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Systematic Review

Efficacy of indocyanine green fluorescence for the identification of non-palpable breast tumours: systematic review

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Abstract

Background: Accurate tumour localization is crucial for precise surgical targeting and complete tumour removal. Indocyanine green fluorescence, an increasingly used technique in oncological surgery, has shown promise in localizing non-palpable breast tumours. The aim of this systematic review was to describe the efficacy of indocyanine green fluorescence for the identification of non-palpable breast tumours.

Methods: A systematic literature search was performed in PubMed, Embase, and the Cochrane Library, including studies from 2012 to 2023. Studies reporting the proportion of breast tumours identified using indocyanine green fluorescence were included. The quality of the studies and their risk of bias were appraised using the Methodological Index for Non-Randomized Studies ('MINORS') tool. The following outcomes were collected: identification rate, clear resection margins, specimen volume, operative time, re-operation rate, adverse events, and complications.

Results: In total, 2061 articles were screened for eligibility, resulting in 11 studies, with 366 patients included: two RCTs, three non-randomized comparative studies, four single-arm studies, and two case reports. All studies achieved a 100 per cent tumour identification rate with indocyanine green fluorescence, except for one study, with an identification rate of 87 per cent (13/15). Clear resection margins were found in 88–100 per cent of all patients. Reoperation rates ranged from 0.0 to 5.4 per cent and no complications or adverse events related to indocyanine green occurred.

Conclusion: Indocyanine green fluorescence has substantial theoretical advantages compared with current routine localization methods. Although a limited number of studies were available, the current literature suggests that indocyanine green fluorescence is a useful, accurate, and safe technique for the intraoperative localization of non-palpable breast tumours, with equivalent efficacy compared with other localization techniques, potentially reducing tumour-positive margins.

Introduction

The implementation of mammographic screening programmes and improvement of diagnostic methods have led to an increase in the diagnosis of early-stage breast cancer[1]. Such tumours are often small, non-palpable lesions. Breast-conserving surgery (BCS) followed by radiotherapy has been shown to be a safe alternative to a mastectomy and, currently, BCS is the preferred and most common treatment option for early-stage breast cancer[2]. The main goal of BCS is to attain safe and accurate removal of tumours while preserving satisfying breast cosmetic appearance and minimizing postoperative complications.

Accurate intraoperative localization techniques are essential for safe and successful surgical excision. During the past decades, different localization techniques for the detection of non-palpable breast tumours have been developed and evaluated. Depending on surgeons' preferences, expertise, and availability, localization methods vary among institutions. Currently, evidence-based methods include wire-guided localization (WGL), ultrasound-guided surgery, radioactive iodine or magnesium seed localization, and charcoal (or carbon) tattooing[3–6]. All techniques have several disadvantages, including patient discomfort (for example additional hospital visits and burdensome interventions), limited scheduling flexibility due to preoperative (time-consuming) tumour localization, displacement or migration of the marker, permanent skin pigmentation, high(er) medical costs, and exposure of patients and caregivers to radiation[4,7,8].

A promising novel technique for the localization of non-palpable breast tumours is the use of indocyanine green (ICG) fluorescence. ICG is a fluorescent dye, which is already

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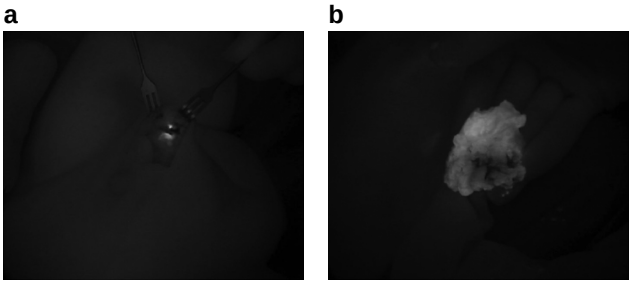

Fig. 1 Intraoperative images of indocyanine green-guided tumour localization after ultrasound-guided intratumoral indocyanine green injection

Intraoperative images of an example patient demonstrating the ICG-fluorescent tumour. **a** *In vivo*. **b** *Ex vivo*.

applied for various other medical indications (for example assessment of cardiac output, ophthalmic angiography, and liver surgery). In the context of breast cancer, ICG is used for sentinel lymph node (SLN) mapping, replacing existing SLN mapping techniques[9–12]. ICG is a safe and inexpensive contrast agent, providing continuous visual feedback regarding its localization when injected into a tumour before surgery[13,14]. Due to its obvious advantages, ICG is becoming increasingly popular in the field of oncological surgery. To perform ICG-guided tumour localization, ICG is injected intratumorally or peritumorally (or intravenously) after general anaesthesia and is detected using a near-infrared fluorescence imaging system (*Fig. 1*). Several studies have described the use of ICG for localization of non-palpable breast tumours and have reported promising results[15–18].

