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Use of indocyanine green fluorescence compared to radioisotope for sentinel lymph node biopsy in early-stage breast cancer: systematic review and meta-analysis

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ABSTRACT

Background: In early-stage breast cancer, indocyanine green (ICG)-fluorescence based sentinel lymph node (SLN) detection is being considered. This is a meta-analysis of SLN detection rates and sensitivity of ICG-fluorescence compared to radioisotope (RI), to evaluate its clinical applicability.
Data sources: Systematic review of full-text articles from PubMed and Scopus, of women with early breast cancer who underwent SLN mapping using ICG and RI concurrently was performed. The meta-analysis was performed using the Mantel–Haenszel method.
Results: 2301 patients from 19 studies were included. No significant difference was observed between ICG and RI for SLN detection (OR0.90,95%CI0.66–1.24) or sensitivity (OR1.23,95%CI0.73–2.05) with heterogeneity between studies ($I^2 = 58\%, P = 0.003$). Sensitivity of dual mapping (ICG + RI) was significantly better compared to single mapping with RI (OR3.69,95%CI1.79–7.62) or ICG (OR3.32,95%CI1.52–7.24) alone with no heterogeneity between studies ($I^2 = 0\%, P = 0.004$).
Conclusion: ICG-fluorescence could complement RI method or provide alternative in centers with poor accessibility to RI lymphoscintigraphy.

Introduction

The diagnosis and treatment of early breast cancer has significantly improved over the last few decades due to better screening methods, risk prediction models, better understanding of gene expressions, along with treatment focused on tumor biology. Amidst this, the axillary lymph node status remains one of the most important prognostic indicators in breast cancer.[1] The increase in detection rate of early stage breast cancer has heightened the importance of minimally invasive management of the axilla, given associated morbidity with unnecessary axillary dissection. Sentinel lymph node (SLN) is defined as the first lymph nodes that receive the lymphatic drainage from the primary tumor during its spread. Based on this theory, sentinel lymph node biopsy (SLNB) was the first step towards reducing the extent of radical axillary surgery in breast cancer. Randomized, controlled trials have shown that five-year overall survival in patients with a negative SLN who do not undergo axillary dissection is indistinguishable from that in comparable patients who do undergo axillary dissection, making it possible to avoid axillary dissection under specific conditions.[2,3] In current practice, SLNB remains the standard of care for patients with early stage breast cancer with clinically and radiologically node negative axillae.[4]

In 1993, radioisotope technetium-99 m (RI) was the first method used to map SLN,[5] followed by patent blue V dye (BD) the following year.[6] Both these methods had a steep learning curve, accounting for the low detection rates during the first few years. Currently, in most institutions the standard of care for SLN mapping is the combination technique used with dual tracer involving RI and BD.[4,7,8] The combined use of RI and BD guarantees higher detection rates >95%, low false-negative rates 5–7%, when compared to using these methods independently.[4,8–10] Applicability of RI is limited to large-volume centers with available RI facilities and nuclear medicine. The BD method is cost-effective, but it has drawbacks, such as a low detection rate, allergic reaction and staining.[11,12] Researchers have developed novel tracers to overcome these drawbacks. Innovative tracers such as indocyanine green (ICG), superparamagnetic iron oxide (SPIO), and microbubbles have been explored with ICG being favored by most researchers.[7,13]

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ICG have been used in assessing liver function, cardiac output and free flap perfusion.[7] In 2005 Kitai et al.,[14] first introduced ICG for fluorescence visualization of lymphatic channels and SLNB in breast cancer. ICG is a low molecular weight organic molecule completely bound to plasma proteins; it fluoresces in the near-infrared wavelength.[11] Following ICG injection into the breast, near infrared fluorescent imaging system visualizes subcutaneous lymphatic flow and allows the surgeon to directly observe the axillary fluorescent SLN.[12] Ahmed et al.,[13] in their systematic review, showed ICG was superior to BD in all studies in terms of SLN detection rates and comparable to RI. Two further meta-analyses,[12,15] including latest meta-analysis in 2017 by Sugie et al.,[12] demonstrated overall and tumor-positive SLN detection rates for the ICG fluorescence were comparable or superior to the RI method. Further studies have since been performed worldwide, adding to the pool data of ICG based SLN detection rates. Given its early days, there is no consensus on the best protocol for its clinical use in SLNB and how it will complement or replace other traditional methods of SLN mapping. In this review, we performed an updated meta-analysis to analyze SLN detection rates of ICG fluorescence compared to standard RI method and evaluated its clinically applicability.[5]

Methods

Protocol and registration

This systematic review was conducted according to guidelines set by Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).

Eligibility criteria

Inclusion criteria for the meta-analysis were studies evaluating women with early breast cancer who underwent SLN mapping using ICG and RI concurrently, with a documented SLN detection rate for both modalities. Retrospective and prospective studies were included. All studies published in English language with more than ten patients from January 2005 to June 2019 were included in this review. Editorial comments, case reports, reviews, meta-analyses and studies comparing other modalities including BD for SLN mapping were excluded.

Search strategy

Two investigators (JG and CY) performed the systematic search through PubMed (Table 1), Scopus (Table 2) and Cochrane library June 14, 2019. The two investigators screened publication titles and abstracts independently. Duplicate studies were excluded. Discrepancies between the two investigators were resolved following discussion with third investigator (ML). Full-text articles were then reviewed. An additional snowball search found three studies.

Data extraction and methodological quality

The following data were extracted from the selected studies: authors, year of publication, country of origin, type of study, number of participants, tracers, injected volumes, concentration and location, patient and tumor characteristics, detection device, ICG-related adverse reactions, average number of detected SLNs, number of patients with successful fluorescence imaging, measures of test performance of ICG fluorescence-guided SLNB including true-positive, and false-negative results.

The quality of each study was evaluated using the updated quality assessment tool for diagnostic accuracy studies (QUADAS-2). QUADAS-2 is the current version of QUADAS and the tool recommend for use in systematic reviews to evaluate the risk of bias and applicability of primary diagnostic accuracy studies.[16]

Statistical analysis

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 Review Manager software. The heterogeneity was considered significant at $I^2 > 50\%$ or $P < 0.05$. The publication bias of the studies included was explored by visual inspection of its funnel plot.

Results

Efficacy of ICG compared to RI in SLN detection

The literature search identified a total of ninety-five articles, out of which forty-one articles were read in full (Fig. 1). Having excluded twenty-two articles, nineteen studies[1,4,8,9,17–31] were included in the systematic review and meta-analysis. Only eleven studies[1,4,8,9,19,20,22,25,26,28,29] of those included in the analysis provided data for tumor positive SLN detection rates.

