Original Article

Second echelon node predicts metastatic involvement of additional axillary nodes following sentinel node biopsy in early breast cancer

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Abstract

BACKGROUND: In many patients with early breast cancer, the sentinel lymph node (SLN) is the sole site of regional nodal metastasis. This subgroup of patients may not benefit from completion axillary lymph node dissection (CALND). **AIMS:** This pilot study evaluates the status of 2nd echelon (station) lymph nodes in the axilla as a predictor of additional positive nodes in the axilla in the presence of sentinel node metastasis. **SETTINGS AND DESIGN:** Cross-sectional study of 40 breast cancer patients. **MATERIALS AND METHODS:** Forty patients with invasive breast cancer underwent SLN biopsy followed by 2nd echelon lymph node biopsy in the same sitting. SLN mapping was performed using a combined technique of isosulfan blue and 99 mTc-sulfur colloid. SLNs (Station I) were defined as blue and/or hot nodes. These nodes were then injected with 0.1 ml of blue dye using a fine needle and their efferent lymphatic was traced to identify the Station II nodes. Then a complete ALND was performed. All the specimens were sent separately for histopathological evaluation. **RESULTS**: SLNs (Station I nodes) were successfully identified in 98% (39/40) patients. Of the 17 patients with a positive SLN, 8 (47%) patients had no further positive nodes in the axilla, 9 (53%) patients had additional metastasis in nonsentinel lymph nodes upon CALND. Station II nodes were identified in 76% (13/17) patients with a positive SLN. Station II nodes accurately predicted the status of the remaining axilla in 92% patients (12/13). **STATISTICAL ANALYSIS**: We calculated the Sensitivity, Negative predictive value, Positive predictive value, False negative rate and Identification rate. **CONCLUSION**: Station II nodes may predict metastatic involvement of additional nodes in the axilla.

Key words: Axillary lymph node dissection, breast cancer, sentinel node (station I node), second echelon (station) node

Introduction

The sentinel lymph node (SLN) concept is based on the belief that all the lymphatic from the tumor bearing area drain first into the sentinel node. If this is identified and subjected to histopathology examination, it reflects the status of rest of axilla.[1-6] Thus, we may abandon the full axillary dissection in breast cancer, if SLN is negative. It results in fewer ALND (axillary lymph node dissection) related complications; shorter hospital stays and cost reduction.[7]

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Even in patients with metastasis in SLN, a third to half of the patients with invasive breast cancer, SLN is the only lymph node involved.[1,3,4] It is not certain whether complete axillary lymph node dissection is justified in such patients? We propose to examine the second echelon lymph nodes draining the sentinel node to find out whether they can predict the involvement of rest of axillary lymph nodes. The term "second echelon node" has been interchangeably used with "Station II Node" in this article. Assuming a sequential spread of metastasis we label the SLN as "Station I" and the higher nodes as "Station II" [Figure 1].

Materials and Methods

From June 2001 to Nov 2004, 40 (age 30-76 years) patients with cytological/biopsy proven operable breast cancer (T1-T3 / N0-N1) were enrolled into this study. We took ethical clearance from "Dean's Research Protocol Committee". We excluded patients treated by previous radiotherapy / Chemotherapy or previous axillary surgery or excision biopsy of tumor or those with a history of tuberculosis. The sentinel node mapping was carried by a combination of isosulfan blue dye and Tc-99 sulphur colloid (combined technique) in 26 patients and isosulfan blue in 14 patients. In combination technique 0.4 mCi of Tc99 m sulphur colloid was injected in subareolar region three hours before the operation.