Therefore, the aim of this review was to systematically assess the literature and summarize data describing the efficacy of ICG fluorescence for localization of non-palpable tumours in patients with an indication for BCS.

Methods

This systematic review followed the PRISMA recommendations (www.prisma-statement.org) and was registered in PROSPERO, the international prospective register of systematic reviews (registration no. CRD42021265168), before conducting the research (1 July 2021). The main outcome of interest was the proportion of breast tumours identified using ICG fluorescence.

Search strategy

A systematic and comprehensive literature search was performed in three electronic databases: PubMed, Embase, and the Cochrane Library. Articles were included that were published from inception until May 2023 (*Fig. 2*). Two authors (B.A.M.J. and C.A.B.) and a clinical librarian (Nienke van der Werf, Knowledge and Information Centre, St Antonius Hospital, Nieuwegein, The Netherlands) designed an extensive search strategy (*Table S1*), which included the following essential components: 'breast tumour' and 'indocyanine green', with all relevant synonyms for both search terms. Duplicate articles were removed.

Study selection

The title and abstract of all studies were independently screened by two authors (B.A.M.J. and C.A.B.) for evaluation of inclusion and exclusion criteria. Studies were included when they addressed the intraoperative tumour localization rate in BCS. Tumour localization was defined as the identification of a breast tumour using intraoperative molecular imaging while the tumour was still present in the breast (that is before surgical removal). There were no restrictions on study design, considering the limited available literature on this innovative subject. Exclusion criteria included ICG used for purposes other than primary breast tumour localization, full text not available, written languages other than English or Dutch, review articles, conference abstracts, editorials and letters, and studies only including animals. A cross-reference check of reference lists of included articles and excluded reviews was performed to identify additional studies.

Quality assessment

The quality of each study was evaluated by two authors (B.A.M.J. and C.A.B.) independently, using the Methodological Index for Non-Randomized Studies (MINORS) tool (*Table S2*)[19]. The risk of bias was assessed by evaluating 8 items for non-comparative studies and 12 items for comparative studies. Items were scored as follows: 0, if not reported; 1, when reported, but inadequate; and 2, when reported and adequate. Higher scores reflect less risk of bias. The maximum MINORS score is 16 for non-comparative studies and 24 for comparative studies. For non-comparative studies, the cut-off points were less than or equal to 8 for poor quality, 9–14 for moderate quality, and 15–16 for good quality. For comparative studies, the cut-off points were less than or equal to 14, 15–22, and 22–24 respectively.

Funding sources were checked and reported (if applicable) for all included studies (see *Table 1*).

Data extraction and statistical analysis

Two authors (B.A.M.J. and C.A.B.) independently extracted data from the selected articles using a standardized data extraction table that was pilot-tested and improved accordingly. The following data were extracted: study and patient characteristics (*Table 1*), localization methods (*Table S3*), and study outcomes (*Table 2*). Outcomes of interest were identification rate, rate of tumour-free resection margins, specimen volume (ml), duration of operation (min), reoperation rate, adverse events, and complications.

Discrepancies in the screening process, data extraction, and quality assessment were resolved by discussion until consensus was reached. A third author (A.E.H.) was involved if no consensus was reached.

Descriptive statistics were used to describe characteristics and outcomes.

Compliance with ethical standards

This study was in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

Search results

The literature search was performed on 12 May 2023 and yielded 2061 studies. Of these, 676 duplicates were removed before screening (*Fig. 2*). The remaining 1385 articles were screened regarding their titles and abstracts, with the full text of 15 of these articles being assessed. The most common reason for record exclusion was a focus on ICG fluorescence for SLN biopsies. A total of 11 articles met the inclusion criteria and were selected for inclusion[15–18,21–24]. A cross-reference check revealed no additional articles. Additional and missing data were requested from the corresponding authors of three articles[17,21,22].