The nineteen studies evaluated 2301 patients from ten countries around the world. Sixteen were observational studies[4,8,9,17–26,28–30] and three were randomized controlled trials (RCT).[1,27,31] Twelve observational studies[8,9,17–22,24–26,28] used dual tracer with ICG and RI for SLN detection. Four observational studies[4,23,29,30] and one RCT[31] used triple tracer with ICG, RI and BD for SLN detection. One RCT[27] compared triple tracer (ICG + RI + BD) versus dual tracer (ICG + RI), another RCT[1] compared hybrid triple tracer (ICG + RI + BD) versus single tracer (RI) while the other RCT[31] compared triple tracer ICG:HSA + RI + BD versus ICG + RI + BD (Table 3). The dose, volume, concentration of ICG ranged from 0.025 mg to 10 mg, 0.2 ml–2 ml, 0.05 mM–5.4 mM respectively, injected either periareolar and/or peritumoral location. For detection, nine studies used PDE,[4,9,17,18,21,22,25,26,29] five used Mini-FLARE,[23,27,28,30,31] others used various fluorescence imaging

Table 1
Search strategy for PubMed.

(((“green, indocyanine”[MeSH Terms] OR “indocyanine” OR “fluorescence”[MeSH Terms]) AND ((“breast cancer” OR “neoplasm, breast”[MeSH Terms]) AND (“lymph node, sentinel”[MeSH Terms] OR “node, sentinel”[MeSH Terms]))))

Table 2
Search strategy for Scopus.

(INDEXTERMS(“indocyanine green”) OR INDEXTERMS(“fluorescence”) AND INDEXTERMS(“neoplasm, breast”) OR breast cancer) AND (INDEXTERMS(“node, sentinel”) OR INDEXTERMS(“lymph node, sentinel”))

systems namely visual navigator,[1] SPY elite,[8] IC-View,[19] Karl Storz VITOM camera[20] and Karl Storz IMAGE-S camera.[24] (Table 4).The mean number of SLNs removed ranged from 1.31 to 3.8 for ICG and 1.35–2.3 for RI (Table 3). Based on the nineteen studies, overall detection rate for SLN using ICG fluorescence ranged from 81.9% to 100% and for RI ranged from 85% to 100%. The detection rate for tumor-positive SLNs (sensitivity) based on the eleven studies which reported them ranged from 65.2% to 100% for ICG and 76.9–100% for the RI method (Table 3).[1,4,8,9,19,20,22,25,26,28,29] Based on four studies, the false negative rate (FNR) ranged from 0.0% to 34.7% for ICG and 0.0%–23.1% for RI.[4,8,19,22] Dual mapping ICG/RI method increased the sensitivity to >91.3% and decreased FNR to <8.7% compared to single modality with either RI or ICG (Table 3).[1,4,8,9,19,20,22,25,26,28,29]

Quality assessment and meta-analysis

The QUASAD-2 graphically represents risk of bias and applicability of each study (Table 5). During patient selection, 47% of studies had low risk of bias and 37% of studies had unclear risk of bias. Out of the selected studies, 58% had high risk of bias in carrying out the ICG (index) test and 42% high risk of bias in carrying out RI (reference) test. With regards to applicability of the studies, 90%, 53%, 100% of the studies had low concerns in patient selection, ICG (index test) and RI (reference test), respectively (Fig. 2).

This meta-analysis revealed no significant difference in SLN detection between ICG and RI in either the fixed effects model [odds ratio (OR) 0.90, 95% confidence interval (CI) 0.66–1.24] (Fig. 3) or the random effects model (OR 0.93, 95% CI 0.47–1.83) (Fig. 4). There was heterogeneity of SLN detection rate observed between nineteen studies, with $I^2 = 58\%$, $P = 0.003$. There was no significant difference between ICG and RI for tumor positive SLN detection (sensitivity) in either the fixed effects model (OR 1.23, 95% CI 0.73–2.05) (Fig. 5) or the random effects model (OR 1.17, 95% CI 0.43–3.17) (Fig. 6). There was heterogeneity of sensitivity observed between eleven studies, with $I^2 = 41\%$, $P = 0.09$. Dual mapping with ICG/RI was significantly better compared to single mapping with RI (OR 3.69, 95% CI 1.79–7.62) (Fig. 7) or single mapping with ICG (OR 3.32, 95% CI 1.52–7.24) in the fixed effects model (Fig. 8) with no heterogeneity between studies. ($I^2 = 0\%$, $P = 0.87$, P = 0.78). Visual symmetry of the funnel plots suggest no publication bias for SLN detection (Fig. 9) or sensitivity (Fig. 10) observed in the nineteen studies included in the meta-analysis.

Discussion

SLN detection, sensitivity, false negative rate (FNR) for ICG compared to RI

ICG have been one of the newer techniques favored by researches in view of complementing or replacing existing standard SLN detection methods of RI and BD. In this meta-analysis, the SLN detection rate using ICG ranged from 81.9% to 100% and sensitivity of ICG ranged from 65.2% to 100%. This is a much lower range compared to previous meta-analysis.[12,15] The SLN detection rate and sensitivity for RI remains comparable to previous reports.[12,15] Dual mapping ICG + RI method increased the sensitivity to >91.3% compared to single mapping with either RI or ICG.

The National Surgical Adjuvant Breast and Bowel Project randomized Phase 3 trial B32 results showed a 9.8% FNR with use of dual mapping (RI + BD) and is the current accepted standard.[32] Zhang et al.,[15] in their meta-analysis based on six studies showed a FNR of 8% using ICG fluorescence as single modality. In our meta-analysis out of the eleven studies[1,4,8,9,19,20,22,25,26,28,29] which evaluated sensitivity of ICG, only nine had completion axillary lymph node dissection (ALND)[1,4,8,18,19,22,24,25,27] and four of these studies stated FNR ranging from 0.0% to 34.7% for ICG and 0.0%–23.1% for RI.[4,8,19,22] Two other studies stated FNR for ICG,[20,28] however it is

