“Predictability and Reliability of Sentinel Lymph Node Biopsy in Women with Early Breast Cancer using patent blue dye”

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Abstract:
Introduction: Sentinel lymph node (SLN) is the first draining lymph node. Data regarding sentinel lymph node (SLN) biopsy technique are scarce in India and there are no studies done using patent blue dye. Present study was done to know whether blue dye technique alone can be used to detect SLN with high accuracy so that unnecessary axillary dissection and subsequent complication can be avoided.

Material and methods: A pilot study of 35 patients of histopathologically diagnosed patients of early breast cancer included in our study. Site, amount and quantity of dye injected were noted. The breast tissue along with axillary lymph nodes and sentinel lymph nodes were sent separately for histopathological examination. The presence and absence of metastases in sentinel nodes and axillary nodes were compared.

Results: SLN identification rate in our study is 94.2% with Sensitivity was 100%, Specificity 87.5%, Predictive value of positive test 20%, Predictive value of negative test 100%, false negative rate 0%, false positive rate 12.5%, Overall Accuracy-87.87%. Nearly 30% of patients of Breast Cancer could have been saved of unnecessary axillary dissection if sentinel lymph node biopsy technique is used.

Conclusion: SLN can be used confidently in patients of early breast cancer and axilla can be spared of unnecessary dissection provided frozen section biopsy is available thereby minimizing morbidity associated with MRM.

Introduction:
In early cases of breast cancer modified radical mastectomy (MRM) is widely adopted surgery, where axillary lymph node dissection (ALND) is done irrespective of histologic evidence of metastasis in axillary lymph nodes, where as in women with clinically node negative operable breast cancer, incidence of nodal metastasis is only 30%, and axillary lymph node dissection is associated with significant morbidity. Since ALND is associated with high morbidity, the main objective of present study is to find out whether sentinel lymph node biopsy using blue dye alone could be an alternative to axillary lymph node dissection in operable cases of breast cancer, thus avoiding unnecessary axillary dissection. So our study intends to find out sensitivity, specificity and accuracy of sentinel lymph node biopsy using vital blue dye. Study also aims comparing predictability of sentinel lymph node biopsy with that of standard axillary lymph node clearance and intends to know the limitations of SLN biopsy. Several studies have used different dyes like isosulfan blue dye, methylene blue dye, and technetium 99 dyes singly or in combination. But in our validation study we have used...
patent blue V dye which has been used minimally in SLN biopsy technique.

**Material and Methods:**
Study group consisted of 120 patients presenting to The Breast Clinic, in Department Of Surgery, RabindraNath Tagore Medical College, Udaipur Rajasthan India with histopathological evidence of breast cancer. We included 35 patients of early breast cancer whose axilla were clinically and radiologically negative. Ethical committee clearance for the study and individual patient consent was taken before each procedure. Study period was from from July 2008 to July 2011. Inclusion criteria included patients with early breast cancer who are clinically and radiologically negative axilla i.e patients in stage I and stage II (T1NOMO, T2NOMO and T3NOMO). We also included patients with prior surgical biopsy of tumor and multicentric tumor location, as these were earlier considered putative contraindications and now have been disproved. Exclusion criteria included Carcinoma breast with axillary involvement, Multi focal Disease, Prior axillary surgery, patients with infections or trauma to the lymphatics, Pregnant and Lactating mothers and patients with carcinoma breast on neoadjuvant chemotherapy. Base line investigations included complete blood count, Blood urea, Serum creatinine, FNAC of breast lump, Chest X-ray and Electrocardiogram (ECG) and Urine routine. Patients were staged according to standard tumor lymph node and metastasis (TNM) staging proposed by American Joint commission on cancer (AJCC) cancer staging manual, 6th edition.

**Procedure:**
Under general anesthesia and with aseptic precautions 3-5ml of vital blue dye 2% was injected periareolar region intradermally using hypodermic needle few minutes (5 to 10 minutes) before surgery and incision is taken in anterior axillary crease (Fig 1). Skin subcutaneous tissue dissected and blue stained lymphatics are identified (Fig 2). Tracing of dye along the stained lymphatics identifies Sentinel Lymph node (Fig 3). These nodes along with perilymphatic fat tissue were dissected (Fig 4) and were labeled separately. Then planned procedure Modified Radical Mastectomy (MRM) was performed. The breast tissue along with axillary lymph nodes and sentinel lymph nodes were sent separately for histopathological examination. The presence (Fig 5) and absence (Fig 6) of metastases in sentinel nodes and axillary nodes were compared. Statistical analysis was carried out with 2x2 Contingency Table to know sensitivity, specificity, accuracy and positive predictive value.

