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Original Research Article

Methylene blue dye as a single tracer for sentinel lymph node biopsy in early breast cancer

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ABSTRACT

Background: Sentinel lymph node biopsy (SLNB) can reduce the morbidity of axillary lymph node dissection (ALND) in clinically node-negative breast cancer, but dual-tracer SLNB requires nuclear medicine infrastructure that is unavailable in many Indian hospitals. We evaluated 1% methylene blue dye (MBD) as a single tracer for SLNB in a resource-constrained setting.
Methods: In this prospective observational study, 30 women with clinically node-negative, early-stage breast cancer (T1-T2, N0, M0) underwent periareolar injection of 3 ml of 1% MBD during modified radical mastectomy. Sentinel lymph node(s) were identified and harvested separately during axillary dissection, followed by complete axillary lymph node dissection in all patients during our unit's learning phase for histopathological validation.
Results: A sentinel node was identified in 29 of 30 patients (96.67%). Among these 29 patients, sensitivity was 88.89%, specificity 95.00%, positive predictive value 88.89%, negative predictive value 95.00%, and overall accuracy 93.10%. The false-negative rate was 11.11%. No anaphylaxis, skin necrosis, allergic reaction, or wound complication occurred. Three patients (10.0%) developed transient skin tattooing that resolved spontaneously.
Conclusions: MBD-only SLNB achieved a high identification rate and diagnostic accuracy during the learning phase, with no major adverse events. It may provide a pragmatic, low-cost alternative for axillary staging where radioisotope-based SLNB is unavailable. Larger multicentre studies are required before ALND can be safely omitted after a negative MBD-only SLNB.

Keywords: Sentinel lymph node biopsy, Methylene blue dye, Breast cancer, Axillary lymph node dissection, Resource-constrained settings

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer among Indian women, with an age-adjusted incidence of 25.8 per 100,000 and a rising trend attributable in part to improved detection.[1] Axillary staging remains central to prognosis and treatment planning, because axillary nodal metastasis is the single strongest prognostic factor in early breast cancer. For decades, complete axillary lymph node dissection (ALND) was performed in every patient irrespective of nodal status, at the cost of substantial morbidity, including lymphoedema in up to 30% of patients, axillary web syndrome, chronic pain, and restricted shoulder movement.[2]

Sentinel lymph node biopsy (SLNB) emerged as a minimally invasive alternative, built on the principle that the first node or nodes draining a tumour predict the status of the remaining nodal basin.[3,4] Combining a radioisotope tracer with a blue dye - the dual-tracer technique - is now regarded as the reference standard, achieving identification rates above 95% and false-negative rates below 5% in experienced hands.[5] That standard, however, depends on nuclear medicine infrastructure - gamma

cameras, gamma probes, radiation safety protocols, and trained physicists - that is confined in India to a handful of dedicated cancer institutes. Most government teaching hospitals still perform ALND in every operable patient by default, imposing avoidable morbidity on women who are ultimately found to be node-negative.[6]

Methylene blue dye (MBD) offers a practical way around this bottleneck. It is inexpensive, already stocked in most operating theatres, needs no specialised equipment, and stains lymphatic channels and nodes a visible blue within minutes of injection. Its reported anaphylaxis rate of 0-1% also compares favourably with the 1-3% rates reported for patent blue dye and isosulfan blue.[7] Several international series have evaluated MBD as a standalone tracer, reporting identification rates of 85-97% and sensitivities of 87-94%, and a systematic review and meta-analysis has since corroborated these figures across pooled data.[8-14] Indian data, and particularly data from Rajasthan, remain scarce.

We therefore conducted a prospective observational study at SMS Hospital, Jaipur, to determine the sentinel node identification rate, diagnostic accuracy, and safety profile of 1% MBD used as a single tracer for SLNB in women with early-stage, clinically node-negative breast cancer. Concurrent complete axillary lymph node dissection was used as the histopathological reference standard during our unit's learning phase.

METHODS

Study design, setting, and period

This was a hospital-based, prospective observational study conducted in the department of general surgery, S. M. S. Medical College and attached Hospitals, Jaipur, a tertiary referral and teaching centre in Rajasthan, India. Patients were recruited between October 2024 and July 2025, following approval by the institutional ethics review committee.