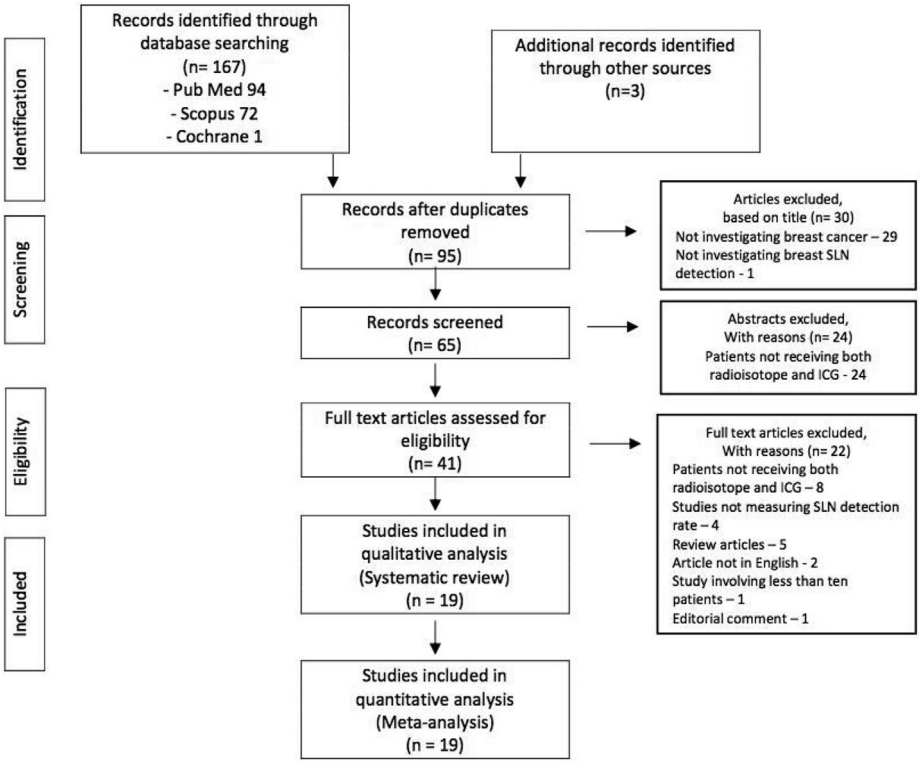

Fig. 1. Flow diagram showing identification of studies for inclusion in this meta-analysis according to PRISMA guidelines.

Table 3
Study and patient characteristics, sentinel lymph node detection rates and sensitivities of all included studies.

Author, Year, Country	Study	Number of SLNs	Number of patients	Tracers	Mean no. of SLNs detected per patient	SLN detection rate	Positive SLNs detection rate (sensitivity)	False negative rate (FNR)	Dual tracer (ICG + RI) sensitivity	Axillary lymph node dissection
Ballardini et al.[17] 2013, Italy	Cohort	246	134	ICG + RI	1.83 ICG 1.72 RI	100% ICG 99.3% RI	NA	NA	NA	no
Grischke et al.[9] 2015, Germany	Cohort	162	105	ICG + RI	1.31 ICG 1.50 RI	88.5% ICG 98.0% RI	92.6% ICG 100% RI	NA	100%	no
Hojo et al.[18] 2010, Japan	Cohort	NA	29 (group 2)	ICG + RI	3.8 ICG 2.0 RI	93.1% ICG 100% RI	NA	NA	NA	yes
Hutteman et al.[31] 2011, Netherlands	RCT	25	18	ICG + RI + BD vs ICG:HSA + RI + BD	1.4 both groups	100% ICG 92% RI	NA	NA	NA	yes
Jung et al.[1] 2014, Korea	RCT	NA	43	ICG + RI + BD vs. RI	3.4 (ICG + RI + BD) 2.3 (RI)	100%ICG 100% RI	100% ICG 100% RI	NA	100%	yes
Mazouni et al.[8] 2018, France	Cohort	NA	122	ICG + RI	2.0 ICG 2.0 RI	81.9% ICG 96.7% RI	65.2% ICG 91.3% RI	34.8%ICG 8.7% RI	>91.3%	yes
Mieog et al.[30] 2011, Netherlands	Cohort	35	24	ICG:HSA + RI + BD	NA	100% ICG 100% RI	NA	NA	NA	yes
Murawa et al.[19] 2009, Poland	Cohort	NA	20 (group 2)	ICG + RI	1.75 ICG 1.35 RI	100% ICG 85% RI	92.3% ICG 76.9% RI	7.7% ICG 23.1% RI	>92.3%	yes
Papathemelis et al.[20] 2018, Germany	Cohort (retrospective)	220	99	ICG + RI	2.2 ICG 1.7 RI	98% ICG 98% RI	100% ICG 95.2% RI	0.0% ICG[a] 4.8% RI[a]	100%	no
Polom et al.[21] 2012, Poland	Cohort (retrospective)	113	49	ICG + RI vs ICH:HSA + RI	2.0 ICG 2.0 RI	97.9% ICG 100% RI	NA	NA	NA	no
Rauch et al.[4] 2017, Austria	Cohort	249	98	ICG + RI+ BD	2.3 ICG 2.3 RI	95% ICG 99% RI	86.4% ICG 100% RI	16.6% ICG 0% RI	100%	yes
Samorani et al.[22] 2015, Italy	Cohort	589	301	ICG + RI	2.0 ICG 2.0 RI	98.7% ICG 95.3%RI	100% ICG 87.0% RI	0% ICG 13.0% RI	100%	yes
Schaafsma et al.[23] 2013, Netherlands	Cohort	48	32	ICG:RI + BD	ICG	100% ICG 100% RI	NA	NA	NA	no
Sorrentino et al.[24] 2018, Italy	Cohort	82	71	ICG + RI	1.1 ICG 1.0 RI	92.7% ICG 95.1% RI	NA	NA	NA	yes
Sugie et al.[25] 2016, Japan	Cohort	NA	821	ICG + RI	2.3 ICG 1.7 RI	97.2% ICG 97.0% RI	93.3% ICG 90.0%% RI	NA	97.2%	yes
Valente et al.[26] 2019, USA	Cohort	235	92	ICG + RI	2.4 ICG 2.2 RI	97.8% ICG 96.7% RI	100% ICG 95.8% RI	NA	100%	no
van der Vorst et al.[27] 2012, Netherlands	RCT	37	24	ICG + RI vs. ICG + RI + BD	1.6 (ICG + RI) 1.5 (ICG + RI + BD)	95.8% (ICG) 95.8% (RI)	NA	NA	NA	yes
Verbeek et al.[28] 2014, Netherlands	Cohort	177	95	ICG + RI	1.9 (ICG or RI)	98.9% ICG 97.9% RI	100% ICG 93.8% RI	0% ICG[a]	NA	no
Wishart et al.[29] 2012, United Kingdom	Cohort	201	100	ICG + RI + BD	1.93 (ICG) 1.5 (RI)	100% (ICG) 91.3% (RI)	100% (ICG) 100% (RI)	NA	100%	no

not a true FNR as the completion ALND was not performed. Dual mapping with ICG + RI method decreased FNR <8.7%. This demonstrates superiority of dual mapping with ICG + RI over dual mapping with RI/BD,[32] which is current standard practice. In addition, dual mapping with ICG + RI was significantly better compared to single mapping with RI or ICG. These findings certainly threaten the current gold standard and provides the basis to move towards dual mapping with ICG/RI, if validated by larger RCTs.