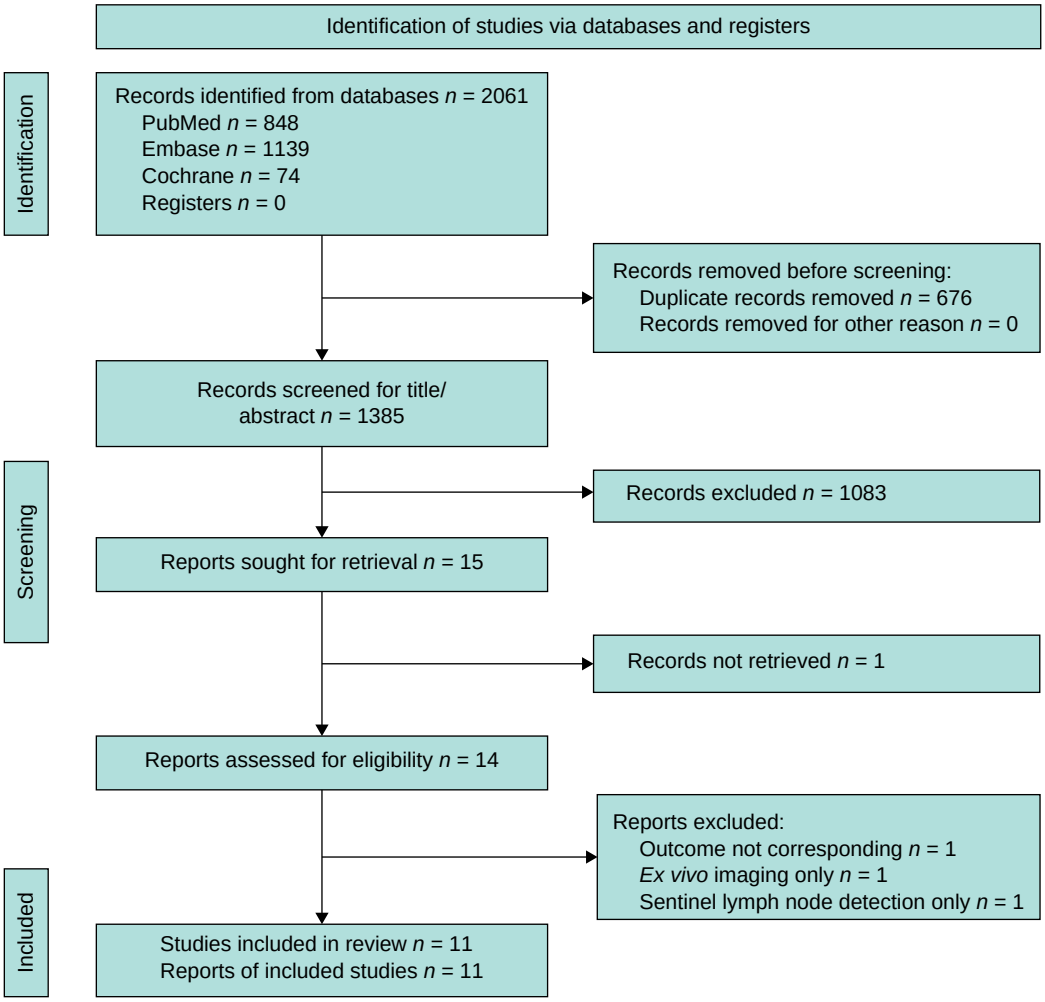

Fig. 2 PRISMA flow diagram

Flow chart of literature search and screening process.

Study characteristics and quality of evidence

All included studies were published between 2012 and 2023 (*Table 1*); they included five comparative studies (two RCTs[16,20], one non-randomized three-arm clinical trial[24], one cohort study with a historical control group[17], and one retrospective study with a control group[22]), three single-arm clinical trials[21,23,26], one single-arm retrospective cohort study[18], and two case reports[15,25]. One study was conducted across four medical centres[20], another study involved two medical institutions[24], and all others were single-centre studies. After evaluation using the MINORS tool, the quality of included studies ranged from 5 to 10 for non-comparative studies and from 11 to 17 for comparative studies (*Table S2*). Overall, six studies were classified as low quality and five as moderate quality.

Five studies received funding for their research project[17,20,21,24,26]. The funding sources had no role in collection, analysis and interpretation of the data, or submission of the manuscript.

Study populations

The 11 included studies evaluated 366 patients who underwent ICG-guided BCS for 366 breast tumours (*Table 1*). All patients were women aged between 24 and 79 years old. In one study[23], 6 of 12 patients had palpable lesions; all other studies only included patients with non-palpable lesions. Most lesions were biopsy-proven invasive carcinoma (311, 85.0 per cent). The five comparative studies compared ICG fluorescence with the use of ultrasonography, guidewire, or charcoal as the localization method.

Indocyanine green localization methods

Most studies (9 of 11) used an ICG solution for injection, with dosages ranging from 0.2 to 2 ml (0.5 to 10 mg). Two studies used an ICG/hyaluronic acid (0.1 mg ICG and 4 mg/2 ml hyaluronic acid) mixture for injection (*Table S3*)[20,24]. Most studies (9 of 11) performed an ultrasound-guided ICG injection in the centre of the lesion or in the tumour resection surface. Two studies administered an intravenous ICG injection at 2 or 24 h before surgery at a dose of 5 mg/kg[23,26]. *Table S3* lists the different camera systems used across the studies.