Sample size

Sample size was calculated using the formula at a 95% confidence level (Z=1.96), assuming a sentinel node identification rate of 94.74% based on prior published data (p=0.9474, q=0.0526), and an acceptable absolute error of 8%.

$$n = Z^2pq/e^2$$

This yielded a sample size of 30 patients.

Eligibility criteria

Women aged 18 years or older with histologically confirmed, clinically node-negative breast carcinoma (T1-T2, N0, M0) who provided written informed consent were eligible. Patients were excluded if they had prior axillary surgery or radiotherapy, a clinically node-positive or metastatic axilla (stage III-IV disease), a known allergy to methylene blue dye, or inflammatory breast carcinoma. Male patients were excluded, as the study was restricted to female patients with breast cancer.

Pre-operative work-up

All patients underwent clinical breast and axillary examination, routine haematological and biochemical investigations, chest radiography, ultrasonography of the bilateral breasts, axillae, and abdomen, mammography where indicated, and core needle biopsy or fine-needle aspiration cytology for histological confirmation. An intradermal test dose of 0.1 ml was administered 24 hours before surgery to screen for methylene blue hypersensitivity.

Operative technique

Under general anaesthesia, 3 ml of 1% methylene blue dye was injected into the periareolar tissue. After a dwell time of 5–10 minutes, modified radical mastectomy was performed. During axillary dissection, the blue-stained lymphatic channels were traced and the sentinel lymph node(s) were identified and harvested separately before completion of the axillary dissection. Complete axillary lymph node dissection involving levels I, II and III was subsequently performed, and the remaining axillary lymph nodes were removed as part of the MRM. Sentinel lymph node specimens and the remaining axillary lymph node specimen were submitted separately for histopathological examination. As this represented the learning phase of SLNB for our unit, concurrent complete axillary lymph node dissection was performed in all patients and served as the histopathological reference standard for calculating sensitivity, specificity, and false-negative rate.

Histopathological examination

All sentinel lymph node specimens and the remaining axillary lymph node specimen obtained during complete axillary lymph node dissection were submitted separately and examined by an experienced pathologist. Sections were stained with haematoxylin and eosin. Nodal deposits were classified as macrometastasis (>2 mm), micrometastasis (0.2-2 mm), or isolated tumour cells (<0.2 mm or <200 cells).

Outcome measures and statistical analysis

The primary outcomes were the sentinel node identification rate and the diagnostic accuracy of MBD-only SLNB, expressed as sensitivity, specificity, positive and negative predictive value, and false-negative rate, calculated against ALND histopathology using a standard 2×2 contingency table with 95% confidence intervals. Secondary outcomes were the number of sentinel nodes retrieved, the type of nodal metastasis, and adverse effects of MBD injection. Data were entered in Microsoft Excel

and analysed using statistical package for the social sciences (SPSS) version 25.0 (IBM Corp, Armonk, NY). Categorical variables are reported as frequencies and percentages; continuous variables are reported as mean ± standard deviation or median (range) as appropriate.

Ethical considerations

The study protocol was approved by the Institutional Ethics Review Committee of S. M. S. Medical College and Hospital, Jaipur. Written informed consent was obtained from every participant in her preferred language after the objectives, risks, benefits, and voluntary nature of participation had been explained. Patient confidentiality was maintained throughout, and all procedures conformed to the 2013 revision of the Declaration of Helsinki.

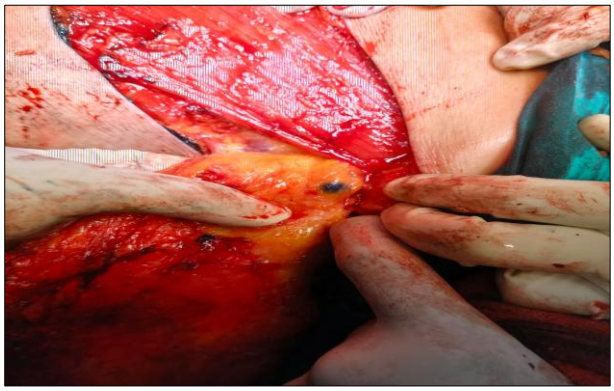

Figure 1: Intraoperative photograph showing a methylene blue-stained sentinel lymph node identified in the axilla after periareolar injection of 1% methylene blue dye.

