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Review Article

# Using indocyanine green and methylene blue for axillary reverse mapping in modified radical mastectomy for breast cancer

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MRMModified Radical Mastectomy
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MBMethylene Blue
ALNDAxillary Lymph Node Dissection
SLNB: Sentinel Lymph Node Biopsy

ABSTRACT

**Introduction:** Axillary Reverse Mapping (ARM) is a method used to identify the lymph nodes (known as ARM nodes) and lymphatic vessels of the upper limb that pass through the axillary region during breast cancer surgery. It is also known as DUAL mapping or SPLIT mapping. The main goal of ARM is to decrease the occurrence of arm lymphedema. This is based on the hypothesis that the ARM procedure reveals an alternative lymphatic pathway originating from the arm.
To assess the rates of intraoperative identification and metastasis of ARM nodes.
**Results:** The ARM node identification rate was 83.33%. In our study, the visualization rate in the methylene blue plus ICG group was significantly higher at 95.8% (23 out of 24) compared to 75% (18 out of 24) in the methylene blue alone group.
**Conclusion:** Using methylene blue combined with ICG for ARM achieves a higher identification rate of ARM nodes compared to methylene blue alone, making it a promising technique for surgical treatment in patients with invasive breast cancer.



## 1. Introduction

The advancement of novel indicators for oncogene activity demonstrated potential in informing treatment approaches for breast cancer. The status of axillary lymph nodes plays a pivotal role in determining treatment strategies and remains a key prognostic indicator for patient outcomes.[1] Currently, axillary lymph node dissection (ALND) is the standard treatment for patients with metastatic axillary lymph nodes. However, ALND is associated with significant complications, including upper extremity lymphedema (ranging from 7% to 77%)[Figures 1 and 2], arm/shoulder dysfunction, paresthesia, and discomfort.[2] For patients with clinically node-negative axilla, sentinel lymph node biopsy (SLNB) is recommended, which significantly reduces complications compared to ALND. Despite this, SLNB alone has been shown to cause lymphedema in 7% of cases.[3]

In 2007, Thompson first described axillary reverse mapping (ARM), also referred to as SPLIT mapping or dual mapping technique, to identify the lymph nodes (termed ARM nodes) and lymphatic vessels of the upper limb that traverse the axillary region during axillary surgery in breast cancer patients.[4] This technique involves injecting a tracer into the upper arm just before surgery to identify the ARM nodes, which is similar to the process used for the sentinel lymph node (SLN) that drains the tumor. The primary objective of ARM is to reduce the occurrence of arm lymphedema by identifying and preserving an alternative lymphatic pathway from the arm.

During ALND or SLNB, the ARM procedure can be utilized to decrease the incidence of upper extremity lymphedema by maintaining the lymphatic drainage of the arm. ARM has been performed using three visualization

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methods: radioisotopes, fluorescence dye, and blue dye. The most commonly used method is blue dye, while the use of fluorescent indocyanine green (ICG) dye has been reported in only five studies.[5–9] In this study, we will evaluate the effect of the combined use of blue dye and ICG during modified radical mastectomy (MRM) in breast cancer patients.

Aim of the study was to evaluate the impact of using a combination of fluorescence dye and methylene blue during axillary reverse mapping (ARM) and to assess the feasibility of implementing ARM in breast cancer patients undergoing modified radical mastectomy (MRM).

