

# Sentinel lymph node biopsy using indocyanine green fluorescence in early-stage breast cancer: a meta-analysis

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**Abstract** Sentinel lymph node (SLN) biopsy using indocyanine green (ICG) fluorescence is safe and has a high detection rate for SLNs. However, the results of this novel technique are heterogeneous. The objective of this meta-analysis was to evaluate the diagnostic performance of the ICG fluorescence method compared with the standard radioisotope (RI) method. All eligible studies were identified from 2005 through 2015. A proportion meta-analysis was performed using a fixed effects and/or random effects model based on the study heterogeneity. A total of 12 studies met the inclusion criteria and included 1736 women. There was no significant difference between ICG fluorescence and RI for SLN detection using either the fixed effects model [odds ratio (OR) 1.29, 95% confidence interval (CI) 0.87–1.90] or the random effects model (OR 1.32, 95% CI 0.54–3.18). There were seven studies reporting the detection rate for tumor-positive SLN. The ICG fluorescence method was significantly better than the RI method in the fixed effects model (OR 1.87, 95% CI 1.00–3.49) for staging axilla. However, there was no difference in the random effects model (OR

1.90, 95% CI 0.74–4.86). There was study outcome heterogeneity for the detection of SLN but not for tumor-positive SLN. There was no publication bias observed in the studies included. The ICG fluorescence method has valid diagnostic performance for SLN detection and shows a trend toward better axilla staging compared with the RI method. ICG fluorescence is a useful alternative to RI for SLN biopsy.

**Keywords** Indocyanine green · Fluorescence · Radioisotope · Sentinel lymph node · Breast cancer

## Introduction

Axillary lymph node dissection has been traditionally used to evaluate axillary involvement and local control in women with node-negative breast cancer. However, conventional axillary dissection is associated with considerable morbidity, including lymphedema, pain, and shoulder stiffness. The sentinel lymph node (SLN) is defined as the first lymph node receiving lymphatic drainage from the breast and represents the actual nodal status. SLN biopsy is currently the standard of care for staging the axilla and decreases morbidity after lymphadenectomy [1, 2]. Previous studies have demonstrated that SLN biopsies using radioisotopes (RI) show a high detection rate, and the dual method involving RI and blue dye (BD) is considered the standard method [3–5]. However, the RI method requires expensive equipment, authorized radiation protection areas, and access of the nuclear medicine department to RI. These logistic and legislative issues limit SLN biopsy using RI to high-volume centers in developed countries. Although the BD method is cost-effective, there are limitations, including a low detection rate and requirements regarding physician skill and experience [6].

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A novel method using indocyanine green (ICG) fluorescence was developed in 2005 to address these problems [7]. ICG is a widely used reagent in clinical practice for the assessment of liver function and cardiac output. The near-infrared (NIR) fluorescence imaging system utilizes the characteristic fluorescence spectra of ICG within an optical window. NIR/ICG fluorescence imaging visualizes subcutaneous lymphatic flow and allows the surgeon to directly observe the axillary fluorescent SLN [8]. Previous clinical studies demonstrated that the ICG fluorescence method was consistently superior to the BD method for SLN detection. Furthermore, both the overall and tumor-positive SLN detection rates for the ICG fluorescence method are comparable or superior to the RI method. However, the superiority of the overall and tumor-positive SLN detection rates for the ICG fluorescence method remains uncertain due to small-sample studies and technical heterogeneity.

In this study, we conducted a meta-analysis to assess the diagnostic utility of ICG fluorescence for SLN detection and axilla staging compared with the standard RI method.

## Materials and methods

A systematic review of publications was performed using PubMed and the Cochrane Library. All relevant studies were published between January 2005 and December 2015. The medial subject heading (MESH) terms were the following: indocyanine green, sentinel lymph node biopsy, and breast neoplasms. The inclusion criteria were women with early breast cancer who underwent SLN mapping using ICG and RI concurrently. The studies included reported the procedural detection rate for SLN using each modality. Reviews, meta-analyses, abstracts, commentaries, letters, duplicated publications, studies on ICG titration, and studies with fewer than 10 patients were excluded.