**Results :**
Clinicopathological profile of study patients is depicted in Table 1. Among the total of 35 patients studied 28.5 % of patients were in fourth decade of life, 34.2% were in fifth decade, 25.7% patients were in sixth decade and 11.4% of patients belonged to seventh decade. 29/35 patients (82.8%) had tumor T2NOMO (Stage II A), 4/35 patients had T3NOMO (Stage II B) (11.4%) and few patients, 2/35, (5.7%) presented with tumor less than 2 cms. Mean tumor size was 3.5 cms. Upper outer quadrant was most common site (42.8%) of tumor location, followed by Lower outer quadrant (31.4%), Lower inner quadrant (8.5%), Upper inner quadrant (5%) and Central (11.4%). We were able to localize Sentinel lymph node in 33 patients out of 35 patients with detection rate of 94.28 %. We detected 2 SLN in 19 (54.2%), single lymph node in 12 patients (34.2) and 3 lymph nodes in 2 patients (5.7 %). Only in two cases we were unable to identify sentinel lymph nodes. When the amount of dye injected was 3ml sentinel node was not localized in 2 cases (5.7%) but node was easily localized.
when quantity of dye was 4ml as in 8 cases (22.8%) and 5ml as in 25 cases (71.4%) ( Table 2). Thus average quantity of dye needed to localize sentinel node was between 3-5ml. When the status of Axillary lymph nodes was compared to Sentinel lymph nodes it is clear that in 28/33 cases (84.8%) axilla was negative and there were no axillary metastasis ( Table 3). In 5/33 (15.1%) cases sentinel lymph node harboured metastatic tumor though only in one case (1/33, i.e 3%), axillary lymph node beyond sentinel harboured metastasis and in rest (4/5) axilla was negative. There was no single case axillary lymph node was positive with negative sentinel lymph node. In our study Sensitivity was 100%, Specificity 87.5%, Predictive value of positive test 20%, Predictive value of negative test 100%, false negative rate 0%, false positive rate 12.5 %, Overall Accuracy- 87.87%.

Discussion:
The status of axillary lymph node remains the most important predictor of survival in women with invasive breast cancer and the status is used for making treatment decision. Various methods of predicting axillary lymph node status has been described including clinical assessment, radiological and operative procedures. Axillary lymph node dissection was earlier considered to be the gold standard for predicting the axillary lymph node status. Axillary lymph node dissection may be associated with significant morbidity such as post-operative pain in arm, chronic lymphedema of involved arm, neuropathy of arm, seroma formation, painful neuroma formation, frozen shoulder syndrome or local wound complications. Developing techniques that make axillary procedure more conservative and less morbid will improve quality of life of patients. Pain in the arm and lymphoedema are the vestigial remnant of MRM.

No doubt MRM improves quantity of life but at the cost of quality of life. Indian patients differ in their clinicopathological profile the western experience and guidelines cannot be directly extrapolated to the Indian Scenario without addressing the issues pertaining to the patient profile, availability of expertise, training, quality control and cost factors.

In present study we conducted validation study on predictability and reliability of sentinel lymph node using blue dye alone. Among 120 patients who were operated for breast cancer 35 (29.1%) patients were included whose axilla was clinically negative. In our study although number of patients included in the study is small (N= 35) it is comparable to studies done by Krag et al04(N=22) ,Borgstein et al05(N=33), Pijpiers et al06(N=34), Ikeda et al07(N=29), Motta C et al08(N=54), Seenu and Bassi et al09(N=40) from AIIMS, New Delhi .These validation studies done earlier in spite of being small in sample size have well established the role of SLN biopsy.

Majority of patients were of middle age and belonged to fourth decade of life (34.2%).Mean age of presentation was 49.2 years (range 35 to 75 years). Patients with less than 50 years were 22/35(62.8%) and more than 50 years were 13/35(37.1%). Patients presented in our study were having large tumor with majority in T2NOMO and T3NOMO. The distribution of size of tumor (T) in our study is contrastingly different to western studies where majority of patients belong to T1 stage04, 10, 11, 12, 13, 14, 15. However when compared to published Indian studies done by Parmer et al01 at Tata memorial, Mumbai (T2=73%) Somashekar et al16 at Manipal Hospital (T1=13%, T2=83%), Deo et al17 at AIIMS New Delhi (mean tumor size=3 cms), Bassi and Seenu M et al09 from AIIMS New Delhi (T1=0%,T2=87.5%) this distribution appears to appear to be normal considering Indian scenario.
In India majority of patients present with locally advanced disease and positive axillary nodes due to socioeconomic factors, lack of access to quality health care and absence of an effective screening program. Contrary to reported western figures of 70% node negative early breast cancer, only minority of Indian patients are node negative and majority 60 to 70% of breast cancer patients in India present with locally advanced node positive disease. This could possibly explain discrepancy when compare our patients profile with western statistics.