One could argue triple modality would further increase sensitivity and decrease FNR. A RCT performed by van der Vorst et al.,[27] comparing two groups of patients receiving RI + ICG versus RI + ICG + BD showed no additional benefit of using BD with ICG. ICG outperformed BD in all patients. In fact, they demostrated that BD may interfere with NIR fluorescence imaging by absorbing the fluorescent light and thereby decreasing detection of ICG positive nodes. The brightness of SLN and the signal-to-background ratio was much higher in the group of patients without BD, even though this wasn't statistically significant. Contrary to this Yuan et al.,[33] in 2018 performed a single center RCT in China enrolling 471 patients comparing RI + BD and with ICG + BD. This study was excluded from the review as there were no patients receiving both RI and ICG. This study showed no significant difference between patients receiving RI + BD and ICG + BD with regards to sensitivity (94.5% vs 92.5%), and FNR (5.6% vs 7.5%). Interestingly they went on to state that ICG can be used as an alternative to RI in SLNB in conjunction with BD further challenging current treatment paradigm.

Difference in administration of ICG and detection

Over the past 15 years researchers have continued to experiment various concentrations, dosages, administration sites and detection methods to optimise ICG utility. In a few studies there seems to be a discrepancy between the concentration and the dose of ICG mentioned (Table 4). Polom et al.,[21] stated during the preparation the concentration was 10 mg/dl. During the procedure they stated that each patient received 1 ml equivalent to 10 mg. If this prepared concentration was used and 1 ml was given the patient would have received 0.1 mg. Rauch et al.,[4] mentioned 2 ml of 0.5% ICG solution was used equivalent to 1 mg. If this concentration was used and 2 ml were given the patient would have received 10 mg.

The optimal dosage, volume and concentration have not been standardised. The most common volume and concentration used in nine studies[4,9,17,19,22,24–26,29] is 1–2 ml of 6.4 mM, ten-fold higher than optimal range. This is likely due to the ease of preparation of dilution of 25 mg ICG vial in 5 ml of sterile water. Mieog et al.,[30] reported an optimal injection dose of ICG:HSA (human serum albumin) ranged between 1.6 ml of 0.4–0.8 mM with other studies favoring 0.5 mM ICG alone.[27,28,31] The hybrid combination is not standard practice and the presence of HSA could alter its efficacy. Hutteman et al.,[31] in their RCT comparing ICG:HSA versus ICG alone demonstrated no direct benefit of premixing ICG with HSA prior to injection for SLN mapping in breast cancer patients, thereby reducing the cost and complexity of the procedure. Studies have shown that counterintuitively, higher injected ICG concentrations actually lead to worse detectability because of fluorophore "quenching."[28,34] That is, at too high a concentration, photons emitted by ICG are reabsorbed and are therefore not detectable. Verbeek et al.,[28] also showed that high concentration can also lead to increased flow to second-tier nodes, as reflected in the higher number of "SLNs" seen with higher injected doses. This highlights the importance of using optimal ICG concentration to prevent excising unnecessary nodes thus reducing patient morbidity. An argument can be made that visualization of lymphatic vessels are dose dependent. However, Murawa et al.,[19] demonstrated that although lymphatic vessels were visualised more clearly at higher doses, this did not increase the sensitivity of SLN detection because the tissue contrast appeared to decrease.

Periareolar ICG injection seems to be favored in twelve studies[1,4,8,19,20,23–27,31] over periareolar and/or peritumoral injection[9,17,18,21,22,28,30] given subcutaneously or intradermally between 1 and 4 sites. Detection using PDE[4,9,17,18,21,22,25,26,29] and Mini-FLARE[23,27,28,30,31] near-infrared (NIR) fluorescence techniques remain most common. A few recent studies[20,24] have advocated for full high definition laparoscopic camera coupled near-infrared system enabling to precisely identify fluorescent lymph nodes, distinguishing them from surrounding tissues. This would avoid unnecessary resection of peri-nodal fluorescent tissue which could lead to excision of other non-sentinel nodes and lymphatics resulting in increased morbidity. Moreover, the possibility of an intuitive switch between white light and ICG fluorescence avoids repeated lengthy interruptions of the surgical procedure for dimming the light to identify fluorescent nodes and lymphatic vessels.

Clinical applicability, advantages and disadvantages of ICG

Primary focus of SLNB is to identify those patients in whom the axillary nodes are clinically and radiologically negative but truly pathological and tumor positive leading to upstaging of disease. On the other hand, an essential intention of the sentinel node technique is to reduce the surgical trauma to the axilla and to minimize the removal of uninvolved lymph nodes. From this perspective using dual tracer method and removing unaffected nodes would be a disadvantage.[20] However, our study shows dual mapping with ICG/RI method increased the sensitivity to >91.3% and decreased FNR <8.7% compared to single modality with either RI or ICG. It is therefore important that surgeons understand the fine balance in this technique, which have implications on patient morbidity, upstaging of disease and adjuvant therapy.

Many advantages and its applicability of ICG have been highlighted compared to other modalities. ICG is cheap, quick, does not require any special licensing, storage, or handling procedures[11] and therefore particularly attractive to hospitals unable to work with radioactive isotopes and certain regional centers. The reoccurring average cost of the RI based method range between $331 – $420 per patient,[9,35] whereas the ICG method cost $5 - $111 per patient.[9,35,36] ICG initial investment for the operating unit could range from $76700 (PDE) - $270000 (SPY Elite)[36]; however a RI setup cost for a hospital without nuclear medicine facility already available would be more hefty. Compared to RI, it avoids exposing patients or health workers to ionizing radiation and remove the need for patients to undergo a preoperative injection in the breast and additional visits to the radiology department prior to surgery.[13] Moreover, these radioisotopes are a by-product of a contracting nuclear industry, and supply might become unpredictable with more widespread usage, particularly within emerging economies of Asia.[37] Multiple occasions of global shortage of technetium-99 m had been encountered and its poor sustainability highlights the potential problems and the need for exploration of alternatives. Non-radioactive agents such as ICG is warranted to maintain

BD, blue dye; *HSA*, human serum albumin; *ICG*, indocyanine green; *NA*, not applicable; *RI*, radioactive isotope, *RCT*, randomized control trial; *SLN*, Sentinel lymph node; *USA*, United States America.

[a] not true false negative rate given axillary lymph node dissection not performed.

Table 4
Indocyanine green-fluorescence dosing, concentration and device data for all included studies.