The quality of cohort studies was assessed according to the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [9]. Six items of the amended STROBE statement were considered relevant for the quality evaluation. The risk bias tool described in the Cochrane handbook was used to assess the suitability of randomized controlled studies [10].

The meta-analysis was performed using the Mantel-Haenszel method for calculating the weighted pooled odds ratio using a fixed effects and/or random effects model based on the study heterogeneity. The statistical heterogeneity among studies was evaluated using  $I^2$  statistics and  $P$  values using the meta package available within the statistical software R. The heterogeneity was considered significant at  $I^2 > 50\%$  or  $P < 0.05$ . The publication bias of the

studies included was explored by Egger's regression test, which was performed using the meta package within the statistical software R.

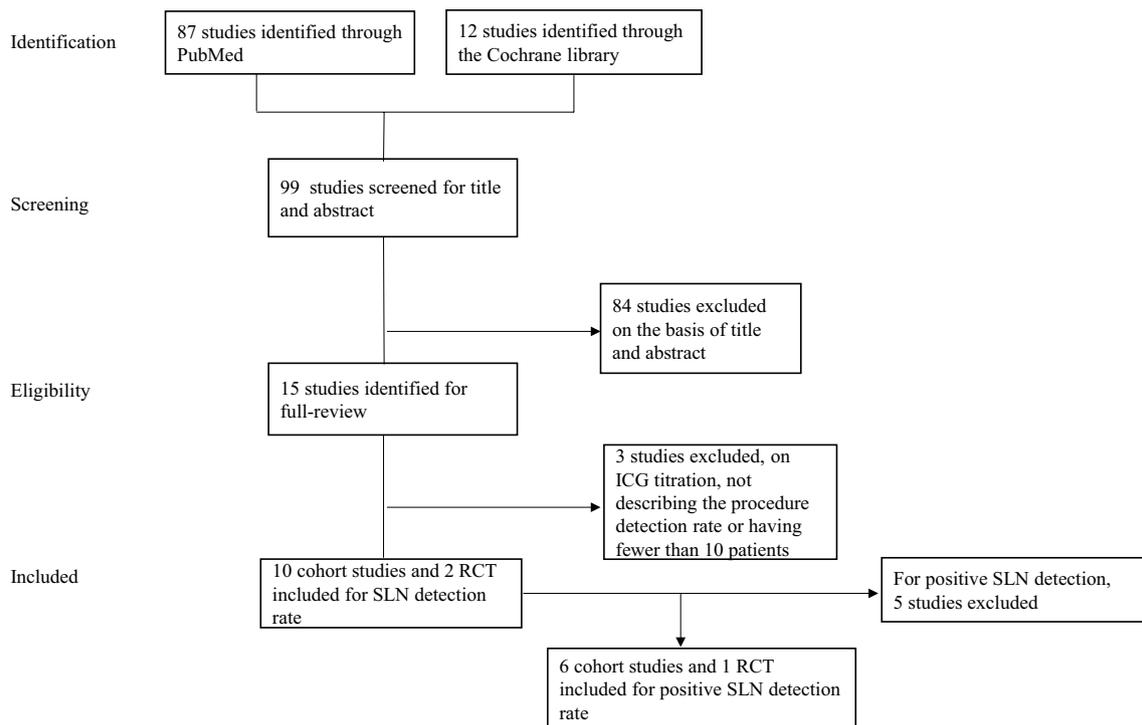
## Results

The literature search identified 87 studies in PubMed and 12 additional studies in the Cochrane Library. Of the 99 studies, 84 were excluded for the following reasons: not using RI concurrently, review or commentary format, language other than English, animal studies, and studies of other cancers. There were 15 studies under full review that were included, and 3 other studies were excluded because of the use of other surgical techniques and ICG titration, small sample size, and no reported data for the procedure detection rate. Thus, 12 studies were included in the present analysis. There were 7 studies with data for tumor-positive SLN detection used to evaluate the utility for staging axilla (Fig. 1).