Main outcomes

Different definitions for tumour identification rate were reported, but all described the localization of breast tumours using ICG fluorescence imaging (*Table 2*). One study observed an identification rate of 86.7 per cent (13 of 15)[24]; all other studies achieved 100 per cent retrieval of tumours using the ICG method (*Table 2*).

Table 1 Main characteristics of included studies and patients

Reference	Year	Study design	No. of included patients	Control group	Age (years), mean (range)	Pathology results	Tumour size (cm), mean (range)
Aydogan *et al.*[15]	2012	Case report	2	N/A	59 (46–72)	IDC $n = 1$ DCIS $n = 1$	–*
Bang *et al.*[20]†	2023	Two-arm RCT	ICG group: 52 Control group: 56	0.3 to 1.0 ml activated charcoal (Charcotrace™, 40 mg/ml)	ICG group: 49.5 (28–70) Control group: 49 (31–75)	ICG group: benign $n = 12$, malignant $n = 40$ Control group: benign $n = 15$, malignant $n = 41$	ICG group: 1.0 (0.3–2.4) Control group: 1.1 (0.1–3.0)
Francini *et al.*[21]‡	2020	Single-arm clinical trial	10	N/A	–*	–*	–*
Guo *et al.*[22]	2022	Retrospective	ICG group: 42 Control group: 36	Ultrasound guided	ICG group: 48.6 (24–78)§ Control group: 51.4 (28–79)§	Invasive $n = 78$	ICG group: 1.36 (0.5–2.4) Control group: 1.48 (0.7–2.2)
Keating *et al.*[23]	2016	Single-arm clinical trial	12¶	N/A	60 (44–70)	IDC $n = 9$ ILC $n = 3$	1.7 (0.7–2.6)
Kim *et al.*[24]†	2021	Three-arm clinical trial	ICG group 1: 15# ICG group 2: 15# Control group: 14	0.3 to 1.0 ml activated charcoal (Charcotrace™, 40 mg/ml)	ICG group 1: 47.4 (37–57) ICG group 2: 44.2 (26–76) Control group: 52.2 (39–68)	ICG group 1: benign $n = 13$, malignant $n = 2$ ICG group 2: benign $n = 13$, malignant $n = 2$ Control group: benign $n = 13$, malignant $n = 1$	ICG group 1: 1.2 (0.6–2.8) ICG group 2: 1.3 (0.6–2.7) Control group: 1.0 (0.4–2.7)
Lee *et al.*[17]**	2021	Cohort with a historical control group	ICG group: 114 Control group: 300	Ultrasound-guided skin marking	ICG group: 52.43 Control group: 54.73	ICG group: DCIS $n = 12$, invasive $n = 102$ Control group: DCIS $n = 38$, invasive $n = 262$	ICG group: 1.3 (0.1–5.0) Control group: 1.6 (0.1–7.5)
Liu *et al.*[18]	2016	Retrospective	56	N/A	53.8 (34–78)	DCIS $n = 6$ Invasive $n = 50$	1.3 (0.6–2.1)
Muraoka *et al.*[25]	2022	Case report	1	N/A	66	DCIS	–*
Tong and Guo[16]	2019	Two-arm RCT	ICG group: 32 Control group: 30	Wire localization	ICG group: 51.3 (26–78) Control group: 53.6 (31–79)	Invasive $n = 62$	ICG group: 1.43 (0.6–2.4) Control group: 1.5 (0.8–2.5)
Wang *et al.*[26]††	2022	Single-arm clinical trial	43	N/A	56.3	DCIS $n = 2$ Invasive $n = 36$ Other (that is mucinous adenocarcinoma or micropapillary carcinoma) $n = 5$	≤2 cm $n = 29$ >2 cm $n = 14$

*Not reported. †Study was funded by Hanlim Pharm. Co., Ltd. ‡Study was funded by the Montpellier-Reine Breast Cancer Association. §Median (range). ¶A total of 6 of 12 tumours were palpable lesions. #ICG group 1, 0.1 ml; and ICG group 2, 0.2 ml. **Study was funded by the National Cancer Center. ††Study was funded by the Science and Technology Achievement Transformation Project (Wuhu Science and Technology Bureau). N/A, not applicable; IDC, invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; ICG, indocyanine green; ILC, invasive lobular carcinoma.