Upper outer quadrant was the most common location of tumor and Sentinel lymph node could be identified in axilla with tumor in any quadrant of breast during operative procedure. We did not look for Internal mammary (IM) sentinel lymph node since <1% of all patients have nodal metastasis isolated to IM nodes and is not recommended. It is also associated with additional morbidity in form of pneumothorax and bleeding.

We used the patent blue dye in our study to identify SLN lymph node. Isosulfan blue is used most commonly as observed in studies and most Asian authors use blue dye (, or patent blue dye or indigo carmine . As patent blue dye is cheap and there are few published studies of SLN biopsy we tried this dye in our study with equally good results.

Various techniques have been described regarding the site of injection before biopsy procedure. Peritumoral, intradermal, subdermal or subareolar routes of injections have been tried with equally good results and comparably low false negative rates. Breast tissue and its overlying skin drains to same few axillary SLNs regardless of the injection site. Among these periareolar site, intradermal injection is favoured by many as it can be injected into subareolar lymphatic plexus of Sappey and drainage is independently of tumor size and requires much lower volume of injection. Periareolar intradermal injection increases identification rates compared to peritumoral injection as demonstrated by studied done by . In our study we used periareolar intradermal technique to inject the dye at 3’o clock, 6’o clock, 9’o clock and 12’o clock positions in all our patients. Our results are similar to other validation studies .

The main purpose of this study was to determine the role of sentinel lymph node biopsy in predicting the axillary lymph node status in patients with early operable breast cancer. The sentinel lymph node biopsy using blue dye has a strong learning curve and has been performed by relatively few surgeons with extensive experience in breast surgery. It is important to understand that presently sentinel lymph node biopsy is still a state of the art procedure only in experienced hands. The American Society of Breast Surgeons recommends that a surgeon must have performed minimum of 20 SLNB procedures on sentinel lymph node before doing SLNB therapeutically which is also endorsed by The American Society of Clinical Onclogy.

In present validation study we detected sentinel lymph node in 32/35 patients using standard patent blue dye. Our identification rate of SLN was 94.5%. It matches with the validation study done by different authors . Our identification rate is better than many authors .

In the present study, we dissected 491 axillary lymph nodes from 35 patients and subjected for histopathological examination for evidence of metastasis. We could identify 56 blue stained sentinel lymph node during the procedure.
during the procedure. The range of SLN detected was
between 1 to 3 with mean identification of 1.6
sentinel lymph node per patient. This finding is in
conjuction with identification rates of several authors
like Giuliano et al\textsuperscript{10}(1.8), Motomura K et al\textsuperscript{26}(1.8),
Cserni et al\textsuperscript{25}(1.3)Cox et al\textsuperscript{20}(1.92), Hill et
al\textsuperscript{21}(2.1),Ikeda et al\textsuperscript{07}(1.95), Albertini et al\textsuperscript{11}(2),
Pijpers et al\textsuperscript{06}(2), Fiemu et al\textsuperscript{14}. However few authors
like Veronessi et al\textsuperscript{13},Winchester DJ et al\textsuperscript{37},Cox et al\textsuperscript{20}
have identified average 3 sentinel lymph nodes per
patient. Even though there is slight discrepancy in
average number of lymph nodes dissected per patient,
majority of validation studies have put forth 2 SLN
per patient which is consistent with our present study
(54.2% patients SLN were 2 with mean of 1.6). Nearly 30% of patients of Breast Cancer could have
been saved of unnecessary axillary dissection if
sentinel lymph node biopsy technique is used.

Our results dictate 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.
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\textsuperscript{38}. It has been suggested that surgeons
should demonstrate an SLN identification rate of
more than or equal to 90% and a false negative rate of
less than 5% before they offer SLNB without
completion axillary dissection\textsuperscript{38}. However, before
SLNB becomes the undisputed standard of care, randomized trials will have to show no difference in
axillary recurrence and overall survival (OAS) between
SLNB alone and SLNB followed by axillary dissection in
patients with negative sentinel node(s).

The results of our study match with various studies
that have been done in the past decade which validate the
reliability of technique. Although in our study 5/33 had
positive SLN and only in one case axilla was involved, even
in these patients there is no need to perform routine axillary
dissection as in 50% of patients, SLN is the only positive
node and they can be managed with radiotherapy\textsuperscript{02}.From the
above comparative study it is clear that SLN biopsy using
blue dye alone has high sensitivity and high specificity with
good positive predictive value. Blue dye along with Tc99m
mapping theoretically increases the accuracy of test but
from various validation studies it is clear that blue dye
 technique alone can be used when Tc99m mapping facility
is not available.