Intraoperative localisation of "Station I and II nodes"

After cleaning and draping of the operative site, 5 ml of isosulfan blue dye was injected peritumorally at 4 sites and then breast was massaged for 5 minutes towards axilla. We gave small incision 2-3 cm in the natural skin crease between axilla and breast mound centred over lateral border of pectoral muscle 10 minutes after blue dye injection [Figure 2]. By blunt and sharp dissection, blue stained lymphatic(s) coming from breast parenchyma were traced to blue node, we

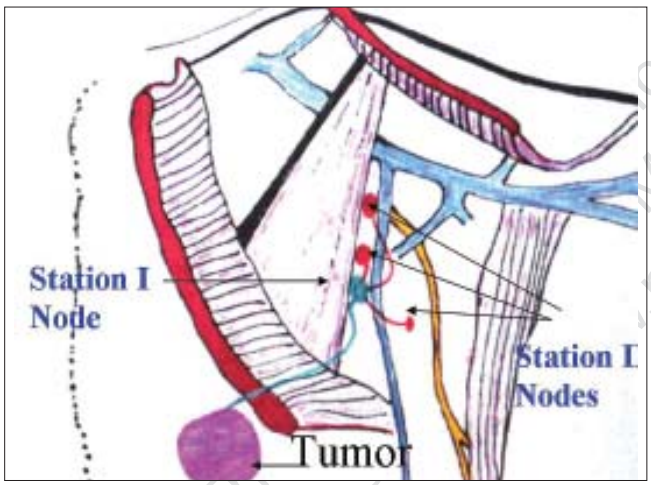

Figure 1: Line diagram to show the concept of "Station I and II nodes" in breast cancer

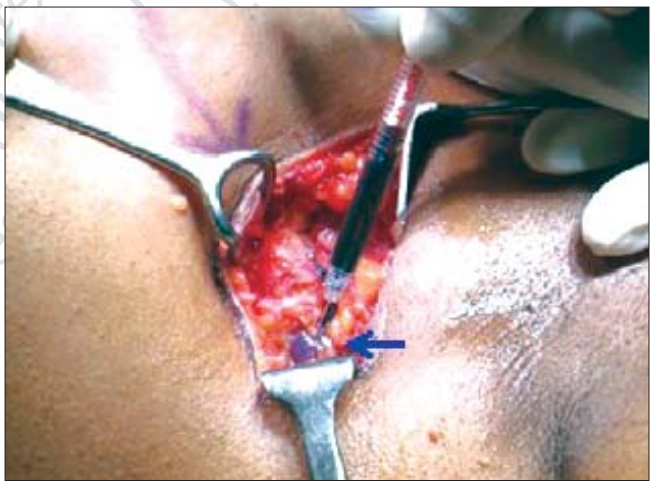

Figure 3: Isosulfan blue dye injection into "Station I node"

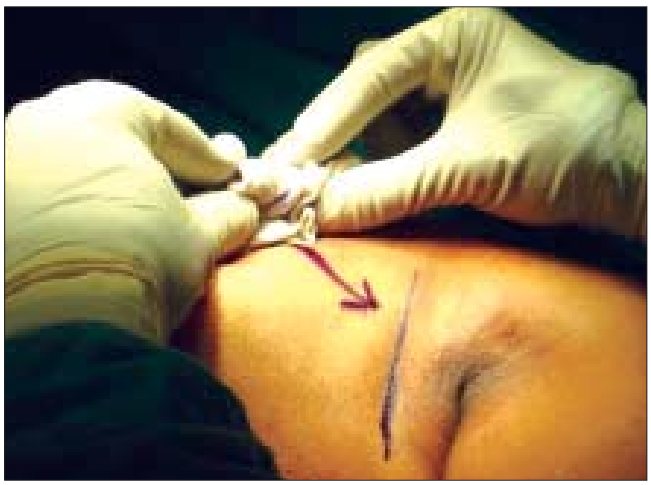

Figure 2: Natural skin crease between breast and axilla where we sited our incision for detection of "Station I lymph node".

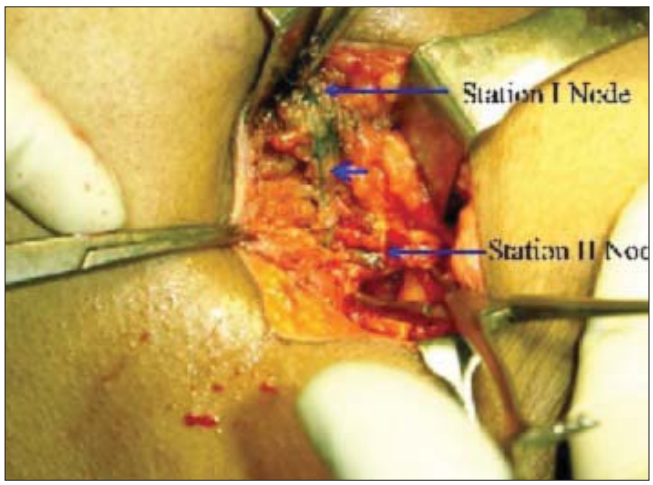

Figure 4: Lymphatic trunk going to "Station II node"