Clear resection margins were reported in 10 studies and were observed in 88–100 per cent of ICG-guided resections. Reoperation rates were reported in only three studies[17,18,23] and ranged from 0.0 to 5.4 per cent for the ICG localization method. With the exception of mild nausea after intravenous ICG injection in one patient, no adverse events or complications were reported by Keating *et al.*[23]. The mean specimen volume, described in four comparative studies[16–18,22], varied from 38.2 to 75.11 ml[17]. Duration of operation for the ICG localization method varied between 4 and 60 min[15–17].

Non-comparative studies

The case report by Aydogan *et al.*[15] described non-palpable lesion localization in two patients, one with a biopsy-proven malignancy and one with suspected malignancy. In both cases, the breast lesion was correctly localized and surgically removed based on ICG fluorescence. Specimen mammography and intraoperative frozen section examination was performed to confirm removal of the lesion. Histopathological examination confirmed invasive ductal carcinoma in the first case and ductal carcinoma *in situ* (DCIS) in the second. There was no tumour involvement at the surgical resection margins.

The case report by Muraoka and Kobayashi[25] described localization of non-palpable and ultrasonographically undetectable microcalcifications in a 66-year-old woman. Preoperative ICG injection enabled clear visualization and accurate resection of the lesion.

Francini *et al.*[21] described the results of a feasibility study including ten patients to determine the reliability of ICG by defining the distance between the ICG hotspot and tumour localization on the histological specimen. The ICG hotspot was in the 'centre' of the resected specimen in six of ten cases, but localization of the lesions was successful in all cases (100 per cent). Patient and tumour characteristics were not reported.

Keating *et al.*[23] performed a pilot clinical trial to evaluate the role of ICG in margin assessment in 12 patients, of whom six had non-palpable tumours. A total of nine patients were

Table 2 Study outcomes

Reference	Identification rate*	Clear resection margins	Specimen volume (ml), mean	Duration of operation (min), mean (range)	Reoperation rate	Adverse events/ complications	Follow-up, mean (range)
Aydogan *et al.*[15]	2/2 (100)	2/2 (100)	–†	47.5 (35–60)	–†	0	1 day
Bang *et al.*[20]	ICG group: 51/51 (100) Control group: 49/53 (92.5)	ICG group: 50/51 (98) Control group: 48/53 (90.6)	–†	–†	–†	ICG group: 0/50 (0) Control group: 16/52 (30.8) skin pigmentation	14–21 days
Francini *et al.*[21]	10/10 (100)	10/10 (100)	–†	–†	–†	0	–†
Guo *et al.*[22]	ICG group: 42/42 (100) Control group: 36/36 (100)	ICG group: 38/42 (90.5) Control group: 30/36 (83.3)	ICG group: 58 Control group: 73	–†	–†	–†	–†
Keating *et al.*[23]	12/12 (100)	12/12 (100)	–†	–†	0	Mild nausea after ICG injection in one patient	14.4 (7–19.2) months
Kim *et al.*[24]	ICG group 1: 13/15 (86.7) ICG group 2: 14/15 (93.3) Control group: 13/14 (92.9)	–†	–†	–†	–†	ICG group 1&2: 0/30 (0) Control group: 9/14 (64.8) skin pigmentation	7–17 days
Lee *et al.*[17]	ICG group: 114/114 (100) Control group: 300/300 (100)	ICG group: 102/114 (89.5) Control group: 225/300 (75)	ICG group: 75.11 Control group: 105.15	ICG group: 13 (4–28)	ICG group: 5/114 (4.4) Control group: 12/300 (4.0)	0	6 months
Liu *et al.*[18]	56/56 (100)	53/56 (94.6)	38.2	–†	3/56 (5.4)‡	–†	19 (6–38) months
Muroaka *et al.*[25]	1/1 (100)	1/1 (100)	–†	–†	0	0	2 days
Tong and Guo[16]	ICG group: 32/32 (100) Control group: 30/30 (100)	ICG group: 28/32 (87.5) Control group: 19/30 (63.3)	ICG group: 56 Control group: 74	ICG group: 31.4 Control group: 33.1	–†	0	–†
Wang *et al.*[26]	43/43 (100)	40/43 (93)	–†	–†	–†	–†	–†