The 12 studies included 1736 patients [11–22]. Their characteristics and technical details are shown in Table 1. There were two single-center randomized controlled studies. One assessed the diagnostic value of BD in combination with RI and ICG [15]. The other study evaluated a multimodal method involving ICG, RI, and BD compared with RI alone [20]. Of the remaining 10 studies, 9 were prospective and one was a retrospective cohort study. Two studies used modified ICG combined with another tracer. One study compared the usefulness of ICG with ICG conjugated to human serum albumin for SLN detection [14]. In the other study, a hybrid tracer of ICG and RI was used for SLN detection and compared with RI [16]. There were 7 studies [12–14, 17, 18, 21, 22] that used a Photo Dynamic Eye (PDE; Hamamatsu Photonics, Hamamatsu, Japan) and 3 that used the Mini-FLARE™ system [15, 16, 19]. IC-view [11] and ICG-F [20] were each conducted in one study.

The quality of the 10 cohort studies and 2 randomized control studies was assessed in the STROBE statement and in the Cochrane handbook, respectively. The 10 cohort studies all stated the following: a study objective, clear inclusion criteria, a standardized technique, a standardized histopathology, and withdrawals from the study. Although no studies conducted patient follow-up examinations, their STROBE score was 5 (Supplement Table 1). The two randomized controlled studies were not blinded. One study had a bias from a small sample size of 24 patients. There were 2 studies that cleared the remaining items, and the mean score was 5.5 (Supplement Table 2).

The 12 studies included reported the overall detection rate for SLN using ICG fluorescence and RI. The rates ranged from 88.6 to 100% and from 85 to 100%,



**Fig. 1** Flow diagram for selection of the studies included

respectively. There were 7 studies that reported the detection rate for tumor-positive SLNs, which ranged from 92.6 to 100% for the ICG fluorescence method and from 76.9 to 100% for the RI method. The number of SLNs removed was 1.5–3.4 for ICG and 1.35–2.3 for RI.

The meta-analysis of SLN detection revealed no significant difference between ICG and RI in either the fixed effects model [odds ratio (OR) 1.29, 95% confidence interval (CI) 0.87–1.90] or the random effects model (OR 1.32, 95% CI 0.54–3.18). There was heterogeneity of SLN detection rate observed, with  $I^2 = 52.5%$ ,  $P = 0.0257$  (Fig. 2). The heterogeneities of tumor-positive SLN detection were low for staging axilla, with  $I^2 = 15.4%$ ,  $P = 0.3162$  (Fig. 3). The ICG fluorescence method was significantly better than the RI method in the fixed effects model (OR 1.87, 95% CI 1.00–3.49) but not in the random effects model (OR 1.90, 95% CI 0.74–4.86).

There was no publication bias observed in the 12 studies included in the meta-analysis (Fig. 4).

## Discussion

This meta-analysis was focused on the direct comparison of the diagnostic performance for ICG fluorescence and RI in SLN biopsy for patients with breast cancer. Although there was no statistically significant difference between ICG

fluorescence and RI for SLN detection, ICG fluorescence showed a trend toward better axilla staging than RI. These results confirm that the ICG fluorescence method is a useful alternative to the standard RI method for SLN biopsy.

ICG is an amphiphilic molecule that quickly binds to plasma albumin and functions as a fluorescent tracer agent on NIR imaging systems [23]. This is the only fluorophore approved for clinical use in Japan, Europe, and the USA. PDE is commercially available and is widely used in the majority of clinical studies. The fluorescence signal is captured by the PDE using a light-emitting diode (LED) producing light at a wavelength of 760 nm in its active state [24]. The detector is a charge-coupled device camera that filters out wavelengths below 820 nm. The other devices such as the Mini-FLARE system<sup>TM</sup> [25] are also used in clinical studies and have achieved high detection rates for SLN.

BD and RI are currently the standard techniques for SLN mapping. ICG is used in clinical practice as the third technique in NIR fluorescence imaging. Several recent meta-analyses demonstrated that ICG-guided SLN biopsy achieved a high detection rate for SLN and was viable for the detection of SLN metastasis [26, 27]. ICG fluorescence was significantly superior to BD for SLN detection in a direct comparison [28]. Ahmed et al. [29] conducted a systematic review and reported that ICG was significantly better than BD for SLN detection (OR 18.7, 95%

