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Low-Cost, Isotope-Free Sentinel Node Mapping with Methylene Blue and Fluorescein in Patients with Early Breast Cancer: Experience from an Indian Tertiary Care Centre

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Abstract: ***Purpose**: Sentinel lymph node biopsy (SLNB) is out of reach in many Indian hospitals, which have neither a radioisotope facility nor a near-infrared camera. We describe our experience with an inexpensive method that needs neither: methylene blue and fluorescein sodium injected together. **Methods**: Forty women with breast cancer and clinically normal axillary nodes were studied. After general anaesthesia was induced, 2 mL of 1% methylene blue and 2 mL of 20% fluorescein sodium were injected around the areola. The surgeon searched for nodes by sight and under a simple ultraviolet lamp. We noted how often a node was found, what each dye contributed, how long each took to show, whether results differed between the first and second halves of the series, how many patients needed axillary clearance, complications, and the surgeon's rating of ease. **Results**: A sentinel node was found in 36 of 40 patients (90.0%; 95% CI 76.9–96.0%). Fluorescein located a node in 35 of these 36 patients and methylene blue in 25. The success rate was 18/20 in both the first and the last 20 patients. Methylene blue became visible at a mean of 24.8 minutes and fluorescein at 33.8 minutes. Thirty-two patients (80.0%) were spared axillary clearance. One patient had minor skin necrosis. Surgeons scored ease of use at 4.05 ± 0.50 out of 5. **Conclusion**: Two cheap dyes and a simple lamp were enough to find the sentinel node in nine of ten patients, and the technique was easy to take up. It is a realistic choice for centres without nuclear medicine or near-infrared imaging. Cost analysis and a head-to-head comparison with the standard method are still required.*

Keywords: Sentinel lymph node biopsy · Breast cancer · Methylene blue · Fluorescein sodium · Low-cost technique, India

1. Introduction

Carcinoma of the breast is now the most frequent cancer among women in India. In 2022 the country recorded an estimated 192,020 new cases and 98,337 deaths [1, 2]. When the axilla feels normal on examination, sentinel lymph node biopsy (SLNB) is preferred over complete axillary lymph node dissection (ALND). Large trials found that cancer outcomes were comparable, while the arm suffered far less [3–5].

The most established way to find the sentinel node combines a radioactive tracer with a blue dye. It depends on a nuclear medicine department, which most district hospitals and many government teaching hospitals in India lack [6]. Indocyanine green (ICG) avoids radiation and performs well, but it requires a near-infrared camera [7]. For this reason, a number of Indian women still undergo a full axillary clearance simply because the better test cannot be done where they are treated.

Fluorescein sodium costs little and glows under an ordinary ultraviolet lamp [8]. Methylene blue is a cheap blue dye. When the two are given together, the surgeon can look for the node in two ways: as a stain in normal light and as a glow under ultraviolet light. Indian studies of this combination are promising, but few centres have reported [9–11]. Most of them concentrate on accuracy. Much less has been said about everyday practice: how much each dye adds, how long the surgeon must wait, how quickly the method can be learned, and how many women avoid a full clearance.

This paper describes our practical experience with this isotope-free, two-dye method in breast cancer with clinically normal axillary nodes.

2. Materials and Methods

Design and setting: We carried out a prospective single-arm study in the Department of Surgical Oncology of a tertiary care centre in India. The Institutional Ethics Committee gave approval, and every patient signed a written consent form. The same patients are also reported in a separate paper that deals with the primary feasibility result and the neoadjuvant chemotherapy subgroup. The present paper concentrates on practical and implementation questions.

Patients: Forty women aged 18 years or above were included. All had biopsy-proven breast carcinoma and clinically normal axillary nodes. The axilla was assessed by ultrasonography, and any suspicious node was sampled by fine-needle aspiration. Tumours were T1 or T2, or operable T3 with no palpable nodes. Nine patients received neoadjuvant chemotherapy for reasons related to the tumour and stayed node-negative throughout.

Sample size: A meta-analysis of fluorescein-guided SLNB gave a pooled identification rate of 93.2% [12]. Using this figure, with 95% confidence and a precision of 8 percentage points, at least 38 patients were required. We enrolled 40.

Technique: We did not use a radioisotope, a gamma probe or a near-infrared camera. With the patient under general anaesthesia, 2 mL of 1% methylene blue and 2 mL of 20% fluorescein sodium were injected around the areola. The incision was made only after at least 10 minutes had passed for methylene blue and 20 minutes for fluorescein. Any axillary node that stained blue, glowed under the ultraviolet lamp, or did both, and that had a visible lymph channel leading to it, was taken as a sentinel node. Each node was labelled as blue only, fluorescent only or both. A completion ALND was done when a sentinel node contained tumour or when no node could be found. Nodal status was reported by the AJCC pN system.