Values are *n*/*n* (%) unless otherwise stated. *Definitions of identification rates: Aydogan *et al.*[15], successfully localized and surgically removed by observing the area of ICG-derived fluorescence; Bang *et al.*[20], successful visualization after localization of the excised specimen; Francini *et al.*[21], occult lesion localization on the skin; Guo *et al.*[22], retrieval of lesions as confirmed with imaging study to the breast specimen; Keating *et al.*[23], breast tumours positively identified using intraoperative molecular imaging *in situ*; Kim *et al.*[24], percentage of patients identified with localized target lesions for surgery; Lee *et al.*[17], not reported; Liu *et al.*[18], identification of ICG-guided localized target lesions; Muroaka *et al.*[25], visualization of injected lesion; Tong and Guo[16], retrieval of lesions as confirmed by imaging of breast specimens; and Wang *et al.*[26], preoperative fluorescence detection of primary tumour. †Not reported. ‡Two re-excisions and one mastectomy. ICG, idocyanine green.

diagnosed with invasive ductal carcinoma and three patients were diagnosed with invasive lobular carcinoma. In all patients, the breast tumour was identified using ICG fluorescence imaging and no tumour was found at the specimen margins.

Liu *et al.*[18] retrospectively analysed 56 patients with non-palpable breast tumours[18]. All lesions (100 per cent) were identified using ICG fluorescence. Re-excision was needed in three of 56 patients (5.4 per cent); two patients required re-excision due to DCIS-positive resection margins and, in one patient, a mastectomy was indicated due to multifocal invasive carcinoma.

Wang *et al.*[26] described the feasibility and accuracy of intravenous ICG injection for tumour localization and margin assessment in 43 patients. The fluorescent ICG was detected in all primary tumours and the surgical margins were free of tumours in 93 per cent (40 of 43) of patients.

Comparative studies

Bang *et al.*[20] recently conducted an RCT to compare the efficacy of an ICG/hyaluronic acid mixture with activated charcoal as the localization method. The study included 104 patients with non-palpable breast lesions, of whom 53 patients underwent ICG-guided BCS and 51 patients were assigned to the control group, utilizing activated charcoal for lesion localization. The localization rates for marking on the excised specimen were 100 per cent (51 of 51) for ICG-guided surgery and 92.5 per cent (49 of 53) for charcoal localization. The localization rates for marking on the breast (that is before surgical incision) were 98.0 per cent (49 of 51) and 88.2 per cent (45 of 53) respectively. No statistically significant differences in accuracy of resection were found. The primary endpoint was accuracy of resection, with 98.0 per cent (50 of 51) negative resection margins in the study group and 90.6 per cent (48 of 53) in the control group.

Guo *et al.*[22] retrospectively analysed 78 patients with non-palpable breast cancer undergoing BCS. Of all excisions, 42 were guided by ICG fluorescence and 36 by ultrasonography. Both resulted in a 100 per cent retrieval of lesions. The rate of clear resection margins was 91 per cent (38 of 42) in the ICG group compared with 83 per cent (30 of 36) in the

ultrasonography group. The mean specimen volume was 58 ml for ICG-guided excisions compared with 73 ml for ultrasound-guided surgery.

In a clinical trial conducted by Kim *et al.*[24], a comparison was made between an ICG/hyaluronic acid mixture (that is 0.01 mg ICG and 4 mg/2 ml hyaluronic acid) and activated charcoal as the localization method. A total of 44 patients, of whom 5 (11.4 per cent) were diagnosed with a malignancy, were assigned to the control group (14 patients), test group 1 (15 patients), or test group 2 (15 patients). Test groups 1 and 2 received 0.1 and 0.2 ml ICG/hyaluronic acid mixture respectively, with identification rates of 87 per cent (13 of 15) and 93 per cent (14 of 15) respectively. Kim *et al.*[24] did not mention whether failed lesion localization related to benign or malignant lesions. The primary outcome of the study by Kim *et al.*[24] was the accuracy of resection (that is the largest diameter of the excised specimen divided by the largest diameter of the preoperative lesion as detected by ultrasonography). The accuracy for test groups 1 and 2 was statistically significantly higher than the accuracy for the control group.

Lee *et al.*[17] described the largest cohort of patients who underwent BCS with ICG localization (114 patients) and compared these results with a retrospectively identified cohort who had undergone ultrasound-guided BCS (300 patients). The study by Lee *et al.*[17] included 50 patients (12.1 per cent) with DCIS and 364 patients (87.9 per cent) with invasive carcinoma. For both methods, the identification rate was 100 per cent. The intraoperative re-resection rate was 8.8 per cent (10 of 114) in the ICG group compared with 23.3 per cent (70 of 300) in the ultrasound-guided skin-marking group. The reoperation rate was 4.4 per cent (5 of 114) for ICG *versus* 4.0 per cent (12 of 300) for ultrasound-guided surgery. The mean resection size of the ICG-guided resections was 75 ml compared with 105 ml in the control group.