called it "Station I node" [Figure 3]. At the same time we noted radioactive count of the nodes with the help of gamma probe [Navigator, USA]. Any lymph node with count more than 10% of the hottest node was considered as SLN and excised. In some patients we experienced difficulty in visualising lymphatic below the skin flap, because of rapid wash out of dye. We tried to solve this problem by reinjecting the isosulfan blue dye peritumorally, so that we could visualise the flow of dye in the lymphatic directly.

After the blue and/or hot "Station I node" was seen, 0.1 ml of isosulfan blue dye with sterile 'Insulin Syringe' with 26G needle, was injected into this node, taking adequate precautions to avoid spillage and back flow of dye [Figure 3]. Following injection, next higher order nodes which were coloured blue, were identified as "Station II nodes" [Figure 4]. These were excised and labelled separately. Subsequently CALND was performed in the standard manner. At the end of CALND the background count was noted with gamma probe to see any residual activity.

Processing of specimens

After removal of the "Station I and II nodes" [Figure 5] and completion of the axillary dissection procedure, surgical specimens were sent to Department of Pathology. The specimens were properly labelled and preserved in 10% formalin. The lymph nodes in CALND specimen were identified by palpation and visual inspection and then bisected. An attending pathologist examined minimum of 3 (range 3 to 5) cross sections of each lymph node (SLN, second echelon and axillary nodes), stained by HandE (Haematoxylin and Eosin stain).

Statistical analysis

We calculated the following indices.

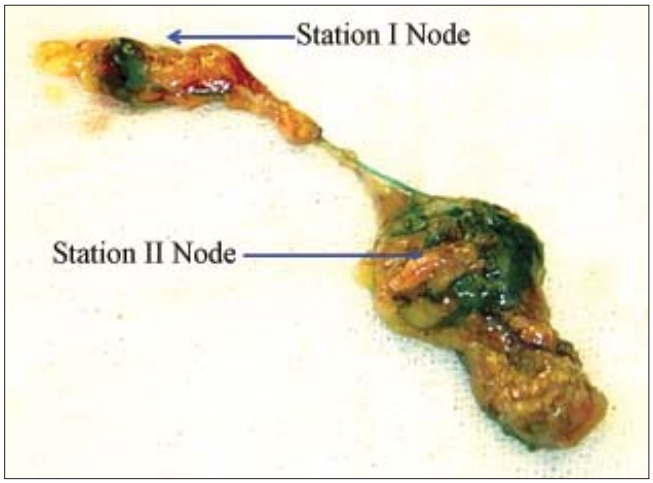

Figure 5: "Station I and II nodes" after removal from axilla

Sensitivity

The ability of the test/procedure to detect target condition (axillary metastasis) or the True positive rate. (True positive/ true positive + false negative).

False negative rate (FNR)

The test is negative in the presence of the disease (False negative/ false negative+ true positive).

Negative Predictive Value (NPV)

The likelihood that a negative test/procedure result indicates the true absence of the disease (True negative/ true negative + false negative).

Accuracy

The rate of concordance with axillary status. (True positive + true negative/ Total).

Results

We studied 35 patients of T2 and 5 patients of T3 stage. SLN was identified in 39 out of 40 patients (98%) [Table 1]. SLN identification by combined method and blue dye method was 100% (26/26) and 93% (13/14) respectively. Mean number of SLNs by combined method and only blue dye were 1.9 (1-6) and 1.5(1-4) respectively. Median of SLN by both methods was one. Average number of nodes found in axillary dissection specimen were 14.2 (9-33). Overall "Station II nodes" were identified in 33 of 39 (85%) patients.