Outcomes: The primary outcome was the sentinel node identification rate. We also measured what each dye contributed (by patient and by node), the interval from injection to visibility, the identification rate in the first 20 and the last 20 patients, the number needing ALND, the type of breast operation, complications, and the surgeon's score for ease of use on a scale of 1 to 5.

Statistics: Counts are given with percentages, and measurements as mean ± standard deviation or range. The identification rate carries a 95% confidence interval (Wilson method). Proportions were compared with Fisher's exact test, and the two dye times with a paired t-test. A p-value under 0.05 was taken as significant.

3. Results

The mean age of the 40 patients was 45.7 ± 9.0 years (Table 1). Breast conservation was done in 38 (95.0%) and mastectomy in 2 (5.0%).

Table 1: Baseline characteristics of the 40 patients

Characteristic	Value (n = 40)
Age, years (mean ± SD)	45.7 ± 9.0 (range 27–68)
Left : right breast	22 (55.0%) : 18 (45.0%)
Clinical T1	10 (25.0%)
Clinical T2	20 (50.0%)
Clinical T3	8 (20.0%)
Clinical T0 / T4b	1 (2.5%) each
Neoadjuvant chemotherapy (node-negative throughout)	9 (22.5%)
Breast conservation : mastectomy	38 (95.0%) : 2 (5.0%)

Identification rate: A sentinel node was found in 36 of 40 patients (90.0%; 95% CI 76.9–96.0%). Altogether 148 nodes were removed from these 36 patients, a mean of 4.1 ± 1.4 per patient (range 2–8).

What each dye contributed: Fluorescein found a node in 35 of the 36 patients (97.2%), and methylene blue in 25 (69.4%). Both dyes were successful in 24 patients, fluorescein alone in 11 and methylene blue alone in 1 (Fig. 1). Counting nodes, fluorescein marked 121 of 148 (81.8%) and methylene blue 57 (38.5%); 30 nodes (20.3%) took up both dyes.

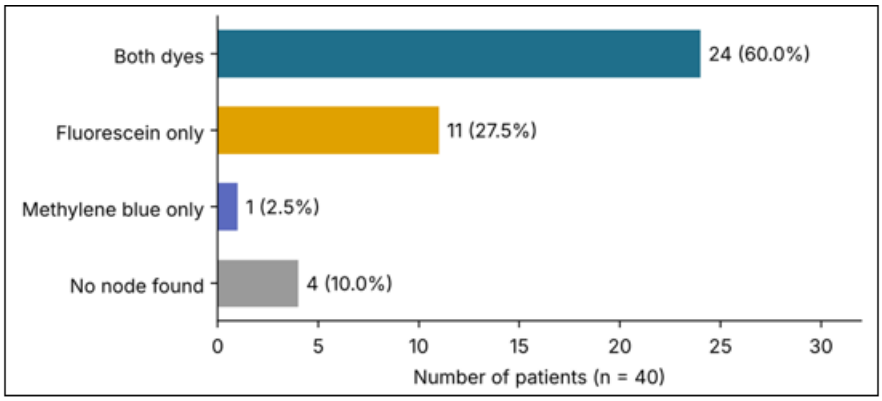


Figure 1: Patient-wise contribution of each dye to sentinel node identification (n = 40)

Time to visibility: Methylene blue marked the node after a mean of 24.8 ± 4.0 minutes (range 15–30; n = 25) and fluorescein after 33.8 ± 4.5 minutes (range 25–40; n = 35). The blue dye was quicker ($p < 0.001$, paired t-test).

First and second halves of the series: A node was found in 18 of the first 20 patients (90.0%) and in 18 of the last 20 (90.0%) ($p = 1.000$). The mean number of nodes removed was 4.1 in each half.

Axillary surgery: Of the 36 patients with a mapped node, 4 (11.1%) had tumour in a sentinel node. Completion ALND was performed in 8 patients (20.0%): 4 for a positive node and 4 because no node was found. The remaining 32 patients (80.0%) were spared axillary clearance. A false-negative rate could not be worked out, as no patient with a negative sentinel node had completion dissection.

Safety and ease of use: One patient (2.5%) had minor skin necrosis at the methylene blue injection site, and it healed with simple dressings. No patient had an allergic reaction, anaphylaxis or any other wound problem. Surgeons scored ease of use at 4.05 ± 0.50 out of 5, and identification was rated good in 39 of 40 patients.