**Table 1** Characteristics and technical details of 12 studies included in meta-analysis

Study	N	Tracer	Device	No. of SLNs removed	Detection rate for SLNs	Detection rate for positive SLNs	FNR
Murawa et al. [11]	20 (group2)	ICG + RI	IC-view	1.73 (ICG) 1.35 (RI)	100% (ICG) 85% (RI)	92.4% (ICG) 76.9% (RI)	7.6% (ICG) 23% (RI)
Hojo et al. [12]	29 (group2)	ICG + RI	PDE	3 (ICG) 2 (RI)	93.1% (ICG) 100% (RI)	NA	NA
Wishart et al. [13]	100 (104 procedures)	ICG + RI + BD	PDE	1.93 (ICG) 1.5 (RI) 1.84 (BD)	100% (ICG) 91.3% (RI) 99% (BD)	100% (ICG) 100% (RI)	NA
Polom et al. [14]	28 (ICG group)	ICG + RI	PDE	2 (ICG) 2 (RI)	96.4% (ICG) 100% (RI)	NA	NA
Van der Vorst et al. [15]	24	ICG + RI vs. ICG + RI + BD	Mini-FLARE	1.6 (ICG + RI) 1.5 (ICG + RI + BD)	95.8% (ICG) 95.8% (RI)	NA	NA
Schaafsma et al. [16]	32	ICG-RI (hybrid) + BD	Mini-FLARE	1.5 (ICG)	100% (ICG) 100% (RI) 88% (BD)	NA	1.5% (ICG + RI)
Ballardini et al. [17]	134	ICG + RI	PDE	NA	100% (ICG) 99.3% (RI)	NA	NA
Samorani et al. [18]	301	ICG + RI	PDE	2.0 (ICG) 2.0 (RI)	98.7% (ICG) 95.3% (RI)	100% (ICG) 87.0% (RI)	NA
Verbeek et al. [19]	95	ICG + RI	Mini-FLARE	1.9 (ICG or RI)	98.9% (ICG) 97.9% (RI)	100% (ICG) 93.8% (RI)	NA
Jung et al. [20]	43	ICG + RI + BD vs. RI	ICG-F	3.4 (ICG + RI + BD) 2.3 (RI)	100% (ICG) 100% (RI)	100% (ICG) 100% (RI)	NA
Grischke et al. [21]	105	ICG + RI	PDE	NA	90.7% (BD) 88.6% (ICG) 98.1% (RI)	92.6% (ICG) 100% (RI)	NA
Sugie et al. [22]	821	ICG + RI	PDE	2.3 (ICG) 1.7 (RI)	97.2% (ICG) 97.0% (RI)	93.3% (ICG) 90% (RI)	NA

FNR false negative rate, NA not applicable, SLN sentinel lymph node, ICG indocyanine green, RI radioisotope, BD blue dye, PDE photodynamic, FLARE the fluorescence-assisted resection and exploration