19 patients had true negative "Station I nodes". Two patients had false negative "Station I node" (SLN). It means that identified SLN was negative for metastasis, but there were metastatic foci in axillary dissection specimen on histopathological examination.

Table 1: Axillary status and "Station I nodes" (n=40)

No. patients	Status of station of I node	Status of station II node	Status of rest of axilla (CALND)
19	(-)	(-)	(-)
1	(-)	Not found	(-)
1	(-)	Not Found	(+)
1	(-)	(-)	(+)
6	(+)	(-)	(-)
5	(+)	(+)	(+)
1	(+)	(-)	(+)
1	(+)	(+)	(-)
2	(+)	Not found	(-)
2	(+)	Not Found	(+)
1	Not Found	Not Found	(+)

CALND - Completion axillary lymph node dissection

The "Station I" correctly identified the disease process in the axilla in 89.5% of the cases. The NPV of the SLN was 90.9% implying the ability to rule out metastasis in axilla in majority of the cases, on the basis of negative SLN results on histopathological examination. The FNR of SLN was 10.5%. The "Station II" had a sensitivity, FNR and NPV 75%, 25 and 90.5% respectively. The accuracy of "Station-I and Station-II" in reflecting axillary status was 94.5 and 96.9% respectively [Table 2].

There were 17 patients with positive "Station I nodes". 8 (47%) had no further disease in axilla upon ALND and 9 (53%) patients had metastases in rest of axilla.

"Station II nodes" were identified in 76% (13/17) of patients with positive "Station I nodes". "Station II nodes" accurately predicted the status in 12/13 patients. In 6 patients, we could not find "Station II Nodes"; we attribute this failure to the reasons given in Table 3.

Overall, there were three patients with false negative results. [Table 4], we have discussed these patients in detail ahead.

Discussion

It is clinically relevant to evaluate the distribution of metastases in SLNs and non-SLNs because an overview of studies has shown that 38-67 percent of patients with breast cancer and positive SLNs have no disease in other non-SLNs.[3,5] In a series of one hundred seventy-four SLN mapping procedures, Giuliano found that 38% of pathologically positive axilla SLN was the only positive node.[1] In an effort to locate the SLN, in a consecutive series of 163 patients with operable breast cancer, Veronesi found sentinel lymph nodes were metastatic in 81 of 160 patients with identifiable sentinel nodes. In 40% of them sentinel lymph nodes were the only metastatic nodes.[3]

Krag in a multicenter trial of 405 patients concluded that there were 60 (60%) patients with only positive SLN of 101 patients with positive axilla.[4] This finding not only strongly supports the SLN concept, but also suggests that axillary dissection can be avoided in such patients. Several investigators Chu *et al*, Reynolds *et al* and Viale *et al* have examined the incidence of non-SLN metastasis in patients with SLN metastasis. [9-11] They found that micro metastasis existing solely in SLNs of patients with clinically lymph node negative T1 disease correlated with the absence or low prevalence of metastasis in other non-SLNs.

Table 2: Sensitivity, false negative rate (FNR) and negative predictive value (NPV) of Sentinel lymph node examination.

Method	Sensitivity	FNR	NPV	Accuracy
Station I (SLN)	89.5% (17/19)	10.5% (2/19)	90.9% (20/22)	94.5% (37/39)
Station II	75% (6/8)	25% (2/8)	90.5% (19/21)	96.9% (31/33)

SLN - Sentinel lymph node

A prospective database including 212 breast cancer patients who underwent sentinel lymph node biopsy followed by completion axillary dissection was reviewed by Sachdev *et al*.[12] A multivariate, logistic, stepwise regression was performed and found that tumor size greater that 2 cm, lymphatic invasion of primary tumor, use of radioisotope to identify the sentinel node and micro metastasis in the sentinel node correlated independently with the metastasis in the non-sentinel lymph node metastasis.