Author, Year, Country	Tracer	ICG dose	ICG volume	ICG concentration	Injected location	Device
Ballardini et al.[17] 2013, Italy	ICG + RI	5 mg	1 ml	5 mg/ml = 6.4 mM	peritumoral or periareolar	PDE
Grischke et al.[9] 2015, Germany	ICG + RI	10 mg	2 ml	5 mg/ml = 6.4 mM	periareolar in the quadrant of the tumor	PDE
Hojo et al.[18] 2010, Japan	ICG + RI	NA	2 ml	NA	peritumoral and periareolar	PDE
Hutteman et al.[31] 2011, Netherlands	ICG + RI + BD vs ICG:HSA + RI + BD	0.62 mg	1.6 ml	0.50 mM	Intradermal and periareolar in four sites	Mini-FLARE
Jung et al.[1] 2014, Korea	ICG + RI + BD vs. RI	0.18 mg	0.3 ml	0.6 mg/ml = 0.77 mM	periareolar	Visual navigator
Mazouni et al.[8] 2018, France	ICG + RI	5 mg	2 ml	2.5 mg/ml = 3.2 mM	periareolar in four sites	SPY elite
Mieog et al.[30] 2011, Netherlands	ICG:HSA + RI + BD	0.07 mg–1.4 mg	1.6 ml	0.05 mM–1 mM	peritumoral or periareolar	Mini-FLARE
Murawa et al.[19] 2009, Poland	ICG + RI	5–15 mg	1–3 ml	5 mg/ml = 6.4 mM	periareolar	IC-view
Papathemelis et al.[20] 2018, Germany	ICG + RI	3.3 mg	0.5 ml	0.77 mmol/L	subcutaneously periareolar in four quadrants	VITOM camera
Polom et al.[21] 2012, Poland	ICG + RI	10 mg[a]	1 ml	10 mg/dl	intradermally peritumoral or periareolar	PDE
Rauch et al.[4] 2017, Austria	ICG + RI+ BD	1 mg[a]	2 ml	5 mg/ml = 6.4 mM	intracutaneously periareolar tissue of the quadrant of lesion	PDE
Samorani et al.[22] 2015, Italy	ICG + RI	2 mg–6 mg	0.4 ml to1.2 ml	5 mg/ml = 6.4 mM	subcutaneously above tumor (unicentric) or periareolar (multicentric)	PDE
Schaafsma et al.[23] 2013, Netherlands	ICG:RI + BD	0·025 mg to 0·050 mg	0.2 ml	0.16 mM–0.32 mM	periareolar	Mini-FLARE
Sorrentino et al.[24] 2018, Italy	ICG + RI	7.5 mg	1.5 ml	5 mg/ml = 6.4 mM	subdermal periareolar	IMAGE-S camera + laparoscope
Sugie et al.[25] 2016, Japan	ICG + RI	5 mg	1 ml	5 mg/ml = 6.4 mM	periareolar	PDE
Valente et al.[26] 2019, USA	ICG + RI	4 mg–5 mg	0.8 ml –1 ml	5 mg/ml = 6.4 mM	subdermal periareolar	PDE
van der Vorst et al.[27] 2012, Netherlands	ICG + RI vs. ICG + RI + BD	0.62 mg	1.6 ml	0.50 mM	four site intradermally and periareolar	Mini-FLARE
Verbeek et al.[28] 2014, Netherlands	ICG + RI	0.62 mg	1.6 ml	0.50 mM = 0.39 mg/ml	periareolar or intradermally peritumoral	Mini-FLARE
Wishart et al.[29] 2012, United Kingdom	ICG + RI + BD	10 mg	2 ml	5 mg/ml	intradermally and subcutaneously periareolar 2OC and 10OC	PDE

BD, blue dye; *ICG*, indocyanine green; *Mini-FLARE*, Mini-fluorescence-assisted resection and exploration; *PDE*, photodynamic eye; *RI*, radioactive isotope.
[a] Discrepancy between published and calculated dose.

Table 5
Quality assessment tool for diagnostic accuracy studies (QUADAS-2) included in analysis.

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Ballardini, et al. [17]	?	☺	☺	☺	☹	☺	☺
Grischke, et al. [9]	?	☹	☹	☹	☺	☺	☺
Hojo, et al. [18]	☹	☺	☺	☺	☺	☺	☺
Hutteman, et al. [31]	☺	?	?	☺	☺	☹	☺
Jung, et al. [1]	☺	?	?	☺	☺	☹	☺
Mazouni, et al. [8]	?	☹	☹	☺	☺	☺	☺
Mieog, et al. [30]	☺	☹	☹	☺	☺	☹	☺
Murawa, et al. [19]	?	☺	☺	☺	☺	☹	☺
Papathemelis, et al. [20]	☺	☺	☺	☹	☹	☺	☺
Polom, et al. [21]	☺	☹	☹	☺	☺	☺	☺
Rauch, et al. [4]	☺	☹	☹	☺	☺	☺	☺
Samorani, et al. [22]	☺	☺	☺	☺	☺	☹	☺
Schaafsma, et al. [23]	☺	☹	☺	☺	☺	☹	☺
Sorrentino, et al. [24]	☹	☹	☺	☺	☺	☹	☺
Sugie, et al. [25]	☹	☹	☺	☹	☺	☺	☺
Valente, et al. [26]	?	☺	☺	☹	☺	☹	☺
van der Vorst, et al. [27]	☺	☹	☹	☺	☺	☺	☺
Verbeek, et al. [28]	?	☹	☹	☺	☺	☺	☺
Wishart, et al. [29]	?	☹	☹	☺	☺	☺	☺

☺ - low ☹ - high ? - unclear

comparable and reproducible sentinel node results instead of resorting to BD, which produces less favourable results compared to RI or ICG alone. Compared to BD, ICG doesn't stain the surgical field and does not cause unnatural skin pigmentation for many months.[27] BD cannot be visualised with overlying tissue where else NIR fluorescence mapping with ICG can be detected through millimetres to a centimeter of overlying tissue.[1,27,37] This allows lymphatic mapping prior to surgery which would decrease time to

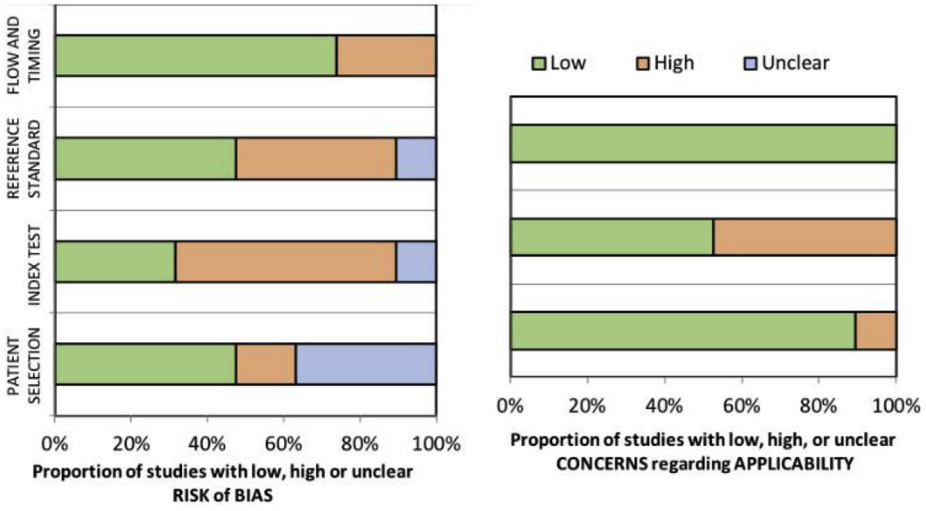

Fig. 2. Quality assessment tool for diagnostic accuracy studies (QUADAS-2) graphical representation.