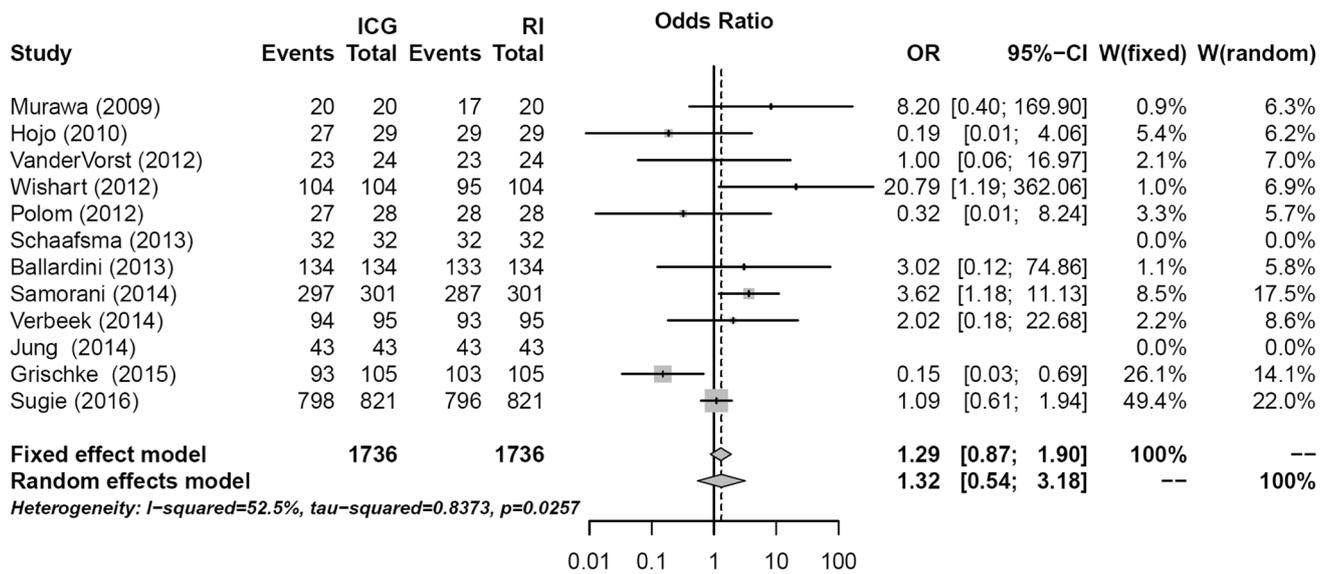


Fig. 2 Sentinel lymph node detection (ICG vs. RI)

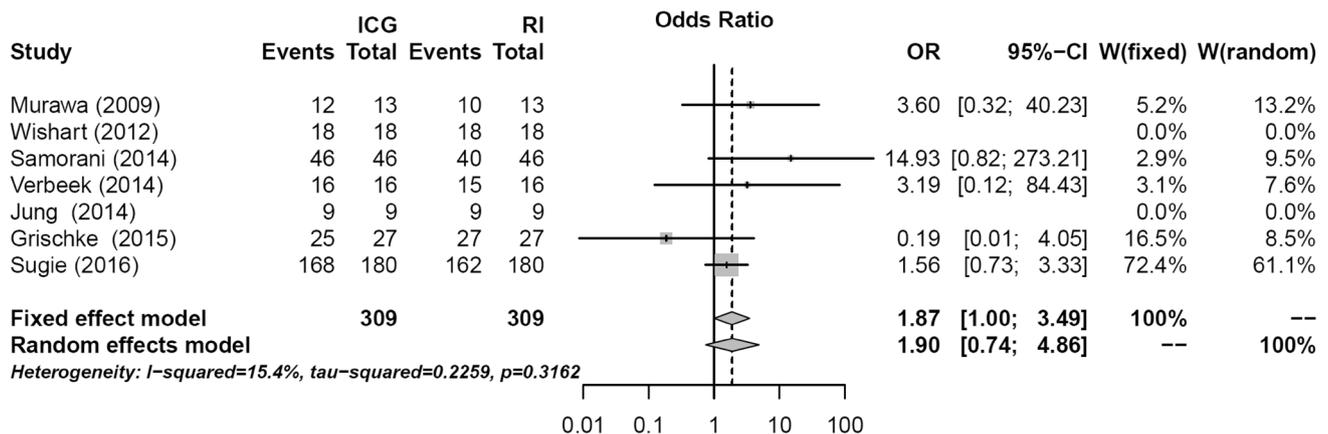


Fig. 3 Tumor-positive sentinel lymph node detection (ICG vs. RI)