No operative technique is free from complication so is
sentinel lymph node biopsy technique. Technique is
associated with dye related complications like temporary
staining of urine with dye, cutaneous staining of dye and
very rarely allergic reaction. Severe allergic reactions are
noted in 0.06% of patients who receive patent blue dye\textsuperscript{39}. In
our study severe allergic reaction was not seen in single
patients but minor changes like staining of urine with dye
and pigmentation of skin was seen two patients. All these
effects of dye were temporary and disappear within few
days. We aknowledge limitation of our study like we did not
compare patent blue dye with other dyes, did not compare
site of injection with identification rate.

In India where large number of patients belongs to low
socio economic status and majority hospitals could not
afford the cost of sentinel lymph node mapping with Tc99m
dye and gamma camera, SLN biopsy using patent blue dye
technique is cheap reliable cost effective alternative to know

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the axillary status of patients and to decide level of dissection. This technique can provide a women fully functional limb, especially in India where many patients are from rural background and are manual laborers thereby improving their work efficiency.

**Conclusion:** Since our study SLN biopsy technique has high sensitivity high specificity and least false negative rate (0%) we conclude that SLN biopsy using vital blue dye is highly accurate reliable and predictable technique. When done by experienced surgeons it can be used confidently in patients of early breast cancer and axilla can be spared of unnecessary dissection provided frozen section biopsy is available thereby minimizing morbidity associated with MRM. It is too early to interpret and change the practice of treating early breast cancer in our set up but looking at results of our study, sentinel lymph node biopsy procedure seems promising and might become standard care of treating early breast cancer in near future.

**Table 1: Clinicopathological profile characters of patients (N=35)**

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>31-40</th>
<th>10 (28.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41-50</td>
<td>12 (34.2)</td>
</tr>
<tr>
<td></td>
<td>51-60</td>
<td>09 (25.7)</td>
</tr>
<tr>
<td></td>
<td>&gt; 61</td>
<td>04 (11.4)</td>
</tr>
<tr>
<td>Stage (TNM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1NOMO</td>
<td>02 (5.7)</td>
<td></td>
</tr>
<tr>
<td>T2NOMO</td>
<td>29 (82.8)</td>
<td></td>
</tr>
<tr>
<td>T3NOMO</td>
<td>04 (11.4)</td>
<td></td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UOQ</td>
<td>15 (42.8)</td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>11 (31.4)</td>
<td></td>
</tr>
<tr>
<td>UIQ</td>
<td>02 (5)</td>
<td></td>
</tr>
<tr>
<td>LIQ</td>
<td>03 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>04 (11.4)</td>
<td></td>
</tr>
<tr>
<td>SLN Nodes Dissected</td>
<td>1</td>
<td>12 (34.2)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19 (54.2)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>02 (5.7)</td>
</tr>
<tr>
<td></td>
<td>Nil</td>
<td>02 (5.7)</td>
</tr>
<tr>
<td>Dye quantity (ml)</td>
<td>3</td>
<td>02 (5.7)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>08 (22.8)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>25 (71.4)</td>
</tr>
</tbody>
</table>

Conti….
### Table 2: Amount of dye injected and Detection of sentinel node (N = 35)

<table>
<thead>
<tr>
<th>Quantity (ml)</th>
<th>No (%)</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2 (5.7)</td>
<td>NO</td>
</tr>
<tr>
<td>4</td>
<td>8 (22.8)</td>
<td>YES</td>
</tr>
<tr>
<td>5</td>
<td>25 (71.4)</td>
<td>YES</td>
</tr>
</tbody>
</table>

### Table 3: Status of Sentinel (SLN) and Axillary lymph nodes (ALN)

<table>
<thead>
<tr>
<th>SLN</th>
<th>ALN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>05</td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
</tr>
</tbody>
</table>
Fig. 1: Technique of Sentinel Lymph Node Biopsy - Intra-dermal subareolar injection of dye. Arrow mark showing location of tumor from where true cut biopsy was taken.

Fig. 2: Tracing of dye along with stained lymphatics identifies Sentinel Lymph node

Fig. 3: Blue Sentinel Lymph Node dissected during operative procedure

Fig. 4: Sentinel lymph node along with perilymphatic fat tissue isolated

Fig. 5: Slide showing nests of Malignant cells in lymph node (SLN positive)
References:


33. Guidelines from the American Society of Breast Surgeons available online at www.breastsurgeons.org/officialstmts/sentinel.html.


