Research Article

Comparison of Indocyanine Green-Guided Fluorescence with Fluorescein Sodium-Based Sentinel Lymph Node Biopsy in Patients with Breast Cancer: A Randomized Controlled Trial

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ABSTRACT

Introduction: Sentinel lymph node biopsy (SLNB) is now the standard for axillary staging in early breast cancer, replacing axillary lymph node dissection due to lower morbidity while preserving diagnostic accuracy. Dual tracer methods using radioisotopes and blue dyes provide high detection rates but are often limited in resource-constrained settings. Indocyanine green (ICG) and fluorescein sodium have emerged as cost-effective alternatives. This study compared the efficacy, operative efficiency, and cost-effectiveness of ICG versus fluorescein sodium as single-dye tracers for SLNB.

Materials and Method: This study was conducted at AIIMS, New Delhi, between April 2020 and November 2021. Twenty female patients with early breast cancer and clinically node-negative axillae were randomized into two groups: Group A (ICG) and Group B (fluorescein sodium). Standard protocols for dye administration, visualization, and sentinel node dissection were followed. Histopathological analysis was performed for all excised nodes. Data were analysed using Chi-square, Fisher's exact, and Mann–Whitney U tests.

Result: Sentinel lymph nodes were identified in all patients with ICG (100%) and in 80% with fluorescein (p=0.47). The mean number of nodes retrieved was similar across groups. Operative time was significantly shorter in the ICG group (24.2 ± 2.56 min) compared with fluorescein (33 ± 4.93 min; p=0.001). However, the mean procedural cost was higher with ICG (INR 14,684.7) versus fluorescein (INR 908.9).

Conclusion: ICG showed higher identification rates and shorter operative time but at greater cost. With affordable near-infrared systems, ICG could serve as a reliable tracer alternative in resource-limited settings.

Keywords*:* Breast cancer, Sentinel lymph node biopsy, Indocyanine green, Fluorescein sodium, Randomized trial

Introduction

Breast cancer is the most common malignancy among women globally, with an estimated 2 million new cases and over 626,000 deaths reported in 2018[1]. It ranks fifth in global cancer-related mortality but remains the leading cause of cancer deaths in low-resource countries.[2] Advancements in early diagnosis and treatment have improved outcomes, with 5-year overall survival (OS) rates now approaching 90%, depending on the stage at diagnosis [3]. Axillary lymph node

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status remains the most important prognostic factor, reducing 5-year OS by up to 40% when involved [4]. Axillary lymph node dissection (ALND), though once the standard, carries morbidities such as lymphedema and sensory deficits. Trials like NSABP B-04 and King's/Cambridge showed no OS benefit for ALND in clinically node-negative (cN0) patients [5][6]. As early detection increased, so did the need for less morbid alternatives—leading to the development of sentinel lymph node biopsy (SLNB). SLNB was first used in melanoma with radiocolloids [7], then introduced in breast cancer using patent blue by Giuliano et al. [8], and later, radiotracer-guided techniques by Krag et al. [9]. Large trials like ACOSOG Z0011 and AMAROS established SLNB as sufficient in selected early breast cancer patients, even with limited nodal involvement [10][11].

Dual tracer techniques using radioisotopes(e.g., ^{99m}Tc-sulfur colloid) and blue dye remain standard due to higher identification rates—rising from 66% with blue dye alone [12] to >90% with dual tracers [9][13]. At AIIMS, New Delhi, initial SLNB programs achieved 94.7% identification with dual tracers and 87.8% with single dye, later improving to 97% [14][15]. However, radioisotope use is limited in many Asian and African settings due to cost (approx. ₹20 lakh or $23,000 USD) and lack of nuclear medicine facilities. To address this, Srivastava et al. pioneered a dual-dye technique using fluorescein sodium and methylene blue, achieving 95% identification and 8% false-negative rates at Tata Memorial Hospital [16]. Subsequent trials at AIIMS showed comparable efficacy between fluorescein + methylene blue and isotope + methylene blue, with SLN identification in 84% of patients. In 2015, AIIMS observed a novel dual-route (intravenous + local) fluorescein technique and compared it to indocyanine green (ICG), a near-infrared dye emitting at 802 nm. Because of its deeper tissue penetration and non-radioactive nature, ICG is gaining popularity as an alternative tracer. Therefore, this study aims to compare the sentinel lymph node identification rates between two single-dye techniques: Indocyanine Green and Fluorescein Sodium.