RESULTS

Thirty women with clinically node-negative, early-stage breast cancer underwent modified radical mastectomy with 1% methylene blue dye-guided sentinel lymph node identification, followed by complete axillary lymph node dissection. Mean age was 44.6±10.2 years (median 43, range 22-68); the largest age group was 41-50 years (36.67%), consistent with the younger presentation typically reported in Indian breast cancer cohorts. Baseline clinicopathological characteristics are summarised in Table 1.

All 30 patients had clinically N0, M0 disease. Twenty-five (83.3%) had T2 tumours and five (16.7%) had T1 tumours. Invasive ductal carcinoma of no special type accounted for 29 of 30 cases (96.67%), and grade III histology predominated (60.0%), reflecting the relatively advanced pathological features seen despite early clinical staging in this population.

A sentinel node was identified in 29 of 30 patients, an identification rate of 96.67% (95% CI 82.8-99.9%; Table 3). The single non-identification was not linked to any deviation from the standard injection technique and most likely reflected patient-level anatomical or technical factors previously shown to affect MBD uptake. The median number of sentinel nodes retrieved per patient was 3 (range 1-7). Nine of the 29 identified sentinel nodes (31.0%) were positive for metastasis, of which three (33.3%) showed macrometastasis and six (66.7%) showed micrometastasis. The diagnostic contingency table is shown in Table 2, and the overall diagnostic performance is summarised in Table 3.

Table 1: Baseline clinicopathological characteristics of study patients (n=30).

Characteristics	Category	Value/N (%)
Age (years)	Mean±SD	44.6±10.2
	Median (range)	43 (22-68)
	41-50 years (largest group)	11 (36.67)
Clinical tumour stage	T1	05 (16.7)
	T2	25 (83.3)
Clinical nodal/ metastatic status	N0, M0	30 (100)
Histology	Invasive ductal carcinoma, no special type	29 (96.67)
	Other histological type	01 (3.33)
Histological grade	Grade III	18 (60.0)
	Grade I/II	12 (40.0)

Table 2: Diagnostic performance of MBD-only SLNB against ALND histopathology (n=29 identified).

Sentinel node status	ALND positive	ALND negative	Total
SLN positive	08 (TP)	01 (FP)	09
SLN negative	01 (FN)	19 (TN)	20
Total	09	20	29

Table 3: Summary diagnostic accuracy parameters.

Parameters	Value (%)	95% CI (%)
Identification rate	96.67	82.8-99.9
Sensitivity	88.89	51.8-99.7
Specificity	95.00	75.1-99.9
Positive predictive value	88.89	51.8-99.7
Negative predictive value	95.00	75.1-99.9
Overall accuracy	93.10	77.2-99.2
False-negative rate	11.11	0.3-48.2

Using histopathological findings from the complete axillary lymph node dissection as the reference standard, MBD-only SLNB correctly classified 27 of 29 patients

(93.10% overall accuracy), with a sensitivity of 88.89%, specificity of 95.00%, positive predictive value of 88.89%, and negative predictive value of 95.00% (Table 3). One false-positive and one false-negative result were recorded, giving a false-negative rate of 11.11%.

No anaphylaxis, allergic reaction, skin necrosis, or wound complication occurred in any patient (Table 4). Three patients (10.0%) developed temporary skin tattooing at the injection site (Figure 2), which resolved completely within three months without further intervention.

Table 4: Adverse effects of methylene blue dye injection (n=30).

Adverse effect	N (%)
Anaphylaxis	0 (0.0)
Allergic reaction	0 (0.0)
Skin necrosis	0 (0.0)
Wound complication	0 (0.0)
Transient skin tattooing (resolved spontaneously)	3 (10.0)
No adverse effect	27 (90.0)

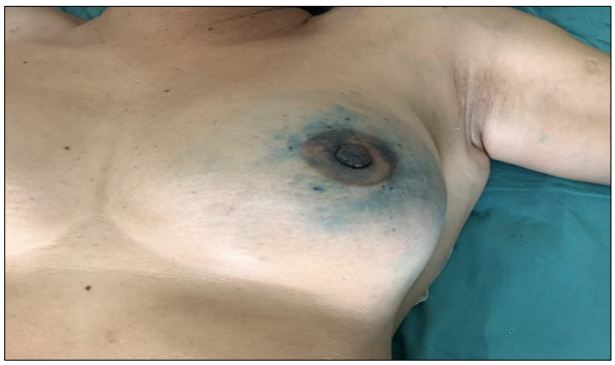

Figure 2: Periareolar skin staining ("tattooing") following intradermal and periareolar injection of 1% methylene blue dye, a transient adverse effect that resolved spontaneously without intervention.