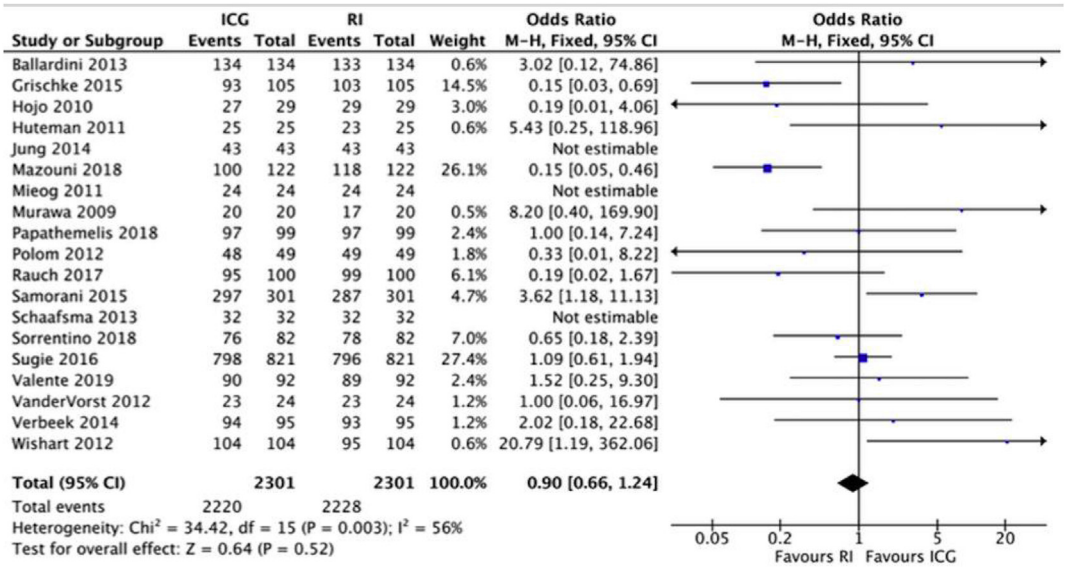

Fig. 3. Forrest plot for SLN detection ICG compared to RI (fixed effect model).

identify SLN.[11,27]

One of the main disadvantages of ICG that has been highlighted is its low molecular weight and hence rapid migration to higher lymph nodes.[11] This is evident in higher mean number of SLNs removed ranged from 1.31 to 3.8 for ICG compared to 1.35–2.3 for RI. Sugie et al.,[12] made a counter argument based on the fact that tumor cells bypassing the first SLN to reach the second or higher echelon nodes occurred in 11.1% of node-positive patients, suggesting that 2.3 is an acceptable number of SLNs to be excised. The other main disadvantage is spillage of ICG during dissection obscuring adequate surgical views. RI could be detected in tissues deeper than 2 cm whereas ICG is difficult to be detected at a depth of more than 1 cm.[1,11,21,27,37] Coufal and Fait[38] suggested that the problem is therefore not that ICG would be insufficiently transported into SLNs, rather, it is tissues' permeability to NIR fluorescence limiting visualization to a depth of 1 cm. German study by Grischke et al.,[9] suggested that Body Mass Index (BMI) > 40 is a limiting factor for using ICG in breast cancer; however in their recent study by Rauch et al.,[4] dedicated at investigating effect of BMI, refuted this claim. ICG also cannot be given to patients with iodine allergy. Side effects of ICG include nausea, vomiting, tachycardia, leucocytosis with no documented life-threatening side effects such as anaphylaxis when compared to BD.[9,25]

Certain practical aspects need to be considered when using ICG for SLNB. Timing of SLNB is critical as time greater than 30 min from the initial injection of ICG may affect movement of ICG to subsequent higher lymph nodes and affect outcome of the technique.[21] Along with this comes the sequence of the procedure in breast conservative surgery. It is important to perform the SLNB prior to wide local excision of tumor in non-mastectomy cases for two reasons. It allows SLNB to be performed within the ideal 30 min. It also prevents spillage of ICG that occurs during dissection of surrounding tissue and inevitable injury to the lymphatics leading to poor visualization of the SLN.[21] Two studies used gamma probe for SLNB prior to using

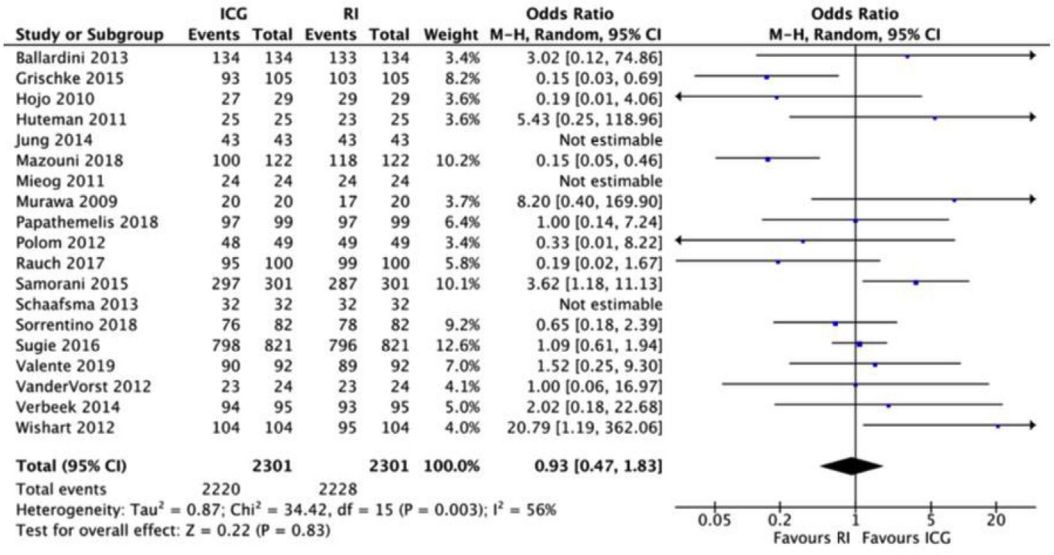

Fig. 4. Forrest plot for SLN detection ICG compared to RI (random effect model).