Reynolds *et al* found both tumor size greater than 2 cm and macrometastatsis (>2 mm) in the sentinel node positively correlated with spread of disease beyond the sentinel node.[10] Similarly Weiser *et al* found tumor size 1 cm or less and SLN metastasis 2 mm or less to be inversely related to non-sentinel lymph nodes metastasis.[13]

Rahusen *et al* described a series of 255 T1 and T2 breast cancer patients, of which, 36% had a positive sentinel node and underwent a full axillary lymph node dissection with non sentinel lymph nodes positive in 46 patients.[14] Patients with a single positive sentinel node and with metastasis less than 1 mm^2 in the sentinel node had significantly less non-sentinel node involvement than patients with more than one positive sentinel node (40% Vs 78%) and patients with macrometastaes (27% Vs 49%).

Guenther *et al* in a study of 46 tumors of breast found 16 only positive SLNs (35%) on H and E staining, while 30 SLNs were positive on immunohistochemistry.[15] ALND was not performed in these patients. They have not noted a single axillary recurrence with a median follow up of 32 months. The lack of axillary recurrence and the duration of follow up support hypothesis that completion ALND may not be necessary for selected patients with positive SLNs.

Recently, Ozmen *et al* were investigated retrospectively clinicopathologic characteristics to determine the factors predicting the status of a SLN biopsy and the metastatic involvement of non-SLNs in 400 consecutive patients with clinical T1/T2 N0 breast cancer. Patients

Table 3: Six patients with failed station II node identification

Site of tumor	Tumor stage	SLN	CALND	Reason
Left LIQ*	T3N0	0	0/14	Dye spilled and staining of tissue
Right LIQ*	T2N0	1/1	0/9	Lymphatic not able to trace from Station I
Central	T2N1	1/1	0/33	Lymphatic not able to trace from Station I
Left UOQ†	T2N1	1/3	3/9	Matted lymph nodes at level –I
Right UOQ†	T2N1	0/1	2/10	Matted nodes at Station -I
Left UOQ†	T2N1	1/1	4/4	Lymphatic not able to trace from Station I

*Lower inner quadrant, †Upper outer quadrant, CALND - Completion axillary lymph node dissection, SLN - Sentinel lymph node

Table 4: Patients with false negative results

Site of primary tumor	Tumour stage	Technique	Station I node	Station II node axilla (CALND)	Rest of
Right UOQ*	T3N1	Blue dye	(+)	(-)	5/11
Right UOQ*	T2N1	Blue dye	(-)	Not found	2/10
Left LOQ†	T3N0	Blue dye	(-)	(-)	1/10

*Upper outer quadrant, †Lower outer quadrant, CALND - Completion axillary lymph node dissection

with tumor size more than 2 cm and lymphovascular invasion were more likely to have positive SLNs in both univariate and multivariate analyses. Among patients with a positive SLN biopsy, those with T2 tumors, macrometastases in SLNs and extracapsular node extension were more likely to have non-SLN metastases.[16]

Wada *et al* in a multivariate analysis of 726 breast cancer patients with stage 0-II, in whom SLNs were successfully identified, revealed that a larger size of the primary tumor (>2.0 cm), presence of lymphatic invasion, larger size of the largest SLN metastasis (>2 mm) and a 100% metastatic rate in the SLNs (number of positive SLNs/number of harvested SLNs) were significantly associated with positive non-SLNs. Even the presence of all of these four factors in combination was insufficient to safely predict omission of ALND.[17]

Investigators at Memorial Sloan-Kettering Cancer Center devised and validated a nomogram for predicting the likelihood of non-SLN metastases. Eight clinicopathologic variables for 200 consecutive breast cancer patients at the University of Texas M. D. Anderson Cancer Center with SLN metastases and CLND were entered into the nomogram. The accuracy of the nomogram to predict non-SLN metastases was assessed by the receiver operating characteristic curve and linear regression analysis. The linear correlation coefficient of the nomogram-predicted probabilities correlated with the observed incidence of non-SLN metastases for all patients. The nomogram may help predict an individual's risk of non-SLN metastases and assist in patient decision making regarding the benefit of CALND.[18] It has been further confirmed by study on 222 patients at a regional teaching hospital in The Netherlands.[19]

Data from validation studies combining sentinel lymphadenectomy and CALND have shown that most of the time the SLN is the only positive node.[5,8]

None of the above studies could reliably predict group of breast cancer patients with positive SLN in which we may possibly omit ALND, based on various factors that might help in predicting the involvement of the non-SLNs. But the question is how to find this patient with only positive sentinel node?