In one RCT[16], 62 patients with invasive carcinoma were included, of whom 32 underwent ICG localization and 30 underwent WGL. In two patients, wire displacement was observed in the operating room. These patients underwent ICG-guided surgery as an alternative. Both techniques resulted in a 100 per cent detection rate for non-palpable lesions. The rate of clear resection margins was 87.5 per cent (28 of 32) in the ICG group and 63.3 per cent (19 of 30) in the control group.

Discussion

This systematic review provides an overview of studies on the novel ICG-based method for tumour localization in BCS. Despite sparse literature and lack of essential details, this review includes 11 studies with 366 patients and suggests that ICG fluorescence provides an effective, accurate, and safe option for intraoperative localization of non-palpable breast tumours during BCS. ICG fluorescence has equivalent identification rates compared with other localization techniques and potentially reduces tumour-positive margins. ICG fluorescence allows more flexible scheduling of surgical procedures, less patient discomfort, reduction of costs, and omission of radioactive agents.

Two studies describe the use of (activated) charcoal as a simple and cheap method for tumour localization[20,24]. A charcoal suspension is injected into the breast lesion and a visible track is created while withdrawing the needle, leaving a tattoo on the skin. Although charcoal localization showed similar detection rates compared with ICG localization, permanent postoperative skin pigmentation is seen in nine of 14 patients (64 per cent)[24]. Also, in recent years, cases of charcoal granulomas have been reported. These lesions might mimic breast cancer during mammographic or ultrasonographic follow-up, resulting in misdiagnoses[27,28]. Therefore, charcoal localization can be of value for communities with low resources, but is inferior to ICG fluorescence in more prosperous communities[29].

Ultrasound-guided surgery is a patient-friendly method as there is no need for additional preoperative interventions. In a recent network meta-analysis[30], including 18 studies, ultrasound-guided surgery showed promising results regarding the reduction of positive margins and reoperation rates in breast cancer patients with non-palpable breast tumours. However, ultrasound-guidance has its restrictions as it is difficult to determine depth of the lesion in patients with large breasts, it has a long learning curve, and it can be hard to reconfirm localization during surgery due to the air layer. Re-excision rates up to 12.5 per cent have been reported[31,32]. In Athanasiou *et al.*[30], ICG fluorescence was described to be equally effective regarding lesion localization, but second best regarding tumour-free surgical margins. After the meta-analysis by Athanasiou *et al.*[30] was published in 2021, three more studies describing the important benefit of ICG fluorescence for tumour localization were published[17,22,24]. These studies showed substantial improvements in terms of tumour-free resection margins, reoperation rates, and resected tissue volume of ICG localization *versus* other localization techniques. One study compared ICG fluorescence with ultrasound-guided surgery[22], showing clear resection margins of 90.5 per cent for ICG compared with 83.3 per cent for ultrasound-guided excisions. The mean resected specimen volume was 58 ml for ICG-guided surgery compared with 73 ml for ultrasound-guided surgery. The mean difference of 15 ml suggests that excised tissue volume can be reduced using the ICG technique, without compromising surgical margin status.

WGL has been the historical gold standard for non-palpable tumour localization. WGL is effective regarding lesion localization, but reoperation rates range from 2.8 to 53 per cent, as a result of tumour-positive resection margins[33,34]. Also, WGL is associated with logistical challenges, wire dislocation, kinking or fracturing, and patient discomfort[3]. In this review, one study compared ICG localization with WGL[16], showing similar localization rates (100 per cent for both techniques).

In the Netherlands, the most commonly used method to localize non-palpable tumours is radioactive seed localization (RSL). This technique includes insertion of a radioactive seed into or near the breast lesion to localize the tumour using a γ probe[35]. The localization rate of RSL is similar to that of WGL[36] and a recent meta-analysis showed superior complete resection and reoperation rates of RSL compared with WGL[37]. With RSL, patients are still subjected to an additional hospital visit for seed insertion, have a risk of seed placement failure or migration, and are exposed to radioactive substances[38]. Moreover, when an axillar SLN procedure with ^{99m}Tc is performed, the radioactive signal of ^{99m}Tc overlaps with the signal of the iodine seed, potentially making it difficult for the surgeon to distinguish between the two radioactive signals. Although no studies have directly compared ICG fluorescence with RSL so far, previously mentioned advantages of ICG fluorescence are substantial and, therefore, it would be valuable to compare this novel technique with the current gold standard.