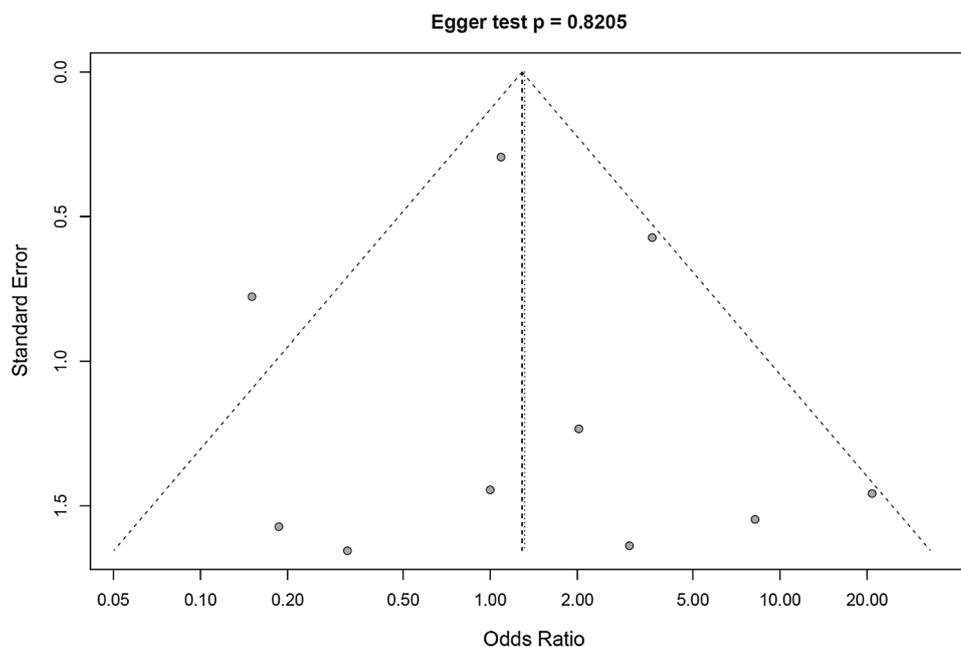
CI 8.63–39.10). However, there was no statistically significant difference between ICG fluorescence and RI for SLN detection. Although there was heterogeneity among studies providing outcome, our study confirms that the ICG fluorescence method achieved a high SLN detection rate and is comparable to the RI method.

It is critical to identify tumor-positive nodes correctly for axillary management. A false-negative study leads to downstaging in the patients with lymph node involvement, and these patients could lose the opportunity to receive adequate systemic therapy. One early study [11] reported that accurate axilla staging was 92.4% for ICG and 76.9% for RI in 13 patients undergoing completion of axillary lymph node dissection (ALND). The largest recent study [22] demonstrated that the detection rate of positive SLNs using ICG was

100%, compared with 90% for RI in 180 patients. Using a fixed effects model, the ICG method had a statistically better detection rate for positive SLNs than did RI without heterogeneity in 5 studies (309 patients). However, the random effect model did not demonstrate statistical significance. The results indicate that the ICG fluorescence method displays a trend toward better axilla staging compared to the RI method.

The endpoint of this meta-analysis was restricted to the detection of overall and tumor-positive SLNs because there are limited data available for clinical relevance such as the false-negative rate, loco-regional recurrence, and overall survival. Only one of the twelve studies examined in the meta-analysis reported a false-negative rate for RI and the ICG fluorescence method after subsequent completion of ALND in all patients, irrespective of SLN involvement

**Fig. 4** Funnel plot of 12 studies of SLN detection included



[11]. There were no studies showing long-term follow-up data for this novel technique and the rate of loco-regional recurrence was not available in published studies. The data from the direct comparison between ICG fluorescence and the RI method might be a surrogate for the clinical impact on prognosis of early breast cancer. However, future studies are required to show reliable data for recurrence and survival in patients undergoing ICG fluorescence-guided SLN biopsy. A second limitation is the sample sizes of the studies included, which ranged from 20 to 821 patients. However, half of the studies enrolled fewer than 50 patients. A third limitation is the quantity of eligible studies for final analysis. Only 12 studies were included because they had to describe the use of RI concurrently with ICG fluorescence and report the procedure detection rate. The fourth limitation to the meta-analysis was the non-uniformity of the ICG fluorescence technique. There was no standardized method of dose and volume administered among included studies. In one study [16], a hybrid tracer of RI conjugated with ICG localized in the SLN and showed high concordance between fluorescence and radioactivity. These technical non-uniformities of the ICG fluorescence method and non-stratification might influence the results of this meta-analysis.

The current standard techniques for SLN mapping are RI and/or the BD method. A nation-wide survey in Japan [30] found that 64% of patients received RI, and the dual method using both RI and BD was performed in 53% of procedures. However, the applicability of RI is limited to high-volume centers. The non-radioactive ICG fluorescence method might be useful in SLN biopsy in small private hospitals and developing countries. The recent trend

toward reducing access to RI also warrants this novel non-radioactive technique as an alternative to the standard RI method.

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**Compliance with ethical standards**

**Conflict of interest** Tomoharu Sugie received honoraria from Hamamatsu Photonics. The other authors declare no conflict of interest.

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