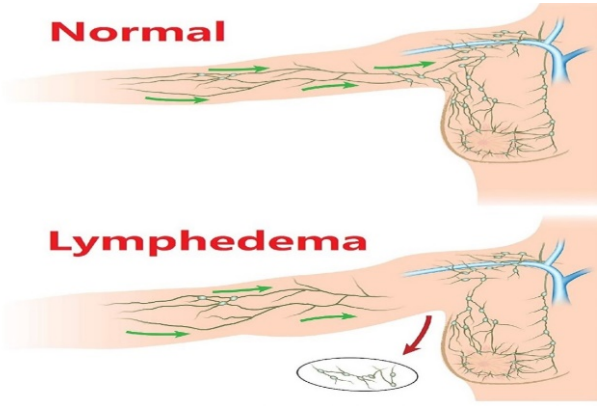


**Figure 1:** Development of lymphedema

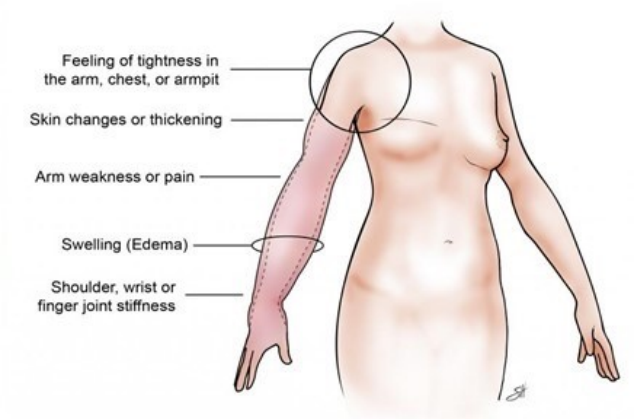


**Figure 2:** Clinical features of lymphedema

### *1.1. Clinico-pathological characteristics of patients*

The clinico-pathological profile of the patients selected in both the groups are shown in the following Table 1.

## 2. Review Literature

The study included 48 patients, divided equally into two groups: the Control group (24 patients) received only methylene blue, while the Experimental group (24 patients) received a combination of indocyanine green (ICG) and methylene blue. This cohort study was conducted from January 2022 to March 2023, enrolling a total of 48 patients who underwent modified radical mastectomy (MRM). These patients were diagnosed with primary breast cancer at clinical stages I-IV (Tis-T3, N0-N3, and M0) at VIMSAR, Burla. Patients were excluded if they had received preoperative radiotherapy or chemotherapy, had bilateral disease, were allergic to methylene blue or indocyanine green (ICG) dye, or had previously undergone axillary dissection. The median age of the study cohort was 55 years, ranging from 40 to 70 years. Informed consent was obtained from each patient prior to surgery.

**Table 1:** Clinico-pathological characteristics of patients taken in the study

| Characteristics | Methylene blue | Methylene blue + ICG |
|---|---|---|
| Median age | 58 (43-68) | 52 (40-70) |
| Her 2: Negative | 14 | 16 |
| Her 2 Positive | 10 | 8 |
| Histological type: Invasive ductal | 12 | 15 |
| Invasive lobular | 8 | 6 |
| Mix (ductal + lobular) | 4 | 3 |
| ER: Positive | 15 | 13 |
| Negative | 9 | 11 |
| PR: Positive | 17 | 12 |
| Negative | 7 | 12 |
| Clinical T stage: T1 | 10 | 11 |
| T2 | 10 | 12 |
| T3 | 2 | 1 |
| Clinical N stage: N0 | 12 | 16 |
| N1 | 10 | 7 |
| N2 | 1 | 1 |
| N3 | 1 | 0 |

For the Control group, 2 mL of methylene blue mixed with 2 mL of normal saline (NS) was injected subcutaneously into the upper inner arm, 4-5 cm from the axillary crease. After injection, the arm was raised for 10 minutes following a 5–6-minute massage of the injection site. In the Experimental group, 2 mL of methylene blue and 5 mg of ICG were injected using the same method[Figure 3]. This injection site was chosen to ensure any permanent tattooing caused by the dye would remain hidden.

The dye was injected prior to dissecting the clavipectoral fascia, either in a single prick or multi-prick fashion. In overlay mode, the procedure aimed to detect any dye leakage. The absence of leakage indicated that no upper limb lymphatics were injured during the procedure.

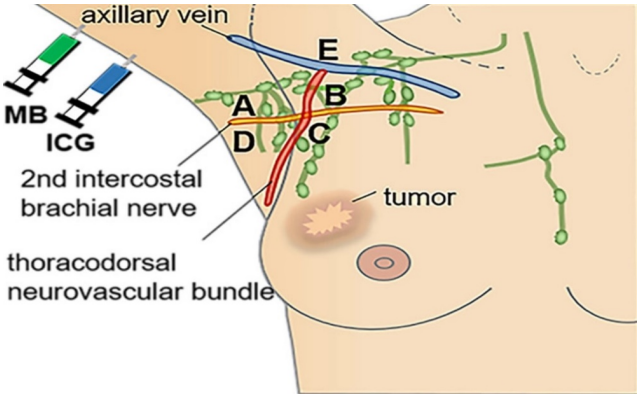


**Figure 3:** Site of dye injection

### 2.1. Axillary lymph node dissection

Based on imaging assessment indicating enlargement of axillary lymph nodes, primary axillary lymph node dissection (ALND) was deemed necessary. During the procedure, axillary reverse mapping (ARM) nodes labeled with methylene blue were visible to the naked eye, while those labeled with indocyanine green (ICG) were visualized using a fluorescence imaging camera. The metastasis rates of ARM nodes and the identification rates of ARM nodes were subsequently evaluated intraoperatively. During the surgery, ARM nodes were initially detected and extracted individually, followed by subsequent examination through pathological tests.[5]