Table 2: Practical outcomes of the isotope-free dual-dye method

Outcome	Result
Sentinel node identified	36/40 (90.0%; 95% CI 76.9–96.0%)
Patients with a node found by fluorescein	35/36 (97.2%)
Patients with a node found by methylene blue	25/36 (69.4%)
Time to visualisation, methylene blue	24.8 ± 4.0 min (15–30)
Time to visualisation, fluorescein	33.8 ± 4.5 min (25–40)
Identification, first 20 vs last 20 patients	18/20 vs 18/20 (p = 1.000)
Patients not needing ALND	32/40 (80.0%)
Breast conservation	38/40 (95.0%)
Complications	1/40 (2.5%), minor
Surgeon-rated ease of use (1–5)	4.05 ± 0.50

4. Discussion

Using two inexpensive dyes and a basic ultraviolet lamp, we found the sentinel node in 90.0% of our patients. This is near the pooled 93.2% reported for fluorescein-guided SLNB [12] and near the figures from Indian groups using the same pair [9–11]. It is a little lower than the roughly 96% usually quoted for radiocolloid with blue dye [13] and the 95–99.8% for ICG [14]. Reaching this level without any radioactive material or near-infrared equipment is the central practical message.

The two dyes were not equal partners. Fluorescein found a node in nearly every patient who mapped, and in 11 patients it worked when methylene blue did not. Methylene blue worked alone in just one. A comparable pattern has been seen when ICG is combined with blue dye [15]. A Chinese study of fluorescein with methylene blue found a node in 92.9% of patients and no serious reactions [16]. In our hands, fluorescein did most of the work, while the blue dye served as a visible back-up and a guide during the wait for fluorescein to appear. A unit able to afford only one dye would probably do best to choose fluorescein, though we did not test that.

The timings help at the operating table. Methylene blue became visible between 15 and 30 minutes and fluorescein between 25 and 40 minutes. A surgeon who sees no blue node early should therefore keep waiting, and should look under the lamp before concluding that mapping has failed.

Results did not differ between the first and second halves of our series (18/20 each). This hints that the method is easy to take up, and agrees with the high ease-of-use score. Even so, there were only four failures, so a true learning curve cannot be judged from 40 cases. Earlier work suggests that about 20 to 30 procedures are needed before results settle [17].

We removed about four nodes per patient, which is rather high. Some glowing nodes may lie one step beyond the true sentinel node. Removing extra nodes is probably harmless and may reduce the chance of missing disease, but it is not clear that fluorescein picks out first-level nodes alone. Nine of our patients had chemotherapy before surgery while staying node-negative. They differ from patients whose nodes turn negative after chemotherapy, the situation that has been studied more often [18–20], and they are described in the separate paper.

One woman developed minor skin necrosis after methylene blue. Skin injury is a recognised but uncommon effect of this dye [21]. The case is a reminder to inject at the right depth and in the right volume. Nothing in our series pointed to a problem from fluorescein.

The chief gain from this approach is access. Eight in ten patients avoided axillary clearance, and 95% kept the breast. Sparing routine dissection protects patients from arm swelling and shoulder stiffness [22]. A randomised trial from an Indian centre judged fluorescein with methylene blue to be more cost-effective than technetium-guided SLNB, with similar detection [9]. We did not measure costs ourselves. Still, the method needs only two easily available dyes, an ultraviolet lamp and the usual theatre instruments. It requires no nuclear medicine team, no radiation safety arrangements and no special licence.

Limitations: This was a single-centre study with a small sample and no comparison group. Because patients with a negative sentinel node did not undergo completion dissection, the false-negative rate is unknown. The comparison of early and later cases is crude and has little power. No formal cost analysis was done. The same patients are also reported in a separate paper covering the primary feasibility result.

5. Conclusion

Methylene blue and fluorescein sodium together offer a simple, cheap and isotope-free route to sentinel lymph node biopsy in breast cancer with clinically normal axillary nodes. A node was found in 90.0% of patients, the method was easy to adopt, most patients avoided a full axillary clearance, and only one minor complication occurred. Fluorescein contributed most of the nodes, and methylene blue appeared sooner. Centres without nuclear medicine or near-infrared imaging can consider this approach. Larger multicentre studies, with a formal cost analysis and a direct comparison with the standard method, are needed.

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Declarations

Ethics approval: This study was approved by the Institutional Ethics Committee of the study centre (Ref. No. 670/MC/EC/2026, dated 29/07/2026). It followed the principles of the Declaration of Helsinki.

Consent to participate: Written informed consent was obtained from all participants.

Consent to publish: Written informed consent for publication was obtained. No identifying patient information or images are included.

Competing interests: The authors declare that they have no competing interests.

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