Despite ICG fluorescence showing localization rates similar to other conventional localization techniques for non-palpable breast tumours[15,18,21–24], and even higher rates of clear resection margins in some studies[16,17], ICG fluorescence has some clinical

limitations. Human breast tumours lack a well-defined capsule, leading to diffusion of the injected fluorescent dye into the tissue surrounding a tumour. This problem may be reduced by choosing the most effective dose and technique for ICG injection and reducing the time from injection to excision. High doses might increase the risk of altered visualization and blurred boundaries between a tumour and its surrounding tissue, whereas a lower dose might reduce tumour fluorescence. Injection volumes reported in the included studies ranged from 0.1 to 2.0 ml. Francini *et al.*[21] suggested 0.3 ml as the most effective dose associated with the least diffusion. The single study that did not report a 100 per cent detection rate for ICG utilized doses of 0.1 ml (detection rate 86.7 per cent) and 0.2 ml (detection rate 93.3 per cent) ICG-hyaluronic acid, administered 1 day or 1 h before surgery, suggesting that 0.2 ml was the most optimal dosage[24]. A subsequent study conducted by the same research group, using 0.2 ml, demonstrated a detection rate of 100 per cent[20]. The optimal dose classified by tumour size has yet to be determined, as well as the optimal timing for incision. Although ICG seems highly capable of localizing breast tumours, its lack of a tumour-specific interaction is a disadvantage. To better discriminate between malignant and normal tissue, conjugation of ICG with tumour-targeting peptides might be a solution[39]. These fluorescence-imaging agents are currently under investigation, to potentially improve outcomes by optimizing tumour-free resections[40]. Another challenge of ICG fluorescence may be the maximum penetration depth of approximately 1 cm[41]. This could cause difficulties in women with deeper-lying tumours. In cases where the lesion depth from the skin was greater than 10 mm, three studies suggested injection of an additional dose of 0.2 ml ICG solution in the overlying subcutaneous tissue to enhance visualization of fluorescence[15,18,22]. Furthermore, this new technique might involve a learning curve. The continuous visual feedback possibly shortens the surgical learning curve; however, this was not clearly described in the included studies. Only one study discussed the learning curve, suggesting that it takes approximately one use for the surgeon to familiarize themselves with this technique[23]. Finally, the requirement of a near-infrared fluorescence imaging device could be a drawback to institutions as the costs are approximately €65 000[23]. Fortunately, with the emerging use of ICG in multiple medical fields, the camera may be used for other indications. Moreover, medical costs of surgical procedures will be reduced by bypassing preoperative preparations in radiology departments and by the minor cost of the dye itself.

Importantly, different approaches for ICG injection were described in the included studies. Four studies[15,16,18,24] examined ICG injections into the centre of the tumour, whereas others evaluated small peritumoral injections[17,21] or systemic (intravenous) ICG[23,26]. Intravenous ICG injection is a non-targeted approach where ICG binds to serum proteins and acts as a macromolecule in the bloodstream, resulting in accumulation in tumour tissue due to relative hypervascularization. In this case ICG provides systemic distribution, potentially leading to enhanced permeability in other vascular structures as well. Consequently, determining the optimal dosage and timing are crucial. Intratumoral ICG injection delivers ICG directly to the targeted tumour, potentially offering more precise localization. According to Lee *et al.*[17], peritumoral injection enables visualization of the surgical resection margin planned by the surgeon[42].

A shortcoming of this review is the low to moderate quality of included studies and, therefore, the results of this review should be interpreted with care. Most studies included a small number of patients and only two RCTs have been performed[16]. Also, heterogeneity in definitions of tumour localization rate and study protocols (that is ICG posology) was observed among studies. Inconsistency in posology resulted in variations in administration, timing, and dosage of ICG, possibly affecting the detection rates.

Although this new technique seems promising when reviewing the existing literature, adequately powered prospective clinical trials are needed to confirm the effectiveness of ICG fluorescence and superiority over conventional localization techniques before safely implementing this novel technique. Also, future research focusing on optimal dosing and administration strategies is essential to standardize protocols in clinical practice.

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Author contributions

Britt A. M. Jansen (Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Visualization, Writing—original draft, Writing—review & editing), Claudia A. Bargon (Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing), Anne E. Huibers (Conceptualization, Methodology, Supervision, Writing—review & editing), Emily L. Postma (Conceptualization, Supervision, Writing—review & editing), Danny A. Young-Afat (Conceptualization, Supervision, Writing—review & editing), Helena M. Verkooijen (Conceptualization, Methodology, Supervision, Writing—review & editing), and Annemiek Doeksen (Conceptualization, Methodology, Supervision, Writing—review & editing).

Disclosure

The authors declare no conflict of interest.

Supplementary material

Supplementary material is available at *BJS Open* online.

Data availability

The search query is presented in *Table S1*. Template data collection forms are available from the corresponding author on request.

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