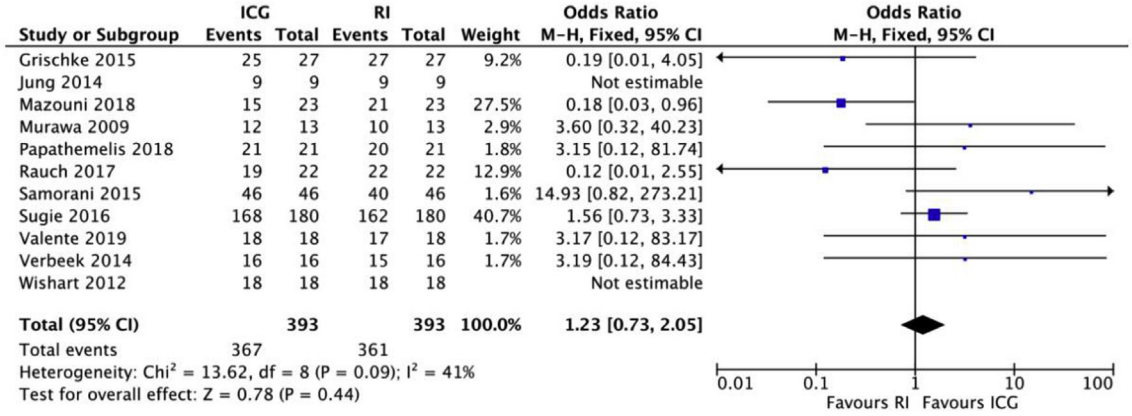

Fig. 5. Forrest plot for tumor positive SLN detection (sensitivity) ICG compared to RI (fixed effect model).

NIR fluorescence.[8,18] To prevent spillage of ICG during harvesting of SLNs it is beneficial to complete the SLNB based on NIR fluorescence mapping prior to using the gamma probe and ensure adequate clipping of all lymphatics during excision of the SLNs.

Quality assessment of studies

During patient selection 47% of studies had low risk of bias and 37% of studies had unclear risk of bias as the method of selection and inclusion criteria were not stated.[8,9,17,19,26,28,29] 58% of the selected studies had high risk of bias in carrying out the ICG (index) test as the gamma probe assisted RI localization was carried out prior to ICG-fluorescence based localization.[8,9,21,23–25,30] 42% of studies had high risk of bias in carrying out RI (reference) test as they used dual mapping with BD[4,27,29] or interchanged between ICG-fluorescence and RI lymphoscintigraphy during the same procedure to achieve best localization.[8,9,21,28,30] With regards to applicability of the studies, 90% and 100% of the studies had low risk of bias in patient selection and RI (reference) test respectively. 47% of studies had high risk of bias in the applying the ICG (index) test due to a variety of reasons which included usage of non-standard formulations containing all three (ICG + BD + RI) tracers,[1] hybrid ICG:HAS tracers,[30,31] dose variation within cohort,[19,22] and image enhancers.[24,26]

Strength and limitations of review

Strength of this review is that it discusses pitfalls to avoid during ICG-fluorescence and its clinical applicability based on all conducted ICG-fluorescence SLNB compared to RI. It provides the first meta-analysis demonstrating dual mapping with ICG + RI was significantly better compared to single mapping with ICG or RI. We performed first systematic review using QUADAS-2 tool recommend for use to evaluate the risk of bias and applicability of primary diagnostic accuracy studies and graphically presented the results for all included studies. One of the main limitations of the review is that the findings are based on the quality of nineteen studies; three RCTs[1,27,31] and sixteen cohort studies[4,8,9,17–26,28–30] which had no long term follow up. Therefore, the end points were restricted to be SLN detection rates and sensitivity of ICG and RI rather that more important outcome measures such as loco-regional recurrence and overall survival rate. Only eleven[1,4,8,9,19,20,22,25,26,28,29] out of nineteen studies measured sensitivity rate. FNRs were based on only four studies[4,8,19,22] out of nine studies which had ALND.[1,4,8,18,19,22,24,25,27] High risk of bias was introduced during the conduction of the ICG test in the studies which included hybrid tracers, gamma probe RI assistance for localization and dose variations within cohort. This was one of the other main limitations which could have had a significant impact on SLN detection rates and sensitivity.

With the introduction of new tracers, the optimal combination

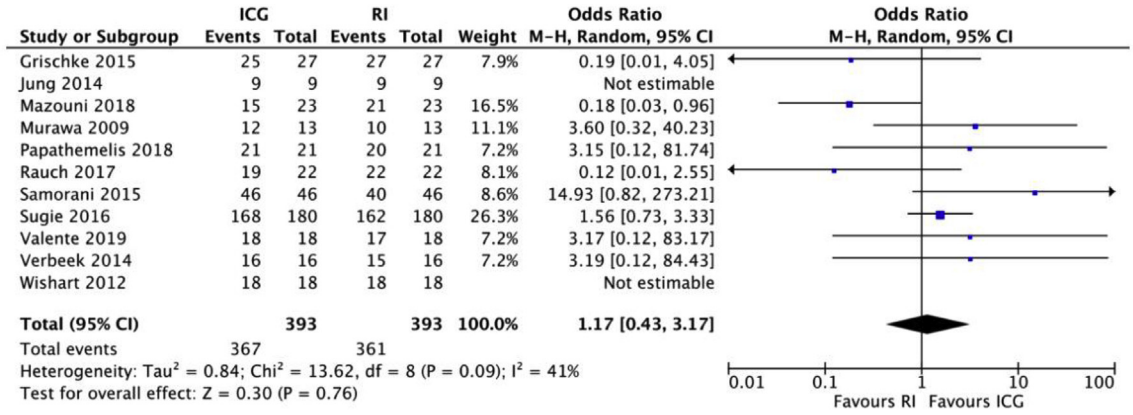

Fig. 6. Forrest plot for tumor positive SLN detection (sensitivity) ICG compared to RI (random effect model).