### 2.2. Pathological examination

Following the revised TNM staging system outlined in the NCCN Clinical Practice Guidelines in Oncology: Breast Cancer (Version 1.2016), all patients underwent evaluation for postoperative TNM staging and molecular profiling. They were categorized into luminal A, luminal B, basal, or overexpressed HER2 types based on molecular profiling, as well as assessed for estrogen/progesterone receptor (ER/PR) status and HER2/neu status.

### 2.3. Statistical analysis

According to the updated TNM staging system delineated in the NCCN Clinical Practice Guidelines in Oncology: Breast Cancer (Version 1.2016),[10] all individuals underwent assessment for postoperative TNM staging and molecular profiling. They were stratified into luminal A, luminal B, basal, or HER2 overexpression subtypes based on molecular profiling, alongside evaluation of estrogen/progesterone receptor (ER/PR) status and HER2/neu expression.

## 3. Results

### 3.1. Dyeing rate

The rate of successful dyeing for ARM nodes overall was 83.33% (40 out of 48). Significant differences were noted in the clinical stages of N0-N1 and N3-N4 between the group receiving methylene blue alone and the group receiving both methylene blue and indocyanine green[Table 2].

### 3.2. Postoperative limb circumference

There was a somewhat lower difference in limb circumference in the Control group compared to the Experimental group from day 1 to day 6. By day 7 and 8, the difference in limb circumference between both groups tended to stabilize. There was no significant disparity noted in the duration of hospital stay between patients in these two groups ($P > 0.05$)[Table 3].

### 3.3. Postoperative injection site induration

Observations were made regarding the diameter of induration, which represents the blue tattooing effect at the injection site. There was a tendency for a larger difference in induration diameter among patients in the Control group compared to those in the Experimental group. However, statistically significant differences ($P < 0.05$) were only observed from day 3 to day 8[Table 4].

### 3.4. Positive lymph node

The incidence of lymph node positivity was higher in the group receiving both Methylene blue and ICG, while it remained inconclusive in the group receiving Methylene blue alone[Table 5].

## 4. Discussion

Indocyanin green (ICG) also known as Tricarbocyanine dye emits light after excitation under infrared light at 806 nm. ICG is highly soluble in water. It binds to beta-lipoproteins, particularly albumin. Since lymph nodes also have high protein content, ICG accumulates in lymph nodes and when near infrared light is illuminated, ICG gets excited and emits green light.[8,11] The optimal concentration of ICG is 3-6 micro gram per mL. ICG dyed lymph node becomes visible 5 minutes after light excitation and remains visible uptup till minutes (1 hour). The transit time of ICG is very less compared to Methylene blue. Elimination of ICG occurs through liver and intra-vascular half-life is 3-4 minutes. Side effects include allergic reactions, skin damage, sub cutaneous nodules and permanent tattooing. The 10% rule of sentinel node states that all nodes with radioactive count of 10% or more of the hottest node ex-vivo should be removed.[9,12,13]

**Table 2:** visualisation rate of the two groups

| Parameters | Methylene blue group | Combination group (MB + ICG) |
|---|---|---|
| Total patients | 24 | 24 |
| Visualization | 18/24 (75%) | 23/24 (95.8%) |
| Lymph node | 23 (95.84%): N0-N1 1 (4.17%): N2-N3 | 22 (91.67%): N0-N1 2 (8.34%): N2-N3 |