Materials and methods:

This study was a two-arm, open-label, parallel-group randomised controlled trial. It was conducted in the Department of Surgical Disciplines at AIIMS, New Delhi, in collaboration with the Departments of Radio-diagnosis, Pathology, and Hospital Administration. The study was conducted from April 23, 2020, to November 2021, after obtaining ethical clearance (IEC no. IECPG-15/27/23.04.2020) and trial registration (CTRI/2020/01/030756).

Based on an expected identification rate of 92% and a non-inferiority margin of 16, the calculated sample size was 46 patients per group (total 92). Due to the COVID-19 pandemic, only 20 patients were enrolled (10 in each group) with IEC approval.

Patients were randomised in a 1:1 ratio into Group A (ICG) or Group B (Fluorescein) using a computer-generated randomisation schedule. Block randomisation with blocks of ten was used. Allocation concealment was ensured using sealed opaque envelopes.

Eligibility Criteria

Inclusion Criteria:
- Female patients with histopathologically confirmed early breast cancer
- Clinically node-negative axilla
- Provided written informed consent

Exclusion Criteria:
- Refusal to consent
- Palpable axillary lymphadenopathy
- Distant metastasis or inflammatory breast cancer
- **Prior breast/axillary surgery, axillary scarring from burns/trauma**
- Multicentric/multifocal tumors
- Prior radiotherapy or chemotherapy
- Past or current tuberculosis of the breast or axilla

Preoperative Assessment

All enrolled patients underwent triple assessment, including detailed history, clinical breast and axillary examination, bilateral mammography, and histopathological confirmation. Sonographic evaluation of axillary lymph nodes was done in all cases. Suspicious nodes on ultrasound were subjected to fine needle aspiration cytology (FNAC) or core needle biopsy. Patients with positive axillary nodal disease were excluded.

Intervention

Group A – Indocyanine Green (ICG)

At the time of anesthesia induction, 2.5 mL (12.5 mg) of ICG was administered intravenously. Simultaneously, 2.5 mL of 1% ICG was injected intradermally in equal halves at peritumoral and peri-areolar locations in the outer quadrants of the breast. In cases with non-palpable tumors, only peri-areolar injection was given. After a gentle massage for 5–7 minutes towards the axilla, lymphatic flow was visualised using an infrared SPY camera system. Skin incision was planned at the point of fluorescence entry into the axilla. Fluorescent lymphatics were followed via blunt and sharp dissection. All fluorescent and/or palpable lymph nodes were excised and labelled as SLNs. Excised nodes were categorised as fluorescent or non-fluorescent.

Group B – Fluorescein

A solution containing 0.1 mL of fluorescein diluted in 4 mL of saline was prepared. At anaesthesia induction, 2 mL was administered intravenously, and the remaining 2 mL was injected intradermally in a manner similar to ICG. A gentle breast massage was done for 5 minutes to facilitate dye migration. Lymphatic pathways were visualised using a handheld cobalt blue light. SLNs were dissected as in Group A, and categorised similarly.

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Surgical and Histopathological Protocol

In both groups, sentinel lymph nodes identified intraoperatively were excised and sent for histopathological

Statistical Analysis

All data were analysed using STATA version 14. Categorical variables were presented as frequencies and percentages. Continuous variables were reported as mean ± standard deviation (SD), median, and range. Group comparisons for categorical variables were done using Chi-square or Fisher's exact test, while continuous variables were analysed using the Mann–Whitney U test for non-parametric data. A p-value of <0.05 was considered statistically significant.

Result

A total of 36 patients were assessed for eligibility between April 2020 and November 2021. Twelve patients received neoadjuvant chemotherapy due to restricted operative

evaluation. Patients with SLN positivity underwent axillary lymph node dissection (ALND), while those with negative SLNB had no further axillary intervention.

services during the COVID-19 pandemic, and four patients had axillary metastasis on FNAC from suspicious nodes detected on ultrasound. These patients were excluded.

The mean age of the study population was 47.65 ± 11.26 years. Group A patients had a mean age of 48.1 ± 15.18 years, and Group B patients had a mean age of 44.4 ± 7.73 years (p=0.27). The mean BMI was comparable between the two groups (27.0 ± 4.64 vs. 26.6 ± 3.03 kg/m²; p=0.19). Forty per cent of patients were postmenopausal. In Group A, half the patients were postmenopausal, while in Group B, 70% were premenopausal (p=0.47). Lesions were more frequently left-sided in Group A (70%) and right-sided in Group B (60%). The majority of tumors were located in the upper outer quadrant (55%). Most patients (95%) presented with T2 disease, while one patient (5%) had Tis (Table 1).