We, in our study, observed that the SLN was the only positive node in 47% of patients with metastatic axilla. In this group of women full axillary dissection is unjustified. For one positive node, we are removing the whole of the lymphnodal group from the axilla. A therapeutic benefit of ALND in patients with metastases has long been hypothesised but has never been proven conclusively. Moreover, we are exposing these women to complications of the nodal dissection. By evaluating "Station II nodes", if we can find axilla with only positive SLN, then we can spare these patients of the morbidity of axillary dissection. There are 19 patients in our study with negative "Station I

node" (SLN) which were doubly confirmed by evaluation of "Station II node", since our concept is based on sequential spread of cancer cells to higher stations (echelons) in an orderly pattern through lymphatic. It is an extrapolation of Sentinel lymph node concept. Upon ALND, it is found that there are 8 patients with no disease in axilla except at SLN. Station II mapping in these patients, could identify 6 patients. These are the only SLN positive patients which may possibly be spared of CALND.

"Station II node" identification is more comprehensive search for nodes for highest risk of involvement. In our study "Station II Nodes" accurately predicted the status of the axilla in 92% of patients in whom we found the "Station II Nodes". There were 3 patients in which "Station I and II nodal status" could not predict the status of axilla accurately, probable explanations for which are (1) we included some patients with N1 disease which lead to blockage of draining lymphatic trunks from the tumor bed and opening of alternative channels for lymph drainage, hence identification of alternate false negative SLN, (2) tumors of larger size (≥T3) could cause overburdening of lymphatic with tumor emboli and lead to false negative SLN.

In one patient [Table 4], 70 year female with T3N1M0 tumor with palpable axillary lymphadenopathy, we found 2 blue lymphatic along lateral border of pectoralis muscle, going towards two separate nodes. We labelled them as "Station Ia node" and "Station Ib node". We did not use isotope injection in this patient. There was some spillage of dye while injecting "Station I nodes" and tissue got stained. In spite of that we were able to trace lymphatic higher nodes and we found a large matted (2.5 x 1.5-cm) partially blue stained node, labelled as "Station II node". Histopathology report showed positive "Station I nodes" (3/3), rest of axilla (5/11) and negative "Station II nodes" (0/1).

In second patient [Table 4] 40 year female, with tumor staged T2 with N1 nodal disease, we found a bunch of four matted lymph nodes, one blue and three associated non blue nodes, after injecting blue dye peritumorally. In this case we could not trace lymphatic for "Station II Nodes". Final report showed negative "Station I Node" (0/1) and negative non blue nodes, sent as level I nodes (0/3) and showed involvement of rest of the axilla (2/10) for metastatic cancer cells.

In third patient [Table 4], 45 year female, staged T3N0M0 for left lower outer quadrant tumor of breast, we used only blue dye for lymphatic mapping. We found one blue node. On injection into it we found three lymphatic going to "Station II Nodes". Histopathology report showed negative "Station I and II Nodes" but positive axilla (1/10). We do not know the reason in this case.

We are of opinion that after some experience will be gained in "Station II Node" mapping, we will be able to find only positive SLN in whole axilla. Then we will think of abandoning ALND in patients with only metastasis to SLN ("Station I nodes").

Presently, our data in early stage tumors of breast cancer, based on "Station II Concept", can not attest to safety of avoiding complete ALND in patients with only metastases to SLN, as number of patients were low to be conclusive and there will be need for randomised controlled trials after the validity of this concept would be proven by large size of study group with low volume disease (T1-2N0). In patients with larger volume of disease, tumor emboli might block the lymphatic leading from tumor to lymph nodes and cause rerouting of the cancer cells and possibly leading to finding of alternate SLNs or 'False Negative SLNs'. This was one of the limitations of our study. In future our focus will be to study this concept on a bigger study group with combined technique in patients with low volume of disease.

Further information on clinical relevance of sentinel lymph node metastasis is likely to gain from data from American College of Surgeons Oncology Group (ACOSOG) study Z0011. This open study randomises patients with low volume disease with positive SLNs to either axillary dissection or observation of axilla.[20,21]

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