**Table 3:** Post-operative limb circumference during the 8 days of follow up

| Days | Me thylene blue (24) | Methylene blue + ICG (24) | t | P |
|---|---|---|---|---|
| D1 | 2.23 ± 0.745 | 2.421 ± 1.131 | 0.805 | 0.312 |
| D2 | 2.49 ± 0.781 | 2.83 ± 1.72 | 1.040 | 0.310 |
| D3 | 2.58 ± 1.101 | 3.159 ± 2.412 | 1.012 | 0.355 |
| D4 | 2.476 ± 1.150 | 2.910 ± 2.390 | 0.786 | 0.501 |
| D5 | 2.410 ± 1.11 | 2.701 ± 2.511 | 0.442 | 0.672 |
| D6 | 2.380 ± 1.085 | 2.396 ± 2.55 | 0.131 | 0.912 |
| D7 | 2.317 ± 1.102 | 2.436 ± 2.444 | 0.44 | 0.89 |
| D8 | 2.054 ± 0.939 | 2.010 ± 2.221 | 0.059 | 0.913 |
| Total days | 11.765 ± 3.152 | 11.432 ± 3.560 | 0.41 | 0.691 |

**Table 4:** Post-operative injection site induration during the 8 days of follow up

| Days | Methylene blue + ICG (24) | Methylene blue (24) | t | P |
|---|---|---|---|---|
| D1 | 2.609 ± 0.467 | 2.427 ± 0.504 | 1.28 | 0.201 |
| D2 | 2.793 ± 0.466 | 2.520 ± 0.499 | 1.609 | 0.15 |
| D3 | 2.756 ± 0.563 | 2.575 ± 0.410 | 2.202 | 0.039 |
| D4 | 2.856 ± 0.458 | 2.531 ± 0.356 | 2.492 | 0.020 |
| D5 | 2.9 ± 0.489 | 2.35 ± 0.316 | 2.635 | 0.013 |
| D6 | 2.771 ± 0.519 | 2.374 ± 0.346 | 2.593 | 0.020 |
| D7 | 2.8 ± 0.480 | 2.408 ± 0.396 | 2.338 | 0.017 |
| D8 | 2.504 ± 0.521 | 2.300± 0.407 | 2.219 | 0.044 |

**Table 5:** Rate of positive lymph node

| Groups | Lymph node positive | Lymph node negative |
|---|---|---|
| Methylene blue + ICG (24) | 14 (58.34%) | 10 (41.67%) |
| Methylene blue (24) | 12 (50%) | 12 (50%) |

Methylene blue, also known as Methylthionium chloride (derivative of Phenothiazine) which is a monoamine oxidase inhibitor, is a dark green color powder that yeilds blue solution in water. The peak light absorption occurs around 670 nm. Side effects include headache, confusion, vomiting, shortness of breath, Serotonin syndrome, and allergic reactions. It also causes urine, sweat, and stool to turn blue to green in color. However, it is cheap, needs simple pre-operative preparation and is non-radioactive. [14,15]

The underlying principle of the ARM technique posits that the lymphatics of the upper extremity are typically not implicated in the metastasis of primary breast tumors. Therefore, identifying and preserving these lymphatics is believed to reduce the risk of lymphedema. [6] However, certain studies suggest that the incidence of metastatic tumors in ARM nodes ranges from 14% to 43%. Currently, most research on the ARM technique focuses on its feasibility and aims to reduce lymphedema incidence by preserving ARM nodes/lymphatics, with less emphasis on safety considerations. [16–18] In this study, our objective is to enhance the ARM procedure by utilizing a combination of ICG and methylene blue dye while prioritizing safety, feasibility, and enhancing the identification rate of ARM nodes.

## 5. Summary

The rate of ARM node identification reached 93.18%. In the Experimental group, visualizing ARM nodes was notably higher [23 out of 24 (95.8%)] compared to the Control group [18 out of 24 (75%)]. This study indicates that the combined use of methylene blue and ICG enhances ARM node identification, especially beneficial for patients with more advanced breast cancer. [19]

There were no significant differences observed in limb circumference between the Experimental and Control groups. However, the diameter of induration at the injection site was notably larger in the Experimental group compared to the Control group.

## 6. Conclusion

The identification rate of ARM nodes using the combination of methylene blue and ICG is significantly higher compared to methylene blue alone. This finding suggests that the combined approach could serve as a valuable tool for surgical therapy in patients with invasive breast cancer.

## 7. Study Limitations

Our study was limited by its small sample size, potentially hindering our ability to detect significant differences in lymphedema incidence between the groups. Large-scale studies are highly recommended to confirm these findings. Additionally, the short-term follow-up of 6 months may not have provided sufficient time to distinguish transient lymphedema caused by acute surgical edema from permanent lymphedema. Therefore, longer follow-up periods are warranted for a more comprehensive evaluation.

## 8. Source of Funding

None.

## 9. Conflict of Interest

None.

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