Table 1. Baseline Demographic and Clinical Characteristics of Study Population

Parameter	Group A: ICG (n=10)	Group B: Fluorescein (n=10)	p-value
Age (years), Mean ± SD	48.1 ± 15.18	44.4 ± 7.73	0.27
BMI (kg/m²), Mean ± SD	27.0 ± 4.64	26.6 ± 3.03	0.19
Menopausal status			
Pre-menopausal	5 (50%)	7 (70%)	0.47
Post-menopausal	5 (50%)	3 (30%)	
Laterality			
Left	7 (70%)	4 (40%)	0.36
Right	3 (30%)	6 (60%)	
Clinical T stage			
Tis	0 (0%)	1 (10%)	1.0
T2	10 (100%)	9 (90%)	

Twelve patients (60%) underwent breast conservation surgery and eight patients (40%) underwent mastectomy. Group A had more breast conservation surgeries (80%) compared to Group B (40%), while mastectomy was more common in Group B (60% vs. 20%), though this difference was not statistically significant (p=0.07). All patients underwent SLNB as the initial axillary procedure. In Group A, three patients (30%) required completion ALND due to positive SLNB, while in Group B, two patients (20%) required ALND (p=0.60) (Table 2).

Invasive ductal carcinoma was the predominant histology (95%), with one patient (5%) having ductal carcinoma in situ. Luminal A was the most common molecular subtype (60%), followed by basal/triple-negative (20%), HER2-enriched (10%), and luminal B (5%). Hormone receptor expression did not differ significantly between the two groups: ER positivity was 80% in Group A and 50% in Group B (p=0.16), PR positivity was

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80% vs. 40% (p=0.67), and HER2 positivity was 20% vs. 30% (p=0.60) (Table 2).

Table 2. Surgical and Histopathological Profile

Parameter	Group A: ICG (n=10)	Group B: Fluorescein (n=10)	p-value
Type of Breast Surgery			
Breast Conservation Surgery	8 (80%)	4 (40%)	0.07
Mastectomy	2 (20%)	6 (60%)	
Axillary Surgery			
SLNB only	7 (70%)	8 (80%)	0.60
SLNB + ALND	3 (30%)	2 (20%)	
Histopathology			
Invasive ductal carcinoma	10 (100%)	9 (90%)	1.0
DCIS	0 (0%)	1 (10%)	
ER positive	8 (80%)	5 (50%)	0.16
PR positive	8 (80%)	4 (40%)	0.67
HER2 positive	2 (20%)	3 (30%)	0.60

Sentinel lymph nodes were identified in all patients in the ICG group (100%) and in 8 of 10 patients in the fluorescein group (80%). Although the identification rate was higher with ICG, the difference was not statistically significant (p=0.47) (Table 3). The total number of SLNs identified was 28 in Group A and 24 in Group B. The mean number of SLNs per patient was 2.8 ± 1.07 in Group A and 2.41 ± 1.56 in Group B, with a median of 3 in both groups (p=0.96). SLN positivity was observed in three patients (30%) in Group A and two patients (20%) in Group B (Table 3).The mean time to perform SLNB was significantly shorter in the ICG group (24.2 ± 2.56 minutes) compared to the fluorescein group (33 ± 4.93 minutes, p=0.001) (Table 3). The cost per procedure was substantially higher with ICG (INR 14,684.7) compared to fluorescein (INR 908.9), owing largely to the cost of the infrared SPY camera used for ICG fluorescence detection. Consumables cost INR 1750 for ICG and INR 360 for fluorescein, while manpower costs were comparable between the two groups (Table 3).