DISCUSSION

This prospective study set out to determine whether 1% methylene blue dye, used without a radioisotope, could deliver sentinel node identification and diagnostic accuracy adequate for routine axillary staging in a resource-constrained Indian tertiary centre. The identification rate of 96.67% sits at the upper end of the range reported for MBD-only SLNB internationally, and closely matches the index study on which our protocol was based, Hermansyah et al, who reported an identification rate of 97.6% using the same 1% concentration and periareolar technique.[15] It also compares favourably with the pooled identification rate of 90-92% reported in a systematic review and meta-analysis of MBD-only SLNB.[14]

Diagnostic accuracy findings were similarly consistent with the published literature. Sensitivity of 88.89% and specificity of 95.00% in the present series are comparable to those reported by Hermansyah et al (91.67% and 96.67%), Brahma et al (92% and 100%), and Golshan and Nakhlis (91.6% and 96.6%).[12,13,15] Our false-negative rate of 11.11% was modestly higher than the sub-10% threshold generally considered acceptable for SLNB, which is not unexpected: this cohort represents our unit's learning phase, and false-negative rates are known to fall as case volume and surgeon experience increase.[16]

The clinicopathological profile of our patients mirrors patterns described elsewhere in India and South Asia - a mean age roughly a decade younger than typical Western cohorts, and a predominance of grade III, T2 disease despite clinically negative nodes. This underscores why accurate, accessible axillary staging matters particularly in this population. Safety findings were reassuring: no anaphylaxis, skin necrosis, or wound complications occurred, and the only adverse effect, transient skin tattooing in three patients, resolved spontaneously. This safety profile is materially better than the 1-3% anaphylaxis rate reported for patent blue dye and isosulfan blue, and supports MBD as a favourable choice where facilities for managing anaphylaxis intraoperatively may be limited.[7]

Taken together, these results support the practical case for MBD-only SLNB in settings that default to ALND because dual-tracer infrastructure is unavailable. Radioisotope-based SLNB requires gamma probe procurement, radiopharmaceutical licensing, and a radiation safety officer - costs that are out of reach for most government hospitals. 1% MBD, by contrast, costs a fraction of this per procedure and needs no additional infrastructure. If validated in larger series, MBD-only SLNB could extend accurate axillary staging, and sparing of unnecessary ALND in node-negative women, well beyond India's handful of specialist cancer centres.

This study has several limitations that should temper interpretation of its findings. The sample size of 30 patients, while adequate for the pre-specified calculation, limits statistical precision; the wide confidence intervals around sensitivity and false-negative rate in particular should be read with caution. This was a single-centre, single-surgical-team study without a concurrent dual-tracer comparison arm, so we cannot directly confirm equivalence to the reference standard within our own data - our comparisons rely instead on historical benchmarks from the published literature. Because this cohort represented a deliberate learning-phase design, in which complete axillary lymph node dissection was performed in every patient after identification and separate harvesting of the sentinel lymph node(s), regardless of sentinel node status, the false-negative rate reported here should be regarded as an upper bound that may improve with further experience, rather than a fixed property of the technique. Long-term oncological outcomes, including regional

recurrence and survival, were not assessed and would require considerably longer follow-up. Larger, multicentre, and where feasible randomised studies, ideally incorporating a direct dual-tracer comparison arm, are needed before ALND can be safely omitted after a negative MBD-only sentinel node biopsy in this population.

CONCLUSION

In this prospective series from a north Indian tertiary centre, 1% methylene blue dye used as a single tracer identified the sentinel node in 96.67% of patients and achieved diagnostic accuracy comparable to published dual-tracer benchmarks, with an excellent safety profile and no major adverse events. These findings, generated during our unit's learning phase, support MBD-only SLNB as a pragmatic, low-cost strategy for extending accurate axillary staging to hospitals that currently rely on routine ALND because dual-tracer infrastructure is unavailable. Confirmation in larger, multicentre studies is needed before this approach can be recommended as a substitute for ALND outside a validated learning protocol.

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