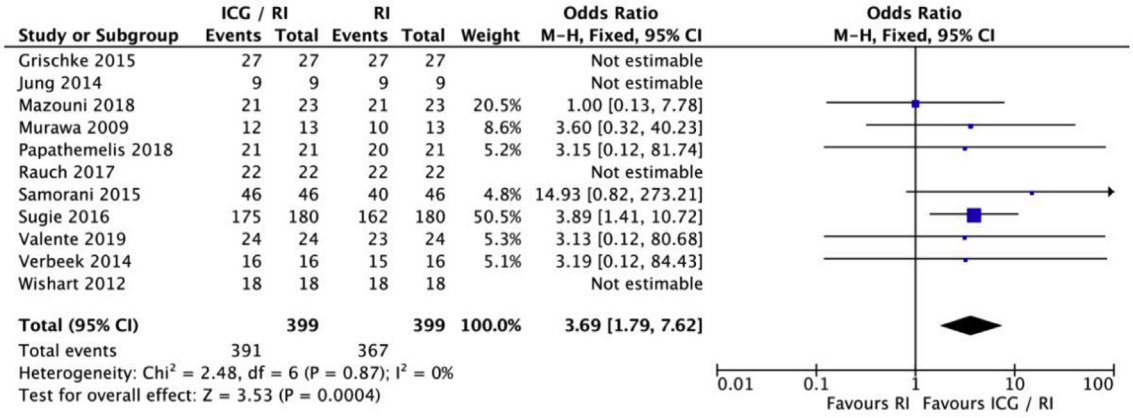

Fig. 7. Forrest plot for tumor positive SLN detection (sensitivity) for dual mapping (ICG + RI) compared to single mapping with RI (fixed effect model).

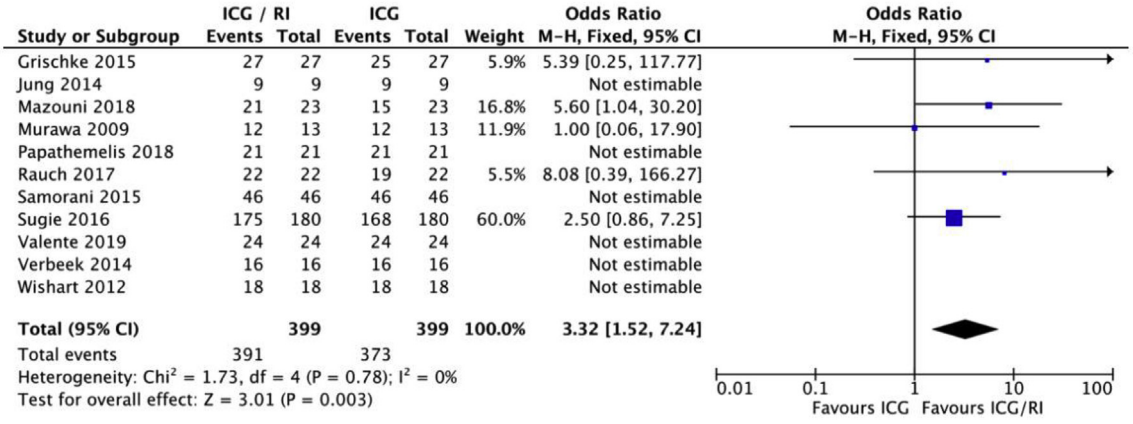

Fig. 8. Forrest plot for tumor positive SLN detection (sensitivity) for dual mapping (ICG + RI) compared to single mapping with ICG (fixed effect model).

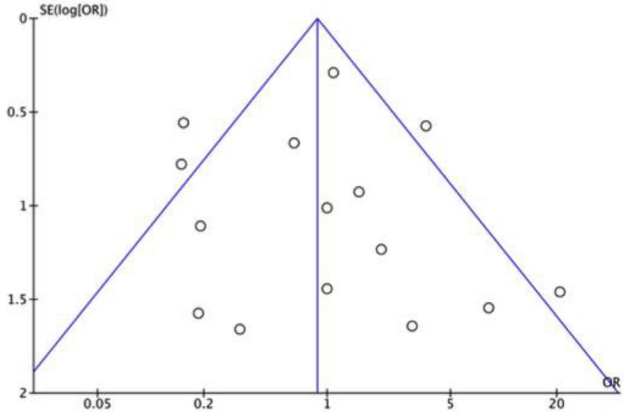

Fig. 9. Funnel plot for SLN detection.

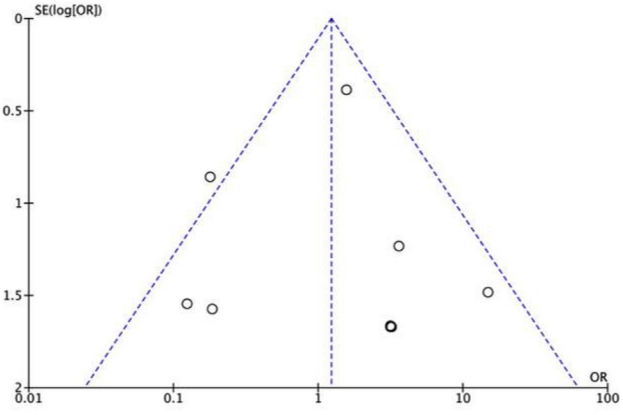

Fig. 10. Funnel plot for sensitivity.

of tracers for SLNB in early breast cancer is constantly under threat. More dual tracer RCTs with different combinations (ICG + RI versus RI + BD, ICG + BD versus RI + BD) would be beneficial to validate the optimal method. Future studies need to focus on FNR for ICG; either as single tracer or dual tracer to provide arguments for change of practice. However, ALND being not standard practice for most patients with early breast cancer calculating FNR would be difficult. Number of ICG-fluorescence cases previously performed by each surgeon involved in the trials needs to be incorporated to adjust for the learning curve. Performing ICG-fluorescence imaging prior to gamma probe RI lymphoscintigraphy and not interchanging between methods whilst performing SLNB will reduce the risk of bias in carrying out the ICG (index) test and improve its applicability. Cost-benefit analysis per patient including the setup cost for these combinations would be of high value for centers willing to trial newer techniques. Patient centered questionnaire focused on convenience of not having to undergo separate preoperative injection, effects of BD tattoo and other side effects could be

measured as secondary outcomes. Most importantly, monitoring these patients and analysing long term outcomes on loco-regional recurrence and overall survival would be valuable.

Conclusion

No statistically significant difference in SLN detection rates and sensitivity between ICG-fluorescence and RI was observed. However, sensitivity of dual mapping (ICG + RI) was significantly better compared to RI or ICG alone. The optimal ICG concentration was tenfold lower than what was used in most studies. ICG-fluorescence could complement RI method or provide alternative in centers with poor accessibility to RI lymphoscintigraphy. Future long-term multicenter RCTs with ICG-fluorescence as single or dual tracer would provide pivotal information for change in practice.

Declaration of competing interest

None.

Acknowledgements

None.

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