Table 3. Sentinel Lymph Node Outcomes and Procedural Parameters

Parameter	Group A: ICG (n=10)	Group B: Fluorescein (n=10)	p-value
SLN Identification Rate	10/10 (100%)	8/10 (80%)	0.47
Total SLNs identified	28	24	0.96
Mean ± SD per patient	2.8 ± 1.07	2.41 ± 1.56	

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Median (range)	3 (1–4)	3 (0–5)	
SLN positivity	3 (30%)	2 (20%)	–
Time for SLNB (min), Mean ± SD	24.2 ± 2.56	33 ± 4.93	0.001
Total cost per procedure (INR)	14,684.7	908.9	–

Discussion

Sentinel lymph node biopsy (SLNB) has emerged as the gold standard for axillary staging in early operable breast cancer with clinically and radiologically negative axillae. The use of dual tracers, typically a radiocolloid and blue dye, has been widely accepted due to consistently high identification rates. However, the reliance on nuclear medicine facilities and concerns regarding allergic reactions to dyes such as Patent Blue Violet (PBV) have prompted the search for alternative agents. In India, methylene blue has been used as a substitute for PBV due to its cost-effectiveness and local availability [17]. In the West, limited availability and risk of anaphylaxis with PBV encouraged the use of indocyanine green (ICG) as an alternative fluorophore. In our study, we evaluated and compared ICG with fluorescein sodium as single tracers for SLNB.

The mean age of our study population was 47.5 years, which is consistent with prior studies from our institute (median 47 years) [18] and similar to other Indian studies reporting presentation between 50–55 years [19, 20]. This is considerably lower than the median age reported in the United States, where SEER data estimate it at 61 years [21].
The mean tumor size in our study was 3.4 cm, which is larger than that reported in Western studies, where 65–84% of early breast cancers were less than 2 cm [21, 22–25]. This observation is consistent with Indian studies where tumors are often bigger in size at presentation, due to late detection and differences in screening practices [26, 27].

The primary endpoint of our study was the identification rate of SLNs. Using ICG, the identification rate was 100%, whereas with fluorescein it was 80%. These results highlight the superior performance of ICG as a single tracer. Our findings align with existing literature. At Tata Memorial Centre, Agrawal et al. reported SLN identification rates of 95% with Tc-99m sulfur colloid plus methylene blue and 97% with ICG plus methylene blue [28]. Yuan et al., in a large randomized trial of 471 patients, reported identification rates of 99.6% with radiotracer plus methylene blue and 99% with ICG plus methylene blue [29]. Valente et al. also demonstrated a 100% identification rate with ICG in 92 patients, compared to 96% with Tc-99m sulfur colloid [30]. Similarly, Verbeek et al. reported an identification rate of 99% in 95 patients using near-infrared ICG fluorescence [31], while Aoyama et al. and Abe et al. documented 100% identification rates in their studies of 312 and 128 patients, respectively [32,33]. Thus, our findings further confirm that ICG is highly reliable as a tracer for SLNB.

In contrast, the SLN identification rate with fluorescein in our study was lower (80%). Previous work from our institute by Srivastava et al. demonstrated the utility of fluorescein sodium in combination with methylene blue, reporting an identification rate of 95% and a lower false-negative rate compared to Tc-99m sulfur colloid [16]. A validation study at SGPGI also found fluorescein to be a cost-effective and effective alternative to isotopes. The lower rate in our study may be attributable to the use of fluorescein as a single agent rather than in combination, and possibly the dilutional concentration used.

The mean time to complete SLNB was significantly shorter with ICG (24.2 minutes) compared to fluorescein (33 minutes). This was likely due to better contrast and visualization with ICG, where fluorescent nodes appear bright white against a black background, whereas fluorescein required cobalt blue light, providing less contrast. Our findings correlate with other studies that ICG improves operative efficiency due to ease of lymphatic visualization [30–33].

Cost analysis revealed a significant difference between the two groups, with per-procedure costs estimated at INR 14,684.7 for ICG versus INR 908.9 for fluorescein. The higher cost for ICG was largely due to the use of the SPY Elite infrared camera system. While the capital cost of such equipment remains a limitation in resource-limited settings, newer and less expensive handheld ICG detection systems are now available, which may improve cost-effectiveness in the future. Moreover, higher procedural volumes could further reduce the per-procedure costs of ICG systems.

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Conclusion

Based on interim results, SLNB using indocyanine green as a single dye technique was found to be non-inferior to fluorescein, with the added advantage of real-time near-infrared imaging. This finding is particularly relevant for developing countries where nuclear medicine facilities are limited. Although the current cost of ICG is higher, the use of newer, more affordable NIR systems and higher procedural volumes could make it more cost-effective. Continued patient recruitment is planned to achieve the target sample size and validate these findings with more sample size.

Limitations

This study had some limitations. The desired sample size could not be achieved due to the COVID-19 pandemic, reducing statistical power. Being a single-centre study, the generalizability of findings is limited. Additionally, the false-negative rate could not be assessed as results were not compared with the dual tracer radioisotope standard.

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