this study 856 cases were selected who had node negative early breast cancer and had not received adjuvant therapy, 856 cases with isolated tumour cells or micrometastasis who did not receive systemic adjuvant

therapy and 995 cases with isolated tumour cells or micrometastasis who received systemic adjuvant therapy. The patients were followed for a median of 5.1 years. The primary end point was disease free survival. The adjusted hazard ratio for disease recurrence among patients with isolated tumour cells without adjuvant therapy as compared to women with node negative disease was 1.5 (95% C.I. 1.15 to 1.94); among patients with micrometastasis was 1.56 (95% C.I. 1.15 to 2.13). Among patients with isolated tumour cells or micrometastasis, hazard ratio was 0.57 (95% C.I. 0.45 to 0.73) in the node positive adjuvant therapy cohort as compared to node positive no adjuvant therapy cohort. The authors concluded that presence of isolated tumour cells or micro-metastasis in sentinel nodes was associated with reduced 5 year disease free survival and addition of systemic adjuvant therapy improves it.²⁶ Two trials (NSABP-32 and ACOSOG Z0010/0011) have been launched in prospective way for validating the importance of micro-metastases and the results are awaited.

Conclusion

Sentinel node mapping with a combination of dye and isotope offers the best results for assessing axillary nodes in early breast cancer. Isotope should be given intradermally at edge of areola with a preoperative lymphoscintigraphy and hot nodes marked on the skin. Hot, blue or any palpable node should be removed for pathological examination. Haematoxylin and eosin staining of 2 mm sections should be used for pathological examination. Immunohistochemical stain with cytokeratin antibody may help in identifying isolated tumor cells/micrometastases in case H&E staining shows a negative node. In presence of micrometastases and isolated tumor cells full completion axillary dissection should be done in addition to systemic adjuvant therapy. Studies have shown less morbidity with SLNB compared to ALND but the comparative effects of these two approaches on tumor recurrence or patient survival are awaited.

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